



Is attachment substrate a prerequisite for mussels to establish on soft-sediment substrate?



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ARTICLE INFO

Keywords:

Attachment substrate
Green-lipped mussels
P. canaliculus
Shellfish restoration
Soft-sediment

ABSTRACT

It is unknown whether the presence of hard substrate is a necessary prerequisite for the attachment and establishment of mussels, especially on soft-sediment habitats where hard substrates are scarce. Therefore, we examined the importance of natural attachment substrates in the form of mussel shell and adult conspecifics for the establishment of juvenile and adult green-lipped mussels (*Perna canaliculus*) on soft-sediment. In field experiments where shell material was added as substrate to soft-sediment it made no difference to the subsequent retention of adult mussels. Laboratory experiments showed that juvenile mussels preferentially sought out, and attached to adult mussels compared to remaining on unmodified soft-sediment. Furthermore, juvenile mussels attached to live adult mussels had higher survival in the presence of a common sea star predator compared to juveniles on unmodified soft-sediment or attached to mussel shell. The results suggest that establishment of mussel beds on soft-sediment requires only adult mussels, which receive sufficient anchorage through attachment to neighbouring adults and in so doing, providing a stable, complex substrate suitable for improving the survival of establishing juvenile mussels by protecting them from sea star predators.

1. Introduction

Epifaunal bivalves such as oysters and mussels anchor themselves to hard substrates through permanent cementation and detachable byssal threads, respectively. This process of attachment helps to reduce the likelihood of dislodgement and transport away from selected environments (Bell and Gosline, 1997; Hunt and Scheibling, 2001) which can result in mortality (Carrington et al., 2009; Petrović and Guichard, 2008). Bivalves are at particular risk of dislodgement during storm events as well as in areas of naturally strong hydrodynamic conditions (Carrington et al., 2009; Denny, 1987; Hunt and Scheibling, 2001; Petrović and Guichard, 2008). The hard substrates which these bivalves attach to include natural cobbles and bedrock, anthropogenic structures, as well as other organisms (Commito et al., 2014; Dankers et al., 2001; Dolmer and Frandsen, 2002; McGrorty et al., 1993; Southgate and Myers, 1985), of which conspecifics are a common substrate (Commito et al., 2014). The gregarious nature of many epifaunal bivalves often leads to the formation of extensive populations, known as beds, which occur both intertidally and subtidally within coastal ecosystems. The attachment of bivalves within these beds not only reduces the risk of dislodgement but has also been shown to reduce the risk of predation by crabs (Leonard et al., 1999). In addition, the complex substrate created by the aggregating bivalves provides a preferred

habitat for settling larvae (Commito, 1987).

For the many species of mussel that inhabit rocky coastal habitats, hard substrates for attachment are abundant. In addition to attaching to conspecifics, these mussels can attach directly to the primary substrate. On soft-sediment habitats, however, hard substrates for attachment are sparse and mussels rarely attach to the primary sediment (Bayne, 1964; Commito et al., 2005) as the byssal threads are often unable to attach to the small grain sizes which would not provide sufficient anchorage. In these environments, mussels rely on rocks, shells, and predominantly conspecifics for attachment (Commito et al., 2014) with experiments showing that recruiting mussels primarily use these attachment materials rather than bare soft-sediment (Commito et al., 2014; van der Heide et al., 2014). Whether by the dislodgement and transportation of adults or the settlement of larvae, the importance of substrates in the establishment of mussel beds on soft-sediment is not clear. The re-establishment of adult northern horse mussels, *Modiolus modiolus*, showed no increase in survival when transplanted onto shell cultch of either high or low relief when compared to bare soft-sediment (Fariñas-Franco et al., 2013). Likewise, the survival of transplanted adult blue mussels, *Mytilus edulis*, was not higher on natural fibre mats made of coir compared to those transplanted directly onto soft-sediment (de Paoli et al., 2015). This suggests that additional attachment substrates aside from conspecifics are likely unnecessary for the establishment of mussel beds

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by adult mussels. In contrast, larval recruitment of both northern horse mussels and blue mussels were both higher in the presence of adults compared with any other available substrate including shell cultch (Commito et al., 2014; Fariñas-Franco et al., 2013). The survival of seed mussels was also shown to be greatest when provided with more complex substrates (Frandsen and Dolmer, 2002). This suggests that on soft-sediment, available attachment substrates may provide critical habitat for establishing larval and juvenile mussels.

Extensive mussel beds of the green-lipped mussel, *Perna canaliculus*, covering over 1300 km² on soft-sediment in the Hauraki Gulf, New Zealand, were nearly extirpated by dredge fishing during the last century (Greenway, 1969; Reid, 1969). Despite the closure of the fishery in 1969 (Paul, 2012), there has been no sign of natural recovery to date. The removal of the adult mussel beds through fishing has subsequently led to the removal of much of the available hard substrate which could have contributed to the lack of recovery in this population. The aim of this study is therefore to determine whether attachment substrates are necessary for the establishment of mussel beds on soft-sediment. This was accomplished using a series of laboratory and field experiments examining particular benefits to establishing adult and juvenile mussels provided with two common attachment substrates found within natural soft-sediment mussel beds, i.e., adult mussels and mussel shell. This study will address the hypotheses of whether; (1) conspecific shell increases the persistence of adult mussels establishing on soft-sediment, (2) conspecific shell and/or adult conspecifics increases the persistence of juvenile mussels establishing on soft-sediment, (3) juvenile mussels establishing on soft-sediment preferentially attach to conspecific shells and/or adults, and (4) the attachment of establishing juvenile mussels to mussel shell and/or adult mussels increases survival in the presence of a common sea star predator. The results of these experiments will help to increase our understanding of why natural recovery fails in depleted mussel populations and will have implications for restoration initiatives in this and other mussel species.

2. Materials and methods

2.1. Field study site

The Hauraki Gulf is located on the northeastern coast of the North Island of New Zealand. Field experiments were conducted in a sheltered coastal embayment on the northern section of Kawau Bay, in the Hauraki Gulf (36° 22' 47" S, 174° 49' 02" E). All experimental plots were situated on fine sand substrate at a depth of 4.1 to 4.9 m below chart datum.

2.2. Mussel sources

Wild juvenile mussels (*P. canaliculus*) are regularly found attached to drifting algae (Alfaro et al., 2010) and were collected from Ninety Mile Beach (35° 02' 08" S, 173° 10' 05" E) and Muriwai Beach (36° 50' 05" S, 174° 27' 59" E) in northern New Zealand. All juvenile mussels (1–5 mm shell length (SL)) that were collected and used in the present study were housed in aquaria supplied with ambient seawater with aeration until utilised in field and laboratory experiments. Adult mussels were most easily obtained from the extensive aquaculture operations in the Hauraki Gulf, where juvenile mussels originating from wild sources at Ninety Mile Beach in northern New Zealand, are grown on suspended long lines until they reach commercial size (Jeffs et al., 1999). All the adult mussels (80–100 mm SL) utilised in these experiments were first cleared of all fouling organisms and kept in aquaria with flow-through seawater until deployment. Given that aquacultured mussels are the most readily available source of mussels for restoration, maintaining a similar size range of adult mussels throughout these experiments ensures the results will be applicable to future restoration initiatives.

2.3. Use of substrate by adult mussels in the field

On 26 November 2013, twenty 0.25 m² (0.5 × 0.5 m) plots were established by divers in the field arranged in five rows, each containing four plots. Each plot was separated by a distance of 1.5 m and marked with a subsurface float. The crossed experimental design consisted of a substrate level of either unmodified soft-sediment or the addition of 60 clean adult mussel shells (80–100 mm SL) which was crossed with a predator exclusion or access level, with a total of five replicates per treatment. This number of adult mussel shells was used to ensure that they would provide sufficient attachment substrate for juvenile mussels in the experiment. Each of the five rows of plots contained one replicate of each treatment arranged in a random order. Predator exclusion plots were enclosed in a lightweight stainless steel frame covered with coarse plastic mesh (20 mm openings). The plastic mesh prevented large mobile predators, such as fish and lobster, from removing and consuming mussels from the plots, while not unduly restricting water flow to the mussels inside. The experimental design did not include an additional control treatment for possible artefacts caused by caging because the primary aim of the caging was to determine whether or not mussels were being lost from the experiment due to emigration or predation. Forty live adult green-lipped mussels were then transplanted into each plot to establish a density of mussels typical of the wide range of densities found in natural beds of these mussels (McLeod, 2009). After 50 days, the number of surviving mussels in each plot was enumerated by divers.

2.4. Use of substrate by juvenile mussels in the field

On 26 November 2013, a total of fifteen 1.5 m² circular plots were established by divers in three rows of five plots at the field site and marked with subsurface floats. The experiment consisted of three substrate treatments; 1) unmodified soft-sediment substrate, 2) addition of ≈ 250 adult mussel shells (80–100 mm SL) and, 3) addition of ≈ 1200 live adult mussels. Quantities of adult mussels and mussel shell ensured the entire plot was covered with the available attachment substrate and the density of live mussels was consistent with the densities found in natural beds of these mussels (McLeod, 2009). The three substrate treatments were each randomly allocated to five of the plots with no more than two of the same substrate treatment per row. In the laboratory, macroalgal material with attached juvenile mussels that had been previously collected from Ninety Mile Beach was divided into fifteen roughly equal bundles. Each bundle weighed 0.318 kg (± 0.013 SE) and based on mussel counts from weighed subsamples of mussel laden algae, each bundle contained on average 5298 mussels (± 353 SE). The bundles were then each enclosed within a biodegradable mesh sock (5–10 mm mesh size), commonly used for the deployment of juvenile mussels on seaweed in aquaculture operations (Jeffs et al., 1999), that helped to maintain the pre-measured quantities of juvenile mussels during transport. Bundles were transported to the site and secured by divers to the centre of each of the 15 experimental plots with a stainless steel pin driven 10 cm into the sediment. The mesh socks also helped to ensure the macroalgae with attached juvenile mussels remained within the positioned plot. The mesh size used did not unduly inhibit the movement of the juvenile mussels into and out of the sock, allowing the juvenile mussels to freely disperse onto the plot. After a period of 44 days each plot was surveyed by haphazardly placing a 0.0625 m² quadrat within each quarter of the plot and quantifying the number of remaining juvenile mussels in situ (1–5 mm SL) within each of the four quadrats for each plot. Divers haphazardly placed the quadrat by releasing the frame from 1.5 m above the plot and allowing it to fall to the seabed. Clear visibility at this site allowed divers to observe the entire quadrat and identify the presence of mussels > 1 mm SL.

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