Improving the efficiency of mussel reef restoration

By

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy in Marine Science, the University of Auckland, 2022
Abstract

The translocation of shellfish from aquaculture to sites on the seafloor is a popular method used in the restoration of shellfish reefs. Globally, there is interest in developing the practice to restore function to degraded nearshore systems such as benthic habitat, water quality regulation, and sediment stabilisation. In New Zealand, the translocation of adult green-lipped mussels (*Perna canaliculus*) to restore lost subtidal reefs has so far been a slow and expensive process, demonstrating a need to make the practice more efficient to achieve large scale restoration goals (e.g., >1000 km² of restored reef habitat). Through multiple field and lab experiments, this body of research investigated the efficacy of translocating sub-adult and juvenile mussels to increase the number of mussels translocated per kg harvested (Chapter 2, 3), the importance of mussel source to improve translocated mussel survival (Chapter 3), and timing deployments to limit predation and improve translocated mussel survival (Chapter 4). Experimentally translocated sub-adult and juvenile mussels consistently exhibited high mortality (60 – 100% loss) in the hours-to-days following transfer to the seafloor. These losses were often attributed to removals by mobile predator species, like Australasian snapper (*Pagrus auratus*) and New Zealand eagle rays (*Myliobatis tenuicaudatus*), who visited experimentally translocated mussels within 24 hours after placement at experimental sites. High mussel mortalities quickly demonstrated that restoration practice will need to account for mussel losses before more efficient practice can be scaled up. This study provides evidence that this may eventually rely on the selection or culture of sub-adult and juvenile mussels from resistant stock (e.g., with crush-resistant shells and/or numerous attachment threads) to improve mussel resilience against translocation stresses from harvest, transport, and transfer to the seafloor. Further improvement to mussel survival following transfer to the seafloor will depend on planning translocations for times of year, or at sites, where predator abundance is low. Overall, the results of this thesis suggest that efficient mussel reef
restoration practice is attainable but will require further study of the nuanced relationships
among mussel physiology, reef-building behaviour following transfer to the seafloor, and the
community of organisms likely to be attracted to restored reefs.
In loving memory of my Honey June

Dr. June Evelyn Braverman

31st May 1933 – 17th October 2019
Acknowledgements

I would like to take this space to offer my deep gratitude to the colourful cadre of people that helped make this thesis possible.

First, I would like to thank my supervisors Dr. Andrew Jeffs and Dr. Jenny Hillman. Your patient guidance, support, and honesty have been invaluable through countless drafts, ramblings, and rabbit holes. Thank you for giving me this opportunity to grow and learn. I am not only a better scientist, but a better person for it. I am forever grateful for y’all’s tutelage.

To my colleagues (staff and students) across the institute of marine science, I am extremely thankful to all of the helping hands that contributed the success of this project both in the field and in the lab. Thank you to Eliana Ferretti, Kelsey Miller, Dr. Peter Schlegel, Mallory Sea, Sophie Roberts, Dr. Timothy Haggitt, Louise Wilson, Benn Hanns, Brad Skelton, and Dr. Carina Sim-Smith who willingly jumped into the murky “depths” of the Mahurangi Harbour. Thank you to Stefan Spreitzenbarth, Peter Browne, Derek Sauer, Will McKay, Paul Caiger and Esther Stuck all of whom took the time to skipper me about and clean a healthy amount of mud off of the lab vessels. Thank you to Dallas LaFont, Luis Nahmad, Celia Balemi, Errol Murray, Daria Bell, Jess Smith, Richard Scott, Olivia Lord and Alyssa Ward for all of your help collecting and sorting mussels. Thank you to the shellfish reef restoration lab for all of your support and input. Thank you to Dr. Maria Mugica for your guidance and help in all things lab-related. Thank you to Jimmy Rapson for your electronic wizardry and for taking the time to build a fleet of timelapse cameras. Thank you to Jaime Rowntree for constant words of encouragement, hugs, yeah-nahs, strangely salty liquorice, and for enabling my caffeine habit. A big thank you to Martha Stafford for pulling significant weight around lab in Jaime’s absence.
I also want to thank a number of people for greasing the wheels of daily life. To Natalie Keane and her family, Ashley Flood, Cameron Cope, Hayley Nessia, Harry Allard, Amy Weller, Fiona MacKechnie, Evan Bare, Caleb Arellano, Catheline Froehlich, Trinity Livingston, Sam Ladewig, Caitlin Blain, Julie Hope, Stefano Schenone, Selwyn Collins, Carolin Nieder, Ohad Peleg and his family for making this process more playful than painful.

I want to offer a very special thank you to Ross Dockery, the Dockery family, and Dudley from Aotea Marine Farms for their time, wisdom, and generosity. Much of this work would not have been possible without your incredible support. Thank you also to Mike Moy, North Island Mussels LTD and David Blythe, Gold Ridge Farms.

Thank you to the anonymous donor that made the first year of this research possible and to the Lou and Iris Fisher Charitable Trust for continuing to support the vision of restoring the Hauraki Gulf.

Lastly, I would like to thank my family and friends, near and far, for their unwavering support in my pursuit of this life-long dream. Thank you to greenhouse, you all have been my home away from home and an oft needed reprieve from academic life. A special thank you to Lindsay Duncan for housing us as I wrote up. Thank you to Bianca Yeager for your enduring friendship, support, and countless SBSTs. A very special thank you to Millie Perocheau for the wonderful frontispieces and for your gracious help picking, counting, and shifting mussels with blissful tranquility despite my tendency to sweat the small stuff. Finally, a massive thank you to my nomadic family (Mom, Dad, Nat), to which I owe my love for community, science, and the ocean.
Collectively, y’all supported me through this PhD and for this I am profoundly grateful.
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Glossary of terms

**Clump:** Two or more mussels connected by byssus attachment threads (Côté & Jelnikar 1999).

**Conservation translocation:** The intentional movement of animals to restore populations (IUCN/SSC 2013).

**Ecosystem Damage:** Acute and obvious harmful impact upon an ecosystem such as selective logging, road building, poaching, or invasions of non-native species (Society for Ecological Restoration 2021).

**Defaunation:** The loss or depletion of animal species from ecological communities (Seddon et al. 2014).

**Degradation:** Chronic human impacts resulting in the loss of biodiversity and the disruption of an ecosystem’s structure, composition, and functionality. Examples include long-term grazing impacts, long-term overfishing or hunting pressure, and persistent invasions by non-native species (Society for Ecological Restoration 2021).

**Destruction:** The most severe level of impact, when degradation or damage removes all macroscopic life, ruining the physical environment. Ecosystems are destroyed by such activities as land clearing, urbanization, coastal erosion, and mining (Society for Ecological Restoration 2021).

**Ecosystem:** A biological community of interacting organisms and their physical environment (Hillman 2018).

**Ecological restoration:** Any activity or process that assists the recovery of historic ecological function to an ecosystem that has been degraded, damaged, or destroyed (Gann et al. 2019).

**Habitat:** An area characterized by specific environmental conditions and biogenic features (Hillman 2018).
**Seeding density**: The number of individual shellfish introduced within a given area for aquaculture or restoration (Capelle et al. 2016b).

**Shellfish reef**: Physical structural features in coastal waters created through the aggregation and accumulation of bivalve molluscs, such as oysters and mussels (Fitzsimons et al. 2019).

**Spat**: Broadly refers to an early juvenile shellfish that has recently transitioned from a pelagic to a benthic environment and attached to a surface (Fitzsimons et al. 2019).

**Translocation**: The deliberate movement of organisms from one site to another where the primary objective is a conservation benefit (Berger-Tal et al. 2020).
CHAPTER ONE

General introduction

“Do good and throw it in the sea” - Palestinian proverb
Active restoration

Active restoration is a conservation intervention that can be used to assist (Norris 2001) or stimulate (Schulte et al. 2009) processes of natural recovery following the removal of a degradative stressor (Gann et al. 2019). A common form of active restoration in marine environments involves the reintroduction of lost or diminished species to a degraded ecosystem (Seddon et al. 2014; Fitzsimons et al. 2019). Species reintroductions are especially common in the recovery of habitat-forming species, such as seagrass (Paling et al. 2009), mangroves (Lewis III 2009), corals (Barton et al. 2017), and shellfish (Fitzsimons et al. 2019). Successful reintroductions of habitat-forming species can be a particularly effective means of restoring degraded areas as they typically play multiple roles in an ecosystem functioning as shelter, substrate and prey for other species (Fitzsimons et al. 2019).

Reintroductions of habitat-forming species in marine systems can be achieved by providing a missing function (e.g., augmentation of available settlement substrate, Fitzsimons et al. 2019) or through the intentional release of organisms, which is also known as a conservation translocation (Griffith et al. 1989; IUCN/SSC 2013).

Conservation translocations can be an effective way to establish, re-establish, or augment a population to areas where they once occurred (Griffith et al. 1989; Schulte et al. 2009; IUCN/SSC 2013). Although they appear straightforward (i.e., move species from point A to point B), translocations can be difficult to develop in practice (Lindburg 1992) and may report low initial success rates due to the loss of translocated individuals (e.g., only 26% of 116 terrestrial reintroductions were classified as successful, Fischer & Lindenmayer 2000). While the causes that lead to loss of translocated individuals can vary by species, location, and time, the process of moving species from one area to another usually imparts physical stresses that limit the ability of a species to adapt and survive in a novel environment (Moseby et al. 2015; Robinson et al. 2021). In addition to the stress of being captured or
harvested and transported, translocated species must also adjust to a suite of novel conditions in their new environment, including different levels of predator exposure, habitat quality, and adequate quality and/or quantity of food (Seddon et al. 2014; Moseby et al. 2015; Fig. 1).

Adequate management of the risks or stresses species encounter during the translocation process can improve reintroduction success (IUCN/SSC 2013; Robinson et al. 2021). The identification of these stressors often requires a range of knowledge bases (e.g., biology, ecology, aquaculture, policy) to form initial risk assessments prior to reintroductions, but may also rely on careful observation of how species respond to novel conditions in the days to months following translocation (e.g., in response to differences in turbidity, Mcleod et al. 2012). Rapid identification of factors that contribute to or restrict

![Diagram](image.png)

**Figure 1:** Diagram depicting the stresses encountered during the translocation process. In this scenario, mussels are harvested from a farm at Point A and translocated to a restoration site at Point B. Mussels experience physical stress during, (i) harvest, transport, and (ii) deployment. Once deployed to the seabed, the energetic costs of (iii) movement and reattachment add to stresses from, (iv) lack of adequate quality or quantity of food, (v) the exposure to a novel environment, and (vi) exposure to novel predators.
survival can lead to improved efficiency and efficacy of the translocation process (Robinson et al. 2021).

Shellfish reef restoration

Shellfish reef restoration is rapidly gaining traction as a method for recovering both structure and function to degraded marine ecosystems (Fitzsimons et al. 2019; zu Ermgassen et al. 2020; Fig. 2). Shellfish reefs form complex, three-dimensional structures either by cementing (e.g., oysters, MacDonald et al. 2010) or temporarily attaching (e.g., mussels, Brenner & Buck 2010) to substrate and/or conspecifics across a wide range of environments (e.g., rocky coastline, soft-sediments, coastal wetlands; Fitzsimons et al. 2019; Renzi et al. 2019). They also frequently produce shell material and accumulate organic matter from their feeding activities that can support diverse communities (McLeod et al. 2014; Sea et al. 2021). These reefs perform key functions and/or services in nearshore ecosystems acting as:

- habitat for benthic and demersal fauna (McLeod et al. 2014; Gilby et al. 2021)
- substrate for biofouling communities (Buschbaum & Saier 2001)
- prey for larger reef species such as octopus (Mather 1991) or rays (Michael 1993)
- regulators of water quality that help with:
  - the reduction of turbidity (Grabowski & Peterson 2007)
  - denitrification (Hillman et al. 2021)
  - carbon sequestration (Fodrie et al. 2017)
  - benthic-pelagic coupling (Porter et al. 2004)
- dissipators of wave energy as seen with oyster breakwaters (Scyphers et al. 2011)
- sediment contributors and stabilizers (Kent et al. 2017)

The global loss of shellfish reefs (e.g., up to 85% for oyster reefs, Beck et al. 2009) and the increased awareness of their importance have catalysed efforts to restore these habitat-forming species to their historic ranges. To date, interest in shellfish reef conservation
and restoration is growing across most continents (Toone et al. 2021). While the goal of shellfish reef restoration is to establish a persistent population, the methods used vary by species, tidal zones, and substrate types at restoration sites (Fitzsimons et al. 2019). For example, the successful establishment of oyster reefs over soft-sediments may rely on the addition of hard substrate to initiate the settlement of pelagic larvae (e.g., artificial reefs, Schulte et al. 2009; Fig. 3A) and/or the deployment of nursery-reared spat or adults pre-attached to recycled shell cultch (Scyphers et al. 2011). The techniques used for mussel reef restoration are less well-developed and have primarily relied on the translocation of populations from aquaculture farms to sites on the seafloor (de Paoli et al. 2015; Capelle et al. 2019; Schotanus et al. 2020a; Wilcox et al. 2018; Fig. 3B).

Mussel reef restoration

Mussels have the capacity to build reefs in a range of coastal environments from rocky shorelines to soft-sediment seafloor (Gosling 1992). In soft-sediment environments mussels typically establish reef structures by attaching to conspecifics to form interconnected clumps (Commito & Dankers 2001; Commito et al. 2014). Networks of these clumps go on to create

![Diagram of ecosystem services provided by shellfish reef ecosystems.](image)

**Figure 2:** Examples of the ecosystem services provided by shellfish reef ecosystems. Source: Restoration guidelines for shellfish reefs, Fig 2.1, Fitzsimons et al. 2019.
low-lying reef structures that provide habitat to unique assemblages of species (McLeod et al. 2014). Unlike the process of restoring oyster reefs, some mussel reefs can be directly reintroduced into soft-sediment systems without the extra costs and logistical complexity involved with the inclusion of hard substrate (e.g., shell cultch or artificial reefs) to provide a benthic substrate for the attachment of the shellfish (Wilcox et al. 2018). The ability for mussels to self-organize into reef structures makes them ideal candidates for restoring ecological function to degraded soft-sediment systems with relatively minimal effort.

Under natural circumstances, mussel reefs establish over soft-sediments when a clump becomes dislodged from settlement substrata and disperses over adjacent soft-sediments providing a stable founding population to which others can recruit (Commito & Dankers 2001; Commito et al. 2014; Fig. 4A). Mussels cannot settle directly onto soft-sediments as they are unable to attach themselves to small sediment particles, even at the larval stage (Commito & Dankers 2001). Over time, mussels form into reef networks that have the capacity to recruit pelagic larvae, recruit juvenile mussels after undergoing

Figure 3: (A) Artificial reefs deployed at Helen Wood Park, Mobile Bay, Alabama to attract pelagic oyster larvae as they settle out from their pelagic dispersal phase. (B) Adult green-lipped mussels being spread from a barge over a subtidal restoration site in the Hauraki Gulf, New Zealand. Sources: (A) http://coastalresilience.org/wp-content/uploads/2016/10/RestoreVolunteers.jpg, (B) Shaun Lee, Revive our Gulf
secondary settlement (Comitto et al. 2014), and function as biogenic substrata for the reattachment of dislodged adults (Comitto et al. 2016). Over time mussel reefs grow outward from these initial clumps to form more complex reef systems (Comitto & Dankers 2001; van de Koppel et al. 2012). Mussel reef restoration replicates this natural reef-building processes (Fig. 4A) by harvesting mussels from natal growth substrata (e.g., aquaculture growth lines) and translocating them to a restoration location on the seafloor (de Paoli et al. 2015; Wilcox et al. 2018; Fig. 4B). Within hours, mussels that have been translocated and

Figure 4: (Top) Natural process of mussel reef establishment over soft-sediments; (A) a clump of mussels is dislodged from substrata, (B) becomes established over soft-sediment, self-organizing with other clumps to (C) form a reef structure over time. (Bottom) Current mussel translocation process where mussels are (“A”) harvested from long-line aquaculture and (“B”) spread across the seabed where they (“C”) naturally self-organize into reef structures. Sources: Top diagram redrawn from Dynamics of spatial and temporal complexity in European and North American soft-bottom mussel beds, Fig. 2.1, Commi to & Dankers 2011.
loosely spread on soft sediment benthic habitats will naturally find and attach to nearby
con specifics (Wilcox et al. 2018). In the time it takes for loose mussels to scrub and attach to
one another they are often vulnerable to novel predators and changes to their environment (de
Paoli et al. 2015; Capelle et al. 2016a). For example, intertidal restoration of the blue mussel,
*Mytilus edulis*, reefs has been limited by crab predation and daily tides that quickly disperse
transplanted mussels, preventing reef-building processes from taking place (de Paoli et al.
2015; Schotanus et al. 2020b).

The successful establishment of restored mussel reefs, *id est* when they become self-
sustaining, has yet to be defined for large (e.g., > m²) translocations. Initial translocation
successes and failures have been linked to seeding density (i.e., the number of mussels spread
across a given area, Capelle et al. 2016), exposure to novel predators (Capelle et al. 2019;
Wilcox & Jeffs 2020), and the ability for mussels to contend with the hydrodynamics of the
system in question (e.g., wave action, Capelle et al. 2019; subtidal currents, Wilcox et al.
2018). Recent restoration efforts of intertidal blue mussel reefs have mitigated the effects of
predation (Capelle et al. 2016) and hydrodynamic dislodgement using engineering measures
such as fences and breakwaters (Capelle et al. 2019; Schotanus et al. 2020b). While
impractical for restoration at larger scales (i.e., > m²), these engineering measures can help
identify whether different life stages of mussels (e.g., juveniles and sub-adults) can survive
translocation to a novel environment.

**The New Zealand context**

“The seafloor is like a garden or a paddock – if it is healthy, then the whole system can be
healthy.” - Anonymous, *Sea Change - Tai Timu Tai Pari* 2017

The Hauraki Gulf/Tīkapa Moana (referred to hereafter as the Hauraki Gulf) is the body of
water around the North Island of New Zealand whose boundaries extend from the eastern
coastline of the Auckland region to the eastern coastline of the Coromandel Peninsula (Sea
The Hauraki Gulf includes a marine park that covers 1.2 million hectares and includes over 50 islands, all of which support a diverse range of endemic coastal marine habitats and species (Sea Change 2017). This region is culturally significant and has remained a hub of human activity since the initial settlement of New Zealand/Aotearoa (referred to hereafter as New Zealand; King 2003; Fig. 5A). Conservation translocations are not new to the Hauraki Gulf, with some of the islands used for an assortment of terrestrial reintroductions conducted to protect threatened or endangered endemic species, such as the little spotted kiwi (Apteryx owenii, Colbourne & Robertson 1997), Duvaucel’s gecko (Hoplodactylus duvauceli, van Winkel et al. 2010) and giant wētāpunga (Deinacrida spp., Watts et al. 2020).

The seas around the Hauraki Gulf host a number of reefscapes created by volcanic rock, macroalgae, soft-sediments, and bivalves (to name a few) that support a rich mixture of sea life, including dense populations of migratory seabirds (Gill 2010), dozens of fish species (McMillan et al. 2011), and resident marine megafauna (e.g., Bryde’s whale, Balaenoptera edeni/brydei, Baker et al. 2009). A number of research projects are currently underway to reintroduce habitat-forming species to nearshore waters including kelp (e.g., Ecklonia radiata), the New Zealand horse mussel (Atrina zelandica), and the endemic green-lipped mussel (Perna canaliculus).

**Green-lipped mussel, Perna canaliculus, Gmelin 1791**

Green-lipped mussels (also referred to as Greenshell™, kuku, or kūtai, Jeffs et al. 1999; Paul 2012) are one of the largest species in the Mytilidae family, growing to over 240 mm in shell length (Jeffs et al. 1999). They are ubiquitous along the New Zealand coastline and can be found “attached to rock faces, wharf piles…among algal holdfasts in the intertidal, and in deeper water…over mud or sand” (from Morton & Miller 1973 in Jeffs et al. 1999). Green-
lipped mussels are also a multi-million dollar aquaculture species in New Zealand, having generated $336 million in revenue for 2019-2020 (Aquaculture New Zealand 2020).

Dense reefs of inter-connected green-lipped mussels once formed an extensive habitat in the Hauraki Gulf, covering an estimated 1500 km² of soft sediment seafloor (Paul 2012; Fig. 5A). These mussel reefs formed an important component of the Hauraki Gulf ecosystem, functioning as habitat and a critical regulator of water quality through the transfer of suspended particles and nutrients from the water column to the seafloor (a.k.a. benthic-pelagic coupling; Porter et al. 2004; Smaal et al. 2019). This habitat supported diverse communities of invertebrates, small fish, and likely acted as nursery grounds for

Figure 5: (A) Map of the Hauraki Gulf showing the historic mussel reef distribution and areas dredged by a historic mussel fishery (From Paul 2012, redrawn from Reid 1969). (B) Example of a subtidal mussel reef and (C) some of the species commonly found associated with mussel reefs. Sources: (A) A history of the Firth of Thames dredge fishery for mussels: use and abuse of a coastal resource, Fig. 5, Paul 2012.; (B, C) Mussel reefs on soft sediments: a severely reduced but important habitat for macroinvertebrates and fishes in New Zealand, Fig. 2, McLeod et al. 2014.
economically-important species such as Australasian snapper, *Pagrus auratus* (McLeod et al. 2014; Parsons et al. 2014; Fig. 5B,C).

Throughout the early 1900s, mussel reefs throughout the Hauraki Gulf supported an extensive commercial dredge fishery (Paul 2012). However, as technology advanced and demand for mussels grew, mussel harvesting outpaced the ability for reefs to naturally regenerate, ultimately leading to extensive loss of subtidal habitat and the collapse of the Hauraki Gulf commercial dredge fishery in 1969 (Paul 2012). Despite the closure of the commercial dredge fishery and the subsequent rapid establishment of mussel farms in the Hauraki Gulf throughout the 1970s and 1980s, wild benthic populations of mussels have not recovered to historically dredged areas (Paul 2012; McLeod et al. 2012). Within the last decade, a group of community leaders identified the lack of mussel reef recovery and the need to pursue restoration for the benefit of the Hauraki Gulf ecosystem (Sea Change 2017; Sim-Smith & Tukua 2019). This attention has led to the Hauraki Gulf Forum, a statutory body with oversight of the Hauraki Gulf Marine Park, to propose a goal of restoring 1000 km$^2$ of mussel reefs within the Hauraki Gulf (Hauraki Gulf Forum 2019).

**Improving the efficiency of mussel reef restoration**

Since 2013, more than 200 t of adult (>70 mm shell length (SL)) green-lipped mussels have been deployed to areas of soft sediment seafloor in the Hauraki Gulf in an effort to restore mussel reefs in the region (Wilcox et al. 2018; van Kampen 2017; Sim-Smith & Tukua 2019). To date, restoration efforts have relied on large (7 - 70 t) donations or purchase of adult mussels from the green-lipped mussel aquaculture industry, which remains the only source of mussels used in translocations (Wilcox et al. 2018; Sim-Smith & Tukua 2019). Although a large number of mussels have already been translocated for restoration, collectively they only cover a tiny fraction of the historic range of mussel reefs within the Hauraki Gulf or the proposed 1000 km$^2$ goal. Furthermore, experimental translocations have
been limited by costs in time (e.g., to obtain permits and meet biosecurity requirements) and money (e.g., to pay for transport and permitting costs). While the survival of experimental restored beds suggest that restoration is attainable, the restoration process requires further optimisation to reduce logistical operating costs.

Two key strategies that could improve restoration efficiency is to (1) increase the number of mussels translocated per kilogram harvested, and (2) decrease the time to culture mussels to a size suitable for restoration. This efficiency can be created through the incorporation of juvenile (10–30 mm SL) and/or sub-adult (30–70 mm SL) mussels into the restoration pipeline. The translocation of juvenile mussels is common practice in European bottom-culture mussel aquaculture industry (Capelle et al. 2017), which suggests the potential to include them in restoration efforts in New Zealand. The use of small mussels would improve restoration efficiency by greatly increasing the number of mussels translocated per kilogram (i.e., 10 – 15 times) and reducing the time to culture mussels to a size suitable for restoration (i.e., < 1 year to juvenile/sub-adult sizes compared to 2-3 years for adults; Fig. 6).

Figure 6: Adult green lipped mussel (approximately 100 mm in shell length) compared to multiple, smaller juvenile and sub-adult mussels (20 – 40 mm in shell length).
Like their cultivated adult counterparts, juvenile (10 – 30 mm SL) and sub-adult (30 – 70 mm SL) green-lipped mussels are raised from spat (i.e., juveniles of <10 mm SL) at marine farms throughout New Zealand (Jeffs et al. 1999). Roughly 80% of green-lipped mussel spat used in New Zealand is sourced from the wild by harvesting drifting filamentous algae encrusted with spat (Jeffs et al. 2018). However, in areas with naturally high abundance of larvae, spat are also collected on ropes suspended in the water column (Jeffs et al. 1999; South 2018). Spat-collecting farms may have the capacity to improve restoration efficiency by collecting and outgrowing mussels from a single source (Ross Dockery, Aotea Mussels Ltd, pers. comm.).

**Study site**

The Mahurangi Harbour is a semi-enclosed body of water that connects the Mahurangi River and its catchment with the greater Hauraki Gulf (Harris 1993; Macaskill & Martin 2004; Fig. 7). The harbour historically supported populations of New Zealand horse mussel that were likely lost due to increases in suspended sediment as a result of land-use changes in the harbour catchment (Halliday & Cummings 2009). Interest in restoring ecological functionality to this harbour system has led to the deployment of adult green-lipped mussel reefs since 2017, some of which have continued to survive in the harbour for multiple years, which indicates the suitability of the environment for supporting mussels (Hillman et al. 2021; Sea et al. 2021). Additional observations of adult green-lipped mussels on vessel mooring lines, oyster farms, and some intertidal rock faces in the Mahurangi Harbour suggests the existence of a larval supply from outside the harbour or restored reefs, which is critical for long-term restoration success. The Mahurangi Harbour holds an additional benefit of being well-studied and possesses long-term datasets on water quality, sediment characteristics, and benthic communities (e.g., Cummings et al. 2003). It is also a relatively sheltered site from coastal winds and swells that is readily accessible from University of
Auckland research facilities making it a convenient site for research. Specific details of the research sites used in this study are described in more detail in association with the research methods for each experimental component of this thesis.

Figure 7: Map of the Mahurangi Harbour (yellow star on inset map), New Zealand.
Study aims

“Kia kaha, kia māia, ki te tiaki i ēnei taonga tuku iho hei oranga mo ngā uri
whakatipu/Be strong, be steadfast, and nurture those treasures handed down from the ancestors, for us to build up.” One Gulf one message, Sea Change - Tai Timu Tai Pari
2017

Before restoration efficiency can be improved, it is necessary to establish:

1. Whether juvenile and sub-adult mussels will survive translocation to soft-sediment seafloor, as is currently done with adults (Chapter 2: Considering juvenile and sub-adult mussels for restoration, published in Restoration Ecology).

2. How the source of sub-adult mussels used for translocation affects their subsequent survival following transfer to the seafloor (Chapter 3: The importance of mussel stock selection to improve transplantation efficiency, published in Restoration Ecology).

3. Whether there is a temporal relationship between predator presence and mussel survival following transfer of adult mussels to the seafloor (Chapter 4: Timing mussel deployments to improve restoration efficiency, in prep).

The overall aim of the research presented in this thesis is to improve the efficiency of mussel reef restoration through adjusting mussel size, source, and timing of restoration. Although there are guidelines for the best practice methods for restoring mussel reefs (e.g., Fitzsimons et al. 2019), there is a need to refine the practice so that it is as efficient as it is effective. This thesis is presented in a journal manuscript format and while the chapter elements (Chapters 1 – 4) do include some redundancy in content, they help to present the individual elements of this research, the conclusions of which are brought together in the general discussion (Chapter 5).
Abstract

Widespread resource extraction and habitat degradation have severely reduced functionally-important subtidal mussel reefs globally. While methods for restoring oyster reefs are becoming increasingly well-established, the development of techniques for the effective restoration of mussel reefs remain in their infancy and face biological and logistical challenges. This study investigated the potential use of sub-adult and juvenile green-lipped mussels (*Perna canaliculus*) for mussel reef restoration with the aim of understanding factors related to sub-adult and juvenile mussel survival after transfer to the seafloor. Small-scale (m$^2$) field experiments were conducted subtidally in a New Zealand harbour to assess sub-adult and juvenile mussel survival after translocation to soft-sediment seafloor, the efficacy of biodegradable substrate to support reef development, and whether juvenile mussel survival was related to changes in seeding density. Results demonstrated survival of cultured sub-adult and juvenile mussels after transfer to soft-sediment seafloor only when completely protected from mobile predators. Attachment to biodegradable substrate alone was insufficient to prevent the loss of cultured juvenile mussels, while ~80% of wild sub-adult mussels survived translocation to the seafloor without predator protection - indicating a certain level of resilience. Changes in seeding density failed to prevent loss of cultured juvenile mussels. This study supports further consideration for incorporating cultured sub-adult and juvenile mussels into restoration, provided sub-adult and juvenile mussels can be protected until they become established as adults.
Introduction

Restoration of marine habitats is a necessary management intervention to address the widespread degradation of coastal ecosystems (Beck et al. 2011; Paul 2012; McLeod et al. 2019; Fitzsimons et. al. 2019). Lack of natural recovery and an increased awareness of the ecosystem services provided by shellfish reefs e.g., regulation of water quality (Paul 2012; Nelson et al. 2004), energy transfer (Fodrie et al. 2017), dissipation of wave energy (Chowdhury et al. 2019), provision of food sources (Paul 2012), and provision of habitat to diverse faunal assemblages (McLeod et al. 2019) have prompted global efforts to pursue active seafloor restoration to improve ecosystem health and function (e.g., Crassostrea virginica, Chesapeake Bay, North Carolina, U.S.A., Schulte et al. 2009; Ostrea angasi & Mytilus galloprovincialis, Port Phillip Bay, Australia; Hancock et al., 2019; Mytilus edulis, Wadden Sea, Netherlands, Schotanus et al. 2020). Although methods for restoring lost oyster reefs have received considerable attention, techniques used to restore subtidal mussel reefs have only recently (< 10 years) been investigated as an alternative management intervention to address lost or degraded mussel reef habitat (Jeffs and zu Ermagassen 2019).

The Hauraki Gulf of the North Island of New Zealand once supported an estimated 1500 km² of subtidal green-lipped mussel (Perna canaliculus, Gmelin 1791) reefs that were nearly eradicated by a historic dredge fishery (estimated from Reid, 1969). After the commercial dredge fishery landings reached zero in 1969, subtidal green-lipped mussel reefs failed to re-establish naturally in the Hauraki Gulf, underlining the need to implement a management intervention (McLeod et al. 2014; Paul 2012; Wilcox et al. 2017). Although exact causes for the lack of natural recovery remain unclear, they were likely a combination of little to no natural recruitment, lack of suitable settlement habitat, and increased sediment loads from changes in land-use practices (McLeod et al. 2012; Paul 2012).
Since 2013, community-led groups have deployed more than 150 t of cultured (i.e., farm-raised) adult mussels (70-100 mm shell length (SL)) to the seafloor as an active management intervention to re-establish lost mussel reef habitat (Sim-Smith & Tukua 2019; Wilcox et al. 2017). This strategy mimics bottom-culture practices in Europe (Capelle et al. 2016) and mussel reef restoration efforts that repopulate degraded soft-sediment systems by seeding Mytilid species across the seafloor where they naturally self-organize (Committ et al. 2014, 2016) into large (100 - 1000 m²), spatially complex aggregations (e.g., *Mytilus edulis*, Wadden Sea, Netherlands; de Paoli et al. 2015; *Mytilus galloprovincialis*, Port Phillip Bay, Australia; Hancock et. al. 2019). Current intertidal and subtidal mussel reef restoration efforts encounter significant costs associated with the logistics of harvest, transport, satisfaction of biosecurity protocols, and deployment to the seafloor (de Paoli et al., 2015; Wilcox et al. 2017; Jeffs and zu Ermagassen 2019). Furthermore, restored mussel reefs have suffered large losses due to pressures caused by predators, storm events, and a lack of recruitment (de Paoli et al., 2015; Wilcox et al. 2017). While current methods have restored adult mussel (70 – 100 mm SL) reefs in New Zealand that continue to persist 3 – 4 years post-deployment, longer term survival remains unknown and methods that aid the process are worth investigating to achieve larger scale (>1000 km²) restoration goals (Wilcox et al., 2017).

Sub-adult (30 -70 mm SL) and juvenile (10-30 mm SL) mussels are worth considering to improve restoration efficiency (e.g., cost and time-saving) as they allow for greater numbers of individuals to be relocated to restoration areas (i.e., more individuals kg⁻¹ harvested), take less time to culture than adults, and may be more adaptable when relocated into a new environment (Hickman 1979). In addition, juvenile mussels are between the size where they engage in migratory drifting behaviour (i.e., enabling movement out of a designated restoration site; up to 5 - 6 mm SL) and the size at which they reach sexual
maturity (>27 mm SL; Alfaro et al. 2011). Migratory drifting of smaller mussels (i.e., <10 mm SL) limits their use to rehabilitate degraded sites; whereas mussels greater than 10 mm in SL are considered too large to engage in this behaviour (Buchanan & Babcock 1997). Sub-adult mussels are between the size of early sexual maturity (i.e., ~27 mm SL) and the average size of adults used in past restoration efforts (i.e., 70-120 mm SL; Wilcox et al. 2017).

The use of sub-adult and juvenile mussels for restoration may be limited by external factors such as predation (Paul-Burke & Burke 2016; Wilcox & Jeffs 2019), hydrodynamic dislodgement (de Paoli et al. 2015; Capelle et al. 2016), or the availability of a suitable substrate (Wilcox & Jeffs 2017). In wild populations, sub-adult and juvenile mytilid species minimize predator access and dislodgement by forming an inter-connected network through mutual attachment of byssal threads to stable substrate and/or conspecifics (Bertolini et al. 2018). The addition of materials that promote this clumping behaviour may help support sub-adult and juvenile mussels to become established over soft-sediments (Wilcox & Jeffs 2017; Capelle et al. 2019; Schotanus et al. 2020). Biodegradable materials can serve as a temporary base that anchors translocated sub-adult and juvenile mussels to the seafloor without the onerous logistics (e.g., permitting, collection, transport, satisfaction of biosecurity protocols, and deployment) that come with the use of permanent hard substrate, such as recycled shell and limestone rubble (Hancock et al. 2019).

Although sub-adult and juvenile green-lipped mussels are a potentially attractive cost and timesaving alternative to adults in restoration efforts, they may require different methods of deployment to survive translocation onto soft-sediment seafloor. This study was comprised of a number of small-scale (m²; 100 – 2000 mussels per group) experiments designed to provide a quick (<1 yr) preliminary assessment of the major factors that may limit the use of sub-adult (30 – 70 mm SL) and juvenile (10 - 30 mm SL) green-lipped mussels in seafloor
restoration efforts (Fig. 1). The specific hypotheses tested in this study were: (A) sub-adult mussels would survive translocation to soft-sediment regardless of protection from predators or hydrodynamic dislodgement, (B) survival of translocated sub-adult mussels would improve with prior attachment to a biodegradable substrate, (C) survival of juvenile mussels would improve with prior attachment to a biodegradable substrate, (D) survival of juvenile mussels would improve with prior attachment to substrate of different sizes, structures, and (E) survival of juvenile mussels would improve by optimizing seeding density.

Methods

Study area

This study was conducted at two semi-sheltered sites within the Mahurangi Harbour, New Zealand, from March 2019 - February 2020 (Fig. 2). The primary experimental site (Site One) was shallow, ranging from 4 - 10 m depth located midway up the harbour. For experiment A, a second shallow site (4 m, Site Two) located closer to the harbour entrance was added to compare potential site differences on experimental cage replicates. Both sites were characterised by similarly mixed mud-sand soft-sediment seafloor. This harbour has

![Figure 1: Conceptual diagram describing the assessment process, starting with the trial of sub-adult mussel survival over subtidal soft-sediments. Each box represents a single field experiment, with the exception of juvenile mussel survival over soft-sediment and biodegradable substrate which were tested over two separate deployments. Arrows depict the sequence of experiments conducted between March 2019 – February 2020.](image)
has been extensively monitored and is considered a suitable proxy to test restoration techniques at degraded sub-tidal areas that historically supported shellfish reefs.

The mussels used in this study were sourced primarily from a nursery farm (referred to hereafter as cultured mussels) that collects and ongrows wild settling larvae on suspended longlines in Aotea Harbour, New Zealand (Aotea Marine Farm, 38° 0' 25.21" S, 174° 49' 41.61" E). In two cases (specified below) mussels came from alternate sources: once from a natural intertidal mussel reef at Pakiri Beach (36° 15' 28.41" S, 174° 4' 53.38" E; referred to

![Figure 2: Map of the Mahurangi Harbour, New Zealand. Black dots represent site locations. Site one varied in depth between 4-10 m. Site 2 was at a mean depth of 4 m and was used to supplement results from the sub-adult cage study at Site 1.](image-url)
hereafter as wild mussels) where sub-adult mussels were hand-collected, and once from a floating upwelling culture system (hereafter FLUPSY; cultured juvenile mussels) located near the experimental site in Mahurangi Harbour. All mussel collections for the study were conducted in accordance with the Ministry of Primary Industries (MPI) special permit 679.

(A) Survival of sub-adult mussels in the absence of predators

In March 2019 the survival of cultured sub-adult mussels over soft-sediment was compared among three levels of predator exclusion following transfer from suspended aquaculture to soft-sediment seafloor. Loose cultured mussels (mean SL = 33.5 ± 0.2 SE mm) were collected and transported to the restoration site overnight in a refrigerated truck (12-14 °C). Mussels were counted into 18 groups of 200 mussels apiece and assigned to one of three treatments: caged, fenced (cages without tops), or uncaged. The quantity of mussels used in these experiments was larger than those used in past field studies (25 mussels, McLeod et al. 2012; 40 mussels, Wilcox & Jeffs 2018) and was considered sufficiently representative of optimal densities found in naturally-forming groups of mussels (Snover & Commito, 1998).

In all treatments individual mussels were placed directly on the seafloor (Fig. 3).

- Caged treatments were used to assess mussel survival over soft-sediments in the absence of large mobile predators (e.g., crabs, sea stars, and fish), reduced flow across mussel groups, and mussel migration from the plot (Wilcox 2017). Cages were constructed from rectangular frames of 10 mm diameter steel rod covered with 20 mm plastic mesh (Fig. 3A; cage dimensions: 0.5 × 0.5 × 0.2 m [L × W × H]).

- Fenced treatments acted as a procedural control for the influence of the roof on mussel survival (i.e., reduction of flow across mussel groups, deposition of sediment, and attachment to experimental treatment structures). Fenced treatments followed the same design to cages but without a top (Fig. 3B; dimensions: 0.5 × 0.5 × 0.2 m [L × W × H]).
Uncaged treatments involved no physical structure and were used to represent current restoration methods that seed mussels directly to soft-sediment seafloor (Fig. 3C).

Scuba divers deployed six replicates from each treatment (i.e., 6 caged, 6 fenced, 6 uncaged) at equal spacing and in a randomised sequence around each of three 20 m diameter circles on the seafloor. Circular arrays were selected to ensure sufficient isolation of groups so that treatment effects (e.g., cages acting as new habitat attracting roving predators) would be minimized for all groups. One week after the deployment of experimental materials to Site One, divers deployed four groups of 200 mussels each under cages at Site Two to test the effect of location on sub-adult mussel survival. No other experimental treatments (e.g., fenced, uncaged) were deployed at Site Two due to a lack of mussel availability.

At 20 days, when no mussels were found at fenced or uncaged treatments these materials were recovered. Caged groups at both sites were monitored 20 days and 50 days post-deployment of site one to document changes in mussel abundance over time. For caged groups, divers temporarily removed cages and recorded individual videos for each group of mussels from less than 1 m away with a GoPro 3+ action camera. This method obtained 360° views of each mussel group, ending with a vertical top-down shot to avoid physically removing mussels to obtain abundance counts. To assess the accuracy and precision of abundance estimates from video sampling, video recordings were taken (360° around each group of mussels less than 1 m away with a GoPro 3+ action camera ending with a vertical

![Figure 3](A) Example of predator exclusion cage deployed with relevant experiments, (B) fenced treatments (i.e., cages without a top), and (C) control treatments without any structure.
top-down shot) of six groups of mussels with known abundances (i.e., 1 group each of 50, 60, 70, 80, 90, and 100 mussels) in a large outdoor seawater tank. Counts from top-down images of groups of mussels captured, on average, $77 \pm 2\%$ of the true mussel abundance, which was better than frames from side profiles.

After 50 days, divers permanently removed cages from mussel groups to assess mussel survival after becoming newly exposed. The experiment was checked 14 days later. When no mussels were found, the experiment was ended and all remaining materials were recovered. Individual mussels in each group were counted from top-down images of mussel groups to produce estimates of mussel abundance 20 and 50 days after initial deployment (ImageJ 1.50i NIH).

(B) Survival of sub-adult mussels attached to biodegradable substrate

In June 2019, the survival of wild sub-adult mussels pre-attached to a biodegradable substrate was compared to those pre-attached to conspecifics (without substrate) to determine whether pre-attachment to biodegradable substrate could improve mussel survival following transfer to soft-sediment. Wild sub-adult green-lipped mussels (mean SL = 29.4 ± 0.5 mm SE) were hand collected (to avoid dislodgment of associated mussels) from a local intertidal mussel reef. When possible, byssus threads were severed with a knife to avoid disturbing clumps and byssus glands. Four hours after collection, 12 groups of mussels were measured out with a 1 L plastic scoop (mean = 235 ± 39 SD mussels) and a random subgroup of twenty individuals from each group was measured to obtain an estimate of initial mussel SL for each group.

Six groups of mussels were transferred to six 0.5 × 0.5 m squares of biodegradable substrate (0.02 m thickness; BC400JR Cirtex BioCoir™ erosion control mat; referred to hereafter as BioCoir; Fig. 4A), while the remaining six groups were transferred to six trays filled with soft-sediment only so that individuals would attach to one another with byssus threads and not to the trays. All groups were held in tanks supplied with unfiltered seawater
(tank dimensions: 1.8 m diameter, 0.58 m depth, 1500 L capacity) for approximately 48 hr to attach to BioCoir and/or conspecifics in a controlled environment (i.e., without predators or tidal currents that may limit attachment).

Following the attachment period, all groups were transferred to the seafloor at Site One. Divers arranged two adjacent rows of six groups apiece with groups of mussels randomly assigned to locations 2 m apart within each row so that potential predator effects would be similar across all groups. Divers secured BioCoir mats using steel rods that were inserted flush with the sediment surface at each corner to prevent dislodgement by tidal currents, whilst reducing any increased structure. Sixty days after deployment, divers recovered all remaining mussels and mats and counted all live mussels in each group. The shell lengths of a random subgroup of 20 mussels from each group were measured to estimate growth between deployment and recovery.

(C) Survival of juvenile mussels attached to biodegradable substrate

In October 2019, experiment B was repeated for smaller cultured juvenile mussels to determine whether juvenile mussels would survive transfer to soft-sediment with and without biodegradable substrate. Cultured juvenile green-lipped mussels were obtained from the nursery farm in Aotea Harbour and sorted into two size classes based on shell length: less than 10 mm (mean SL = 6.9 ± 0.6 mm SE) and greater than 10 mm (mean SL = 12.5 ± 1.0 mm).

Mussels were detached from seeded culture lines and counted out into twelve groups of 100 mussels apiece (due to mussel availability in these size classes), measured (SL; 24 groups in total) and randomly assigned to the two treatments: pre-attachment to conspecifics and pre-attachment to biodegradable substrate. Eight groups of mussels from each size class (16 total) were placed on individual 0.25 × 0.25 m BioCoir mats (smaller sections of material were used as groups of juvenile mussels were substantially smaller than sub-adult groups),
while the remaining four groups from each size class (8 total) were transferred to trays of soft-sediment. The unbalanced division of groups related to a lack in availability of mussels > 10 mm SL but was considered to be sufficient to test the effectiveness of prior attachment of mussels to BioCoir mats. Groups of mussels were again given approximately 48 hr to attach to BioCoir and/or conspecifics in a controlled environment (i.e., without predators or tidal currents that may limit attachment).

Following the pre-attachment period, all 24 groups were transferred to the seafloor at Site One. Divers arrayed groups of mussels 0.2 m apart in a grid pattern (six rows of four groups) so that potential predator effects would be similar across all groups. Groups of juvenile mussels pre-attached to BioCoir mats were secured with steel wire stakes (0.25 m long, 1.6 mm diameter) inserted flush to the sediment surface to prevent dislodgement by tidal currents. The experiment was checked 17 days after deployment and no mussels were found, so the experiment was ended and all remaining materials recovered.

**D** Survival of juvenile mussels attached to different sizes and structures of biodegradable substrate

The survival of cultured juvenile mussels was compared across three kinds of biodegradable substrate to examine whether prior attachment to primary settlement substrate could improve mussel survival following transfer to soft-sediment compared to juvenile mussels pre-attached to two different sizes of BioCoir mats.

In May 2019, eight coir rope bundles (25 m long, 4 mm diameter rope wound into 1 m long bundles; Fig. 4B) were vertically suspended at the nursery farm until collected mussel larvae settled and grew to juvenile shell length (>10 mm). In November 2019, all bundles were recovered with attached juvenile green-lipped mussels (mean SL = 15.4 ± 1.3 mm). All visible juvenile mussels on each bundle were counted. Twelve mussels were randomly selected and measured from each bundle.
During bundle recovery, loose juvenile green-lipped mussels (mean SL = 12.9 ± 0.2 mm) were collected from culture lines at the nursery farm. Eighteen groups of mussels were counted out, measured, and assigned to one of two mat sizes. Different numbers of mussels were used between the BioCoir mat sizes to maintain the proportion of mussels in relation to substrate area.

- Twelve groups of mussels were measured out with a 0.5 L plastic scoop (mean = 478 ± 49 mussels) and then placed on individual 0.5 × 0.5 m BioCoir mats
- Six groups of mussels were counted (mean = 2000 ± 2 mussels) and placed on individual 1 × 1 m BioCoir mats

Five hours after harvest, all mussels were transferred to tanks for approximately 48 hr to attach to BioCoir and/or conspecifics in a controlled environment (i.e., without predators or tidal currents that may limit attachment).

**Figure 4:** Example of biodegradable substrates, (A) cultured juvenile mussels attached to a 0.25 × 0.25 m section of biodegradable matting with the wire stakes used to secure it in the sediment in the field, and (B) a 1 m long bundle of 25 m of coir rope with attached cultured juvenile mussels.
Following the pre-attachment period, all groups of mussels were transferred to Site One. Bundles were placed in a single row 1 m apart and secured to the seafloor with steel rod stakes flush with the bundle without disturbing attached mussels. All twelve 0.5 × 0.5 m squares were placed in a row 1 m apart and secured to the seafloor with wire stakes flush to the sediment surface. Every other 0.5 × 0.5 m BioCoir mat was covered with a cage (same cages as in A) as a procedural control for predators, hydrodynamic dislodgement, and migration outside the plot. All six 1 × 1 m BioCoir mats were placed in an adjacent row (2 m between rows) 0.5 m apart and secured to the seafloor with wire stakes flush to the sediment surface. Linear arrays were selected so that potential predator effects would be similar across all groups.

To document potential causes for mussel losses, divers deployed a single programmable time-lapse camera (CoralCam™; Greene et al. 2020) on a 10 cm stand adjacent to one 1 × 1 m substrate with the camera programmed to record one image every five minutes during peak daylight hours (0720 – 1920 at this time of year). Twenty-two days after deployment divers recovered all bundles and uncaged BioCoir mats and counted remaining mussels to document mussel survival. Due to weather and current conditions, caged groups could not be recovered until eighty-four days after deployment. All recovered live mussels were counted and measured.

Predator presence was recorded from still images of known mussel predators (Australasian snapper, Pagrus auratus, Forster 1801; Usmar 2012; and New Zealand eagle ray, Myliobatis tenuicaudatus, Hector 1877; Michael 1993) recorded by the time-lapse camera. Predators were counted and, when photo quality allowed, body lengths of individual snapper were estimated by comparing the head-height-to-eye-diameter ratio (Richardson et al. 2015).

(E) Survival of juvenile mussels in relation to seeding density
In February 2020, the survival of cultured juvenile mussels on BioCoir was compared at three different densities to determine if mussel losses were a factor of seeding density (mussels/m²). Juvenile green-lipped mussels (mean SL = 12.0 ± 0.6 mm) were collected from a floating upwelling system (FLUPSY; i.e., cultured) located near the experimental site. Fifteen groups of 200 mussels apiece were counted out and evenly spread out over three randomly assigned treatments:

- Low-density groups consisted of 0.25 × 0.25 m BioCoir mats.
- Mid-density groups consisted of a 0.12 × 0.12 m area within 0.25 × 0.25 m BioCoir mats.
- High-density groups consisted of a 0.06 × 0.06 m area within 0.25 × 0.25 m BioCoir mats.

Mid-density was based on mean juvenile mussel densities employed in European bottom culture practices (i.e., 3189 mussels/m²; Capelle et al. 2016); while low (800 mussels/m²) and high (12,800 mussels/m²) density treatments were based on halving and doubling areas occupied by mussel groups, respectively. To prevent substrate from falling apart, which may have influenced the outcome of experiment C, sections were secured to a 0.25 × 0.25 m square section of plastic mesh (20 mm mesh) with wire ties. All groups were monitored for one hour to ensure mussels did not migrate from prescribed areas before being transferred to a channel in the FLUPSY that circulated unfiltered seawater (channel dimensions (L × W × Depth): 2 × 0.6 × 0.5 m) and given 24 hr to attach to BioCoir in a controlled environment (i.e., without predators or tidal currents that may limit attachment). Groups were then checked to ensure densities were consistent before being transferred to Site One and randomly placed in a single row 1 m apart and secured to the seafloor with wire stakes flush to the sediment surface. The experiment was checked eight days after deployment and no mussels were found, so the experiment was ended and all remaining materials recovered.
Statistical analyses

For each experiment, the difference was taken between mussel abundances at deployment and recovery and divided by the number of days deployed to obtain corrected mussel loss (i.e. average mussel loss per day).

Separate student $t$-tests were used to assess changes in the abundance of deployed mussels and shell length for all experiments. Paired $t$-tests were selected for comparisons where groups of mussels were related, such as mussel abundance or shell length for individual groups between dates. Independent $t$-tests were selected for comparisons between groups of unrelated mussels, such as mussel abundance and shell lengths between sites or substrate types. Paired and independent $t$-tests were only used to compare results within and not between separate experimental deployments. All statistics were carried out using IBM SPSS Statistics 25.

Results

(A) Survival of sub-adult mussels in the absence of predators

Twenty days following deployment to the seafloor all mussels were absent from uncaged ($n = 6$) and fenced ($n = 6$) treatments at Site One.

For caged treatments ($n = 10$) survival estimates from videos were nearly equal between the first 20 days ($51 \pm 1$ SE %; $102 \pm 6$ SD mussels) and 50 days ($51 \pm 5$ SE %; $101 \pm 24$ SD mussels) after deployment ($t(5) = 0.05, p = 0.962$). Corrected mussel loss was greater at 20 days ($5 \pm 0.1$ SE mussels/day) than 50 days ($2 \pm 0.2$ SE mussels/day; Fig. 5). Estimated survival of mussels in caged treatments at Site Two was $46 \pm 6$ SE % after 50 days on the seafloor, which was not significantly different from Site One. Estimated abundances of caged mussel groups at the time of experimental cage removal (day 50) were equal between Site One ($101 \pm 24$ SD mussels) and Site Two ($114 \pm 23$ SD mussels; $t(5) = -1.03, p = 0.35$).
Fourteen days after divers removed the cages, mussels were completely absent from all ten groups of mussels across both sites.

**Figure 5:** Boxplot comparing mussel loss per day for different experimental deployment periods for three experiments deploying sub-adult and juvenile mussels into the same site in the Mahurangi Harbour (Initial Mussel Abundance – Final Mussel abundance / Days deployed). Blue interquartile range box indicates the middle 50% of the data with the red line indicating data median. Black error bars account for top and bottom 50% of data with red crosses indicating outliers. Experiments C and E had no mussel survival so are not included in the graph. Experiments (A) and (B) represent sub-adult mussel survival whereas experiment (D) pertains to juvenile (Jv) mussels.
(B) Survival of sub-adult mussels attached to biodegradable substrate

Sixty days following deployment to the seafloor, mean survival of wild sub-adult mussels pre-attached to biodegradable substrate (79 ± 9 SE %) equalled those attached to conspecifics (79 ± 8 SE %; t(9) = -0.027, p = 0.98). Corrected mussel loss was equal between sub-adult mussels pre-attached to substrate (0.8 ± 0.2 SE mussels/day) and those attached to conspecifics (0.8 ± 0.3 SE mussels/day; Fig. 5).

On average, mussels deployed on substrate increased shell length by 1.8 ± 0.7 SE mm between deployment (mean SL = 29.6 ± 0.7 SE mm) and recovery (mean SL = 31.4 ± 0.7 SE mm); which was less (but not significantly so) than mussels placed on soft-sediment which increased shell length by 2.6 ± 0.7 SE mm SL between deployment (mean SL = 29.2 ± 0.8 SE mm) and recovery (mean SL = 31.8 ± 0.7 SE mm; t(218) = -0.384, p = 0.701).

(C) Survival of juvenile mussels attached to biodegradable substrate

Seventeen days after deployment, none of the 2400 juvenile mussels deployed remained for all groups (n = 24) on 0.25 × 0.25 m biodegradable substrate or those attached to conspecifics.

(D) Survival of juvenile mussels attached to different sizes and structures of biodegradable substrate

Between May 2019 and October 2019, coir rope bundles collected an average of 255 ± 25 SD mussels with an average shell length of 15.4 ± 1.3 SE mm at the nursery farm. Twenty-one days after deployment at Site One, nine juvenile mussels remained across all coir rope bundles.

Juvenile mussels were absent from all uncaged biodegradable substrate sections (n = 12; 0.5 × 0.5 m and 1 × 1 m) twenty-one days after deployment. Eighty-four days after deployment, 25 ± 5 SE % of all caged groups of mussels remained (114 ± 21 SD mussels). When corrected for days deployed, caged juveniles lost an average 4 ± 0.29 SE mussels/day
Mussels recovered from cages increased shell length by an average of $13.5 \pm 1.0$ SE mm between deployment (mean SL = $12.9 \pm 0.2$ SE mm) and recovery (mean SL = $26.8 \pm 0.8$ SE mm).

The time-lapse camera deployed to one of the $1 \times 1$ m biodegradable substrates captured images of Australasian snapper and a New Zealand eagle ray visiting the group within 24 hr of seafloor deployment. Time-lapse footage captured 561 images over 5 days with the presence of likely predators of juvenile mussels identified in 125 images (22%). Snapper appeared the most, seen in 124 of the 125 predator images. Calculations estimated the mean total length of snapper to be $261.9 \pm 38.0$ SE mm from a selection of 22 images (the other 102 photos were unusable).

**(E) Survival of juvenile mussels in relation to seeding density**

Eight days after deployment, none of the deployed 3000 juvenile mussels remained across all groups ($n = 15$) of seeding densities.

**Discussion**

This study suggests that cultured sub-adult and juvenile mussels can survive direct translocation to soft-sediment seafloor but require additional measures, especially predator exclusion, to allow them to become established – possibly – until they reach an adult size. When corrected for days deployed, mussel loss per day suggested that cultured sub-adult mussels suffered high mortality immediately after translocation to the seafloor but decreased over time, whereas cultured juvenile mussels protected from mobile predators continued to sustain high losses over the course of their deployment. High survival rates of unprotected wild sub-adult mussels greatly contrasted with the complete loss of unprotected cultured sub-adult and juvenile mussels. The poor survival of cultured juveniles indicates that cultured juvenile mussels are particularly sensitive to translocation to the seafloor and are unsuitable for initial restoration of degraded areas. In contrast, wild intertidal sub-adult mussels are
more resilient than cultured sub-adult mussels of a similar size (~30 mm shell length) to predation and/or hydrodynamic dislodgement following translocation. The characteristics of wild intertidal mussels that limit predation or dislodgement of mussels may be related to differences in growth histories, which has implications for how mussels are grown for restoration.

High sustained loss of juvenile mussels, even with protection from predators (caged), may restrict their use for the initial establishment of subtidal mussel reefs. The growth observed in surviving cultured juvenile (4.51 ± 0.31 mm/month; caged) and wild sub-adult (0.98 ± 0.43 mm/month; uncaged) mussels (similar to previously measured allometric growth rates for subtidal [6 mm/month] and intertidal [3 mm/month] green-lipped mussels; Hickman 1979) suggested that mussels were not totally constrained by food availability and may have been differentially affected by alterations to their environment (e.g., differences in the amount of suspended sediment; Gui et al., 2016). At smaller sizes, juvenile mussels possess a high energy demand for growth (Hickman, 1979) but lack fully developed gill structures that allow for efficient food capture compared to their sub-adult and adult counterparts (Gui et al., 2016). This trade-off may restrict their ability to cope with the stresses of establishing reef habitat in subtidal areas dominated by soft sediments prone to resuspension.

Wild intertidal mussels and cultured subtidal mussels possess different growth histories that result in distinct physiological and morphological variations, which may have influenced some the results of substrate use by sub-adult mussels (Hickman 1979). After settlement on intertidal rock substrata, wild mussels are exposed to wide thermal extremes, starvation periods, and wave action that results in slower (stunted) growth; thicker, heavier shells (Beadman et al. 2003); and more numerous byssus threads (Hickman 1979; wa Kangeri et al. 2014). In contrast, after settlement on spat rope, cultured mussels are exposed to narrow thermal extremes and a relatively constant food source provided by subtidal currents that
result in faster growth; longer, thinner shells; and fewer byssus threads (provided there are
less perturbations during development; Hickman 1979; wa Kangeri et al. 2014). Thicker
shells and stronger byssal attachment increase resistance to physical removal, handling, and
consumption by mobile predators - which may explain the differences in survival of the
mussels from wild versus cultured sources observed in this study (Beadman et al. 2003;
Freeman 2007).

Biodegradable substrates (coir matting and coir rope) were ineffective at preventing
cultured juvenile mussel loss, limiting their use as a tool for restoration; a result consistent
with intertidal restoration efforts using blue mussels (de Paoli et al. 2015; Schotanus et al.,
2020). Our method aimed to improve mussel survival by including a period of time that
allowed mussels to attach to biodegradable substrates or conspecifics prior to transfer onto
soft-sediments (a strategy similar to pre-seeding shell material with oyster larvae for oyster
reef restoration; Fitzsimons et al. 2019) but this made no difference to the outcome of
experiments. Changes in substrate area and seeding density were equally ineffective in
preventing the near complete removal of cultured juvenile mussels. At a small-scale, the coir
mats and rope bundles provided little more than an attachment surface for translocated
cultured mussels and did not prevent snapper or rays from removing translocated mussels
within the first 24 hr following experimental set-up.

One potential limitation of this study was the scale at which the experimental
materials and mussels were deployed. Large-scale deployments of mussels for restoration
often rely on significant tonnages of mussels deployed to areas that occupy 20 – 100 m².
However, the use of small, discrete groups of mussels (100 – 2000 mussels) deployed in grid-
like arrays suitably represented naturally forming clumps in wild mussel populations while
avoiding repeated losses of large tonnages of mussels. Furthermore, there is little evidence to
suggest that the mussels used in this study were any more at risk of predation than they would have been at a larger reef, as similarly observed by Bertolini et al. (2020).

Over the course of this study, cultured sub-adult and juvenile mussels were consistently absent after placement on the seafloor regardless of shell length, location, time of year, or pre-attachment to biodegradable substrate or conspecifics. Time-lapse images provided evidence that removal by mobile fish predators contributed to the consistent loss of cultured mussels beginning immediately after transfer to the seafloor. However, the single time-lapse camera employed in this study only captured images at a single mussel group every five minutes during daylight hours and failed to capture images at night, leaving large gaps in the amount of predator activity that could be related to mussel losses at other groups. Mobile sea stars have previously been reported as greatly limiting the persistence of both sub-adult (Paul-Burke & Burke 2016) and adult green-lipped mussels (eleven-armed sea star, *Coscinasterias muricata*, Verrill, 1867; Wilcox and Jeffs 2019) at some of the sites used in restoration efforts in New Zealand, with a relatively slow but ongoing predation pressure. In contrast, the predatory impact of mobile fish predators thwarted the successful establishment of the cultured sub-adult and juvenile mussels by quick and thorough removals. Regardless of the identity of the predator involved, the sustained predator pressure observed over multiple days coupled with daily tidal currents was enough to ensure that all exposed juvenile mussels and shell fragments were absent upon recovery.

While cages were effective for preventing fish predation, they are impractical to implement on a large-scale for mussel reef restoration. There is a need to further investigate alternative predator deterents that can be implemented at a larger scale (> m²) if sub-adult and juvenile mussels are to be considered further for mussel reef restoration. In aquaculture impacted by diving duck predation, mussel farmers have deployed large nets around culture lines to reduce predator pressure (Varennes et al. 2013), however, this is costly, logistically
complex, and likely impractical for mussel reef restoration. Restoration trials at the Wadden Sea have used breakwaters and fences to limit crab predation, however, as we found with the first cage experiment, this is ineffective for sub-adult or juvenile mussels that are consumed by fish (Schotanus et al., 2020). Future work that considers a mixture of deterrents (e.g., physical barriers and acoustic deterrent devices) or a temporal component (e.g., temporary physical barriers until mussels reach adult sizes; time of year) offer potential avenues to improve survival and growth of sub-adult and juvenile mussels used in restoration.

Predation may not be the only factor preventing sub-adult and juvenile mussel survival. The losses of caged sub-adult (50% after 50 days over soft-sediment) and caged juvenile cultured mussels (75% after 84 days when attached to BioCoir mats) in separate experiments indicate that factors other than predation cause losses of translocated mussels. Stress of translocations, sedimentation, changes in food availability, thermohaline stress, and conspecific smothering are all examples of factors that may have contributed to sub-adult and juvenile mussel mortality under cages. Any long-term success of restored beds will need to identify these causes to reduce significant potential losses, but only once an effective predator deterrent technique is developed.

Sub-adult and juvenile mussels present a potential alternative to aid the restoration process but require further study to understand their specific habitat requirements and factors related to their persistence. This study provided a preliminary assessment that shows that cultured sub-adult and juvenile mussels will initially survive transfer to soft-sediments but require physical barriers to prevent predation. High survival of wild versus cultured sub-adult mussels suggests that some element of morphology (e.g., shell thickness, quantity of byssus threads) or physiology (e.g., food capture efficiency) is required to achieve significant survivorship. Future work that investigates biological or artificial means to exclude or deter
predators (e.g., predator resistance in wild mussels) as a way to overcome predator losses of restored mussels may provide alternative strategies to advance mussel reef restoration.
CHAPTER THREE

The importance of stock selection for improving transplantation efficiency

Abstract

The restoration of shellfish reefs to soft-sediment environments often relies on the translocation of donor stock, typically from aquaculture, to the seafloor. In New Zealand, sub-adult green-lipped mussels (*Perna canaliculus*) grown on subtidal aquaculture long-lines are being considered over adult conspecifics to increase restoration efficiency but are currently limited by predation and hydrodynamic dislodgment following transfer to the seafloor. In this study, the survival of sub-adult mussels from five different sources representing a range of growth conditions (i.e., subtidal aquaculture long-lines 1 & 2, subtidal shellfish aquaculture baskets 1, wild intertidal reefs 1 & 2) were compared across separate translocations (1 & 2) to see if careful consideration of stock source could improve sub-adult mussel survival following transfer to the seafloor. Mussel morphology (shell strength, attachment thread structure) and mussel clumping behaviour (Perimeter:Area ratios, clump densities, clump complexity) were compared among populations to explain relationships among prior stock growth conditions, mussel size, and survival. Following experimental translocations, high survival (> 90%) was correlated with groups of mussels from stock with high shell compression strength (subtidal aquaculture long-line 2, 95.5 ± 1.2 SE %), greater attachment thread number (aquaculture basket 1, 92.3 ± 1.5 SE %), or some combination of the two (wild intertidal reef 2, 99.3 ± SE 0.3%). High survival was closely related to decreases in Perimeter:Area ratios of mussel clumps and increases in patch complexity. This study supports further consideration for incorporating sub-adult mussels into restoration provided they are selected from sources that produce individuals with the resistant characteristics outlined in this study.
Introduction

The restoration of shellfish reefs is rapidly gaining traction as an effective conservation intervention that can be used to resist the degradation of nearshore habitat (Bayraktarov et al. 2020) and recover ecosystem function (e.g., eastern oyster, *Crassostrea virginica*, Schulte et al. 2009; blue mussel, *Mytilus edulis*, de Paoli et al. 2015; green-lipped mussel, *Perna canaliculus*, Wilcox et al. 2018). While restoration actions are often designed to assist natural recovery processes (e.g., promoting settlement and recruitment of oyster larvae to reef materials, Schulte et al. 2009), some degraded ecosystems require “substantial intervention to compensate for natural recovery potential” (Gann et al. 2019). Since the natural settlement, recruitment, and establishment of shellfish to soft-sediment systems often requires the presence of conspecifics (e.g., blue mussels, Commis et al. 2014; Commis et al. 2016), these interventions frequently rely on the translocation of a pioneer population, typically from aquaculture, to the seafloor (e.g., blue mussels, de Paoli et al. 2015, Capelle et al. 2019, Schotanus et al. 2020a, Schotanus et al. 2020b; green-lipped mussels, Wilcox et al. 2018). In New Zealand, mussel reef restoration has so far relied on the transfer of adult mussels (70 – 100 mm shell length (SL)) from subtidal aquaculture long-lines to restoration sites where they naturally organize into reef structures (Wilcox et al. 2018). Although effective, the use of adult mussels incurs significant costs both in time and money in the establishment of restored reefs, underlining the need to develop more viable alternatives to progress restoration efforts (Wilcox et al. 2018). Since this process was partly modelled after European seafloor culture practices that translocate juvenile or ‘seed’ mussels (blue mussels, Capelle et al. 2016) to subtidal culture sites, there is the potential to employ smaller mussels to improve restoration efficiency by increasing the number of mussels harvested per kilogram (10 – 15×) and decreasing culture time (< 1 yr) before translocation to restoration sites.
The use of juvenile (10 – 30 mm SL) and sub-adult (30 – 70 mm SL) mussels from long-line stock for large-scale restoration in New Zealand is often restricted by post-translocation predation (e.g., Australasian snapper, Pagrus auratus, and New Zealand eagle ray, Myliobatis tenuicaudatus; Alder et al. 2020) and hydrodynamic dislodgement similar to the tidal flow that limited the establishment of restored intertidal blue mussel reefs (de Paoli et al. 2015). Conversely, the high survival (> 80%) of wild intertidal sub-adult mussels during small-scale experimental translocations (Alder et al. 2020) suggested that characteristics developed in an intertidal environment, such as stronger shells and more numerous and thick byssus attachment threads (Hickman 1979), may have improved resistance to subsequent predation and dislodgement. At small (m²) scales, long-line juvenile and sub-adult mussel losses can be mitigated by engineering measures such as predator exclusion cages (e.g., green-lipped mussels, Alder et al. 2020) or breakwaters that lower environmental stressors to promote organization into reef structures (e.g., for intertidal blue mussels, Capelle et al. 2019; Schotanus et al. 2020a; Schotanus et al. 2020b). However for restoration efforts at larger (> km²) scales, the deployment of engineering measures is both difficult and impractical (Schotanus et al. 2020a), highlighting the need to investigate methods that emphasize natural resistance to external threats (Alder et al. 2020).

The translocation of wild mussel populations receives little to no consideration in mussel reef restoration in New Zealand due to the historically heavy exploitation of wild reefs, the negative effects associated with removals (i.e., loss of habitat, depletion of larval sources, degradation of seafloor or intertidal reef) and the direct access to mussels from a well-established long-line aquaculture industry (Wilcox et al. 2018). While the use of cultured mussels avoids detrimental environmental impacts, they may be less well-suited for restoration. Since these mussels are raised for the purpose of rapid production, they tend to develop weaker shells and fewer, thinner attachment threads compared with wild intertidal
conspecifics (Hickman 1979). For juvenile and sub-adult mussels, these features may reduce their capacity to contend with the stresses encountered during harvest (e.g., physical detachment from growth substrate), transport (e.g., prolonged starvation and exposure during transport to restoration sites, being crushed by conspecifics), and translocation (e.g., changes in food supply, post-transplant exposure to novel predators, energetic costs of conspecific reattachment). Furthermore, since natural resistance to predation and dislodgement builds when mussels form clumps (≥ 2 mussels attached by byssus threads; Côté & Jelnikar 1999) and clump networks (i.e., structured aggregation of mussel clumps; de Jager et al. 2020), small differences in individual mussel characteristics, combined with the expression of clumping behaviour, can have mounting consequences for the function and long-term resilience of reef systems (de Paoli et al. 2017).

The use of sub-adult mussels will fail to improve mussel restoration efficiency unless methods to mitigate predation and/or dislodgement immediately following translocation are refined (Alder et al. 2020). Solving this issue will rely, in part, on the selection of sub-adult mussels from stock capable of withstanding translocation pressures. In this study, survival following experimental translocations was compared among five different populations of mussels to determine the effect of stock source on restoration success. Features characteristic of mussel resistance to predation and hydrodynamic dislodgement (i.e., shell strength, attachment thread number and diameter, clumping behaviour) were compared among five different source populations (3 from aquaculture, 2 from wild intertidal reefs) to explain variations in survival. The primary aim of this study was to determine whether there would be differences in survival among mussels from different source populations when experimentally translocated to the seafloor as they would be for restoration. A secondary aim was to determine whether variations in morphological and behavioural characteristics among
populations corresponded to variations in survival of mussels translocated to a restoration site.

**Methods**

**Mussel sources**

Approximately 1450 sub-adult mussels (33 – 53 mm mean shell length (SL)) were collected from five source populations to represent three main growing conditions: subtidal aquaculture long-lines (x2), subtidal shellfish aquaculture baskets (x1), and wild intertidal reefs (x2).

Source populations 25-75 km from the translocation site were selected to keep collection and transport times relatively consistent among all populations and translocations (Fig. 1).

![Figure 1: Map of mussel populations (coloured squares) sampled to compare survival following translocation to the seafloor at the subtidal restoration site (yellow asterisk). Long-line mussels came from two separate long-line aquaculture farms whereas intertidal wild mussels came from two separate naturally occurring populations.](image)
each population, mussels were detached by hand from their substrata and transferred to holding systems circulating unfiltered seawater within 12 hours of collection. Due to the nature of collections, some mussels lost byssal threads during detachment from substrata and conspecifics.

Subtidal aquaculture long-lines
Subtidal aquaculture long-line mussels are raised from wild-caught spat that come attached to seaweed that wash up on beaches or are passively collected on suspended long-lines. These mussels are then seeded onto fibrous polypropylene long-lines which are then suspended between large floats following a modified Japanese long-line design (Skelton & Jeffs 2020; Fig. 2A). These farms are typically developed in semi-sheltered areas near the coastline (usually in 5 - 10 m of water) where there is sufficient water movement to maximize food delivery to mussels while minimizing physical disturbances that might restrict rapid growth (Jeffs et al. 1999; wa Kangeri et al. 2014). While these farms attract a range of fish species, there is evidence that predators like the Australasian snapper habitually consume juvenile and sub-adult mussels by detaching them from long-lines (Morrisey et al. 2006). The long-line mussels used in this study were randomly collected from two commercial mussel farms (long-line 1 and 2, 6.5 km apart; Fig. 1) during intermediate seeding of lines where sub-adult mussels are thinned and re-seeded to optimal mussel densities (approximately 200-280 mussels m\(^{-1}\) of long-line, Aquaculture New Zealand, 2020).

Subtidal shellfish aquaculture baskets
Subtidal aquaculture basket mussels originated from a wild settlement into plastic baskets (Hexcyl Pro 1014 Oyster Baskets, 10mm mesh, 732 × 270 × 140, length × width × height) used to improve meat conditions of undersized oysters before delivery to market (similar to the floating baskets used by Rankin et al. 2018, Fig. 2B). These baskets were suspended in a sheltered, shallow (<5 m) subtidal area of the Kaipara Harbour where wave energy is used to
swing the baskets to “rumble” oysters to stunt excessive shell growth and promote meat formation (Rankin et al. 2018). Given the construction of these baskets, mussels were assumed to be protected from large roving predators that might be encountered at long-line farms (e.g., Australasian snapper) or wild intertidal reefs (e.g., reef sea star, *Stichaster australis*; Paine 1971). The mussels used in this study were randomly collected by farmers during routine maintenance that removed mussels to promote oyster growth.

*Wild intertidal reefs*

Wild intertidal mussels originate from spat that naturally settle and attach to conspecifics and/or rocky substrata within the littoral zone (Jeffs et al., 1999). Intertidal reefs, like the ones visited in this study, can occur along exposed areas of coastline subject to daily physical disturbance caused by tidal surf (Fig. 2C & 2D). Intertidal 1 was located at a rock outcrop on the western coastline of the North island of New Zealand and is regularly subjected to continuous swells and predation by reef sea stars (Paine 1971; Fig. 1). Intertidal 2 was

*Figure 2: Examples of the different mussel sources. (A) Sub-adult mussels attached to subtidal aquaculture long-lines. (B) Example of plastic shellfish aquaculture baskets used to “rumble” shellfish to improve meat quality before delivery to market. (C) Intertidal wild mussel reef 1 (Muriwai Beach). (D) Intertidal wild mussel reef 2 (Pakiri Beach).*
located at a rocky promontory along the eastern coastline of the north island at an exposed point regularly subjected to intense wave action. The collection of wild mussels from both reefs occurred haphazardly throughout the entire reef at each location to minimize collection pressure on pre-formed clumps of mussels.

**Field comparison of survival**

For field comparisons of survival, mussels from each population were partitioned into seven groups of 200 mussels each (1400 total per population) and held in individual plastic baskets (6 mm mesh) that remained submerged in holding systems until translocation to the seafloor. The quantity of mussels used in field comparisons was considered a sufficient representation of the numbers observed in naturally-occurring clumps of mussels (blue mussels, Snover & Commito 1998; green-lipped mussels, Alder et al. 2020). Note that groups refer to the sets of 200 mussels (n = 7) from each population (n = 5), while clumps refer to aggregates of ≥ 2 mussels attached by byssus threads (Côté & Jelnikar 1999) occurring within each group.

Survival comparisons among populations were conducted across two separate experimental translocations within the Mahurangi Harbour in the North Island of New Zealand in July 2020 (translocation 1: long-line 1, intertidal 1, aquaculture basket 1) and September 2020 (translocation 2: long-line 2, intertidal 2). Given the logistical constraints (e.g., distance from source, access to marine farms, access to intertidal populations) of collecting from multiple populations in a timely manner, two separate translocations were considered sufficient to examine the relationship between growth environment and survival. Both translocations occurred within New Zealand winter months, which are similar in terms of water temperature and clarity. Each translocation lasted one week and recorded changes to clump morphology before (deployment) and after (recovery) transfer to the seafloor for each group. One week was considered sufficient to document initial survival of deployed mussels.
as previous studies in the same harbour suggested the highest losses of sub-adult mussels occur within the first week following transfer to the seafloor (Alder et al. 2020).

The study site was shallow (< 4 m) and characterised by mixed mud-sand soft-sediment. Previous work has documented the ubiquitous presence of Australasian snapper (Usmar, 2012) and New Zealand eagle rays (Alder et al., 2020) at locations adjacent to the study site. This harbour once supported extensive New Zealand horse mussel (*Atrina zelandica*) reefs which were likely lost to excessive sediment loading (Cummings et al., 2003) and is considered a representative ecosystem (i.e., it resembles sites within the range of historic mussel reefs; McLeod et al., 2012) within which to test methods for restoring mussel reefs over soft-sediments.

Previous work found that loose, unprotected sub-adult and juvenile mussels disappear within one week of being placed at experimental sites (Alder et al., 2020). To improve initial post-deployment survival, the mussels in this study were provided a clumping period prior to deployment. To account for differences in holding times, mussels from each group were physically detached from conspecifics or the sides of their holding baskets four days prior to each translocation to measure SL and then returned to holding baskets. On the day of deployment, all seven mussel groups from each population were carefully removed from their respective holding baskets (maintaining the integrity of any clumps that had formed following initial detachment) and photographed next to a scale to document clump morphology at deployment (including clump abundance per group) before being transferred to the seafloor by scuba divers. Divers arranged the groups of mussels into a modified randomized complete block design with rows of seven groups apiece separated by 1 m spacings to keep potential predator and hydrodynamic effects similar across the study site. Each group was placed adjacent to, but not touching, a pre-marked guideline in a plot approximately 25 cm × 25 cm. This design minimized differences in experimental set-up and
allowed for comparisons of survival and clump morphology between the two translocation events.

Seven days after deployment, all remaining groups of mussels were recovered and photographed next to a scale to document clump abundance and morphology. Care was taken during recovery to maintain the integrity of formed clumps. Due to differences in turbidity between sampling dates, top-down photos were taken of each mussel clump once recovered from the seafloor to ensure that the clumps could always be seen in the photos. Clumps were re-positioned by the same person who recovered them in a manner similar to how they were encountered at the experimental site (i.e., with the majority of valves pointed up). All live mussels were counted and their SL measured. The number of live mussels present at recovery was divided by the initial number (200) to obtain percent survival.

**Mussel clump morphology**

Surface areas and perimeters of mussel clumps from each mussel group were measured from top-down digital images before and after deployment by tracing the edge of each clump-substrate boundary using Image J (1.50 I NIH). Although this ‘top-down’ technique fails to account for vertical and three-dimensional complexity to gain true profiles, it was considered sufficient to obtain complexity values that could be compared among groups (Commito & Rusignuolo 2000). When more than one clump was present in a group, the areas and perimeters for all clumps were summed to create a total mussel area and perimeter for the group. Perimeter:Area (P:A) ratios were calculated for each group by dividing the total perimeter by the total surface area (Capelle et al. 2019). Mussel group densities were calculated by dividing the total number of mussels in each group by the total surface area of the group.

Fractal dimensions ($D$) were calculated for each group at recovery for a measure of final clump complexity. Given the variation in clumped and loose mussels prior to
deployment (but not at recovery), initial fractal dimensions were not obtained. Complexity was selected as a measure to compare discrete structural differences of mussel clumps among populations that might not be captured by changes in density or P:A. Fractal dimensions were calculated using a grid-boundary method (Sugihara & May 1990) following the protocol used to establish the fractal patterns of mussel clump outlines developed by Snover and Commito (1998). In this protocol, seven different sized grids (with the number of squares in each grid equal to: \(2^0, 2^1, 2^2, 2^3, 2^4, 2^5, 2^6\)) were overlaid on top-down digital images (1:1 scale) of each group using the grid overlay plug-in in ImageJ. The number of squares with a mussel-substrate boundary was counted for each grid square size. \(D\) was the negative value of slopes obtained from linear regressions performed on a double log scale of grid square side length against the number of squares containing boundaries (e.g., Appendix A in Bertolini et al. 2017). All \(D\) values were converted to absolute values for the purpose of the analysis and visual presentation.

**Mussel morphology**

For laboratory comparisons of byssus and shell properties, fifty mussels from each of the five populations were cleaned of biofouling and their external byssus threads cut at the shell margin so that newly secreted threads could be distinguished (note: only mussels with pre-existing visible threads were selected for laboratory comparisons). Mussels were numbered with non-toxic paint and placed onto plastic trays (0.46 × 0.36 × 0.025 m, L × W × H) submerged in tanks circulating unfiltered seawater. These mussels were left for one week to naturally self-organize and reattach to plastic trays and/or conspecifics. Mussels were then carefully detached from trays and conspecifics with a scalpel by severing threads near the byssus attachment plaques to keep newly secreted byssus intact. Mussel shell length, width, and height (to the nearest mm) were measured for each mussel prior to dissections of byssus threads. Following dissections, wet shell mass and wet flesh mass were recorded once
drained of excess seawater. A wet mussel condition index was calculated for each mussel by dividing the wet flesh mass by the whole wet mass (shell and flesh; Hickman & Illingworth 1980).

Due to the logistic difficulties of accessing mussel populations for sampling, including regional COVID-19 restrictions, mussels from each population were harvested and held for different amounts of time prior to translocation (10-38 days) and laboratory analysis (7-29 days). This affected the sample size used in laboratory analysis of byssus properties, resulting in the comparison of 25 mussels from translocation 1 populations and 50 mussels from translocation 2 populations.

Byssus abundance and diameter
Mussels can resist dislodgment and predation following transfer to soft-sediment seafloor by attaching to conspecifics with byssus threads secreted by the pedal gland at the base of the mussel foot (Bell & Gosline 1996). Over time, the base of secreted threads overlay and compact into laminar sheaths to form a collagenous central stem that provides further anchorage support to individual threads (Price 1983). Therefore, byssus properties were assessed to compare relative attachment capabilities among populations of mussels. For this, a minimum of three byssus thread diameters were measured (to the nearest mm) above where the threads widen at their base near the point of their attachment to the central stem (Fig. S1). To standardize byssus thread counts between mussels dissected at different times, only byssus threads present in the last 2 mm of the central stem (referred to hereafter as byssus abundance) were counted, as threads in this portion of stem were deemed to have formed since collection. In cases where stems were absent due to natural variations in the timing of byssus formation, all threads were counted. When present, the diameter and length of the central stem was measured (to the nearest mm) for each dissected mussel. All dissection work
was done using a dissection microscope with a digital camera attachment (Micro Capture Ver7.9, Nikon SMZ800; Nikon Corporation, Tokyo, Japan).

*Shell compression strength*

Observations of the most common mussel predators in New Zealand, especially snapper and eagle rays, show they consume the mussels by crushing the shells with their jaws (Michael 1993; Usmar 2012). Therefore, mussel shell compression strength was tested for 50 mussels from each population by recording the peak compression force (N-mm²) at the point of the first major failure (Fig. S2). One empty shell from each dissected mussel was compressed (interior face of shell pointing down) in a saline water bath (36 PSU) using a load tester (Bluehill Compression Test, Instron E3000; Instron Testing Systems, Norwood, Massachusetts, USA) programmed to extend a circular steel rod (diameter 13 mm) at 10 mm per minute onto the highest point on the external surface of the shell. Mussel shells were positioned such that the load compressed the highest point near the middle of each mussel shell (Fig. S2).

*Data analyses*

General linear models (GLMs) were used to relate variations in clump morphology to survival, mussel morphology to shell compression strength, and mussel morphology to byssus abundance and diameter. GLMs were not conducted categorically to compare differences between translocations (1 and 2) but were instead conducted as a single backwards selection multiple regression that allowed for investigation of interactions between multiple metrics of clump and mussel morphology. Since the mussels used for laboratory analysis were not used in field experiments, the data from lab and field comparisons were analysed separately. Note, metrics for clump morphology were used to explain variations in survival among populations while metrics of mussel morphology were used to explain variations among populations regardless of survival.
Multicollinearity was assessed using variance inflation factors (VIF) which help to explain how much of the variance of an independent variable is influenced by its interaction or correlation with other independent variables. Variables with VIF values greater than 10 were reviewed for their inclusion and one was selected for further analysis based on highest correlation (Pearson’s r) with survival (Table S1). VIF scores were recalculated for the remaining variables to verify all values were less than 10 (Table S1). The VIF curated list of predictors was used as explanatory variables for survival in the GLMs, with the most parsimonious final models generated using a backward selection procedure based on Akaike’s Information Criterion (AIC) scores.

A one-way analysis of variance (ANOVA) was used to test for differences in mean shell compression strength and byssus properties (i.e., diameter of stem, number and diameter of threads) among populations. Shell length was more closely related to population and did not change significantly for any population over the course of the translocation period. For this reason, shell lengths at deployment and recovery were excluded from analysis of clump morphology and compared with separate one-way ANOVAs. Holding time was initially included in the GLMs for survival, shell strength, and byssus properties as a potential covariate but failed to demonstrate any significant effects within or between populations and so was removed from subsequent analyses. Relationships between shell length, shell mass, and shell compression strength were visualized with linear models.

To meet the assumptions of normality for all cases, data were appropriately transformed when needed and tested for heterogeneity of variance using Levene’s tests. A Bonferroni post-hoc test was selected when p-values were significant (p < 0.05). All analyses were conducted using IBM SPSS Statistics 26.

Results

Field comparison of survival
One week after each translocation to the seafloor, the survival of mussels was significantly different when compared among the five populations ($R^2 = 0.91$, $F_{(4,34)} = 76.38$, $p < 0.001$; Table 1; Fig. 3A). Groups with high survival (> 90%) came from intertidal 2 (99.3 ± 0.3 SE)

Figure 3: (A) Mussel survival and (B) clump complexity, $D$, upon recovery after a one-week deployment on the seafloor. Survival is represented on the y-axis and was calculated by dividing the final number of mussels in each group ($n = 7$) by the initial amount ($n = 200$) and converted to percentage. $D$ is a quantitative measure of patch complexity ranging from 1 (least complex) to 2 (most complex). Lower case letters indicate significant differences based on post-hoc analyses of group means from a general linear model of survival among populations at recovery. Numbers next to names on the x-axis and vertical dotted lines indicate which populations belonged to translocation 1 (left) and translocation 2 (right).
%, long-line 2 (95.5 ± 1.2 SE %), and aquaculture basket 1 (92.3 ± 1.5 SE %) populations. Intermediate survival (50-75%) was observed in groups from the intertidal 1 (67.1 ± 0.1 SE %) population, and the lowest survival (< 50%) was observed for groups from the long-line 1 (39.9 ± 0.1 SE %) population (Fig. 3A).

**Table 1**: Predictive factors of survival related to mussel clump morphology, shell compressive strength, byssus abundance, and byssus diameter based on separate backward selection multiple regressions. Akaike’s Information Criterion (AIC) were used to select the most parsimonious models.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>R²</th>
<th>AIC</th>
<th>Predictors</th>
<th>Standardized β coefficient</th>
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**Mussel clump morphology**

Upon recovery, clump complexity (\(D\)) was significantly different among populations \((F_{(4,33)} = 5.20, p < 0.001\)) with the highest complexity values generated for mussel groups from long-line 2 (1.39 ± 0.02 SE) and similar, low complexity values for aquaculture basket (1.34 SE 0.02) and both intertidal populations (intertidal 1, 1.29 ± 0.02 SE; intertidal 2, 1.28 ± 0.01 SE; Fig. 3B). There were no significant differences in P:A ratios among populations at deployment \((F_{(4,34)} = 1.21, p = 0.326; \text{Fig. 4A})\) but became significantly different among populations at recovery \((F_{(4,34)} = 24.57, < 0.001; \text{Fig. 4B})\). At recovery, high survival groups demonstrated lower P:A values than at deployment, whereas low survival groups demonstrated higher P:A values than at deployment. Mussel densities were significantly different among populations at deployment \((F_{(4,34)} = 19.21, p < 0.001; \text{Fig. 4C})\) and recovery \((F_{(4,34)} = 13.31, p < 0.001; \text{Fig. 4D})\). Mussel densities decreased similarly for all groups such that the same significant differences among populations at deployment were seen among populations at recovery. Although the initial number of mussels was equal across each group for all populations, small differences in shell lengths and the ratio of loose:clumped mussels at deployment resulted in substantial variation in the areas occupied by groups from different sources at both deployment and recovery. The highest clump densities were recorded for mussels from the intertidal 1 population at both deployment (0.64 ± 0.03 SD mussels·cm\(^{-2}\)) and recovery (0.58 ± 0.04 SD mussels·cm\(^{-2}\)). The lowest clump densities were recorded for mussels from the long-line 2 at both deployment (0.31 ± 0.02 SD mussels·cm\(^{-2}\)) and recovery (0.27 ± 0.01 SD mussels·cm\(^{-2}\); Fig. 3D). GLM results of the most parsimonious model indicated that survival was best predicted by P:A ratios at recovery, clump complexity (\(D\)) at recovery, and group densities at both deployment and recovery (Table 1).
Figure 4: Perimeter:Area ratios and mussel density at deployment (A,C) and recovery (B,D) after a one-week deployment on the seafloor. P:A is the quotient resulting from the division of total group perimeter and total group surface area. Mussel density is the quotient resulting from the division of the total number of mussels in a group by the total group surface area. Lower case letters indicate significant differences based on post-hoc analyses of group means from a general linear model of survival among populations at recovery. Numbers next to names on the x-axis and vertical dotted lines indicate which populations belonged to translocation 1 (left) and translocation 2 (right).
Mussel morphology

Byssus abundance and diameter
Byssus abundance (13.8 ± 6.9 SD threads, $R^2 = 0.24$, $F_{(5,172)} = 10.38$, $p < 0.001$; Fig. 5A) and stem diameter (0.87 ± 0.04 SE mm, $R^2 = 0.47$, $F_{(5,172)} = 29.47$, $p < 0.001$; Fig. 5B) were significantly higher for mussels raised in aquaculture baskets compared with all other populations. GLM results indicated that byssus abundance was best predicted by stem diameter, shell length, population, stem length, and wet condition index (Table 1). Byssus diameter was thickest for mussels from intertidal 2 (0.23 ± 0.01 SE mm) and thinnest for mussels from long-line populations (0.14 ± 0.01 SE mm; $R^2 = 0.56$, $F_{(3,172)} = 72.01$, $p < 0.001$; Fig. 5C). GLM results indicated that byssus diameter was best predicted by population, shell length, and stem diameter (Table 1).

Shell compression strength
Shell compression strength was significantly different among populations ($R^2 = 0.58$, $F_{(4,249)} = 84.97$, $p < 0.001$; Fig. 5D). Mussels from the intertidal 2 population had the highest mean compression strength (375.5 ± 23.2 SE N·mm$^{-2}$) followed by mussels from the long-line 2 population (212.4 ± 11.6 SE N·mm$^{-2}$), which shared a distribution similar to long-line 1 (159.6 ± 6.9 SE N·mm$^{-2}$) and intertidal 1 (197.7 ± SE 14.0 N·mm$^{-2}$) populations, but greater than mussels from aquaculture baskets (149.5 ± 11.7 SE N·mm$^{-2}$). GLM results indicated that shell compression strength was best predicted by shell mass, shell length, population, and wet condition index (Table 1).

Shell characteristics
Mean shell length was significantly different among populations at deployment ($F_{(4,34)} = 40.13$, $p < 0.001$) and recovery ($F_{(4,33)} = 70.12$, $p < 0.001$). Long-line 2 mussels had the highest mean SL between deployment and recovery (52.18 ± 0.92 SE mm) which was different from all other populations (all $p < 0.05$). Intertidal 1 mussels had the lowest mean
Figure 5: Morphological characteristics of mussels from the five source populations: (A) byssus thread abundance, (B) stem diameter, (C) byssus thread diameter, and (D) shell compressive strength. Numbers next to names and dotted lines indicate which populations belonged to translocation 1 (left) and translocation 2 (right). Lower case letters indicate significant differences based on post-hoc analyses of group means from a general linear model of survival among populations at recovery. Numbers next to names on the x-axis and vertical dotted lines indicate which populations belonged to translocation 1 (left) and translocation 2 (right).
SL between deployment and recovery (33.46 ± 0.68 SE mm) which were similar to aquaculture basket mussels (37.77 ± 0.95 SE mm). Intertidal 2 (42.34 ± 0.65 SE mm) and long-line 1 (44.77 ± 1.05 SE mm) mussels had equal shell lengths at deployment and recovery. Positive linear relationships were observed between shell mass and shell compression strength for all mussels (Fig. 6A). Logarithmic relationships between shell mass and shell length were observed for all mussels with slight differences observed in the distribution between mussels from long-line and intertidal environments (Fig. 6B).

Figure 6: Relationships between (A) shell compressive strength (N·mm⁻²), and (B) shell length (mm) as a function of shell mass (g). Trendlines represent averages based on type of growth environment (subtidal long-lines, subtidal aquaculture baskets, intertidal reefs).
Discussion

The restoration of biogenic habitat depends on careful consideration of larval, seed, and adult stock sources (Schulte et al. 2009; Fitzsimons et al. 2019). For restoration that relies on translocations of populations from aquaculture, careful stock selection that accounts for growth conditions and size may improve survival and restoration efficiency (Alder et al. 2020) while avoiding the use of costly engineering interventions (Capelle et al. 2019; Schotanus et al. 2020a, Schotanus et al. 2020b). In this study, variations in post-translocation survival among five populations of mussels were related to, or explained by, differences observed in attachment thread morphology, shell characteristics, and two aspects of clumping behaviour (P:A ratio, $D$) – properties that change with source growth conditions as a result of natural variations in degrees of predation and hydrodynamic stress (Beadman et al. 2003; Brazee & Carrington 2006). High survival (> 90%) corresponded with sub-adult mussels from populations that formed large, well-connected clumps partly mediated by the expression of numerous attachment threads (aquaculture basket 1), strong shells (long-line 2), or some combination of the two (intertidal 2). Increases in shell length positively correlated with shell strength and supported the discrepancies in survival for mussels from aquaculture long-line (i.e., long-line 1, 43 mm SL, 40% survival vs. long-line 2, 52 mm SL, 96% survival) and wild intertidal (i.e., intertidal 1, 33 mm SL, 67% survival vs. intertidal 2, 42 mm SL, 99% survival) populations. This study demonstrates that phenotypic differences, likely the result of a combination of environmental conditions (e.g., hydrodynamics, predation) and growth conditions (subtidal aquaculture long-lines, subtidal shellfish aquaculture baskets, wild intertidal reef), may influence mussel survival following translocation to a novel environment. These data suggest that smaller sub-adult mussels can achieve initial survival comparable to the adult mussels currently used for restoration (Wilcox et al. 2018) and have the capacity to progress efficient restoration practice.
The high survival of aquaculture basket mussels suggests that modifications to aquaculture growing conditions can be made to raise mussels well-suited to overcome the pressures that currently limit the use of sub-adult mussels for restoration (Alder et al. 2020). While mussels raised intertidally or to larger shell lengths on aquaculture long-lines (i.e., long-line 2, adult mussels) may develop heavier, stronger shells (i.e., requiring predators to use more force to crush mussels: green-lipped mussels Hickman 1979; blue mussels, Beadman et al. 2003), the survival of 92% of mussels sourced from aquaculture baskets after a one-week translocation to soft-sediment seafloor suggests that an enhanced capacity to produce attachment threads can be equally effective at resisting post-transplant exposure to predators and/or alterations to hydrodynamic regimes. In blue mussels, attachment strength can increase up to 2.9% for every thread produced (Garner & Litvaitis 2013), meaning mussels with more numerous attachments to conspecifics are more resistant to forcible removal (e.g., by large roving predators, Alder et al. 2020) or dislodgement (e.g., by hydrodynamic stress, wa Kangeri et al. 2014). The regular agitation of mussels during their growth in aquaculture baskets likely induced an enhanced ability to produce significantly more attachment threads (as seen with blue mussels in wa Kangeri et al. 2014) than long-line or intertidal conspecifics. Future work should consider whether these conditions can be replicated to raise mussels for restoration and, if so, whether it is economically and/or logistically feasible for restoration at larger scales (> m²).

The variations in clump complexity observed among the high-survival groups in this study suggested that byssus properties (i.e., number, thickness) influence the structural arrangement of mussel clumps when transferred to soft-sediment seafloor, possibly through different modes of conspecific attachment. Upon recovery of mussel groups from each translocation, divers noted more numerous byssus threads for conspecifics of aquaculture basket and intertidal populations, compared to both long-line groups. These observations
could be explained, in part, by similar, low-complexity clump arrangements of aquaculture basket and intertidal groups compared with more complex clump arrangements from long-line 2 mussels at recovery. While the differences in shell lengths likely contributed to differences in complexity, this set of observations indicates that mussels from intertidal and aquaculture basket populations may have also continued to produce attachment threads amongst conspecifics over the course of each translocation resulting in more compact, well-connected clumps. Since reattachment to conspecifics requires a substantial energetic investment (i.e., doubling metabolic rate, Lurman et al. 2013) and is a crucial first step for the development of a mussel reef, failure to continue attachment thread production following transfer to the seafloor could have negative consequences for the long-term formation and function of clump networks in larger reefs (> 1 Ha; de Jager et al. 2020). The ability of mussels to form clumps as part of a larger network in natural reef systems underpins resilience to disturbance (van de Koppel et al. 2008; de Paoli et al. 2017; Capelle et al. 2019) so understanding how to facilitate this behaviour is key for improving the survival of translocated mussels to achieve more efficient restoration practice (Schotanus et al. 2020b).

The recovery of small clumps from the low-survival groups provides additional explanation of how characteristics of individual mussels can influence the emergence of traits at the clump level (as seen with blue mussels in van de Koppel et al. 2005). The mussels from low survival groups (translocation 1) were collectively smaller (33 - 43 mm SL) than the mussels deployed in translocation 2 (42 - 52 mm SL) and possessed weaker shells with lower mass. Coupled with a lack of attachment threads, these features may have made the smaller mussels more vulnerable to dislodgment prior to conspecific attachment (a process that can take many hours, Capelle et al. 2019) thus inhibiting the emergence of additional resistance in mussel clumps. Green-lipped mussels are one of the largest mytilid species currently targeted for restoration (Toone et al. 2021). This means that they may be more resistant than smaller
species to hydrodynamic dispersal following transfer of adults to the seafloor. For smaller species or life stages (i.e., juveniles or sub-adults), it is possible that higher survivorship can be achieved by facilitating the formation of clumps prior to or immediately after transfer to the seafloor. Future restoration efforts should consider mussel size, deployment density (as in Schotanus et al. 2020b), harvesting mussels in clumps rather than stripping them off the aquaculture lines as individuals, and/or providing mussels a period of time to reattach to conspecifics prior to seafloor transfer (i.e., time in holding tanks between collection from aquaculture and deployment to the seafloor).

The comparison of attachment thread properties and shell characteristics in this study facilitated a comparison of the importance of prior growing environment to mussel survival following translocation for restoration. The use of small numbers of mussels in this study provided a unique opportunity to conduct precise comparisons of prior growing conditions on survival while avoiding unnecessary losses that may have resulted from a large-scale effort. The careful selection of source populations also ensured limited genetic variability among populations of mussels (as reported by Apte & Gardner 2001) so that the variations in survival observed could be more closely linked to morphology. As restoration increases in scale (e.g., number of mussels translocated, length of deployment), continued assessment of the relationships between individual mussel resistance and their effectiveness in establishing mussel reefs will help to inform best practice.

This study found that differences in prior growth conditions hold implications for mussel survival when experimentally translocated to the seafloor (primary aim). Differences in survival corresponded with variations in behavioural characteristics and were related to morphological differences (secondary aim) meaning the indicators used in this study (i.e., attachment thread properties, size, shell strength) can be used for future stock selection. While shell strength is a valuable characteristic for resisting predation, the importance of
attachment threads in building mussel reefs, or the ability to produce threads quickly after mussels are deployed to the seafloor, should not be overlooked. This study provides evidence that careful stock selection based on indicators of individual resistance to predation and hydrodynamic dislodgement can be used to improve restoration efficiency using smaller mussels and supports additional considerations for how mussels are raised for use in restoration.
Table S1: VIF Values generated for all explanatory variables (Initial) and after adjusting for multicollinearity (Final). Correlated variable pairs are shown in italics. The variable with the most predictive power was included for further analysis. Note shell length at deployment was not included in the analysis of survival due to a high correlation with clump density at deployment (Pearson’s r = -0.71).

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Figure S1: Photograph showing mussel central byssus stem giving rise to byssus threads. Inset photo indicates where byssus stems were dissected from each mussel. Dots represent the individual byssus threads that were counted within the first 2 mm of stem as measured from the point of attachment to the foot. Solid lines represent the position of the measurements (to the nearest mm) of stem length, as well as the diameters of the stem and byssus threads.
Figure S2: Experimental set up for assessing shell compressive strength of mussel shells using an Instron E3000 load tester. (A) Empty mussel valves were submerged in a metal dish containing seawater (36 PSU) with the interior side of the shell facing down. Mussel shells were positioned beneath a circular steel rod (x diameter) at the highest point on the shell and the compressive analyser was programmed to extend at a rate of 10 mm per minute until just past the point of the first major failure. (B) A typical compressive strength trace for a mussel shell produced from a bluehill compression test carried out by the Instron E3000.
CHAPTER FOUR

Timing mussel deployments to limit predation and improve restoration efficiency
Abstract:
Predators are a major factor limiting the survival of newly translocated species and can contribute to low rates of reintroduction success in both terrestrial and aquatic ecosystems. In this study, experimental deployments were conducted over a ten-month period (April 2021 – January 2022) to relate temporal variations in predator abundance, size, and environmental parameters (water temperature, rainfall, days +/- full moon, turbidity, wind speed, wind direction) to variations in the survival of translocated green-lipped mussels, *Perna canaliculus*. Predator counts from timelapse images gathered for the first four days after each deployment were used as a measure of predator pressure. Timelapse images (n = 8561) allowed for a census of 2371 individuals from 10 different mobile species, 5 of which were known bivalve predators (Australasian snapper, New Zealand eagle ray, rig shark, octopus, and an unidentifiable ray species), with Australasian snapper contributing to 98% of the species observed. At the end of the study, mussel survival ranged between 0 ± 0 SE % to 56 ± 8 SE % and was best predicted by changes in the total number of predators and turbidity among deployments ($R^2 = 0.455$). Patterns in predator abundance were best explained by time of year and did not share strong correlations among environmental parameters (Rho = 0.015). These results suggest that translocated mussel survival immediately after transfer to the seafloor can be improved up to 56% if deployments are planned for cooler times of year (June – November in Australasia) when predator presence is low.
Introduction

In marine systems, the reintroduction of habitat-forming species is an increasingly popular option to restore lost biogenic habitats (Fitzsimons et al. 2019; Bayraktarov et al. 2020). Reintroductions frequently rely on translocations whereby individuals of a species are deliberately moved from one area to another to recover or augment a diminished population (IUCN SSC 2013). While such translocations appear straightforward conceptually, they are difficult to implement in practice (Lindburg 1992) and may have low initial success rates (e.g., 26% success out of 116 reintroductions reviewed, Fischer & Lindmayer 2000). The loss of newly translocated individuals (referred to hereafter as founders) is a key issue for reintroductions of species across both terrestrial (Moseby et al. 2015; Robinson et al. 2020) and aquatic (de Paoli et al. 2015; Wilcox & Jeffs 2019; Alder et al. 2020) ecosystems. This can be especially apparent in heavily degraded areas, such as reclaimed mines or dredged seafloor (Bradshaw 2000; Walker & Roger 2008; Seddon 2014). Upon introduction to a disturbed area, founders must immediately acclimate to a suite of stressors, such as limited food or nutrient availability (Boldt-Burisch et al. 2015), abiotic disturbance (e.g., wave action; de Paoli et al. 2015), and exposure to novel predators (e.g., eleven-armed sea stars, Coscinasterias muricata, Wilcox et al., 2018). While methods to mitigate these pressures vary by species, site, and context, an understanding of the stressors at this stage in the process can help to improve translocation practice (e.g., deployment strategies) by increasing founder survival (IUCN/SSC 2013; Wilcox et al. 2018; Robinson et al. 2021).

Mitigating the removal of founders by novel predators is a major challenge for restoration practitioners (Moseby et al. 2015; Robinson et al. 2020). In terrestrial ecosystems, reintroductions are sometimes planned for predator-free areas such as islands or fenced peninsulas, which can greatly improve founder survival (Seddon et al. 2014; Moseby et al. 2015). Similarly, in marine ecosystems physical barriers such as fences (e.g., Schotanus et al.
2020a,b) or cages (Alder et al. 2020) can be used to reduce predator access to founders. For habitat-forming species, such as mussels, the absence or reduction of predator activity reduces founder losses and enables processes of natural establishment (Balke et al. 2014; Capelle et al. 2019; Schotanus et al. 2020a,b). However, since the use of physical barriers (temporary or permanent) often adds substantial costs to an already expensive process (Lindburg 1992) and is likely impractical for large-scale (> m²) projects, there is a need to consider alternative, more efficient and more cost-effective approaches to mitigate the predation of founders.

Improving founder response to novel stressors and identifying means of naturally mitigating or avoiding predator pressure may be a more viable method to improve founder survival than relying on physical deterrents (Moseby et al. 2015, Ross et al. 2019, Robinson et al. 2020). For shellfish translocations, this could mean selecting founders with predator-resistant traits (Alder et al. 2021), or the simpler method of identifying times of the year with relatively fewer or less active predators (IUCN/SSC 2013). Among terrestrial reintroductions, there is evidence that planning restoration activities for certain times of the year can yield different levels or rates of success. For example, scheduling prescribed burns of tallgrass prairie habitats in the late growing season can yield an eleven-fold increase of flowering prairie plants compared to burns conducted at other times in the year (Copeland et al. 2002). Less is known about how time-of-year affects the outcome of shellfish reintroductions, although it has been suggested that the reintroduction of species like oysters or kelp should target times of year when larval recruitment is high (Maathuis et al. 2020; McAfee & Connell 2020). Temporal variations in predator or grazer (e.g., in the case of kelp translocations, Oróstica et al. 2014) abundance may also be an important factor to consider when planning marine species translocations, however, empirical data is still needed to confirm this proposition. For example, green-lipped mussels, Perna canaliculus, translocated to the
seafloor showed marked seasonal differences in predation by eleven-armed seastars at one site within the Hauraki Gulf (Wilcox & Jeffs 2019),

At some green-lipped mussel reef restoration sites in New Zealand, predation has limited both the long-term (25% survival after 2 years; Wilcox et al. 2018) and short term (0% survival after 1 week; Alder et al. 2020) survival of translocated individuals. Predation has also stymied efforts to make the restoration process more efficient using subadult (30-50 mm shell length (SL)) and juvenile (10-30 mm SL) mussels (Alder et al. 2020). While smaller mussels would greatly increase the number of individuals translocated per kg (10 – 15 × more than adults), they are more vulnerable to predators like Australasian snapper (Pagrus auratus) and New Zealand eagle rays (Myliobatis tenuicaudatus) than their adult counterparts (70 – 100 mm SL; Wilcox et al. 2018; Alder et al. 2020). In temperate marine ecosystems, such as New Zealand, temporal variations in environmental parameters (e.g., water temperature or storm events), play a significant role in the distribution (Perry et al. 2018), composition (Llompart et al. 2013), and diet (Usmar 2012) of nearshore fish communities. For example, Australasian snapper migrate to deeper waters during the cooler months (Parsons et al. 2014) and may switch feeding strategies (Usmar 2012). Similarly, New Zealand eagle rays alter foraging behaviour, becoming more frequent in shallow water during warmer months (Thrush et al. 1994; Hines et al. 1997). This observation indicates that there may be a naturally occurring opportunity to translocate and establish mussels during cooler months where predation intensity is lower, meaning smaller mussels can be translocated with fewer initial losses to predators (Fig. 1). This can provide sufficient time for the mussels to become well established at restoration sites and more resistant to any subsequent increase in predator activity.

In this study, experimental translocations of mussels were conducted at the same locations at different times of the year to assess temporal relationships among mussel loss,
The primary aim of this study was to determine whether variations in mussel survival corresponded with variations in predator abundance and if discrepancies could be explained by variations in environmental variables across the period of each deployment. The goal of this research was to assess whether knowledge of these variations can be used to determine optimal timing of deployments to increase survival rates for future restoration activities. The specific hypotheses tested in this study were: (1) variations in mussel loss would be best predicted by the total number of potential predators observed during the course of each deployment, and (2) the number of predator observations would be lowest during cooler months.

**Figure 1:** Timeline of past experimental translocations in the Mahurangi Harbour New Zealand and outcomes using juvenile (10 – 30 mm shell length) and subadult (30 – 50 mm shell length) mussels. Red shading indicates times of the year with high mortality. Green shading indicates a potential ‘window of opportunity’ where initial survival of translocated mussels is high. Yellow shading indicates transition months. Mortality data was gathered from Alder et al. 2020, 2021.
Methods

Experimental timeline and study area

To test whether mussel survival corresponds with variations in predator abundance and environmental parameters, a series of identical experimental translocations were carried out every 6 weeks during the transition to cooler months in Australasia (April – July 2021) and again three months later during the transition to warmer months (November 2021 - January 2022) in the Mahurangi Harbour, New Zealand. The study area was shallow (4 - 5 m depth) and characterised by mixed mud-sand soft-sediment. Each experimental translocation was conducted at the same three sites (all within 100 m of each other) located near the centre of the harbour, which were in the vicinity of previous research activity translocating mussels to the seafloor (Alder et al. 2020, 2021; Fig. 2). This harbour once supported extensive New Zealand horse mussel reefs (*Atrina zelandica*, Cummings et al. 2003) and is considered a representative ecosystem within which to test methods for restoring mussel reefs over soft-sediment (i.e., it resembles degraded sites within the range of historic mussel reefs, McLeod et al. 2012).

Experimental set-up

Prior to each experimental translocation, adult mussels (99 ± 0.6 mm SE SL) from aquaculture farms were completely detached from growth substrate (i.e., aquaculture long-lines) and/or conspecifics and separated into 15 experimental groups of 25 mussels each. All groups were taken from seawater and deployed to the seafloor within 24 hours. At each of the three experimental sites, five experimental groups were placed in a row on the seafloor by SCUBA divers with each group separated by 1 m (Fig. 2). Groups were placed next to a 10 m guideline stretched between two metal stakes driven into the seafloor and arranged from east-west to align with tidal current flow.

At the ends of each line, a programmable timelapse camera (2 per site, 6 per
Figure 1: (Top) Map of three experimental site locations (black dots) within the Mahurangi Harbour, New Zealand (indicated by the yellow star on the inset map).
(Bottom) Top-down view of experimental set-up at each site (n = 3), squares indicate the position of each group of 25 mussels deployed to the seafloor.
deployment; CoralCam, Green et al. 2020) was attached to a separate metal stake so the camera was 25 cm above the seafloor and pointed at end mussel groups. This field of view allowed cameras to capture images of predator and non-predator species in the vicinity of the mussel groups positioned 1 – 2 m away from the end of the line. This arrangement helped to standardise depth-of-field and minimized any potential hydrodynamic disturbance of the mussels. Cameras were programmed to take one image every 10 minutes between 12:00 – 17:59 on the date of deployment (day 0) and 07:00 – 17:59 for the next four days (day 1 – 4). This daily operating period was selected to account for differences in day lengths across the study period. As this study aimed to detect the presence of mobile predators, two cameras taking images at 10-minute intervals was considered sufficient to detect predator activity around mussel groups for the first four days after their placement on the seafloor (Alder et al. 2020).

**Mussel survival**

After one week, all remaining mussels and materials were recovered. Any pair of empty and intact valves (i.e., attached at the hinge and without flesh) found within 3 m of each experimental site were also recovered and counted as a proxy for non-predator related mortality, even if they may have been scavenged by a visiting predator species. Mussel survival and the proportion of mussels lost to non-predator related causes were obtained for each group by dividing the number of live mussels or whole shells at recovery by the total number of mussels at deployment (n = 25).

**Image analysis of community composition and snapper size**

All captured digital images were assessed chronologically and categorised based on depth of field by the same reviewer. All images with < 1 m of visibility (i.e., where adjacent mussel groups were not visible) were categorised as turbidity obscured images. All images with 2 m of visibility (i.e. where the closest two mussel groups separated by 1 m spacings were visible)
were categorised as clear images (Supplementary Fig. 1). For both turbidity obscured and clear images, all visible mobile species were counted and identified to the lowest taxonomic level. Counts followed a MaxN approach (similar to Priede & Merrett 1996) where all identifiable species present within 2 m of the camera were recorded. Hourly species counts were calculated for each site by summing the total counts of each species captured across the twelve images taken for each hour (i.e., one picture per camera every 10 minutes, 00:00 – 00:59, two cameras per site). Mobile species were classified as potential predators or non-predators of mussels based on previous knowledge of feeding preferences.

When image quality allowed, the body lengths of individual Australasian snapper were estimated by comparing head-height-to-eye-diameter ratios (Richardson et al., 2015). All snapper body lengths and relative abundances were recorded for each deployment and separated into size groups according to life-stage (based on classifications by Parsons et al. 2014): small juvenile, 60-120 mm fork length (FL), 0-1 yr; large juvenile, 120-230 mm FL, 1-3 yr; adult >230 mm FL, 3+ yr. The allometry used to estimate the length of fish from images varies species-to-species and has only been defined for the Australasian snapper observed in this study. As such, only the estimated lengths of Australasian snapper were obtained.

**Environmental parameters**

To account for differences in environmental variables that may influence mussel loss and predator abundance during the course of the study period (Perry et al. 2018, Rueda et al. 2019), six environmental parameters (based on daily totals and means) were selected and compared among deployments using a principal components analysis (PCA): water temperature (°C), daily rainfall (mm), days before/after full moon, turbidity, wind speed (m/s), and wind direction (degrees). Rainfall, wind speed, and wind direction were used as a proxy for storm events and wave action that are known to influence fish assemblage structure
(Friedlander & Parrish 1998) or hydrodynamic dislodgement of mussel clumps (de Paoli et al. 2015) over the course of each week. Since predator species like Australasian snapper are visual hunters and suspended sediment can impact mussel filtration rates, the number of turbidity obscured images recorded for each deployment was included as a proxy for water column turbidity. The number of days before/after full moon was also included as larger tides can contribute to the dislodgement of shellfish placed on the seafloor and lunar phases are known to influence animal activity (e.g., in temperate fish, Hartill et al. 2003; Hanson et al. 2008). Daily water temperature data was obtained from seatemperature.info. All other environmental data for the period of each deployment was obtained from the NIWA CliFlo database (https://cliflo.niwa.co.nz/).

**Data analyses**

*Comparing mussel survival among deployments*

Separate GLMs were used to assess variations in mussel survival and the proportion of whole shells recovered among deployments (fixed 5 levels) and sites (random, 3 levels). To account for the potential influence of the physical presence of the timelapse camera stands on mussel survival, a GLM was used to assess variations in mussel loss among group positions within each site (fixed, 3 levels: next to camera, second from camera, and middle of the row), site (random, 3 levels), and deployment (random, 5 levels).

*Comparing community composition and Australasian snapper size among deployments*

Variations in community compositions based on hourly species counts were assessed among deployments (fixed 5 levels: April, June, July, November, January) and sites (fixed 3 levels: Site 1-3) using a permutational analysis of variance (PERMANOVA; 999 permutations). A similarity of percent contribution (SIMPER) routine was run to determine which species contributed most to variations observed among deployments. Since differences in the number
of turbidity obscured images directly contributes to the number of possible counts for each deployment, a general linear model (GLM) was used to assess the proportion of turbidity obscured images among deployments (fixed 5 levels) and days (fixed 5 levels: day 0 - 4). To assess whether the daily number of turbidity obscured images influenced the number of predators observed among each deployment, a Pearson’s correlation coefficient was used to correlate the total number of daily turbidity obscured images to summed daily predator counts at each site across all deployments. A single GLM was used to compare the proportion of snapper size classes among deployments (fixed 5 levels).

**Relating community composition to environmental parameters among deployments**

To account for differences in data resolution among species counts and environmental parameters (i.e., hourly counts versus daily averages or totals), daily species counts were generated by summing total hourly counts from each species for every site (1 - 3) and day (0 - 4). To test if patterns in species compositions correlated with differences in environmental parameters among the first four days of each deployment (i.e., timelapse operating period), a series of multivariate analyses were selected. A RELATE routine was first used to test for similarities among resemblance matrices for daily community compositions and environmental parameters. This routine determines how well the relationships in the biological resemblance matrix correlate with data in the environmental resemblance matrix based on Spearman rank coefficients. Rho values close to 1 indicate that the patterns are quite similar between both matrices. To assess correlations among species compositions and specific environmental parameters, a biota and environmental (BEST) routine using a BIOENV method was selected (based on 999 permutations). This routine searches through all possible combinations of environmental factors to determine which set best explains patterns the species compositions among the deployments. A distance-based linear model (DistLM) was then used to explain the degree of variation each environmental variable contributed to
patterns in the species compositions. DistLM is similar to stepwise multiple regression where models are selected based on Akaike’s Information Criterion (AIC). This analysis used marginal tests to investigate the contribution of specific environmental parameters to patterns in species community composition among deployments.

*Predicting causes of mussel loss among deployments*

General linear models were used to compare species compositions, the proportion of whole mussel shells, and environmental parameters against final mussel survival among all deployments. The GLMs were not conducted categorically to compare differences among deployments (1-3) but were instead conducted as a single backwards selection multiple regression that allowed for the investigation of interactions between potential stressors that could contribute to mussel loss.

Multicollinearity was assessed using variance inflation factors (VIFs) which help to explain how much of the variance of an independent variable is influenced by its interaction or correlation with other independent variables. Only variables with VIF values \( \leq 10 \) were included in the analysis (Supplementary Table 1). Of the related/correlated variables, one was selected for inclusion in further analysis based on highest correlation (Pearson’s r) with mussel survival (Supplementary Table 1). The VIF curated list of predictors was used as explanatory variables for survival in GLMs, with the most parsimonious final models generated using a backward selection procedure based on AIC scores.

To meet the assumptions of normality for all cases, data were appropriately transformed when needed and tested for heterogeneity of variance using Levene’s tests. Bonferroni post-hoc tests were conducted when \( p \)-values were significant (\( p < 0.05 \)). All GLMs were conducted using IBM SPSS statistics 27. All multivariate data analyses (PERMANOVA, SIMPER, PCA, RELATE, BEST, DistLM) were conducted in Primer-E v7.
Results

Mussel survival among deployments

One week after each translocation to the seafloor, mussel survival was significantly different among deployments (ArcSin transformation GLM, $F_{(3,73)} = 14.7, p = 0.001$; Fig. 3). Post-hoc analyses revealed a 0 - 50% increase in survivorship between warmer (April, January) and cooler (June, July, November) months (e.g., $0 \pm 0$ SE % survival in January versus $56 \pm 8.4$ SE % survival in July). Significant differences in the proportion of mussels lost to non-predator related causes were detected among deployments (ArcSin transformation GLM, $F_{(4,73)} = 5.1, p = 0.001$), sites ($F_{(4,73)} = 3.8, p = 0.029$), and the interaction between the two ($F_{(8,73)} = 3.3, p = 0.003$). Post-hoc analyses revealed that this was driven a lack of mussel

![Graph: Mussel survival after a one-week deployment on the seafloor at different times of year. Survival is represented on the y-axis. Error bars represent the standard error of survival at each site for each deployment. Lower case letters indicate significant differences based on post-hoc analyses of group means from a general linear model of mussel survival among groups at recovery.](image)
mortality that could be related to natural causes during the January deployment (0 ± 0 SE %) compared with all other deployments (8.6 ± 1.1 SE %). Mussel survival ($F_{(2,73)} = 0.3, p = 0.742$) and the proportion of mussels lost to non-predator related causes ($F_{(2,73)} = 0.2, p = 0.847$) were not significantly different among locations within sites for each deployment.

Comparing community composition and Australasian snapper size among deployments

A total of 10,115 digital timelapse images were captured from five separate deployments between April 2021 and January 2022. Of the images gathered, 8561 fell within the standardised operating period (day 0: 12:00 – 17:00; day 1 -4: 07:00 – 17:00) with 4300 classified as clear and 4261 classified as turbidity obscured images. From all images with identifiable mobile species, a total of 2371 individuals from 10 different species were counted. Australasian snapper (n = 2324), New Zealand eagle ray (n = 10), common Sydney octopus (n = 3, Octopus tetricus), an unidentified ray species (n = 3), and a spotted estuary smooth-hound (a.k.a. Rig Shark, n = 1, Mustelus lenticulatus) were all identified as potential predators since all are commonly known to consume bivalves (Michael 1993, Anderson 1997, King & Clark 2010, Usmar 2012). Non-predator species were based on species not commonly known to consume adult bivalves and included parore (n = 12, Girella tricuspidata), trevally (n = 8, Pseudocaranx dentex), koheru (n = 8, Decapterus koheru), spotty (n = 1, Notolabrus celidotus), and a bluefin gurnard (n = 1, Chelidonichthys kumu) (Russell 1983, Sazima 1998, Raubenheimer et al. 2005; Supplementary Fig. 2). Comparisons of community compositions based on hourly counts detected significant differences among deployments ($\log(x+1)$ transformation, Pseudo-$F_{(4,728)} = 26.2, p = 0.001$), sites (Pseudo-$F_{(2,728)} = 9.2, p = 0.002$), and the interaction between the two (Pseudo-$F_{(8,728)} = 5.7, p = 0.001$). Pairwise tests detected a decrease in species counts over the entire course of the study (April 2021 – January 2022), which encompassed a transition from Australasian autumn (April), to
winter (June, July), to spring (November), and into the middle of Australasian summer (January) \((p = 0.001; \text{Fig. 4A})\).

**Figure 4:** (A) The total number of predators vs. non-predators counted at each site (S1-3) for each deployment (April 2021 – January 2022). Count refers to the total mobile species counts for each month summed for all sites. Counts from each site were based on the summed species counts between cameras at the same site. Error bars represent the standard error of counts for each deployment. Lower case letters indicate significant differences based on post-hoc analyses of group means from a permutational multivariate analysis of variance (PERMANOVA) of predator counts among deployments. (B) Percent contribution of species to image counts across all deployments. Color gradients represent predatory (brown) and non-predatory (green) species.
Of the species counted, 2341 were of potential predator species and 30 were of non-predator species, with Australasian snapper accounting for 98% of the individuals observed (Fig. 4B). Of the snapper measured, adult (61.1 ± 0.9 SE %) and large juveniles (38.4 ± 0.9 SE %) accounted for the majority of sightings, which remained consistent among deployments (square-root transformation, F(4,384) = 1.4, p = 0.243; Fig. 5).

There were no clear daily patterns that emerged in the summed hourly counts of all predator species among deployments, however, observations appeared to correlate with incoming tides (Fig. 6). While the proportion of turbidity obscured images were significantly different among deployments (Square root transformation, F(4,74) = 23.8, p = 0.0001), days

![Figure 5: The percent contribution of different life-stages of Australasian snapper, Pagrus auratus, to all measured individuals from each deployment. Length estimations were made using head height:eye diameter ratios (Richardson et al. 2015). Sizes were binned based on ontogenetic life stage. Snapper size classes were based on Parsons et al. 2014: small juvenile 60-120 mm fork length (FL), 0-1 yr; large juvenile, 120-230 mm FL, 1-3 yr; adult >230 mm FL, 3+ yr.](image)

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Figure 6: Summed predator counts across the first four days following transfer to the seafloor. Bars represent the total predator counts from all three sites (S1-S3). Sites are clustered along the x-axis (between tick marks) for each time point (represented as hours). Vertical dashed lines indicate the ends of operating periods, i.e. when cameras shut off overnight. Red and blue triangles indicate low and high tides respectively.
(F\(_{4,74}\) = 20.1, \(p = 0.0001\)), and the interaction between the two (F\(_{16,74}\) = 24.1, \(p = 0.0001\); Fig. 7), there were no significant correlations detected when compared against summed daily predator counts (Pearson’s correlation coefficient = -0.055, \(p = 0.642\)). Post-hoc analyses revealed significantly more turbidity obscured images were recorded for warmer months (April, January) compared to cooler months (June, July, November).

Relating community composition to environmental parameters among deployments

Comparisons of environmental parameters demonstrated that differences in water temperature and the total number of turbid images contributed to most of the variation among each deployment period (i.e., days within each deployment; Fig. 8A). Comparisons among normalised daily environmental parameters and log(x + 1) transformed predator species counts did not reveal any significant correlations (RELATE, Rho = 0.015). Of the environmental parameters examined, the number of turbidity obscured images and water temperature were most closely related to patterns in species abundance (BEST). Overall, environmental parameters explained 18.7% of the total variation observed in patterns of species counts across all deployments, however, only water temperature demonstrated any significant relationship with the patterns observed in predator species detections (DistLM, pseudo-F = 7.4 \(p = 0.001\)). An ordination based on a distance-based redundancy analysis (dbRDA) indicated that the number of turbidity obscured images and water temperature helped drive 17.1% and 1.5% of the total variation respectively (8B).

Predicting causes of mussel loss among deployments

An assessment of the factors likely to predict mussel survival following transfer to the seafloor revealed that mussel survival was best predicted (R\(^2\) = 0.455) by the total number of predators observed, the total number of turbidity obscured images, and mean gust direction for each deployment (Table 1).
Figure 7: Total proportion of turbidity obscured images from all images captured during the operational period (day 0: 12:00 – 17:00, day 1-4: 07:00-17:00) recorded at each site (1-3) for day 0 (deployment) and the first four days after each deployment.
Figure 8: (A) Principal coordinate analysis of daily (days 0 – 4) environmental parameters among deployments. Objects that are ordinated closer together are more similar than those ordinated further apart. The first two axes account for 35.5% (PC1) and 24.1% (PC2) of the total variation respectively. (B) Distance based redundancy analysis (dbRDA) of a log-transformed Bray-Curtis similarity matrix of mobile predator species community data. The first two axes account for 91.5% (dbRDA1) and 8.2% (dbRDA2) of the total variation among the fitted model for mobile species community composition respectively. Mobile predator species community composition data (based on log(x+1) transformed counts) are shown for each deployment.
Table 1: Predictive factors of mussel loss among deployments based on a backwards selection multiple regression. Akaike’s Information Criterion (AIC) was used to select the most parsimonious models.

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<th>Dependent variable</th>
<th>Model</th>
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<th>Predictors</th>
<th>Standardized $\beta$ coefficient</th>
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Discussion

The predation of founders remains a key risk for species reintroductions (Wilcox et al. 2018; Schotanus et al. 2020a) and can hamper efforts to make the restoration process more efficient if left unmitigated (Alder et al. 2020; Esquivel-Muelbert et al. 2021). For habitat-forming species like mussels or oysters, translocation success can be improved by providing founders with a sufficient period of respite from predator stress to allow them to establish in new habitats and acclimate to the local environmental conditions (Balke et al. 2014; Capelle et al. 2019; Schotanus et al. 2020a; Fivash et al. 2021). In the days-to-weeks following the translocation of mussels to the seafloor, mussels undergo a period of re-orientation where they form permanent attachments to conspecifics via the deployment of anchoring byssus threads (Wilcox et al. 2018; Alder et al. 2021). This period can take many hours (Capelle et al. 2019), leaving unattached mussels more vulnerable to removals by mobile predators (Alder et al. 2020). While the temporary addition of physical barriers (like cages, Alder et al. 2020) can shield founders from predators, the use of these structures contributes to additional operational and logistical costs that do not introduce efficiency into large-scale restoration (Alder et al. 2020; Schotanus et al. 2020a,b). In this study, the use of digital timelapse photography revealed that predatory species encounter newly translocated mussels within 24 hours after placement on the seafloor, regardless of the time of year. Variations in predator abundance were more closely related to time of year (as indicated by the relationship with water temperature) than environmental parameters. This suggests that for some restoration sites with a high abundance of predators, predator mitigation strategies will need to be in place prior to deployment to ensure survival of translocated individuals. The results from this study suggest that the impact of predation can be partially mitigated by timing mussel translocations for cooler times of year (June – November in Australasia) when fewer predator species are present and when water column turbidity is low, which together can improve the
survival of translocated mussels by 25-50%. This finding suggests that careful consideration of the timing of mussel deployments is a crucial factor for developing more efficient restoration practice.

Although a number of predator species were identified over the course of this study, the dominance of Australasian snapper in predator counts among deployments was the most useful indicator for how temporal fluctuations in predator numbers and activity correspond with translocated mussel survival. These fluctuations were likely a result of changes in predator numbers due to differing environmental conditions, which is known to influence foraging activity of snapper (Usmar 2012; Parsons et al. 2014). Like some temperate fish species, snapper will sometimes undergo seasonal migrations to deeper water during winter months (Jones 1988; Coles & Tarr 1990; Desmond et al. 2002; Jaureguizar et al. 2004; Parsons et al. 2014). This could help explain the smaller number of individuals observed among deployments during the cooler months of this study (e.g., June, July). Interestingly, very few snapper were observed during the warmer November and January deployments. While this could have been a factor of an increased number of turbidity obscured images, the high abundance of snapper counted in April (which had a similar proportion of turbidity obscured images) suggested a potential shift in temporal predator activity. The relationship among the number of turbidity obscured images and predator abundance provides some evidence that these factors interact to influence mussel survival. Although restored green-lipped mussels can withstand a wide range in turbidity levels (McLeod et al. 2012), the high proportion of turbidity obscured images in the warmer months of this study suggests that mussels had to contend with high levels of turbid conditions. This could have impacted their ability to feed possibly contributing to higher natural losses and the subsequent attraction of nearby predators.
For Australasian snapper, differences in water temperature have been linked to changes in growth rates and spawning behaviour, which usually occurs between October and March once surface waters exceed 15 – 16 °C (Parsons et al. 2014). This can also lead adult snapper to migrate considerable distances (>100 km, Parsons et al. 2014) to spawning aggregations at preferred locations, one of which was located within 20 km of the experimental site (Kawau Island, Zeldis & Francis 1998). Following spawning activity, some fish increase foraging activity to replenish depleted energy reserves (as seen in Atlantic cod, Gaudus morhua, Fordham & Trippel 1999). This could help explain the large schools of snapper observed visiting mussel groups during the April deployment (just after spawning period) and on day 1 of the January deployment (during the spawning period), both of which had the highest amounts of mussel mortality. The absence of predators during the November deployment suggests potential differences in foraging behaviour across the spawning period, however more work is needed to verify this. Future work that looks to assess optimal timing for mussel translocations may also want to consider the proximity of restoration sites to locations of known spawning aggregations of mussel predators and the times of year when these spawning aggregations of predators are occurring.

Aside from temporal considerations, the spatial arrangement of restoration sites may have direct implications for the species attracted to restored reefs and founder survival (as seen with fish communities at restored oyster reefs, Garbowski et al. 2005). In this study, both mussel survival and predator detections appeared related to site, suggesting decisions in the placement of mussels within a restoration site can influence the number of predators attracted to restored reefs. Previous research in the same harbour has demonstrated that some snapper exhibit resident behaviour, occupying a small home range of 100 – 1000 m (Hartill et al. 2003). This could help explain differences in the abundance of snapper observed among sites, which were separated by 100 m spacings. A resident population could also help explain
the lack of any difference in snapper sizes across all deployments. This suggests that some of the experimental sites used in this study were situated outside of the home range of resident snapper and/or influenced the mechanisms that attract snapper to restored reefs (e.g., direction and flow of an odour plume from perished mussels). Future work should consider how different locations within the same system might differ in the level of potential predator activity and how these might contribute to founder survival.

Spatiotemporal variations in predator activity may have wider implications for the establishment of mussel reefs beyond the direct consumption of newly translocated mussels. Over the course of this study, there were several instances where mussel groups shifted location immediately after being visited by groups of snapper, i.e., up to a meter or more among timelapse images. While hydrodynamic dislodgment cannot be ruled out and it is unlikely that mussels would have moved themselves, it is possible that snapper redistributed groups of interconnected mussels while foraging. This redistribution may have facilitated further mussel loss by leaving small clumps or individuals more exposed and preventing mussels from forming into larger, more well-connected clumps. As the density of mussels deployed to an area of seafloor can influence the rate at which mussels find and attach to one another (Bertolini et al. 2017), the redistribution of clumps immediately after mussels are transferred to the seafloor (i.e., within 24 hours) may hold implications for the longer-term establishment of reef structures. While substrate and local hydrodynamic conditions largely mediate the formation of reef structures (e.g., for blue mussels, *Mytilus edulis*, van de Koppel et al. 2008), future work should consider how changes to the spatial arrangement of mussels after transfer to the seafloor impact future mussel reef resilience (e.g., to disturbances, de Paoli et al. 2017) and persistence (Wilcox et al. 2018).

Addressing the causes of reintroduced species loss immediately after translocation can be a major hurdle for restoration practitioners to overcome (IUCN/SSC 2013; Moseby et al.}

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The use of programmable timelapse photography in this study allowed for fine-scale observations of the presence of mobile predators around translocated mussel groups over multiple days. When addressing the causes for mussel loss, this approach may be preferred over diver surveys or baited remote underwater videos (BRUVs), both of which can influence fish behaviour and overlook daily patterns in fish activity (Colton & Swearer 2010). Although the operating period for the timelapse cameras used in this study missed nocturnal predator activity, they still managed to capture temporal variations in predator presence that could be compared across multiple time points and related to mussel loss. Furthermore, the use of this specific type of timelapse technology (CoralCam, Greene et al. 2019) was more cost-effective than other off-the-shelf systems (e.g., CoralCam = $100 NZD vs. CamDO ≥ $2800 NZD), meaning it can be scaled up across multiple deployments and used by multiple restoration groups. This technology may prove to be useful for future restoration site selections that check for predator presence prior to the translocation of mussels to the seafloor. Additional research should consider whether this kind of technology can be incorporated into monitoring efforts before-and-after mussel reintroductions to better understand the degree of impact predators have at multiple deployment scales (i.e., m² versus km²).

Reducing the loss of mussels immediately after transfer to the seafloor is critical for the development of more efficient mussel reef restoration methods (Alder et al. 2020). In this study, correlations among mussel survival and predator abundance provided encouraging evidence that reintroduction success can be improved when temporal variations in predator activity are taken into consideration, particularly during cooler times of year. The use of digital timelapse images proved to be a useful tool in gaining a rapid and cost-effective assessment of the species and abundance of predators present that was comparable across multiple time points. These results provide evidence that future efforts will need to consider
the impacts of predation in the design of reintroduction plans and that the translocation of mussels for restoration should occur during cooler times of the year (Australasian June – November) to optimize mussel survivorship.
**Supplementary Table 1:** VIF values generated for explanatory variables. Correlated variable pairs are shown in italics. The variable with the highest Pearson correlation coefficient with survival was included in further analysis.

<table>
<thead>
<tr>
<th>Model</th>
<th>Explanatory variable</th>
<th>VIF</th>
<th>Pearson correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total predators</td>
<td>2.205</td>
<td>-0.135</td>
</tr>
<tr>
<td></td>
<td>Whole shell abundance</td>
<td>1.314</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>Water temperature</td>
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<tr>
<td></td>
<td>Total rainfall</td>
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<tr>
<td></td>
<td>Wind direction</td>
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</tr>
<tr>
<td></td>
<td>Total turbid images</td>
<td>23.78</td>
<td>-0.622</td>
</tr>
<tr>
<td></td>
<td>Wind speed</td>
<td>264.121</td>
<td>0.093</td>
</tr>
<tr>
<td>2</td>
<td>Total predators</td>
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<tr>
<td></td>
<td>Whole shell abundance</td>
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<tr>
<td></td>
<td>Wind direction</td>
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<td></td>
<td>Wind speed</td>
<td>3.27</td>
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<td></td>
<td>Water temperature</td>
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<td></td>
<td>Total turbid images</td>
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</tr>
<tr>
<td>3</td>
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<td>Wind speed</td>
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<tr>
<td></td>
<td>Total turbid images</td>
<td>1.777</td>
<td>-0.622</td>
</tr>
</tbody>
</table>
**Supplementary Figure 1:** A series of photos from the same group (1 m distance from camera) taken within the same hour to demonstrate clear (A,B) and turbidity obscured (C,D) images.
Supplementary Figure 2: Examples of a subset of the predator and non-predator species identified from timelapse images.
CHAPTER FIVE

General discussion
Summary of findings

The principal aim of the research presented in this thesis was to investigate ways to increase the efficiency of mussel reef restoration methods. The majority of this research (Chapters 2 & 3) investigated the use of sub-adult and juvenile green-lipped mussels (*Perna canaliculus*) for restoration, which would increase the number of mussels translocated per kilogram harvested (10 -15 × more than adults) and decrease their prior cultivation time (< 1 year vs. 2 – 3 years for adults). The disappearance of sub-adult and juvenile mussels, possibly due to high mortality, immediately after placement on the seafloor (i.e., < 1 week) was a persistent issue over the course of this study, observed during multiple experimental field deployments (60 – 100% loss; Chapter 2 – 4). The loss of mussels after translocation to experimental sites prompted investigation into the mitigation of mussel losses using approaches that could be implemented at larger scales (e.g., ≥ 20 m², Chapters 3 & 4). Subsequent research found that post-deployment mussel survival could be mediated through the careful selection of the size and source of mussels, which appeared to be resistant to predation (Chapter 3), and by undertaking deployments of mussels to the seafloor during times of year when predator abundance or activity is low (Chapter 4). Collectively, these results provide strong experimental evidence that increasing the efficiency of restoration will rely on the development of more effective techniques to mitigate mussel mortality following their transfer to the seafloor, especially if sub-adult and juvenile mussels are to be considered further.

Opportunities for using sub-adult and juvenile mussels in restoration

If the high mortality of sub-adult and juvenile mussels following transfer to the seafloor can be mitigated, then they may still present an opportunity for more efficient restoration. For example, ongoing work restoring subtidal mussel reefs in the Marlborough Sounds, New Zealand (Benjamin, unpublished data) and personal observations of restored intertidal mussel
reefs near a spat-collecting farm at Kawhia, New Zealand, suggest that sub-adult and juvenile mussels can form reef structures (m²) that persist for longer (6 months – 2 years, respectively) than the time periods used in this study (1 week – 3 months; Chapters 2 & 3). While the survival of sub-adult mussels at these reefs vary in scale (e.g., in tonnage deployed, area covered), location, and context, future work will need to assess whether higher survival at these reefs can be explained by proximity to growth environments (e.g., for reefs in the Marlborough Sounds, Kawhia, Chapter 3) and/or tidal zone (e.g., for reefs in Kawhia).

However, before sub-adult and juvenile mussels can be used to improve the efficiency of mussel reef restoration in the Hauraki Gulf, further research is needed to address the various causes of mortality following mussel translocations. For example, although predators consumed experimentally translocated mussels over the course of this study (Chapters 2 & 4), hydrodynamic dislodgement (e.g., as seen with blue mussels, Mytilus edulis, in de Paoli et al. 2015; Capelle et al. 2019; Schotanus et al. 2020a,b), and unsuitable habitat conditions may have also contributed to mussel losses and mortality (IUCN/SSC 2013; Wilcox et al. 2018). This is supported by multiple instances where mussel mortality was observed despite the use of protective cages (e.g., 50 % mortality, Chapter 2; 32 % mortality of adult mussels, McLeod et al. 2012) or after accounting for predator pressure (e.g., 60 % mortality, Wilcox 2017) following mussel transfer to the seafloor. These observations provide evidence that a portion of mussel mortality is the result of physiological stressors experienced by mussels during the translocation process, possibly due to excessive handling (e.g., as seen with blue mussels Capelle et al. 2016a), from an inability to acclimate to novel habitats (IUCN/SSC 2013; Wilcox et al. 2018), or some combination of the two. This suggests that considerable improvement to mussel survival and thus translocation efficiency can be made if:

- Harvest and transport of mussels are optimised to minimise physiological stress.
• The predation of mussels can be mitigated following transfer to the seafloor.

• The parameters for suitable mussel habitat are realized.

Reducing physiological stressors during harvest and transport

A number of terrestrial and aquatic species reintroductions (e.g., Banks Peninsula tree wētā, *Hemideina ricta*; Adriatic sturgeon, *Acipenser naccarii*, red-fronted parakeet, *Cyanoramphus novaezelandiae*) have highlighted stress from excessive handling during collection and transport as a major contributor to founder loss following translocation to a novel environment (van Winkel et al. 2010). Excessive handling is also thought to contribute to the high losses of green-lipped mussel spat that limit production potential in New Zealand mussel aquaculture. For example, typically more than 90% of mussel spat are lost shortly after reseeding at coastal mussel farms in New Zealand, which is thought to be partly triggered by translocation stress (South et al. 2021; Supono et al. 2021). Since current mussel restoration in New Zealand is reliant on the use of aquaculture infrastructure (e.g., growth lines, harvest machinery, collection bags, refrigerated trucks), it is likely that the mussels collected and translocated for restoration are similarly affected. For example, mussels collected for production or restoration may be held out of the water for up to 24 hours before they are transferred to growing lines or restoration sites on the seafloor. During this period, mussels are unable to feed (Supono et al. 2020), are at risk of desiccation, hypoxia, and anoxia (Calderwood et al. 2014), and may face thermal extremes they to which they are unaccustomed (Carton et al. 2007). Under stressful conditions, such as harvest and transport, cells in mussel tissues experience elevated production of reactive oxygen species that contribute towards tissue degradation, energy metabolism, and oxidative stress (Nguyen et al. 2020; Delorme et al. 2021). The elevated energy demands of this process likely leads to the depletion of nutritional condition, which can greatly reduce the ability for mussels to reattach to substrates such as growth lines (e.g., 30% decrease in attachment capability, Carton et al.)
or possibly, to find and attach to conspecifics when deployed to the seafloor for restoration (Chapter 3; Calderwood et al. 2014). This may be further exacerbated by poor local feeding and environmental conditions at the destination environment for translocated mussels (Supono et al. 2021), such as degraded sites on the seafloor (Chapter 2; McLeod et al. 2012).

Mussel mortalities during, or as a result of, the translocation process may contribute towards additional mortality following transfer to the seafloor. For example, the equipment used to harvest and transport mussels at large scales usually contribute to a number of mussel mortalities, such as crushed mussels from harvest machinery, before they reach restoration sites (pers. obs., Chapter 2). As a result, crushed mussels generate an odour plume following transfer to the seafloor that attracts nearby predator species, as seen with sea star predation on blue mussels (Drolet & Himmelman 2004). This likely leads to elevated predation on the remaining live mussels (Chapters 2). While some mussel mortalities are unavoidable, reducing stresses (physical and physiological) during translocation is an important additional consideration to enhance the ability of mussels to cope with translocation conditions and, ultimately, to improve restoration outcomes. For example, some traditional Māori translocation techniques of black foot pāua (*Haliotis iris*) incorporate methods to reduce translocation stress and promotes post-translocation survival, such as the translocation in pōhā (bags made from bull kelp used for storing or transporting food) or whilst remaining attached to rocks (Bennett-Jones et al. 2021).

**Mitigating predator impact on translocated mussels**

For some restoration sites, translocated sub-adult and juvenile mussels will encounter high mortality immediately after transfer to the seafloor (e.g., within 24 hours), especially in areas with a high abundance of bivalve predators (Chapter 4). The predation of newly restored
mussels is a widespread issue with significant predator-related mortality observed in a number of previous studies at:

- **New Zealand:**
  - Rotoroa Island (sea stars on adult green-lipped mussels, Wilcox et al. 2018)
  - Marlborough Sounds (sea stars on adult green-lipped mussels at 1 of 7 sites, Benjamin, *unpublished data*)
  - Ohiwa Harbour (sea stars on sub-adult and juvenile green-lipped mussels, pers. comm.; Paul-Burke 2016)

- **Australia:**
  - Peel Harvey Estuary (crab and rays on sub-adult blue mussels, *Mytilus galloprovincialis*, The Nature Conservancy, pers. comm.)
  - Swan-Canning estuary (fish predation on pygmy black mussels, *Xenostrobus securis*, The Nature Conservancy, pers. comm.)

- **Netherlands:**
  - Wadden sea (crab and birds on sub-adult blue mussels, de Paoli et al. 2015)

For mussel reef restoration in the Hauraki Gulf, it is likely that the same predators (e.g., Australasian snapper, *Pagrus auratus*; New Zealand eagle ray, *Myliobatis tenuicaudatus*) observed removing sub-adult and juvenile mussels in the Mahurangi Harbour will also be present at, or near, areas of future restoration (Chapter 4; Parsons et al. 2014; Alder et al. 2020). This means that predator mitigation strategies should be considered prior to large scale translocations of sub-adult and juvenile mussels.

Predator mitigation is a key issue for species reintroductions (Moseby et al. 2015) and if properly managed can benefit the likelihood of translocation success (Robinson et al. 2020). Since large-scale (20 – 50 m²) mussel reef restoration is unlikely to rely on the use of physical barriers, such as cages or fences, to prevent predator access to translocated mussels
(Chapter 2, Schotanus et al. 2020b), there is a need to develop alternative strategies to reduce the impact of predation (Chapters 2 & 3). Future large-scale predator mitigation may eventually rely on the use of predator-resistant mussels (Chapter 3), deploying mussels to sites with naturally low abundances of predators (Chapter 4), deploying mussels in pre-formed clumps (Chapter 2–4, Benjamin unpublished data), careful spatial arrangement of translocated groups of mussels (as seen with juvenile oysters, Macreadie et al. 2011), or even deploying a mixture of sub-adult and juvenile mussels with adult conspecifics (Wilcox & Jeffs 2017).

**Sourcing high resistance sub-adult mussels for restoration**

To determine which mussels should be used for mussel reef restoration, it is useful to speculate on the sources of the first mussels that colonized large areas of soft-sediment seafloor naturally. Since some of the muddy-sand soft-sediment benthic habitats in the Hauraki Gulf have sediment granules too small for settling mussel larvae to attach (Wilcox & Jeffs 2017), the first mussels to naturally establish at these sites (i.e., founder mussels) were likely dislodged from nearby hard substrata. Such a process has been described as occurring for blue mussels over soft-sediments (Commito & Dankers 2001).

Mussels form reefs on hard substrata across a wide range of habitats from the intertidal to the sublittoral (Gosling 1992). In these habitats, groups of founder mussels would have developed a variety of traits to assist with the transition to life on soft-sediment seafloor. For example, some founder mussels may have come from intertidal reefs where regular periods of exposure would have promoted the development of thick, heavy shells providing greater protection from the crushing jaws of snapper or rays (Chapter 3; Garen et al. 2004). In the littoral zone, some founders would have been subject to hydrodynamic forcing (i.e., physical agitation by waves, tides, and currents). This would have made these founder mussels capable of quickly synthesizing numerous attachment threads - a characteristic
important for the formation of tight, protective clumps that limits predator access to individual mussels (Chapter 3; wa Kangeri et al. 2014). Founder mussels may have also established on emergent substrate (e.g., such as rocks, bivalve shells, woody debris, or other biogenic material) where they would have subsequently established over nearby soft-sediment providing an initial foothold in the habitat and providing a refuge for the recruitment of future generations of mussels (Greenway 1969; Commito et al. 2014). Since the translocation of mussels from aquaculture aims to replicate natural reef-building processes, the use of mussels grown in conditions similar to those of the initial founders has the capacity to vastly improve mussel survival and thus the efficiency of future mussel restoration efforts in the Hauraki Gulf.

Should mussel reef restoration continue to rely on the translocation of mussels from aquaculture, the results of this research show that it is worth considering how juvenile and/or sub-adult mussels can be raised to better respond to the stresses of translocation (Chapter 3). In terrestrial and aquatic species reintroductions, improving founder response to translocation pressures, such as predation, is an effective way to improve reintroduction outcomes (i.e., by reducing prey naïvité, Moseby et al. 2015; Ross et al. 2019). In North America, predator avoidance training in translocated fish is common practice due to high mortality following release from hatcheries (Edwards et al. 2021). For example, innate predator avoidance behaviour in hatchery-reared chinook salmon (*Oncorhynchus tschawytscha*) is enhanced by exposing juvenile salmon to a mixture of chemical cues from predators and conspecific fright responses prior to release (Berejikian et al. 2003). Similarly, hatchery-reared juvenile Japanese flounder (*Paralichthys olivaceus*) exposed to predation pressure by sandy shore crabs (*Matuta lunaris*) exhibited significantly higher survival than naïve conspecifics (Hossain et al. 2002). While behavioural training for mussels is unlikely, exposing mussels to predator cues can induce the production of resistant, predator-specific traits. For example,
blue mussels exposed to cues from common littoral crabs (*Carcinus maenas*) that crush mussel prey developed thicker shells, whereas mussels exposed to cues from northern sea stars that pry mussels open developed heavier adductor muscles (Freeman 2007). Future work could consider exposing sub-adult and juvenile green-lipped mussels to ray, snapper, and/or sea star cues to determine whether mussels possess an innate ability to develop specific predator-resistant characteristics, and if so, whether selecting or cultivating young mussels with these traits improves survival on the seafloor (as seen in Chapter 3).

For shellfish like mussels, finding or raising mussels with resistant traits will be dependent on their growth environments (Chapter 3). Given the wide breadth of knowledge presented by mussel aquaculture (e.g., Jeffs et al. 1999; Garen et al. 2004; Capelle et al. 2017) and the use of mussel reef habitats as model systems in ecology (e.g., Commito & Rusignuolo 2001; Hunt & Scheibling 2001; Seitz et al. 2001; van de Koppel et al. 2008, 2012), it is possible to develop grow-out methods for mussels that express preferential physical and behavioural characteristics. For example, changes to stocking density on aquaculture growing lines could be altered to produce mussels with different shell shapes that may correspond with lower or higher resistance to being crushed (Reed 1968; Lauzon-Guay et al. 2005). Personal observations of mussel spat collected and left to grow on aquaculture long-lines without artificial thinning produced thicker, heavier shells than conspecifics re-seeded to optimal densities (such as blue mussels in Lauzon-Guay et al. 2005). While this may not be ideal for aquaculture production, this may be a simple way to grow mussels with more crush-resistant shells. Alternatively, raising mussels intertidally would not only produce mussels with thicker shells (Chapter 3), but may also make them better suited to contend with prolonged periods of exposure and acclimation to a new habitat since they are naturally more resistant to desiccation, being crushed by conspecifics (Chapter 3), and have a longer gut residence time for food (Bayne et al. 1988) – a characteristic that may be important for
mussels that are translocated to areas with reduced quantity or quality of food. Developing restoration-specific mussel culture methods is a longer-term strategy because it may require some modification to existing aquaculture infrastructure, growing mussels under new conditions, and subsequent testing of mussel survival following transfer to the seafloor. Following this, the feasibility of scaling up culture methods to produce enough mussels to meet the demands of large-scale (e.g., multi-tonne) restoration will need to be assessed before this strategy could be considered for more efficient restoration.

In New Zealand, there are a number of intertidal oyster farms that could be modified to raise mussels for restoration (e.g., around 200 farms, Forrest et al. 2007). Future work could consider raising mussels to sub-adult lengths (30 – 40 mm SL) in intertidal oyster baskets and identifying whether these mussels exhibit higher survivorship than long-line conspecifics following transfer to the seafloor (Chapter 3). While this may not shorten the culture period as much as using sub-adult mussels from suspended aquaculture, it may relate to enhanced efficacy of restoration efforts through improved mussel survival, especially for the initial translocated groups of mussels. Furthermore, it would still be faster than the time it takes to raise mussels to the shell length currently used in restoration (e.g., 1 year vs. 2 – 3 years, Jeffs et al. 1999).

The co-culture of mussels and oysters may present an additional option for raising mussels well-suited for restoration (Chapter 3). Since the specific culture of a restoration broodstock is likely to increase project costs, co-culture with another species may present a unique economic opportunity to grow mussels for restoration while simultaneously producing a marketable product (e.g., rumbled oysters, Rankin et al. 2018). Future work should consider whether this method can reliably grow mussels that produce high numbers of byssus threads and if so, whether these methods are capable of producing large tonnages of mussels to be used in restoration.
The culture of restoration-specific mussels may also help mitigate the potential physiological shortcomings of the restored mussel reefs that sustained high mortalities in the months (Chapter 2; de Paoli et al. 2015) to years (e.g., of the mussel losses unaccounted for in Wilcox et al. 2018) following transfer to the seafloor. Since mussels present an opportunity to recover function to degraded areas, they may need to be sufficiently robust to overcome hysteresis effects whereby the trajectory of mussel reef recovery may be different than the processes that led to the initial establishment of historic reefs (Borja et al. 2010; Capelle et al. 2019). This may be especially important when planning restoration for areas with scant evidence of historic subtidal green-lipped mussel reefs, such as the Mahurangi Harbour.

In the Mahurangi Harbour, populations of New Zealand horse mussels, *Atrina zelandica*, once played an analogous role in ecosystem functioning to subtidal green-lipped mussel reefs (e.g., benthic habitat, water filtration, sediment stabilization; Ellis et al. 2002; Fitzsimons et al. 2019). The loss and subsequent lack of recovery of horse mussel reefs to the harbour, possibly as a result of increased sediment loading from land-use changes in the harbour catchment (Cummings et al. 2003), helped prompt experimental restoration efforts using green-lipped mussels (Sim-Smith & Tukua 2019). This is also due, in large part, to the absence of a New Zealand horse mussel fishery and the ready availability of green-lipped mussels from aquaculture for restoration. The multi-year persistence of restored adult green-lipped mussel reefs in this harbour provides evidence that green-lipped mussels can be used to restore function to degraded seafloor (e.g., such as nitrogen fixation, Hillman et al. 2021; Sea et al. 2021) even when translocated outside of their historic range. However, unlike adult mussels, the high mortality of sub-adult and juvenile mussels (e.g., even when protected, Chapter 2; van Kampen 2017) suggest that younger mussel life stages may be less well-suited for the initial establishment of restored reefs, especially in heavily degraded areas. Since historic mussel habitats are similarly degraded (e.g., by commercial dredging, Thrush et al.
1998; Paul 2012), future work should assess the survival of translocated sub-adult and juvenile mussels at or near an existing wild mussel habitat to examine their utility for augmenting diminished populations as this harbour is only representative of a portion the wider coastal system.

**Spatiotemporal avoidance of predators**

Reducing mussel mortality to predators in the hours to days following transfer to the seafloor will be critical for the long-term persistence of mussel reefs. The establishment of mussel reefs relies on the aggregative effects of individual processes (e.g., conspecific attachment) that lead to the formation of small reef structures, like clumps and patches, where individual mussels have higher chances of survival (e.g., safety in numbers; Reimer & Tedengren 2009; van de Koppel et al. 2012). Over time, clumps and patches form hierarchically structured networks that underpin mussel reef resilience to disturbances (e.g., storms, predators, human collections; Committ et al. 2016; de Paoli et al. 2017) and facilitate larval recruitment (Committ et al. 2014), both of which are necessary for the long-term recovery of subtidal mussel reefs. Since the formation of smaller reef structures relies on the presence of conspecifics and occurs in the hours-to-days after mussel deployment to the seafloor (Wilcox et al. 2018), mussel mortality during this period of time can have a magnified impact on restoration outcomes. This means that planning deployments for times of year and/or at sites where predator abundance is low can reduce initial mussel mortality (Chapter 4) and facilitate natural reef building processes when mussels are not deployed in pre-formed clumps.

Since predator assemblages will not be evenly distributed (in space and time) throughout the wider coastal system of the Hauraki Gulf (e.g., among demersal fishes, Leathwick et al. 2006), future work should consider including predator assessments prior to large translocations. This would improve risk assessments of factors likely to contribute to founder losses, which is an important component to include when planning species
reintroductions (IUCN/SSC 2013). The use of timelapse photography is a helpful aid that could be used in preliminary assessments to detect predator species and abundance (Chapter 4). Understanding the kinds of predators attracted to translocated mussels will be important since temporal patterns in predator abundance will be specific to species and site (Wilcox et al. 2018; Benjamin unpublished data; Chapter 4). For example, at restoration sites with a high abundance of Australasian snapper, practitioners should plan translocations for cooler times of year when snapper undergo seasonal migrations to deeper water. This would allow translocated mussels to establish reef structures capable of resisting predation by snapper when they return (Chapter 4; Hartill et al. 2003; Parsons et al. 2014).

The spatial arrangement of mussels deployed to the seafloor, such as placement and isolation from other habitat types, will also influence the species and abundance of predators visiting restored reefs (e.g., as seen for artificial reef communities, Walsh 1985; Seaman Jr. & Sprague 1991). Coupled with an increased understanding of the kinds of species attracted to restored mussel reefs (Sea et al. in review; Benjamin unpublished data; Chapter 4), it may be possible to configure the spatial arrangement of translocated groups of mussels to mitigate predator pressure and increase mussel survival. Networks of small patch reefs are a common feature in larger reef systems (e.g., as seen with blue mussel reefs in soft sediment systems, van de Koppel et al. 2005, 2008; Commoto et al. 2014) and can serve as important refugia for smaller, more vulnerable individuals (Bertolini et al. 2018). For example, juvenile oysters from larger, more continuous reefs were removed more often by sheepshead fish (*Archosargus probatocephalus*) than juveniles located at smaller patch reefs (Macreadie et al. 2011). However, this appeared to vary among the bivalve species observed in the study (e.g., oysters vs. mussels) and predator type (e.g., stone crabs [*Menippe mercenaria*] vs. sheepshead).
To determine how to configure the spatial arrangement of translocated mussels, it might be useful to speculate on the mechanisms that led to the establishment of extensive subtidal mussel reefs over large areas of soft-sediment seafloor in the Hauraki Gulf. One hypothesis of the initial formation of the extensive subtidal mussel reefs in the Hauraki Gulf suggested that a major episode of bush clearance led to a pulse of available settlement substrate in the form of woody detritus that was quickly colonised by larval mussels (Greenway 1969). This patchy network of mussel reefs would have formed the foundation for the future expansion of mussels across the historic 1500 km² range (Paul 2012). It is possible that a similar distribution of restored reefs could improve juvenile and/or sub-adult mussel survival by decentralizing predator pressure and by taking advantage of areas within systems that are naturally absent of predators (e.g., as seen with reef fishes at isolated reefs, Overholtzer-McLeod 2006). This would hold an additional benefit of restoring large areas of seafloor without having to cover the extent with translocated mussels, especially since one tonne of adults mussels covers approximately 10 m² (pers. comm.). Since this may also introduce a scale effect, id est requiring a minimum amount of mussel reefs to be installed before natural processes of reef generation could take effect (as seen with oyster reefs in the Chesapeake Bay, Schulte et al. 2009), future work will need to identify whether relationships exist between reef placement, isolation from other habitats, and mussel survival.

Conclusions

The most efficient form of restoration will facilitate natural reef building processes that allow for the establishment, growth, and persistence of mussel reefs. The research presented in this thesis provides insight into techniques that can be used to achieve this more efficient version of mussel reef restoration and outlines novel factors for practitioners to consider when planning the reintroduction of mussel reefs to degraded sites on the seafloor. While there is still some indication that sub-adult and juvenile mussels from long-line aquaculture can be
used to restore mussel reefs, the additional considerations required to overcome mortality from translocation stress currently limits their capacity to improve restoration efficiency. To reduce sub-adult and juvenile mussel mortalities in the pursuit of more efficient restoration, future work will need to establish methods that minimize physical and physiological harm during the translocation process. This may eventually rely on the culture and/or careful selection of sub-adult and juvenile mussels from resistant populations to promote reef establishment following transfer to the seafloor. Over time, these reefs will provide habitat to diverse communities of fish, some of which may also include a number of natural mussel predators (McLeod et al. 2012; Wilcox et al. 2018). Since predators can have a synergistic effect on the formation of larger spatial structures in reef networks (e.g., in the distribution or patterning of small patch reefs, van de Koppel et al. 2008), future deployment strategies will need to anticipate these interactions using: predator surveys prior to translocation; habitat suitability models that consider time of year, predator species, and predator abundance for site selections; and the deliberate spatial arrangement of restored reefs to reduce potential predator impacts and improve mussel survival. The results from this research hold broad implications for the restoration of degraded marine habitats and demonstrate that careful consideration of the relationships between human (e.g., aquaculture) and natural (e.g., biology and ecology) systems has the potential for progressing the development of nuanced restoration practices that are as effective as they are efficient.
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