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Mussel seed is highly plastic to settling conditions: the influence of waves versus tidal emergence

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ABSTRACT: Phenotypic plasticity is important for organisms to adjust to a new environment. Therefore, the transplantation success of an organism to a new environment can be increased with knowledge of its capacity for phenotypic plasticity in different life stages, and the phenotypic adjustments it needs to make in specific environmental situations. Both the capacity for phenotypic plasticity and the necessary phenotypic adjustments for transplantation were tested in a mesocosm experiment using blue mussels *Mytilus edulis* as a model organism. This study tested (1) to what extent mussel seed coming from collectors in the water column are still capable of adjusting their phenotype, and (2) whether exposure to air or wave action is more important as a driver of phenotypic adjustments for mussels living in intertidal conditions. We found that mussel seed had a high capacity for phenotypic plasticity, and were capable of adjusting their morphology to accommodate different intertidal hydrodynamic conditions. Exposure to air influenced the shell shape, condition, byssal attachment strength and aggregation behaviour, but exposure to waves played the most important role in determining the phenotype of mussels. Wave-exposed mussels grew bigger, rounder, had thicker shells and a stronger byssal attachment strength than mussels exposed to either calm tidal or calm submerged environments. This knowledge is important for selecting a suitable source population and transplantation location.

KEY WORDS: Phenotypic plasticity · Phenotypic adjustment · Intertidal hydrodynamic conditions · Transplantation

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1. INTRODUCTION

Phenotypic plasticity, or the ability of a genotype to develop a phenotype well-adjusted to the environment, can be crucial for an organism to establish and persist in a new environment (Yeh & Price 2004, Fariñas-Franco et al. 2014). Understanding the capacity for organisms to adjust their phenotype to specific aspects of a new environment is essential for increasing the success rate of transplantation projects (Yeh & Price 2004, Weeks et al. 2011, Fariñas-Franco et al. 2014). Organisms may be transplanted to new envi-

ronments for multiple reasons, such as reintroduction of a lost population (e.g. wolves into Yellowstone National Park, USA; Bangs & Fritts 1996), commercial purposes (e.g. mussels in intertidal or subtidal zones; Kamermans et al. 2002) and more recently for the provision of specific ecosystem services (e.g. coastal protection by oysters; Coen et al. 2007, Scyphers et al. 2011).

Transplantation failure may be partly explained by the organisms having a phenotype mismatching the new environment, and a low plastic response (Fariñas-Franco et al. 2014). For transplantation pur-

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poses, it would be ideal to use a donor population that already possesses the phenotypic characteristics typical for the receiving habitat (Preston et al. 2010). However, access to such donor stock is seldom possible, either because they no longer exist, or the removal of individuals from an existing, comparable population would lead to unintended damages (Fariñas-Franco et al. 2014). In such cases, it is important to find a sustainable alternative source stock that possesses a high phenotypic plasticity, making it capable of adjusting to a new environment.

Low phenotypic plasticity can be either an inherent property of the organism, or the result of being in a life stage with a low plastic response. Phenotypic plasticity is likely to be age-dependent and to decline with age (Fischer et al. 2014, Nilsson-Örtman et al. 2015, Chevin & Hoffmann 2017). In their early life stages, organisms receive and interpret environmental cues that determine whether (and how) to invest in energetically expensive phenotypic adjustments that will increase their fitness. Later in life it is often less favorable to invest in phenotypic adjustments, as the costs are too high and the benefits may not be achieved before death (Fischer et al. 2014). Using juvenile life stages with a high potential for phenotypic plasticity (rather than adults) for transplantation purposes may therefore increase transplantation success.

Understanding how organisms adjust to a new environment, and which environmental factors are the drivers of these adjustments can prevent the transplantation of organisms with ill-adjusted phenotypes. However, because of the interactions among multiple environmental factors in natural systems, it is often difficult to disentangle which environmental factors are responsible for certain phenotypes, and to what extent. Experiments under controlled conditions can increase our understanding of an organism's capacity for phenotypic plasticity and the environmental drivers determining its phenotype. This knowledge is important for selecting a suitable source population and transplantation location. To increase transplantation success, it is therefore of utmost importance to gain knowledge about which specific phenotypic adjustments arise as a result of specific environmental factors, and if the age-class used still possesses a sufficiently high capacity for phenotypic plasticity to be able to adjust to these specific environmental conditions. We addressed both questions using blue mussels *Mytilus edulis* as model organisms, given the interest in mussel transplantations to the intertidal zone and the fact that previous intertidal transplantations of mature subtidal mussels have failed due to phenotypic mismatching (de Paoli et al. 2015).

Blue mussels are a typical example of organisms that can occur in a wide range of geographical sites and at different positions on the shore where they are exposed to a wide variety of abiotic and biotic conditions (Steffani & Branch 2003, Telesca et al. 2018). Multiple studies have shown that mussels can inhabit this diverse range of environments due to their phenotypic plasticity (Reimer & Tedengren 1996, Beadman et al. 2003, Telesca et al. 2018). A clear distinction is often made between mussels inhabiting the intertidal versus the subtidal zone. In the intertidal zone, mussels are exposed to the air and tidal flow and, at many exposed shores, also to wind-generated waves. In comparison, hydrodynamic conditions are often less dynamic in the subtidal zone (Gaylord et al. 2003, Carrington et al. 2008). Mussels can inhabit wave-exposed or sheltered shores and occur at different shore elevations varying in emersion time (Brinkman et al. 2002). This broad range of hydrodynamic conditions may result in strong morphological differences between mussels. Compared with intertidal mussels, subtidal mussels have lighter shells and a weaker byssal attachment strength (de Paoli 2017). Mussels on highly wave-exposed shores can have a stronger attachment strength (Witman & Suchanek 1984, Hunt & Scheibling 2001, Carrington et al. 2008), thicker shells (Steffani & Branch 2003) and a more streamlined shell shape (Seed 1968, Steffani & Branch 2003, Fariñas-Franco et al. 2014) than mussels on sheltered shores without high-energy wind-generated waves.

In the Dutch Wadden Sea, transplantations of subtidal mussels to the intertidal mudflats were carried out to restore intertidal mussel beds (Ens & Altling 1996, de Paoli et al. 2015). However, due to their relatively mature stage at transplantation, the subtidal mussels were not able to adjust their morphology to the harsh intertidal conditions, and the mussels failed to settle (de Paoli 2017). Consequently, de Paoli (2017) suggested that juvenile mussels could potentially be a better donor source for intertidal transplantations, as young mussels may still possess a sufficiently high capacity for phenotypic plasticity, making them capable of adjusting to a new environment. In the Eastern Scheldt estuary in the SW delta of the Netherlands, most intertidal mussel transplantations are done for commercial purposes and are mainly situated in sheltered areas (Dame et al. 1991). Mussel farmers are currently increasing their use of seed mussel collectors (SMCs) as a source of mussels rather than dredging the seed from natural beds (Capelle 2017). These SMCs consist of ropes hanging in the water column on which mussel spat can settle.

The commercial techniques available for collecting mussel seed makes it a potential valid and sustainable donor population for transplantation purposes. Mussel seed collected from these SMCs can subsequently be transplanted to either other subtidal areas or intertidal areas (the focus of this study). Moreover, mussels originating from collectors in the water column are normally still relatively young (i.e. 4 mo old) and may therefore be highly plastic (Seed 1968, Fischer et al. 2014, de Paoli 2017). However, it is not fully clear yet (1) to what extent mussels in this age class are capable of adjusting their phenotype, and (2) whether the most important driver of specific phenotypic adjustments for living in intertidal conditions is exposure to waves, or to air.

In this study, we tested the extent to which *Mytilus edulis* seed originating from SMCs in the water column is still capable of adjusting its morphology and behavior to hydrodynamic conditions typical of the intertidal zone. In addition, we tested whether wave exposure or exposure to air is most important in driving specific phenotypic adjustments. A mesocosm experiment was performed to distinguish between phenotypical adjustments induced by exposure to high wave energy (as present in many tidal systems) and those by exposure to air in combination with tidal flow (present in each tidal system). We hypothesized that mussel seed coming from collectors in the water column is highly plastic and still capable of adjusting its morphology to exposure to waves and to air, making it a highly suitable source for intertidal transplantation purposes. We also expected that exposure to waves has a stronger effect on mussel characteristics than exposure to air.

2. MATERIALS AND METHODS

2.1. Experimental setup

The mussel seed used in the present study was harvested in September 2015 from SMC-ropes that had been deployed for 6 mo in the Eastern Scheldt in the Netherlands near Neeltje Jans. To test mussel seed plasticity as a response to different hydrodynamic conditions, mussel seed was placed in tanks and exposed to 3 different hydrodynamic regimes for a period of 4.5 mo (134 d). The 3 hydrodynamic regimes included (1) calm, submerged (CS) conditions, (2) calm, tidal cycle (CT) conditions without waves and (3) wave-exposed, submerged (WS) conditions. Each of these treatments was carried out in triplicate, resulting in 9 experimental units.

CS conditions. The CS condition treatments were carried out in $1 \times 1 \times 1$ m tanks filled with 900 l of seawater. Water motion was created by a continuous in- and outflow of water with a steady flow rate of $6 \text{ l h}^{-1} \text{ tank}^{-1}$.

CT conditions. The CT condition treatments were carried out in tanks similar to the CS treatments. The tidal cycle in this treatment consisted of a sequence of 8 h immersion followed by 4 h exposure to the air twice every day. In this way, the mussels were submerged for 66.7% and exposed to air 33.3% of the day, representing the tidal regime of intertidal commercial mussel plots in the Eastern Scheldt (Capelle et al. 2014), and corresponding to the preferred immersion time of natural intertidal mussel beds in the Wadden Sea presented by Brinkman et al. (2002). Emptying and filling the tanks with water took approximately 2 h for a volume of approximately 900 l. Based on a ground surface of 1×1 m, this translates to an average flow rate of $0.00125 \text{ cm s}^{-1}$. This flow rate was negligible compared to normal tidal currents, which often reach up to 30 cm s^{-1} (Bouma et al. 2005).

WS conditions. The treatment with wave exposure was carried out in a piston-paddle wavetank measuring 85×205 cm that was filled with water to 50 cm, resulting in a water volume of 902 l. In the WS treatment, wave force was created with a wavepaddle moving in a horizontal motion. This created water movement strong enough to sway the seeded mussels, but not strong enough to dislodge them. The continuous movement of the paddle produced waves with a crest height of approximately 5–10 cm. Using an acoustic Doppler velocity meter (ADV) placed in the centre of the wave tank above the mussels, the average maximum orbital velocity in all 3 wavetanks was measured at $0.6 \pm 0.006 \text{ m s}^{-1}$. This orbital velocity can be considered congruent to a high-energy environment according to the Dutch Ministry of Infrastructure and the Environment (Rijkswaterstaat), which considers a high-energy intertidal location as one with an orbital velocity above 0.2 m s^{-1} (Bouma et al. 2006).

All 9 experimental tanks were filled with a layer of sand 10 cm deep to simulate the natural mudflats in the Netherlands. Each tank contained an airstone, 3 cm in diameter, to keep all tanks continuously aerated. In addition, the water levels in all tanks were regulated with an inflow and outflow port, supplying seawater from the Eastern Scheldt with a steady flow rate of $6 \text{ l h}^{-1} \text{ tank}^{-1}$. Although the water of the Eastern Scheldt probably contained some food particles, to ensure sufficient food availability, mussels were fed daily with 50 ml of instant algae (shellfish diet 1800; Reed Mariculture) at a concentration of 2 billion cells

ml⁻¹ tank⁻¹. A data logger (HOBO pendant temperature/light data logger UA-002-08) was installed into every experimental unit to monitor the temperature (°C) and light intensity (lux) every hour. The experiment took place in a glasshouse so that the tanks were exposed to daily light and temperature changes. Average (\pm SE) temperature over all tanks was 10.67 \pm 0.24°C during the day (06:00–17:59 h) and 10.06 \pm 0.21°C at night (18:00–05:59 h). The average light intensity was 1127 \pm 145 lux during the day and 3.5 \pm 1.0 lux at night.

A subsample of 100 SMC mussels was taken to measure initial shell length, width and height and to determine the condition of the mussels before the experiment began. The initial shell thickness was measured for 30 SMC mussels (see Table 1). At the start of the experiment, 5 kg of mussel seed was dispersed homogeneously over 1 m² in the CT and CS tanks. As the bottoms of the WS tanks had different dimensions from the CT and CS tanks, the mussels were seeded in the middle of the wave tank in a rectangle of 0.85 \times 1.20 m to ensure similar mussel densities in each tank.

2.2. Sampling

Final measurements were taken on Day 134, when no further changes in mussel patch shape or location were observed. To do this, 25 mussels from every tank were randomly chosen and measured for byssal attachment strength, mussel condition, and shell length, width, height, and thickness, resulting in a total of 225 mussels.

Byssal attachment strength. The byssal attachment strength of the 25 selected mussels from every tank was measured using a digital gauge with an accuracy of 0.02 N (Zauter FK50). A tick tweezer hanging on fishing wire attached to the digital gauge was carefully clamped around each mussel. The maximum force (in N) needed to detach the mussel was measured by pulling the force meter in a 180° direction from the point of attachment. The detachment force was corrected for the wet weight of each mussel by subtracting the weight of the mussel from the attachment strength (in g) followed by a conversion to N. Byssal attachment strength (in N) was divided by the condition index (CI; in mg cm⁻³) for every mussel to take the potentially strong correlation between byssal attachment strength and the condition of a mussel into account (Babarro et al. 2008, Lachance et al. 2008).

Shell morphology. After detachment, every mussel was individually measured for shell length (L , maxi-

mum distance from the umbo to the opposite shell edge), shell width (W , maximum distance between the left and right closed shells) and shell height (H , maximum dorsal–ventral distance). Measurements were done with a digital caliper with 0.1 mm accuracy (Mitutoyo Absolute IP67). To compare the shell shape between the 3 treatments, linear shell measurements were calculated following the method of Fariñas-Franco et al. (2014): (1) $H:L$ ratio, as a measure of shell elongation; (2) $W:H$ ratio for shell inflation; and (3) $W:L$ ratio, as a measure of shell obesity. Shell thickness was measured in the center of the adductor muscle scar of the right valve using a Hogetex point micrometer 0–25 mm with 0.001 mm accuracy.

Condition and mortality. The ash free dry weight (AFDW) for each mussel was obtained by drying the flesh of the mussel at 70°C and ashing it at 560°C until the difference in weight was less than 1% per 30 min. The CI (in mg cm⁻³) for each mussel was calculated via AFDW l⁻³ (Beukema 1976). Mussels that died during the experiment were counted and removed weekly to prevent pollution of the water and to keep track of mortality.

Patch characteristics. At the end of the experiment, every tank was photographed from above to determine the aggregation behavior of the mussels under different hydrodynamic conditions. Mussel patch characteristics were analyzed in the photographs by converting mussel patches into polygons to calculate the number of patches (NP), the average perimeter (P) to area (A) ratio ($P:A$) of the patches and the within-patch density (WPD), which was calculated as NP / A for each experimental tank, using the program image-J v.1.52a (National Institutes of Health).

2.3. Statistical analysis

All statistical analyses were carried out using R Studio (R Core Team 2018). Prior to model fitting, all data were visually validated for normality ($Q-Q$ plot) and homogeneity of residuals. In all models, the hydrodynamic treatment was used as the predictive factor. To determine if the experimental tank should be incorporated in a model as a random factor, Akaike's information criterion (AIC) was used to compare models with and without random factors, and the best fitting model was selected. Based on the validation for normality and homogeneity of residuals, assumptions and the selection of the best fitting model, linear shell measurements and the $P:A$ ratio of the mussel patches were analyzed using 1-way ANOVA models with a Gaussian distribution. The byssal attachment strength,

the CI data and the attachment strength corrected for CI data (i.e. attachment strength divided by CI) followed the normality and homogeneity assumptions after a log transformation and were further analyzed using 1-way ANOVA. Shell thickness data was log-transformed and analyzed with a linear mixed model (LMM) with the experimental tank as a random factor. Mortality data could not be analyzed with a Gaussian model, and a generalized linear model (GLM) was used with a quasi-Poisson distribution to correct for overdispersion. Pairwise comparisons were made with a Tukey test (package 'multcomp'; Hothorn et al. 2008) for the 1-way ANOVAs and Tukey adjusted comparisons of estimated marginal means (package 'lsmeans'; Lenth 2016) for the LMM and GLM.

3. RESULTS

3.1. Condition and byssal attachment strength

The mean initial CI of a subsample of 100 mussels was $2.21 \pm 0.74 \text{ mg cm}^{-3}$ at the start of the experiment

(Table 1). This CI decreased slightly for mussels placed in the CS (by 12%) or CT conditions (by 18%), but increased in the WS conditions by 61%. The hydrodynamic treatment had a significant effect on the condition of the mussels (1-way ANOVA, $F_{2,220} = 199.7$, $p < 0.001$). The CI was significantly higher for mussels in the WS treatment than for mussels in the CT (Tukey, $p < 0.001$) or CS (Tukey, $p < 0.001$) treatments. The byssal attachment strength was also stronger for mussels in the WS treatment ($p < 0.001$) than for mussels in CT or CS treatments (Tukey, $p < 0.001$). Mussels in the CT tanks had a significantly stronger byssal attachment strength than the mussels in the CS tanks (Tukey, $p < 0.001$). A strong positive correlation was found between byssal attachment strength and the CI of the mussels ($R^2 = 0.49$, $F_{1,213} = 201.5$, $p < 0.001$) (Fig. 1A). However, even after correction for condition (i.e. attachment strength divided by CI), byssal attachment strength was still found to be stronger for mussels exposed to waves (WS) compared with mussels placed in calm conditions (both CT and CS: Tukey, $p < 0.001$). CT mussels also had a stronger attachment strength than CS mussels (Tukey, $p < 0.001$) (Fig. 1B).

Table 1. Average \pm SD values of corporal parameters shell length, width, height, thickness, and condition index (CI) of *Mytilus edulis* mussel seed at arrival ($t = 0$) and after 134 d being subjected to calm submerged (CS), calm tidal cycle (CT) or wave-exposed submerged (WS) conditions. Numbers in parentheses: sample size (no. of mussels measured)

	Hydrodynamic treatment	Shell length (mm)	Shell width (mm)	Shell height (mm)	Shell thickness (mm)	CI (mg cm^{-3})
$t = 0$		31.07 ± 1.42 (100)	10.52 ± 0.79 (100)	15.61 ± 0.89 (100)	0.251 ± 0.026 (30)	2.21 ± 0.74 (100)
$t = 134 \text{ d}$	CS	34.78 ± 3.13 (75)	11.81 ± 1.22 (75)	17.10 ± 1.52 (75)	0.246 ± 0.043 (75)	1.94 ± 0.47 (75)
	CT	34.69 ± 2.57 (75)	12.00 ± 1.12 (75)	17.15 ± 1.28 (75)	0.252 ± 0.035 (75)	1.82 ± 0.45 (75)
	WS	36.00 ± 3.08 (73)	12.57 ± 1.23 (73)	17.66 ± 1.51 (73)	0.273 ± 0.059 (73)	3.63 ± 0.73 (73)

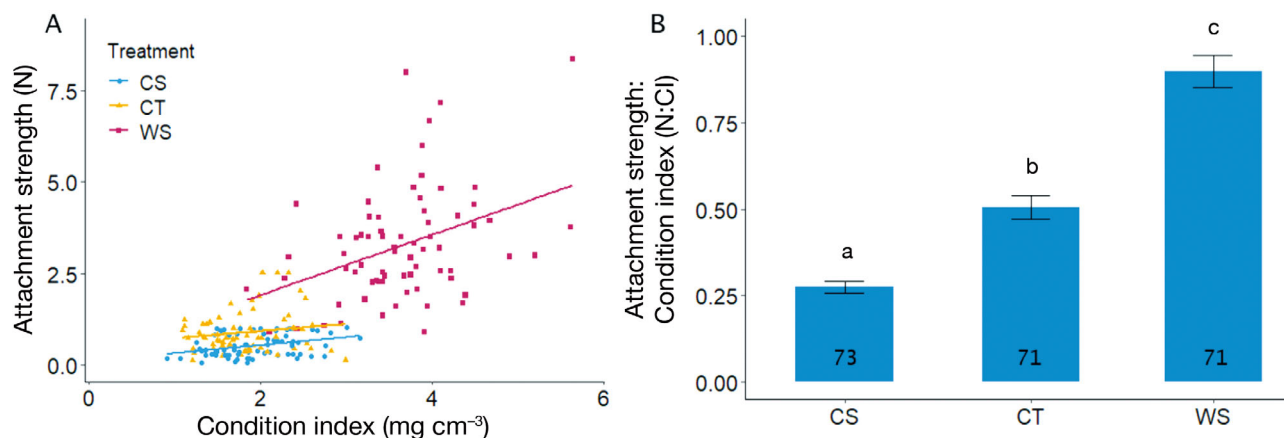


Fig. 1. (A) Correlation between byssal attachment strength and condition index (CI) of *Mytilus edulis* mussels coming from seed mussel collectors subjected to 3 different hydrodynamic treatments: calm submerged (CS), calm tidal cycle (CT), or wave-exposed submerged (WS) conditions. (B) Attachment strength divided by CI per hydrodynamic condition. Numbers inside bars: number of mussels measured; different letters above bars: significant differences among treatments. Data are means \pm SE

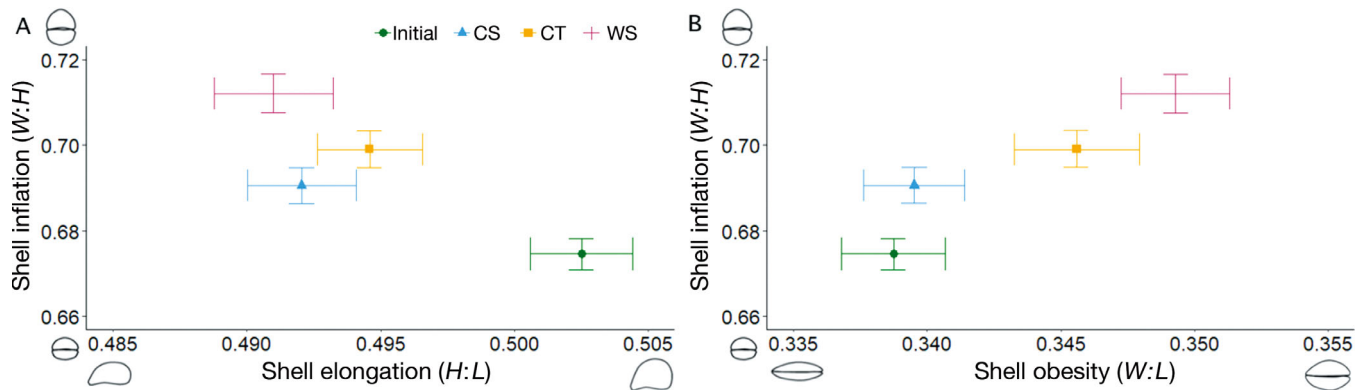


Fig. 2. Linear morphometric plots for *Mytilus edulis* mussels coming from seed mussel collectors. Data are means (\pm SE) of initial measurements ($n = 100$ mussels) and after 134 d being subjected to calm submerged (CS; $n = 74$ mussels), calm tidal cycle (CT; $n = 75$ mussels), and wave-exposed submerged (WS; $n = 73$ mussels) conditions. (A) Width:height ($W:H$) ratio (inflation) against height:length ($H:L$) ratio of mussel shells (elongation); (B) $W:H$ ratio (inflation) against $W:L$ ratio (obesity)

3.2. Shell morphology

Mean (\pm SD) mussel length in the subsample taken prior to the experiment was 31.07 ± 1.42 mm, width was 10.52 ± 0.79 mm, and height was 15.56 ± 0.89 mm (Table 1). Average shell measurements increased in all treatments, but the increase was highest in the WS treatment (Table 1). Hydrodynamic treatment had a significant effect on shell length (1-way ANOVA, $F_{2,220} = 4.546$, $p = 0.012$), height (1-way ANOVA, $F_{2,220} = 3.394$, $p = 0.035$), width (1-way ANOVA, $F_{2,220} = 7.927$, $p < 0.001$), and thickness (LMM, $F_{2,222} = 4.718$, $p = 0.010$). Mussels from the WS treatment had significantly longer (Tukey, $p = 0.033$), higher (Tukey, $p = 0.050$), and wider (Tukey, $p < 0.001$) shells compared to mussels from the CS treatment. Similarly, the shells of mussels coming from CT conditions were shorter (Tukey, $p = 0.020$) and narrower (Tukey, $p = 0.012$) compared with the WS mussels. Linear shell measurements were calculated as in Fariñas-Franco et al. (2014) to get a better insight into how these shell size measurements influence the actual shape of the mussels. The hydrodynamic treatments had a significant effect on the inflation ($W:H$ ratio, 1-way ANOVA, $F_{2,220} = 6.399$, $p = 0.002$) and obesity ($W:L$ ratio, 1-way ANOVA, $F_{2,220} = 5.729$, $p = 0.004$) of the mussel shell, but not on elongation ($H:L$ ratio). The WS mussels formed rounder shells, with a more inflated (Tukey, $p = 0.001$) and obese (Tukey, $p = 0.003$) shape in comparison with mussels from the CS conditions (Fig. 2). WS mussels had a rounder shell shape in comparison with both CS and CT mussels. The average (\pm SD) initial shell thickness at the start of the experiment was 0.251 ± 0.79 mm

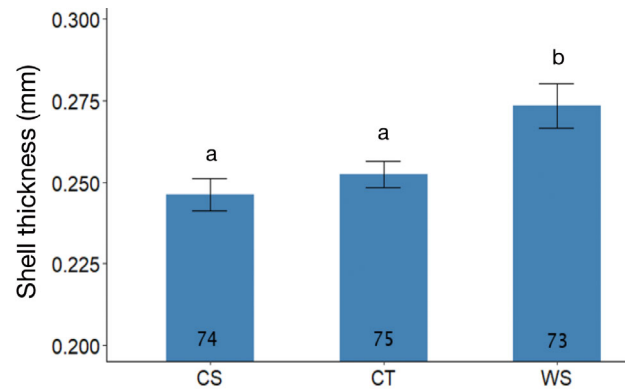


Fig. 3. Mean (\pm SE) shell thickness of the adductor muscle scar of the right valve of *Mytilus edulis* mussels coming from seed mussel collectors subjected to 3 different hydrodynamic treatments: calm submerged (CS), calm tidal cycle (CT), and wave-exposed submerged (WS) conditions. Different letters above bars: significant differences among treatments; numbers inside bars: number of mussels measured

(Table 1, subsample of $n = 30$). WS-exposed mussel seed developed a slightly but significantly thicker shell in comparison with CT (Tukey, $p = 0.022$) and CS (Tukey, $p = 0.012$) mussel seed (Fig. 3).

3.3. Mortality

At the end of the experiment, mean mortality over all treatments was 71.62%. After correction for overdispersion, mortality was significantly higher in the WS treatment than in the CT (GLM, $p = 0.012$) and CS treatments (GLM, $p < 0.001$) (Fig. 4).

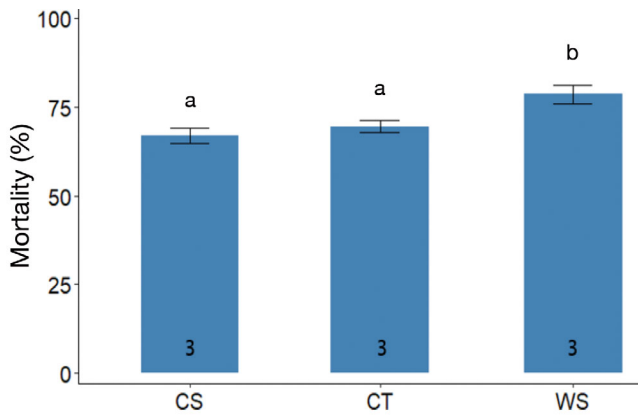


Fig. 4. Mean (\pm SE) mortality of *Mytilus edulis* mussels after 134 d in 3 different hydrodynamic treatments: calm submerged (CS), calm tidal cycle (CT), and wave-exposed submerged (WS) conditions. Numbers inside bars: number of experimental tanks measured; different letters above bars: significant differences among treatments

3.4. Patch characteristics

The pictures taken at the end of the experiment showed that mussels placed in the CT tanks formed significantly smaller patches (i.e. higher $P:A$ ratio) than those placed in CS (Tukey, $p = 0.004$) or WS (Tukey, $p = 0.004$) conditions (Figs. 5A & 6). The WPD was lower in the WS treatment compared with the mussel density in patches of CS tanks (Tukey, $p = 0.008$; Fig. 5B).

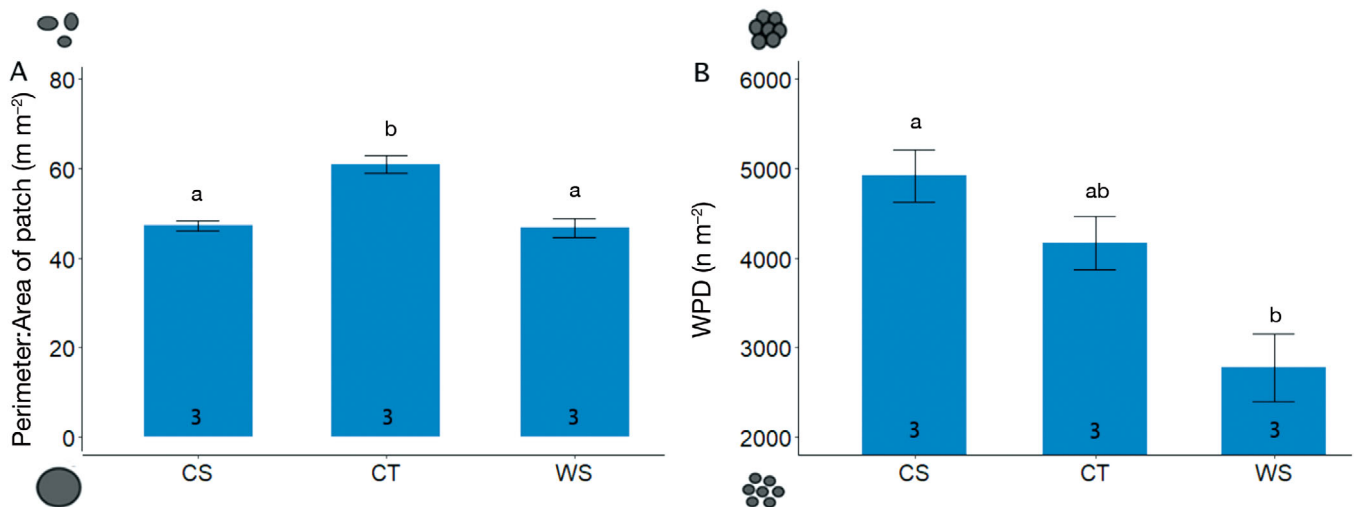


Fig. 5. Different *Mytilus edulis* mussel patch characteristics formed in 3 different hydrodynamic treatments: calm submerged (CS), calm tidal cycle (CT), and wave-exposed submerged (WS) conditions. (A) Average perimeter to area ratio ($m m^{-2}$) of mussel patches per hydrodynamic treatment; (B) within-patch density (WPD) (average number of mussels within mussel patches per hydrodynamic treatment). Numbers inside bars: number of experimental tanks measured; different letters above bars: significant differences among treatments. Data are means \pm SE

4. DISCUSSION

Organisms living in the intertidal zone have to adapt to a complex interplay of multiple environmental drivers, such as regular periods of immersion and exposure to air as well as exposure to high wave energy (Arsenault et al. 2001, Steffani & Branch 2003, Moschino et al. 2015). These environmental stressors can induce phenotypic adaptation, potentially leading to an additive outcome in comparison with isolated effects (Moschino et al. 2015). Under natural conditions, it is often difficult to disentangle which environmental factors are the drivers of phenotypical adjustments. Understanding how specific environmental factors influence phenotypical adjustments can help to identify optimal source populations and suitable locations for transplantation. In this study, we separated 2 hydrodynamic stressors thought to influence the phenotypic expression of intertidal mussels: exposure to wave action (Steffani & Branch 2003, Kandratavicius & Brazeiro 2014) and exposure to air (Moschino et al. 2015). When comparing the effects of exposure to high-energy waves and exposure to air on morphological characteristics of mussels in a mesocosm experiment, we found that exposure to air affected the shell morphology and byssal attachment strength to a lesser extent than exposure to high-energy waves. Interactive effects were not included in this study, as the focus was placed on the individual effects of exposure to waves and air on the

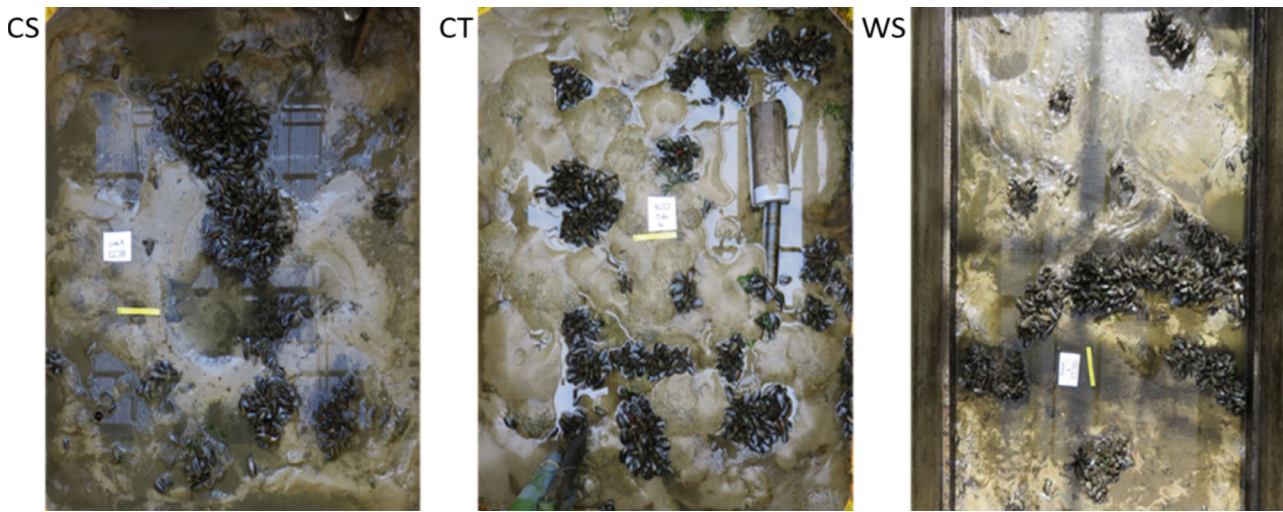


Fig. 6. Photographs of *Mytilus edulis* mussels taken after 134 d of pattern formation under 3 different hydrodynamic treatments: calm submerged (CS), calm tidal cycle (CT), and wave-exposed submerged (WS) conditions, with waves coming from the bottom of the picture

phenotype of the mussels, and because a simulation of interactive effects was not practically feasible. These results suggest that mussel seeds coming from collectors in the water column are capable of adjusting their morphology to hydrodynamic conditions typical of the intertidal zone.

4.1. Factors affecting byssal attachment strength

Mussels growing at natural wave-exposed sites often invest more energy in the production of byssal threads than those in locations with less wave exposure, resulting in stronger attachment strength and thereby a lower chance of dislodgement (Hunt & Scheibling 2001, 2002, Steffani & Branch 2003). This field observation was supported by the present experiment. Byssal attachment strength was strongest for mussels from the WS treatment followed by mussels from the CT treatment and was lowest for mussels in the CS treatment. Byssal attachment strength and condition (i.e. CI) of the mussel were positively correlated. It is possible that orbital water motion in the WS treatment may have resulted in higher food availability, which may have led to a higher CI and byssal attachment strength. Food availability has been shown to have a positive effect on byssus secretion and attachment force in juvenile mussels (Babarro et al. 2008). Mussels from the CT treatment also had higher byssal attachment strength than mussels from the CS treatment. However,

unlike the WS mussels, the CI of the CS mussels decreased during this experiment. Despite the reduction in condition, mussels in the CT tanks produced a stronger attachment strength than mussels from CS tanks. The production of byssal threads is energetically costly (Hawkins & Bayne 1985), suggesting that the increase in byssal attachment strength found in the WS and CT treatment mussels was probably the result of adjustment to hydrodynamic stressors (either exposure to waves or to air) rather than food availability. The stronger attachment strength in mussels in the CT treatment compared with the mussels from the CS tanks could be the result of either exposure to air or to the water motion caused by emptying and filling the tanks with water. However, the average flow rate ($0.00125 \text{ cm s}^{-1}$) during filling and emptying was negligible compared with normal tidal currents, which reach easily up to 30 cm s^{-1} (Bouma et al. 2005). Nevertheless, this 'tidal flow' caused by pumping may still have influenced the attachment strength of the mussels. Alternatively, attachment strength may vary according to the position of the mussel within a patch. Mussels positioned at the edge of a patch have an increased risk of dislodgement and may therefore develop stronger byssus threads than those positioned in the middle of a larger patch (wa Kangeri et al. 2014). The CT tanks contained relatively more edge-positioned mussels than the WS and CS tanks, which may explain the average higher attachment strength.

4.2. Factors affecting condition

The largest mussels with the highest CI were those in the WS treatment, while the mussels with the lowest CI were those in the CT treatment. This supports the conclusions of previous studies, where higher growth rates and CIs were recorded at subtidal and wave-exposed intertidal sites compared with intertidal sheltered sites—presumably because food availability is higher when water velocities are greater and feeding time is not restricted by the tidal cycle (McQuaid & Lindsay 2000, Moschino et al. 2015). In the mesocosm experiment, edible particles may have sunk to the bottom in the CS and CT tanks, while orbital water motion in the WS tanks may have caused continuous resuspension of organic material, thereby causing a higher food availability. Despite the larger energy investment in byssal attachment strength and stronger shells required of mussels in the WS tanks, higher food availability may have resulted in better condition of mussels in the WS tanks. The results of this experiment indicate that an increase in water velocity has a positive effect on mussel growth and condition. However, in some locations with extreme wave exposure, mussel condition and growth can be limited (Harger 1970, Steffani & Branch 2003). High water velocities can inhibit the filtration rates of mussels, reducing their energy availability for growth (Newell et al. 2001). High hydrodynamic forces may also lead to a higher demand for byssus thread production, leaving less energy available for growth. This indicates that an increase in water flow may have a positive effect on mussel condition until feeding efficiency and energy partitioning thresholds are surpassed (Steffani & Branch 2003).

4.3. Factors affecting shell morphology

Mussels on wave-exposed shores often have thicker and narrower shells than mussels on sheltered shores (Steffani & Branch 2003, Babarro & Carrington 2011). An increase in shell thickness can be a protective measure against the destructive effects of hydrodynamic stress (Raubenheimer & Cook 1990). Despite the short duration of the experiment, the mussels exposed to waves showed a slight but significant increase in shell thickness. This increase could be a result of exposure to wave energy but, as the increase was quite small (approximately 8%), biological significance should be interpreted with caution. It would be desirable to conduct a follow-up experiment over a

longer time span to better elucidate biological relevance. The rounder shell shape developed by the mussels in the WS tanks contradicts the results of previous studies showing that mussels at wave-exposed sites tend to have a more narrow shell shape to reduce the effect of hydrodynamic forces (Steffani & Branch 2003, Fariñas-Franco et al. 2014). A possible explanation for this discrepancy might be that the mussels from the WS tanks had rounder shells simply because they had a higher growth rate and CI, forcing them to expand their shells more.

4.4. Factors affecting mussel mortality

In natural populations, mussel mortality can vary tremendously depending on environmental conditions (Petraitis 1995, McQuaid & Lindsay 2000, Capelle et al. 2017). Overall mortality during this experiment was high; approximately 72%. This high mortality rate can be linked to the timing of translocation of the mussels, as mussel condition is known to decrease closer to winter (Nagarajan et al. 2006). Furthermore, mussels can suffer from stress-related loss of condition due to handling during translocation (Calderwood et al. 2014). A 'phenotype–environment mismatch' of mussels transplanted into the experimental tanks may have also played a role. Phenotype–environment mismatch describes the reduction in fitness when an organism specialized to one environment finds itself in an alternative environment (DeWitt et al. 1998). If the mortality rate was a consequence of phenotype–environment mismatching, the lowest mortality would be expected in mussel seeds in the CS treatment (as these originated from collectors in the water column), and highest in the CT and WS tanks. This was, however, only partly the case as mussels in the WS treatment experienced higher mortality than those in the CS treatment, but those in the CT treatment did not. The CI in the WS tanks even increased during the experiment, while a phenotype–environment mismatch should be expected to decrease fitness. It is possible that a phenotype–environment mismatch only played a role at the start of the experiment, and resulted in higher mortality only in the most extreme treatment (exposure to wave energy).

4.5. Factors affecting mussel patch formation

Waves and tides also had a significant effect on mussel patch formation. Mussel seeds have been

shown to actively aggregate into patches within the first few hours after transplantation (van de Koppel et al. 2008). By aggregating into clumps, mussels decrease the risk of dislodgement and predation (Hunt & Scheibling 2001, 2002). By isolating 2 hydrodynamic conditions typical of the intertidal zone (i.e. exposure to waves and exposure to air), this experiment showed that different hydrodynamic conditions result in different mussel patch shapes. The average $P:A$ ratio was significantly higher for mussel patches in the CT tanks than any other treatment. These mussels may simply have had less time to aggregate into patches with a larger area as they were only submerged two-thirds of the time. In the WS tanks, mussels formed a banded pattern perpendicular to the wave direction, while the patches in the CS and CT tanks had a rounder shape. This banded pattern is probably a result of dislodged mussels rolling against mussels behind and reattaching, thereby forming band-shaped patches (van de Koppel et al. 2005).

5. CONCLUSIONS AND IMPLICATIONS

Characterizing how specific morphological differences arise through specific environmental cues can give a better understanding of the capacity for plasticity of an organism at different life stages, and thereby its ability to establish and persist in various environments. Mesocosm experiments such as that carried out in this study may provide mechanistic insight into how an organism can adjust its phenotype when settling, or when transplanted in a new environment. By identifying optimal life stages and source populations for transplantations based on environmental factors and species plasticity, this experimental approach may help to prevent costly mistakes in restoration efforts.

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LITERATURE CITED

- ✦ Arsenault DJ, Marchinko KB, Palmer AR (2001) Precise tuning of barnacle leg length to coastal wave action. *Proc R Soc B* 268:2149–2154
- ✦ Babarro JMF, Carrington E (2011) Byssus secretion of *Mytilus galloprovincialis*: effect of site at macro- and micro-geographical scales within Ria de Vigo (NW Spain). *Mar Ecol Prog Ser* 435:125–140
- ✦ Babarro JMF, Fernández Reiriz MJ, Labarta U (2008) Secretion of byssal threads and attachment strength of *Mytilus galloprovincialis*: the influence of size and food availability. *J Mar Biol Assoc UK* 88:783–791
- Bangs EE, Fritts SH (1996) Reintroducing the gray wolf to central Idaho and Yellowstone National Park. *Wildl Soc Bull* 24:402–413
- ✦ Beadman HA, Caldow RWG, Kaiser MJ, Willows RI (2003) How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. *Mar Biol* 142:487–494
- ✦ Beukema JJ (1976) Biomass and species richness on the macro-benthic animals living on the tidal flats of the Dutch Wadden Sea. *Neth J Sea Res* 10:236–261
- ✦ Bouma TJ, Vries MBD, Low E, Peralta G, Tánzos IC, van de Koppel J, Herman PMJ (2005) Trade-offs related to ecosystem engineering: a case study on stiffness of emerging macrophytes. *Ecology* 86:2187–2199
- Bouma H, De Jong DJ, Twisk F, Wolfstein K (2006) A Dutch ecotope system for coastal waters (zes. 1), to map the potential occurrence of ecological communities in Dutch coastal and transitional waters. Technical Report RIKZ/2005.024. Rijkswaterstaat, Middleburg
- Brinkman AG, Dankers N, Van Stralen M (2002) An analysis of mussel bed habitats in the Dutch Wadden Sea. *Helgol Mar Res* 56:59–75
- ✦ Calderwood J, O'Connor NE, Sigwart JD, Roberts D (2014) Determining optimal duration of seed translocation periods for benthic mussel (*Mytilus edulis*) cultivation using physiological and behavioural measures of stress. *Aquaculture* 434:288–295
- Capelle JJ (2017) Production efficiency of mussel bottom culture. PhD dissertation, Wageningen University
- ✦ Capelle JJ, Wijsman JWM, Schellekens T, van Stralen MR, Herman PMJ, Smaal AC (2014) Spatial organisation and biomass development after relaying of mussel seed. *J Sea Res* 85:395–403
- ✦ Capelle JJ, van Stralen MR, Wijsman JWM, Herman PMJ, Smaal AC (2017) Population dynamics of subtidal blue mussels *Mytilus edulis* and the impact of cultivation. *Aquacult Environ Interact* 9:155–168
- ✦ Carrington E, Moeser GM, Thompson SB, Coutts LC, Craig CA (2008) Mussel attachment on rocky shores: the effect of flow on byssus production. *Integr Comp Biol* 48: 801–807
- ✦ Chevin LM, Hoffmann AA (2017) Evolution of phenotypic plasticity in extreme environments. *Philos Trans R Soc B* 372:20160138
- ✦ Coen LD, Brumbaugh RD, Bushek D, Grizzle R and others (2007) Ecosystem services related to oyster restoration. *Mar Ecol Prog Ser* 341:303–307
- ✦ Dame R, Dankers N, Prins T, Jongasma H, Smaal A (1991) The influence of mussel beds on nutrients in the Western Wadden Sea and Eastern Scheldt estuaries. *Estuaries* 14: 130–138
- ✦ de Paoli H (2017) Restoring mussel bed: a guide on how to

- survive on an intertidal mudflat. PhD dissertation, University of Groningen
- de Paoli H, van de Koppel J, van der Zee E, Kangeri A and others (2015) Processes limiting mussel bed restoration in the Wadden-Sea. *J Sea Res* 103:42–49
- ✦ DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends Ecol Evol* 13:77–81
- Ens BJ, Altling D (1996) The effect of an experimentally created mussel bed on bird densities and food intake of the oystercatcher *Haematopus ostralegus*. *Ardea* 84: 493–507
- Fariñas-Franco JM, Sanderson WG, Roberts D (2014) Phenotypic differences may limit the potential for habitat restoration involving species translocation: a case study of shape ecophenotypes in different populations of *Modiolus modiolus* (Mollusca: Bivalvia). *Aquat Conserv* 94: 76–94
- ✦ Fischer B, van Doorn GS, Dieckmann U, Taborsky B (2014) The evolution of age-dependent plasticity. *Am Nat* 183: 108–125
- ✦ Gaylord B, Denny MW, Koehl MAR (2003) Modulation of wave forces on kelp canopies by alongshore currents. *Limnol Oceanogr* 48:860–871
- Harger JRE (1970) The effect of wave impact on some aspects of the biology of sea mussels. *Veliger* 12:401–414
- ✦ Hawkins AJS, Bayne BL (1985) Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements. *Mar Ecol Prog Ser* 25:181–188
- ✦ Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. *Biom J* 50:346–363
- ✦ Hunt HL, Scheibling RE (2001) Patch dynamics of mussels on rocky shores: integrating process to understand pattern. *Ecology* 82:3213–3231
- Hunt HL, Scheibling RE (2002) Movement and wave dislodgment of mussels on a wave-exposed rocky shore. *Veliger* 45:273–277
- Kamermans P, Brummelhuis E, Smaal A (2002) Use of spat collectors to enhance supply of seed for bottom culture of blue mussels (*Mytilus edulis*) in the Netherlands. *World Aquac* 33:12–15
- KandrataVICIUS N, Brazeiro A (2014) Effects of wave exposure on morphological variation in *Mytilus edulis platensis* (Mollusca, Bivalvia) of the Atlantic Uruguayan coast. *Pan-Am J Aquat Sci* 9:31–38
- ✦ Lachance AA, Myrand B, Tremblay R, Koutitonsky V, Carrington E (2008) Biotic and abiotic factors influencing attachment strength of blue mussels *Mytilus edulis* in suspended culture. *Aquat Biol* 2:119–129
- ✦ Lenth RV (2016) Least-squares means: the R package lsmeans. *J Stat Softw* 69:1–33
- ✦ McQuaid CD, Lindsay TL (2000) Effect of wave exposure on growth and mortality rates of the mussel *Perna perna*: bottom-up regulation of intertidal populations. *Mar Ecol Prog Ser* 206:147–154
- ✦ Moschino V, Bressan M, Cavaleri L, Da Ros L (2015) Shell-shape and morphometric variability in *Mytilus galloprovincialis* from micro-tidal environments: responses to different hydrodynamic drivers. *Mar Ecol* 36:1440–1453
- ✦ Nagarajan R, Lea SEG, Goss-Custard JD (2006) Seasonal variations in mussel, *Mytilus edulis* L. shell thickness and strength and their ecological implications. *J Exp Mar Biol Ecol* 339:241–250
- ✦ Newell CN, Wildish DJ, MacDonald BA (2001) The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. *J Exp Mar Biol Ecol* 262:91–111
- ✦ Nilsson-Örtman V, Rogell B, Stoks R, Johansson F (2015) Ontogenetic changes in genetic variances of age-dependent plasticity along a latitudinal gradient. *Heredity* 115: 366–378
- ✦ Petraitis PS (1995) The role of growth in maintaining spatial dominance by mussels (*Mytilus edulis*). *Ecology* 76: 1337–1346
- ✦ Preston SJ, Harrison A, Lundy M, Roberts D, Beddoe N, Rogowski D (2010) Square pegs in round holes—the implications of shell shape variation on the translocation of adult *Margaritifera margaritifera* (L.). *Aquat Conserv* 20:568–573
- R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Raubenheimer D, Cook P (1990) Effects of exposure to wave action on allocation of resources to shell and meat growth by the subtidal mussel, *Mytilus galloprovincialis*. *J Shellfish Res* 9:87–93
- ✦ Reimer O, Tedengren M (1996) Phenotypical improvement of morphological defences in the mussel *Mytilus edulis* induced by exposure to the predator *Asterias rubens*. *Oikos* 75:383–390
- ✦ Scyphers SB, Powers SP, Heck KL, Byron D (2011) Oyster reefs as natural breakwaters mitigate shoreline loss and facilitate fisheries. *PLOS ONE* 6:e22396
- ✦ Seed R (1968) Factors influencing shell shape in the mussel *Mytilus edulis*. *J Mar Biol Assoc UK* 48:561–584
- ✦ Steffani CN, Branch GM (2003) Growth rate, condition, and shell shape of *Mytilus galloprovincialis*: responses to wave exposure. *Mar Ecol Prog Ser* 246:197–209
- ✦ Telesca L, Michalek K, Sanders T, Peck LS, Thyrring J, Harper EM (2018) Blue mussel shell shape plasticity and natural environments: a quantitative approach. *Sci Rep* 8:2865
- ✦ van de Koppel J, Rietkerk M, Dankers N, Herman PMJ (2005) Scale-dependent feedback and regular spatial patterns in young mussel beds. *Am Nat* 165:E66–E77
- ✦ van de Koppel J, Gascoigne JC, Theraulaz G, Rietkerk M, Mooij WM, Herman PMJ (2008) Experimental evidence for spatial self-organization and its emergent effects in mussel bed ecosystems. *Science* 322:739–742
- wa Kangeri AK, Jansen JM, Barkman BR, Donker JJA, Joppe DJ, Dankers NMJA (2014) Perturbation induced changes in substrate use by the blue mussel, *Mytilus edulis*, in sedimentary systems. *J Sea Res* 85:233–240
- ✦ Weeks AR, Sgro CM, Young AG, Frankham R and others (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evol Appl* 4:709–725
- ✦ Witman J, Suchanek T (1984) Mussels in flow: drag and dislodgement by epizoans. *Mar Ecol Prog Ser* 16:259–268
- ✦ Yeh PJ, Price TD (2004) Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am Nat* 164:531–542