Mud Cockle (*Katelysia* spp.) stock enhancement / restoration

Practical implementation and policy evaluation

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Executive Summary

The Mud Cockle (*Katelysia* spp.) supports an important commercial and recreational fishery in South Australia (SA), however a decrease in catch rate, from ~180 t in 2005 / 06 to less than 20 t in 2009 / 10, resulted in closure of the Section Bank (Port River, South Australia, Australia) fishing zone, one of the three major fishing zones in the state, which remains in place today. At the commencement of this project in 2014, there were concerns that the population density of Mud Cockles at the Section Bank may have been reduced to below the threshold level for successful recruitment, rendering the population effectively "sterile" and constraining natural recovery. While important for commercial production, Mud Cockles also have an important role in maintaining the integrity of the environment by stabilising sediment and reducing turbidity. Hence, it was identified by industry and government sectors that intervention through a re-seeding program may be needed to restore the population and the fishery at the Section Bank.

The primary aim of this study was to develop procedures to restore the Mud Cockle population at the Section Bank to a viable level for natural recruitment. Specifically, methods were developed for transporting and planting hatchery produced Mud Cockle seed into the field to optimise growth and survival. Pilot studies were undertaken to assess transportation and tagging procedures before conducting five trials at two sites (Site A – lower wild cockle population [34° 45' 17.604" S, 138° 30' 31.464" E] and Site B – higher population [34° 45' 18.108" S, 138° 30' 34.488" E]) with the aims of testing planted Mud Cockle apparent survival (number alive in plot) and growth across different seasons, sites, stocking densities, stocking size classes and predator protective methods. Results from the pilot studies indicated that Pacific Oyster spat transportation methods were suitable for Mud Cockle spat and painting one valve of the Mud Cockles with Rosario Pink paint was the most effective method for marking experimental spat. The results of the five field trials indicated that seasonal water temperature had the strongest influence on apparent survival and growth of re-seeded Mud Cockle spat.

Better apparent survival and recovery was observed over winter. However, confounding factors, such as a Pacific Oyster Mortality Syndrome (POMS) outbreak in the area during the 2017/2018 summer which restricted stock movement and measurements, limited the conclusions we could draw from the data. Generally, apparent survival and mortality of re-seeded spat did not differ between sites, re-seeding densities or predator protection methods, however the Mud Cockles tended to grow larger at Site B compared to Site A. Site B, which was located closer to the mangrove region, may have contained a higher organic load than Site A. Alternatively, the higher wild cockle population at Site B may have resulted in a more favourable environment for Mud Cockle growth.

Benefit cost analysis showed that, based on the results of the field trials on spat, at the current market price ($25 / kg), assuming a survival to harvest rate of 9.1% and a growing period of about 90 weeks (7 seasons) from deployment (9.5 mm in shell length) to harvest (30 mm in shell length) or about three years from fertilisation to harvest, the benefit cost ratio would be 1.28:1. The model indicated that harvesting an 11.3 t TACC would provide an annual return of AUD $51,716 after 20 years with an internal rate of return of 38.74% and a payback period of four years. Conversely, if a growing period of four years (from fertilisation to harvest) is required according to the published Mud Cockle field survey results (Cantin, 2010; Tarbath and Gardner, 2015), the benefit cost ratio would drop to 1.02:1, and an annual return for harvesting the 11.3 t TACC would reduce to AUD $5,269 after 20 years with an internal rate of return of 10.97% and a payback period of about 13 years.

The outcomes of this project indicate that Mud Cockle re-seeding at the Section Bank is potentially viable, however, further optimisation of re-seeded Mud Cockle stocking size, stocking density and re-seeding infrastructure for predator protection to increase recovery and survival rates is needed. Future studies are recommended to assess the growth rate of re-seeded Mud Cockles to harvest size, the effectiveness of the re-seeding trial on enhancing natural recruitment over the following years and to better understand the environmental impact of Mud Cockles on the sediment structure. The results of this project will assist in re-establishing the Mud Cockle fishery at the Section Bank and at other depleted areas. Additionally, information from this project will help to inform current and future policy development for the Mud Cockle fishery at the Section Bank.

Keywords: *Katelysia scalarina*, Mud Cockle, South Australia, stock restoration, re-seeding, stock enhancement

Introduction

The collapse of bivalve stocks as a consequence of commercial fishing effort, recreational and commercial watercraft activities, recruitment failure, mass mortality, and habitat degradation is a global issue that impacts potential fishery yields and could compromise the productivity of ecosystems (Arnold, 2001; Joaquim et al., 2008). Clam and cockle species are particularly vulnerable to overfishing given they generally inhabit easily accessible intertidal and shallow subtidal regions and can be simply and economically harvested (Defeo, 2003). Declines in bivalve populations have also had broader ecological consequences, including increased turbidity, increased occurrence and duration of anoxic episodes in bottom waters, and reductions in benthic vegetation and invertebrates, fish and bird species abundances (Phelps, 1994; Jackson et al., 2001; Doall et al., 2008).

Management efforts to limit stock collapse have been implemented in many bivalve fisheries worldwide, but even when harvesting pressure is removed there is no assurance that affected populations will recover to previous levels. Stoner and Ray-Culp (2000) suggested that when population density decreases below a threshold level, recruitment may fail and population recovery may be constrained, rendering the population effectively “sterile”. As a result, natural recovery of the population may be delayed or permanently halted and active intervention may be necessary to restore stock levels to attain reproductive viability. According to Bell et al. (2006), such intervention can involve re-seeding with cultured juveniles to rebuild the spawning-stock biomass of a depleted population to a level that can support fishing. In Japan, the scallop enhancement program has resulted in a stable industry, which has produced annual harvests consistently exceeding maximum historical yields (Kitada and Fujishima, 1997; Uki, 2006). The results from scallop enhancements / restorations in the USA, France and New Zealand have also shown that they enhanced the recruitment and subsequent population size (Dao et al., 1999; Arnold, 2001).

Mud Cockles (*Katelysia* spp.) are a group of commercially and environmentally valuable bivalves and have provided an important resource for both commercial and recreational fishers. In South Australia in 2014, there were ~20 Marine Scalefish Fishery licence holders with Mud Cockle quota. Licence holders with quota entitlements are restricted to taking Mud Cockles within the cockle fishing zones in which they have quota. There are three Mud Cockle fishing zones in South Australia; Port River (Section Bank), Coffin Bay and West Coast. Licence holders who do not hold quota (~600) are permitted to take a maximum of 10 kg per day from these cockle fishing zones for bait purposes only. Additionally, they are permitted to take Mud Cockles outside of these cockle fishing zones without restriction. The quota management system for these species was implemented in 2008, when most of the catch was obtained from the Section Bank and Coffin Bay areas. During the first decade of the 21st century, the harvest of Mud Cockles from the Section Bank decreased steeply from ~180 tonnes in 2005 / 06 to less than 20 tonnes per year in 2009 / 10. Prior to this, both total annual commercial catch and mean annual catch-per-unit-effort had been maintained at consistently high levels from 1998 to 2005 (Dent et al., 2012). Due to concerns for the health of stocks at the Section Bank, the Port River Mud Cockle fishing zone has remained closed to all fishing sectors since the start of the 2011 / 12 financial year.

In the last couple of decades, water quality within the Port River has improved (Wade, 2002; Pfennig, 2008). SA Water and Penrice Soda Pty Ltd, two of the largest releasers of nutrients to Gulf St. Vincent waters, have greatly reduced the quantity and have also improved the quality of their discharge waters. Despite the absence of fishing pressure and the improvement of water quality, there has not yet been any measured recovery of the Mud Cockle population at Section Bank. The remaining Mud Cockle population at the Section Bank might have already reached the critical “sterile” level and in need of intervention.

Given the commercial and environmental importance of Mud Cockles in SA, there is a need to develop methods to help restore depleted populations. Closing the fishing zone and waiting for the population to naturally recover will not be successful if the population size has dropped below the critical “sterile” level, in which case re-seeding will be required. In this study we developed and assessed techniques for transporting and planting hatchery reared Mud Cockles in the field, with the aims of developing a re-seeding method that optimises growth and survival of re-seeded Mud Cockles at the Section Bank and assessing the economic potential of a stock enhancement program.

Objectives

1. Develop optimal methodologies for transporting and planting hatchery produced Mud Cockle (*K. scalarina*) for stock enhancement / restoration at Section Bank.

2. Evaluate post-stocking performance of two hatchery produced Mud Cockle, *K. scalarina* and *K. rhytiphora*, at the Section Bank.

3. Optimise Mud Cockle stock enhancement / restoration strategies for the Section Bank through benefit cost evaluation of different options using the model developed in FRDC project 2008 / 071 - Economic Viability of Pipi (Donax deltoides) Reseeding.

4. Develop a monitoring program that can be incorporated within the existing Mud Cockle fishery stock assessment program to determine the long-term success of stock enhancement / restoration.

5. Transfer knowledge gained from this project to Government fisheries, aquaculture managers and policy makers, and cockle fishers in SA.

Changes to objectives

The project originally proposed to evaluate post-stocking performance of two species of Mud Cockle (*K. rhytiphora* and *K. scalarina*) for re-seeding at the Section Bank. In November 2015 attempts to collect *K. rhytiphora* broodstock at the Section Bank were made. Over two days, a total of only 29 *K. rhytiphora* were found. As a result, the spat production of this species had to be abandoned as this broodstock number did not meet the minimum requirement (>50) for spat production in the PIRSA Mud Cockle release permit. Hence, no post-stocking performance comparison between these two species could be performed. Objective two was changed to "evaluate the post-stocking performance of hatchery produced Mud Cockle, *K. scalarina*, at the Section Bank".

Method

1.1 Experimental Design

1.1.1 Broodstock collection and spat rearing

On two separate occasions (2015 and 2016), Mud Cockle broodstock were collected by a commercial fisher at the Section Bank [34° 45' 18.108" S, 138° 30' 34.488" E] using a commercial Mud Cockle rake with a 2 cm diagonal mesh insert. When the mesh insert was full, contents were sorted and any Mud Cockle > 30 mm was collected. This process was repeated until ~ 200 Mud Cockles were collected on the first occasion and ~100 Mud Cockles on the second occasion.

The project originally proposed to test the viability of two species of Mud Cockle (*K. rhytiphora* and *K. scalarina*) for re-seeding at the Section Bank. However, in November 2015, two attempts to collect *K. rhytiphora* broodstock at the Section Bank [34° 45' 18.108" S, 138° 30' 34.488" E] were made, with a total of only 29 individuals being found. As a result, the spat production of this species had to be abandoned as this broodstock number did not meet the minimum requirement (>50) for spat production in the PIRSA Mud Cockle release permit. Hence, no post-stocking performance comparison between these two species could be performed.

Mud Cockle (*K. scalarina*) spat used in the field trials were successfully cultured at the South Australian Research and Development Institute (SARDI) – Aquatic Sciences Centre according to the methods of Gluis and Li (2014a). Two spat cohorts were produced; the first over the summer of 2014-2015 and the second over the summer of 2015-2016. Collected broodstock, 197 animals for cohort 1 and 104 animals for cohort 2, were spawned naturally. Larvae were reared in a static system until 90% were observed to be competent, at which point they were transferred to a downwelling system for metamorphosis. High survival rates of larvae through metamorphosis and subsequent nursery culture in upwellers was observed. Larvae and spat were fed a diet of four microalgae species, T-Iso, *Pavlova lutheri*, *Chaetoceros calcitrans* and *Chaetoceros muelleri*.

1.1.2 Marker development

For this study, the ability to differentiate between experimental and wild cockles was required. Two preliminary experiments were conducted to assess the suitability of five stains by using post-painting survival and colour retention as key indicators. After seven days, spat painted with synthetic pink paint - MTN 94 RV-279 Rosario Pink (Montana Colours, Barcelona, Spain) on one side showed 100% survival rate and good colour retention (Figure 1). The other painting treatments resulted in either mortality or poor colour retention. Therefore, Rosario Pink was selected as the marker for all the subsequent field trials.

Figure 1: A Mud Cockle (*K. scalarina*) spat 7 days after being painted with MTN 94 RV-279 Rosario Pink (Montana Colours, Barcelona, Spain).

1.1.3 Disease screening and transportation of spat

Within two weeks prior to stocking, for each field trial, 150 spat were assessed for pathological legions via a histopathology examination by Gribbles Veterinary Pathology, Adelaide, SA, Australia, and their disease free status was confirmed.

To ensure transportation did not impact survival of hatchery reared Mud Cockles in the field, a commonly used Pacific Oyster spat transportation protocol (Helm et al., 2004) was tested with the following modifications. Approximately 1,000 Mud Cockle spat were patted dry with paper towel and put into four mesh bags (mesh size = 1 mm^2). The Mud Cockles were placed in an Esky containing two ice-bricks (20 cm x 15 cm x 1cm in size), each wrapped in a thick layer of paper towel. After 24 hours sealed in the Esky, spat were put back into the nursery system and maintained according to the protocol (Gluis and Li, 2014a) developed for other Mud Cockle spat. No mortality was recorded over seven days following containment. This method was therefore applied in the subsequent field trial for transportation of Mud Cockle spat to the Section Bank.

1.1.4 Site selection for field trials

Two potential sites were identified for field trials after consultation industry and a field survey. The field survey was conducted in October 2015 using 0.25 m^2 quadrats. In the study sites, a total of 16 quadrats were randomly allocated. Quadrants were dug to a depth of approximately 10 cm, with all sediment removed from the quadrant sifted through a 2 mm screen. Any cockle / clam larger than 2 mm screen size were collected, counted and returned in situ. Using visual observations, sites were selected with a similar (minimal-none) vegetation covering to reduce the potential impacts on local seagrass and seaweed. The two sites assessed in this study are: Site A - lower existing wild cockle / clam population densities (104 bivalves / m^2); and Site B - higher existing wild cockle / clam population densities (280 bivalves / m^2; Figure 2; GPS coordinates of each site in Table 1).

Figure 2: Map of Section Bank and two trial sites. Site A: Lower existing wild cockle / clam population densities (104 bivalves / m^2); and Site B: higher existing wild cockle / clam population densities (280 bivalves / m^2).

Table 1: GPS coordinates for the two trial sites (Site A and Site B) at Section Bank, confluence of the Port River and Gulf St Vincent, South Australia

Site	Corners	Latitude	Longitude
A	NE	-34.75489	138.50874
	NW	-34.75488	138.50861
	SW	-34.75511	138.50854
	SE	-34.75514	138.5087
B	NE	-34.75503	138.50958
	NW	-34.75499	138.50945
	SW	-34.75521	138.50935
	SE	-34.75525	138.50952

1.1.5 Predator protection method

In this study, trays and frames used for predator protection were modified from plastic plant nursery trays (Garden city plastics, Wingfield, SA, Australia) measuring 530 mm long × 325 mm wide × 80 mm deep. The control frames were made from the top rims of the plant nursery trays with the bottom removed (Figure 3C). The closed trays were constructed by lining the bottom of each tray with 1.7 mm^2 small screen mesh and fixing a lid on the top with 6 mm oyster mesh (Figure 3A). Mesh was secured onto the plant nursery tray with cable ties. The open top trays (only used in the autumn trial 2016) were the same as the closed trays (used in all trials), but without a top oyster mesh lid (Figure 3B). For each site, two ropes (four ropes in the autumn trial 2016), were laid 2 m apart with the trays placed at 1 m intervals along them (Figure 4A) The closed tray and open tray treatments were buried, with the top of each treatment level with the surface, and filled with surrounding sediment (Figure 4). Trays were secured in place with tent pegs.

A. B. C.

Figure 3: Predator protection treatments for field trials. A: Closed tray; B: Open tray; C: Tray frame.

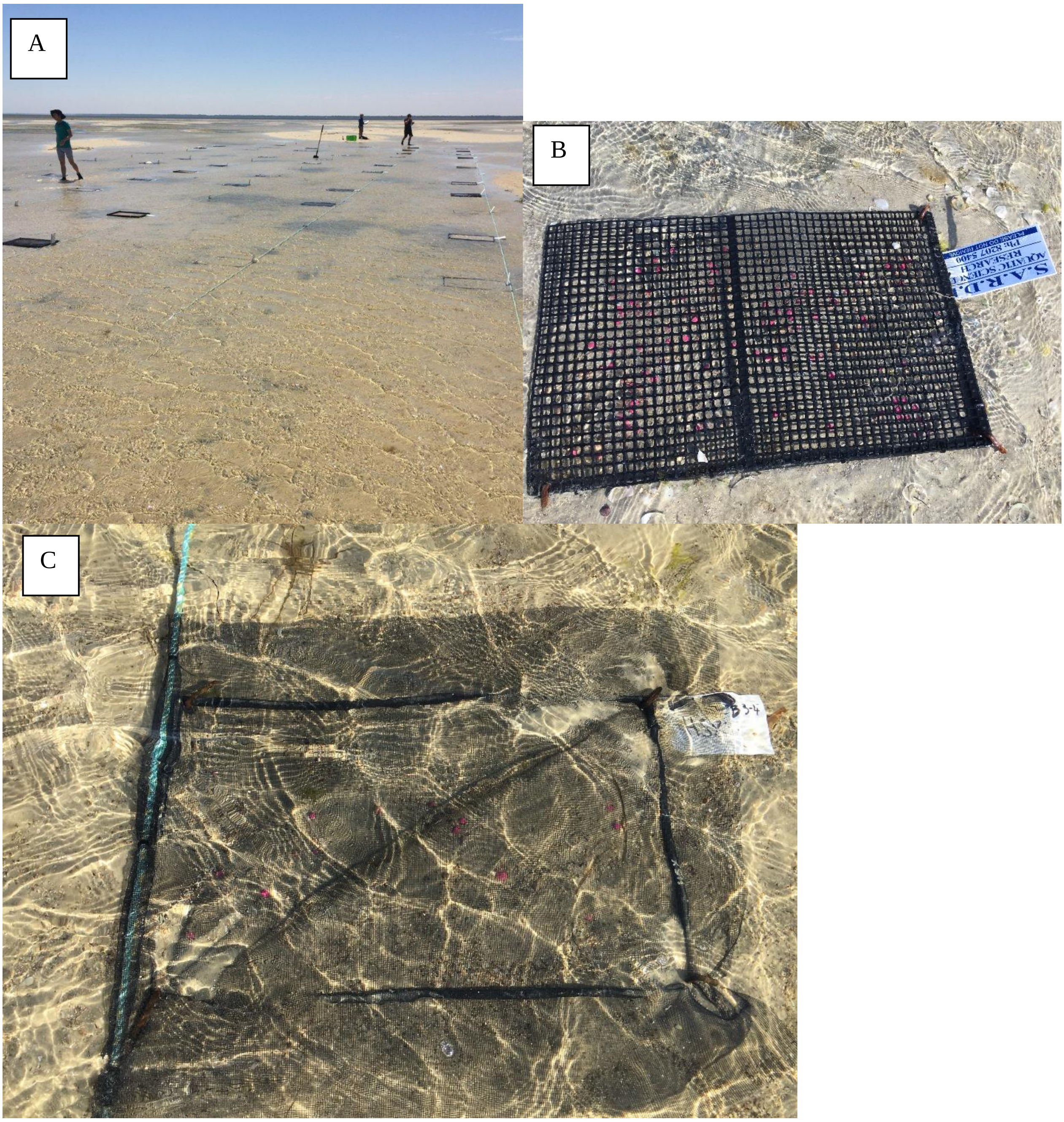


Figure 4: Photos of A) field trial set up, B) closed tray treatment post seeding and C) tray frame treatment with the fine mesh over the top for 24h.

Seeding

Mud Cockle spat were counted and painted with Rosario Pink (Montana Colours, Barcelona, Spain) prior to deployment in the field. The number of spat per treatment and the size of the spat seeded was dependent on the trial (Section 1.2, Table 2). Two size classes of spat were used in the field trials, 3 mm screen size (~ 5.5 mm initial shell length) and 5 mm screen size (~ 9.3 mm initial shell length). Size classes were selected based on common screen sizes other bivalve species (oysters and mussels) are deployed to aquaculture leases. Additionally, Epelbaum et al. (2011) and Dunham et al. (2013) reported high survival in field trials of hatchery reared Basket Cockle (*Clinocardium nuttallii*) ~ 3 mm initial shell length, whereas Gosling (2003) reported the following survival rates for planted Manila Clams (*Ruditapes philippinarum*): 3 mm initial shell length – 34% survival; 10 mm initial shell length – 60% survival and 24 mm initial shell length – 77% survival.. In each trial, painted Mud Cockle spat were seeded by hand, haphazardly on top of the sand in each tray (Figure 4B).

It was observed in the first field trial (Trial 1, section 1.2.1) that some planted Mud Cockle spat were slow to bury into the substrate and were being washed from the plots with the incoming tide. To protect the loss of Mud Cockle spat due to tidal movement and predators at stocking, in all subsequent trials a 1.7 mm^2 screen mesh was used to cover the plots for approximately 24 hours whilst spat burrowed into the sand (Figure 4C).

1.1.6 Mud Cockle sampling

At the end of each trail (see section 1.2) Mud Cockles were sampled from each treatment plot. This involved using a small spade to gently, avoiding damaging any Mud Cockles, dig out the substrate in each tray to a depth of ~ 100 mm and screening the removed sand through a 2 mm screen to capture the Mud Cockles. Bellchambers and Richardson (1995), showed that adult Mud Cockles were incapable of surviving burial at depths greater than 10 cm. Once the Mud Cockles were collected, the sifted sand was used to refill the plot holes.

The sampled Mud Cockles were transported (see section 1.1.3) to the lab at SARDI and frozen at -15 °C within five hours of collection. The Mud Cockles were not lab processed alive due to the time between sampling and processing. A more consistent weight would be obtained by freezing all the Mud Cockles at the same time whilst they were alive as opposed to trying to hold them alive until processing where any that died over this time would have a reduced weight due to water loss. Mud Cockles were thawed prior to the determination of shell length and weight. For apparent survival measurements, all Mud Cockles which contained meat were considered alive at the end of each trial and all empty shells were considered dead.

1.1.8 Water temperature

The seasonal water temperatures reported for each field trial in this report (Figure 7), were compiled from measurements recorded at the closest possible location to the target area using daily satellite sea surface temperature data (IMOS, 2020).

1.1.9 Statistical analysis and Mud Cockle performance

Data is presented as mean ± standard error (SE). SPSS v.26 (IBM, released 2019) was used for all statistical analyses. Data was pre-analysed for normality (Shapiro-Wilk test) and Levene's test for homogeneity of variances. Where data was not normally distributed, it was transformed by square root. In instances where transformation did not normalise the distribution, a non-parametric Kruskal-Wallis test with pairwise comparisons was used on un-transformed data to assess significant ($P \leq 0.05$) differences between treatment means. For normally distributed data, multifactorial ANVOA and Tukey's HSD post hoc test were used to assess significant ($P \leq 0.05$) differences between treatment means. A simple effects test was used to compare significant interactions when present. Linear regression was used to test the relationship between the depth of the anoxic layer (grey sand) and apparent survival and apparent mortality in Trial 5. For this analysis, anoxic layer depth data was normalised via log10 transformation and apparent mortality data was normalised by square root transformation.

Seasonal influences was assessed by compiling data from the field trials (Trials 1 – 5A; section 1.2). High stocking density (1000 individuals / m^2), large size class (5 mm screen sizes), closed tray and tray frame predator protection treatments at Sites A and B were included in the analysis because these factors were consistent across the trials. Mean water temperature data from each trial were calculated using measurements recorded at the closest possible location to the target area using daily satellite sea surface temperature data (Figure 7, IMOS, 2020). A stepwise linear regression was carried out to test if water temperature, site and/or predator protection method significantly predicted Mud Cockle apparent survival, apparent mortality, recovery or growth rate (length and weight). Rates per day were calculated by the value at the end of each trial / days post seeding. Site and predator protection were inputted as binary dummy variables (0, 1; Hardy et al., 1993) Independence of observations was tested through a Dubrin Watson calculation. Normality of residuals (errors) was assessed by a Shapiro-Wilk test. Apparent survival rate, apparent mortality rate and recovery rate were square root transformed and daily growth rate length (DGRL) and daily growth rate weight (DGRW) were $\log_{10}$ transformed to meet the normality assumption. Interactions between factors were assessed in a secondary linear regression model, with the change in r^2 between the two models assessed for significance (DeCoster, 2004).

For each trial, where appropriate, the following methods and formulae were used to calculate the performance of the Mud Cockle re-seeded (Table 2):

- Initial shell lengths and weights were determined by measuring 30 Mud Cockles per size class, randomly selected from the group of graded Mud Cockles for each trial prior to re-seeding (stocking).
- Final shell length and final weight were determined at harvest by measuring all Mud Cockles recovered alive at harvest for Trial 1 (Section 2.1). For the remainder of the trials, the final shell length and final weight were determined by measuring a maximum of 30 randomly selected Mud Cockles recovered alive per replicate or all individuals if less than 30 Mud Cockles were found alive within the replicate. It is important to note that weight was recorded after freezing / thawing, hence due to water loss, the weight recorded in this report would be less than the live weight.
- Daily growth rate for shell length (DGRSL; mm / day) = (final shell length - initial shell length) / days post seeding.
- Daily growth rate for weight (DGRW; g / day) = [final weight (thawed) - initial weight (live)] / days post seeding.
- Recovery (%) = (sum of survivors + sum of mortality) / stocked number × 100
- Apparent survival (%) = the number of Mud Cockles found alive in each plot / stocked number × 100
- Apparent mortality (%) = the number of Mud Cockles found dead in each plot / stocked number × 100
- Whelk predation was measured by enumerating the incidence of drill holes in the shells of the Mud Cockles. Whelk predation (%) = number of dead shells with a drill hole / total number of dead shell × 100
- Benefit cost evaluation was undertaken using the model developed by Phelps et al. (2009).

1.2 Field Trials

To evaluate post-stocking performance of hatchery produced Mud Cockle, five field trials were conducted over a two-year period across seasons at the Section Bank, confluence of the Port River and Gulf St Vincent, South Australia. The design for each trial was modified and improved depending on the outcomes of previous trials. The trials are summarised in Table 2 and detailed below. It was aimed to run each field trial for a season (70 – 90 days), with weather conditions dictating when the Mud Cockle spat could be sampled and hence the number of days each trial was run for.

Table 2: Summary of factors tested (●) or used (✓), trial length, measurements conducted, Mud Cockle performances calculated and rationales for changing the trial design in each field trial at the Section Bank. DGRSL = Daily growth rate for shell length, DGRW = Daily growth rate for weight

Trial	Trial length	Factors	Measurements or Mud Cockle performances (see section?)	Rational for changes to trial design / further comments
1. Re-seeding trial autumn 2016	Site A = 114 days Site B = 87 days	• two size classes (3 & 5 mm screen sizes) • two stocking densities (200 & 1000 individuals / m^2) • three predator protection methods (tray frame, closed tray & open tray) • two sites (Site A & Site B) • 72 plots at each site	• apparent survival • final shell length and DGRSL	• There was not enough time at low tide to collect samples from both sites in a single day. Weather delays meant Mud Cockles from Site A were collected 27 days later. Subsequent trials reduced the number of plots from 144 to 48 to ensure single day collection. This meant size class and density were assessed in separate trials
2. Re-seeding trial winter 2017	73 days	• two stocking densities (200 & 1000 individuals / m^2) • two predator protection methods (tray frame & closed tray) • two sites (Site A & Site B) • 24 plots at each site ✓ size class = 5 mm screen size	• recovery • apparent survival • apparent mortality • snail predation • final shell length and DGRSL • final weight and DGRW	• Open tray predator protection methods was excluded from all subsequent trials given the closed tray performed significantly better. • The 5 mm screen size Mud Cockle spat were selected for this trial as it was predicted that they world have a higher apparent survival and recovery rate than the 3 mm screen size spat. • To protect the loss of Mud Cockle spat due to tidal movement and predators at stocking, in all subsequent trials a 1.7 mm^2 screen mesh was used to cover the plots for approximately 24 hours whilst spat burrowed into the sand.
3. Re-seeding trial spring 2017	86 days	• two size classes (3 & 5 mm screen sizes) • two predator protection methods (tray frame & close tray) • two sites (Site A & Site B) • 24 plots at each site ✓ stocking density = 1000 individuals / m^2.	• recovery • apparent survival • apparent mortality • snail predation • final shell length and DGRSL • final weight and DGRW	• In Trial 2, Mud Cockle stocking density did not significantly impact growth or apparent mortality and more Mud Cockles in the high stocking density treatment were found alive than in the low. For this reason, the high stocking density (1000 individuals / m^2) was used for subsequent trials
4. Re-seeding trial summer 2017 / 2018	157 days	• four predictor protection methods (tray frame, tray frame	• recovery • apparent survival • apparent mortality	• By the time of this trial, Mud Cockles in the hatchery had grown

		with small mesh top, close tray & close tray with small mesh top) • two sites (Site A & Site B) • 24 plots at each site ✓ size class = 5 mm screen size ✓ stocking density = 1000 individuals / m^2	• snail predation • final shell length and DGRSL • final weight and DGRW	beyond the 3 mm screen size, hence size class at stocking was not assessed in this or subsequent trials • The trial was planned to run for 70-90 days. However, due to an outbreak of Pacific Oyster Mortality Syndrome (POMS) in the Port River and the subsequent ban on shellfish removal, the Mud Cockles could not be sampled. Consequently the trial ran for 157 days.
5. Re-seeding trial winter 2018	86 days	*Trial 5A* • four predator protection methods (tray frame, tray frame with small mesh top, close tray & close tray with small mesh top) • two sites (Site A & Site B) • 24 plots at each site • Mud Cockle movement • sediment sizes • anoxic layers *Trial 5B* • Two sites (Original Site B & New Site B • 12 plots at each site • sediment sizes • anoxic layers ✓ size class = 5 mm screen size ✓ stocking density = 1000 individuals / m^2	*Trial 5A* • recovery • apparent survival • apparent mortality • snail predation • final shell length and DGRSL • final weight and DGRW • Mud Cockle numbers in tray frame • Mud Cockle numbers in the area between tray frame and the 1st rectangular quadrant • Mud Cockle numbers in the area between the 1st and 2nd rectangular quadrants • sediment: coarse, medium and fine • depth of sand above the grey (anoxic layer) *Trial 5B* existing vs new plots • recovery • apparent survival • apparent mortality • final shell length • final weight	• To gain a better estimate of recovery and survival, Mud Cockle movement was assessed in this Trial. Time restrictions necessitated that Mud Cockle movements could only be measured for the tray frame treatments. • Sediment size and anoxic layer was assessed to determine if there was a difference between sites and predator protection methods. • In the previous three trials, plots were placed in the same locations at each site. To assess if this continual disturbance of sediment had an impact on the results of the trials, Site A and B were moved directly adjacent (~ 1 m north) for Trial 5A and a secondary trial (Trial 5B) was run to test differences between existing and new plot sites.

			• sediment: coarse, medium and fine • depth of sand above the grey (anoxic layer)	
6. Large scale re-seeding trial summer 2018	Ongoing	• two sites (Site A & Site B) • six 10 m^2 plots per Site, three seeded with Mud Cockles and three not seeded ✓ size class = 5 mm screen size ✓ stocking density = 1000 individuals / m^2 ✓ predator protection method = tray frame	Future monitoring of the re-seeded Mud Cockles will be undertaken through the SARDI / PIRSA Mud Cockle stock assessment program.	• After examining the results from all previous trials, Mud Cockles were re-seeded without a closed tray, as no significant difference was observed in apparent mortality between predator protection methods in the cooler months, higher mortalities in the closed tray treatments in the warmer months (refer to Section 2.6) and low predation by whelks was observed across all treatments.

1.2.1 Trial 1. Re-seeding trial autumn 2016 (27th February - 20th June)

The aim of this multifactorial trial, which was conducted in autumn 2016, was to evaluate the effects of two stocking size classes [3 mm screen size (5.1 ± 0.4 mm initial shell length) and 5 mm screen size (7.1 ± 0.6 mm initial shell length)], two stocking densities (200 and 1000 individuals / m^2) and three predator protection methods [tray frame (control), closed tray, and top open tray (refer to Section 1.1.5)] on the performance of hatchery produced re-seeded Mud Cockles at two sites (Site A and Site B) in the Section Bank, SA (Table 2). Each treatment combination was replicated six times, resulting in 144 plots.

The trial ran for 114 days. During this time the re-seeded Mud Cockles were sampled on two occasions. Site B was harvested on day 87 and Site A was harvested on day 114. There was not enough time at low tide to collect samples from both sites in a single day. Weather delays meant Mud Cockles from Site A were collected 27 days later.

Based on the results of this trial and preliminary data analysis, all subsequent seasonal trials used a total of 48 plots to ensure single day collection at both sites. This meant stocking size and stocking density had to be assessed separately. The closed tray predator protection method performed significantly better than the open tray (Section 2.1), therefore, the open tray method was excluded from all subsequent trials in this project. Additionally, to protect the loss of Mud Cockle spat due to tidal movement and predators at stocking, in all subsequent trials a 1.7 mm^2 screen mesh was used to cover the plots for approximately 24 hours whilst spat burrowed into the sand.

1.2.2 Trial 2. Re-seeding trial winter 2017 (13th June - 25th August)

This trial was initially scheduled to run in winter 2016, however, delays with permits necessitated that the next trial could not take place until the following year.

The aim of this multifactorial trial, which was completed in winter 2017, was to evaluate the effects of two stocking densities (200 and 1000 individuals / m^2) and two predator protection methods [tray frame (control) and closed tray (refer to Section 1.1.5)] on the performance of hatchery produced re-seeded Mud Cockles [5 mm screen size (9.9 ± 1.1 mm initial shell length)] at two sites (Site A and Site B) in the Section Bank, SA

(Table 2). Each treatment combination was replicated six times, resulting in 48 plots. The trial ran for 73 days.

As stated above, to ensure sampling was completed in a single day the total number of plots was reduced to 48, hence size class was not assessed in this trial. The 5 mm screen size Mud Cockle spat were selected for this trial as it was predicted that they world have a higher apparent survival and recovery rate than the 3 mm screen size spat, despite preliminary analysis of Trial 1 indicating no significant difference in apparent survival between size classes.

Preliminary data analysis from this trial indicated that Mud Cockle stocking density did not significantly impact growth or apparent mortality and more Mud Cockles in the high stocking density treatment were found alive than in the low. For this reason, the high stocking density (1000 individuals / m^2) was used for subsequent trials.

1.2.3 Trial 3. Re-seeding trial spring 2017 (11th September - 6th December)

The aim of this multifactorial trial, which was completed in spring 2017, was to evaluate the effects of two stocking size classes [3 mm screen size (5.8 ± 0.6 mm initial shell length) and 5 mm screen size (9.6 ± 0.9 mm initial shell length)] and two predator protection methods (tray frame [control] and closed tray [refer to section 1.1.5]) on the performance of hatchery produced re-seeded Mud Cockles at two sites (Site A and Site B) at a stocking density of 1000 individuals / m^2 in the Section Bank, SA (Table 2). Each treatment combination was replicated six times, resulting in 48 plots. The trial ran for 86 days.

1.2.4 Trial 4. Re-seeding trial summer 2017-2018 (12th December - 18th May)

At the time of this trial, Mud Cockle spat in the hatchery had grown beyond the 3 mm screen size, hence size class at stocking was not assessed in this or subsequent trials.

The aim of this multifactorial trial, which was run in summer 2017-2018 and autumn 2018, was to evaluate the effects of four predator protection methods [tray frame (control), tray frame with small mesh (1.7 mm^2) top, closed tray (refer to Section 1.1.5) and closed tray with small mesh (1.7 mm^2) top] on the performance of hatchery produced re-seeded Mud Cockles [5 mm screen size (10.3 ± 0.8 mm initial shell length)] at two sites (Site A and Site B) at a stocking density of 1000 individuals / m^2 in the Section Bank, SA (Table 2). Each treatment was replicated six times, resulting in 48 plots.

Initially, the trial was planned to run for 70-90 days. However, due to an outbreak of Pacific Oyster Mortality Syndrome (POMS) in the Port River and the subsequent ban on shellfish removal, the Mud Cockles could not be sampled. Consequently the trial ran for 157 days.

1.2.5 Trial 5. Re-seeding trial winter 2018 (3rd July - 27th September)

This trial was split into two parts, Trial 5A and Trial 5B. Trial 5A was set up to compare differences between sites A and B and between predator protection methods, whereas Trial 5B was set up to assess if continual use/disturbance of the same plot locations affected apparent survival, growth and/or sediment characteristics.

The main aim of multifactorial Trial 5A, which was run in winter 2018, was to evaluate the effects of four predator protection methods [tray frame (control), tray frame with small mesh (1.7 mm^2) top, closed tray (refer to Section 1.1.5) and closed tray with small mesh (1.7 mm^2) top] on the performance of hatchery produced re-seeded Mud Cockles [5 mm screen size; 9.5 ± 0.6 mm initial shell length)] at two sites (Site A and Site B) at a stocking density of 1000 individuals / m^2 in the Section Bank, SA (Table 2). Each treatment was replicated six times, resulting in 48 plots. This trial replicated the summer 2018 trial (Trial 4) and could be used to validate the results from the winter 2017 trial (Trial 2).

Additionally, Trial 5A assessed Mud Cockle movement for the tray frame treatment at each site. After the Mud Cockles were sampled from the tray frame plot (see section 1.1.6), a rectangular quadrant (0.5 m larger than then the tray frame at each side; quadrant 1; Figure 5) was centred over the tray frame. All Mud Cockles within the quadrant were captured by using a spade to gently remove the substrate to a depth of ~100 mm and sifting the removed sand through a 2 mm screen. This was then repeated with a second, larger rectangular quadrant (1.0 m larger than then the tray frame at each side; quadrant 2; Figure 5). Once the Mud Cockles were collected, the sifted sand was use to refill the holes.

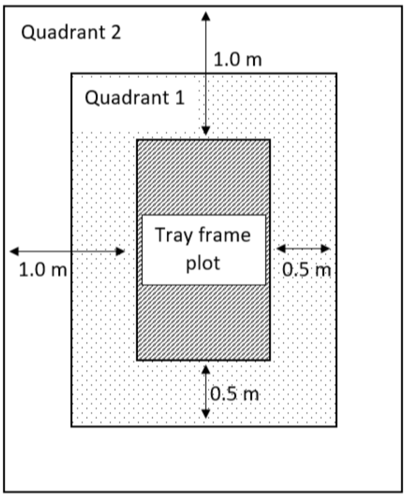


Figure 5: Experimental design used to assess Mud Cockle (*K. scalarina)* movement away from tray frame treatments at Sites A and B in Trial 5A (section 1.2.5).

Trial 5B was to assess if continual use of the same plot areas in the three previous trials (Sections 1.2.2, 1.2.3 and 1.2.4) had an impact on the apparent survival of re-seeded Mud Cockle spat at the Section Bank during the second winter. Throughout the previous three trials (Sections 1.2.2, 1.2.3 and 1.2.4), plots were placed in the same locations at each site. There was a concern that the regular digging and sifting of the sand may have changed the sediment size characteristics. For this reason, plots at Sites A and B were moved directly adjacent (~ 1 m north) of the plot sites used in the previous three trials for each treatment (Figure 6). Additionally, to act as a control, the existing plot sites for Site B were also stocked with six closed tray treatments and six closed tray small mesh (1.7 mm^2) treatments (Figure 6).

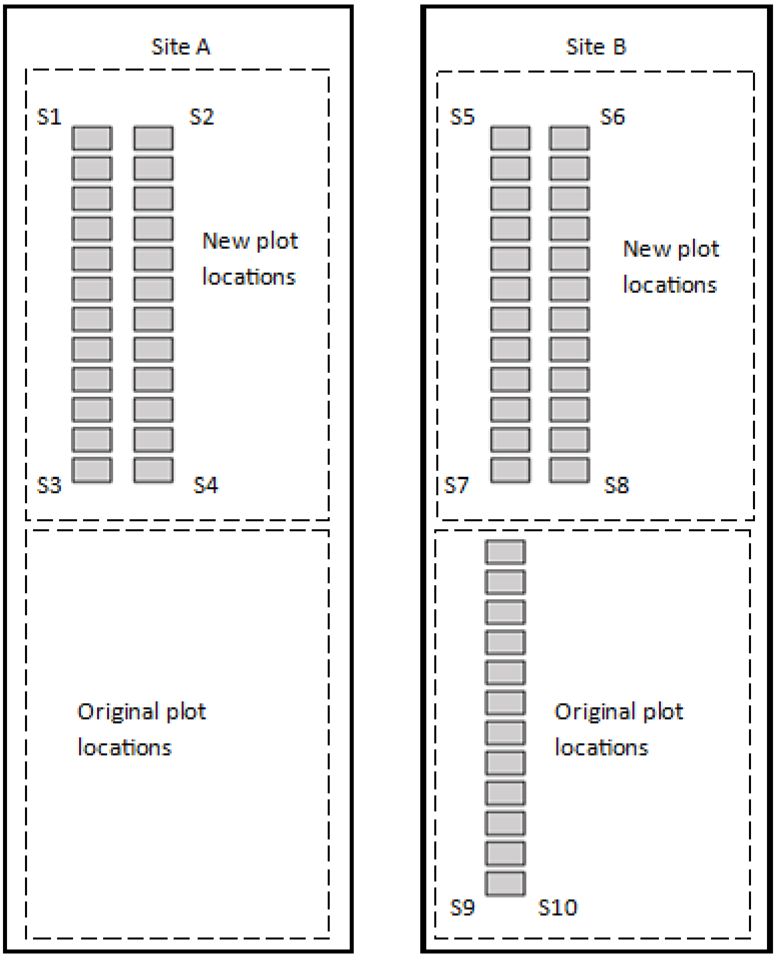


Figure 6: Set up of winter 2018 trial (Trial 5 Section 1.2.5). New plot locations are Site A and Site B used in Trial 5A (24 plots per site), whereas original plot locations are Sites used in previous trials (~1 m south of new plot locations), with the experimental plots represented for Trial 5B (12 plots). Sediment samples were collected from each experimental plot and control sediment sites (S1-10).

 Represents each experimental plot.

Trial 5A and 5B ran for 86 days.

Sediment size was assessed at the end of trials 5A and 5B. A 30 mm diameter tube was used to randomly extract 60 mm of sediment (equalling a 75 g dry-weight sample) from each plot, with three replicates per plot. Ten control samples were collected from outside the plots (adjacent corners; S1-10, Figure 6). Each sample was placed in a labelled zip-lock bag and transported to SARDI-Aquatic Sciences for further analysis. Samples were sifted into three size classes (coarse - > 1000 µm, medium - 250-1000 µm and fine - < 250 µm) using a Mastersizer and the percentage composition of each size class calculated.

In order to estimate if anoxic sand was building up in the tray systems, the depth of sand above the grey (anoxic layer) was recorded at each plot.

1.2.6 Trial 6. Large scale re-seeding trial summer 2018

A large scale re-seeding trial using the methodologies developed in the five previous trials (trials 1-5A) in this project was started in December 2018.

The rationale behind the design of this trial with reference to stocking density and predator protection method were as follows:

- With regard to stocking density, Mud Cockles were re-seeded at 1000 individuals / m^2, as no significant difference (P = 0.444) in apparent survival was observed between the high and low density treatments in the 2016 autumn re-seeding trial (Section 1.2.1). Additionally, this stocking density (high density) had significantly better apparent survival (P = 0.019) and no significant difference in apparent mortality (P = 0.279) in the 2017 winter re-seeding trial (Section 1.2.2).

- With regard to size class, 5 mm screen size Mud Cockles were re-seeded because all spat in the hatchery had grown beyond the 3 mm screen size.

- With regard to predator protection, after examining the results from all previous trials, Mud Cockles were re-seeded without a closed tray. Reasons for this decision are as follows

    - Whilst predator protection method has a significant effect on recovery, survival and mortality rates, it only explained a small percent of the variation in the data (3.6%, 2.3% and 6.9% respectively, refer to Section 2.6).

    - Higher mortality rate in the closed tray over warmer seasons (refer to Section 2.6).

    - Low whelk predation was observed across all treatments.

    - No significant effect of predator protection method on growth (refer to Section 2.6).

At each site (Site A and Site B), six 10 m^2 plots were marked out (SARDI tag placed at each corner) and designated as three experimental plots (re-seeded with Mud Cockles) and three control plots (no re-seeding). The trial was stocked on the 12th December 2018 with 5 mm screen size (9.5 ± 1.0 mm initial shell length) Mud Cockles. The size of the plots (10 m^2) and number of replicates was chosen based on:

- The number of 5 mm screen size Mud Cockles still available in the hatchery. There needed to be enough to have a density of 1000 individuals / m^2 in each experimental treatment.

- Consideration of sampling logistics, i.e. ability to sample the treatments in a single day.

Future monitoring of the re-seeded Mud Cockles will be undertaken through the SARDI / PIRSA Mud Cockle stock assessment program.

1.3 Benefit cost evaluation

The data from the field trials was used to develop a benefit cost evaluation of optimal Mud Cockle stock enhancement / restoration strategies for the Section Bank using the model developed in FRDC project 2008 / 071 (Phelps et al., 2009). In undertaking the benefit cost analysis to assess the potential for Mud Cockle re-seeding, a number of assumptions were made based on the best available information collected from fishery statistics, fisher observations and hatchery experience (Table 3). The benefit cost model requires a market price input. The current market price for Mud Cockles in South Australia is $25.00 / kg (PFD Food Services, Adelaide, July 2019), however if supply were to increase this price would be expected to drop to $15-$20 / kg in line with similar species. To further improve our estimations, multiple simulations were run to assess the effect of variable market prices on the benefit cost ratio.

Table 3: Benefit cost analysis assumptions for Mud Cockle (*K. scalarina*) re-seeding at the Section Bank

Key Assumptions	**Value**	**Rationale**
Target TACC harvest	11.3 t	This was the PIRSA quota for the TACC before the closure of the Section Bank fishery in 2010.
Price of spat	\$0.004 per mm	Based on current commercial sale price of edible oysters of a similar size.[1]
Size at deployment	9.5 mm shell length	Sizes of Mud Cockles used in the field trials.
Size at harvest	30 mm	Based on minimum length at harvest for all South Australian cockle fishing zones except Coffin Bay.
Time from deployment to harvestable size	90 weeks = 7 seasons	Based on mean spat growth rate of 0.035 mm / day observed in all of the field trials in this study.
	137 weeks = 11 seasons	Based on the published Mud Cockle survey information (Cantin, 2010; Tarbath and Gardner, 2015).
Average weight at harvest	9 g	Based on published shell length weight relationships (Cantin, 2010; Tarbath and Gardner, 2015)

[1] Sale price data sourced from Phelps et al. (2009).

Results

2.1 Trial 1. Re-seeding trial autumn 2016 (27th February - 20th June)

Results from Trial 1 are summarised in Table 4. The average water temperature recorded over the duration of Trial 1 was 20.1 ± 3.0 °C (Figure 7).

Table 4: Results (mean ± SE) from Trial 1 for hatchery produced Mud Cockles (*K. scalarina*) re-seeded at the Section Bank in autumn 2016. Small size class = 3mm screen size spat; Large size class = 5 mm screen size spat; High density = 1000 individuals / m^2; Low density = 200 individuals / m^2

	Small size class											
	Site A						**Site B**					
	High density			**Low density**			**High density**			**Low density**		
	Closed Tray	**Open Tray**	**Tray Frame**	**Closed Tray**	**Open Tray**	**Tray Frame**	**Closed Tray**	**Open Tray**	**Tray Frame**	**Closed Tray**	**Open Tray**	**Tray Frame**
Apparent survival (%)	17.62±2.75	6.82±1.81	4.40±0.99	23.51±3.27	13.85±3.15	5.98±1.43	6.22±2.85	6.91±3.24	2.51±1.60	14.96±6.89	12.39±5.23	0.00±0.00
Final shell length (mm)	10.89±0.28	11.5±0.39	12.07±0.37	11.69±0.33	12.03±0.50	12.09±0.33	13.01±0.52	13.43±0.33	10.13±0.54	12.60±1.09	12.65±0.90	NA

	Large size class											
	Site A						**Site B**					
	High density			**Low density**			**High density**			**Low density**		
	Closed Tray	**Open Tray**	**Tray Frame**	**Closed Tray**	**Open Tray**	**Tray Frame**	**Closed Tray**	**Open Tray**	**Tray Frame**	**Closed Tray**	**Open Tray**	**Tray Frame**
Apparent survival (%)	23.49±5.10	7.17±1.14	5.87±2.78	22.22±6.64	12.82±3.92	5.98±2.06	10.71±2.76	3.98±0.76	1.56±0.33	14.10±2.87	6.84±3.73	0.00±0.00
Final shell length (mm)	12.75±0.32	12.87±0.49	12.79±0.41	11.70±0.93	11.39±0.99	13.97±0.83	13.54±0.32	14.53±0.53	12.66±1.12	13.34±0.32	14.25±0.28	NA

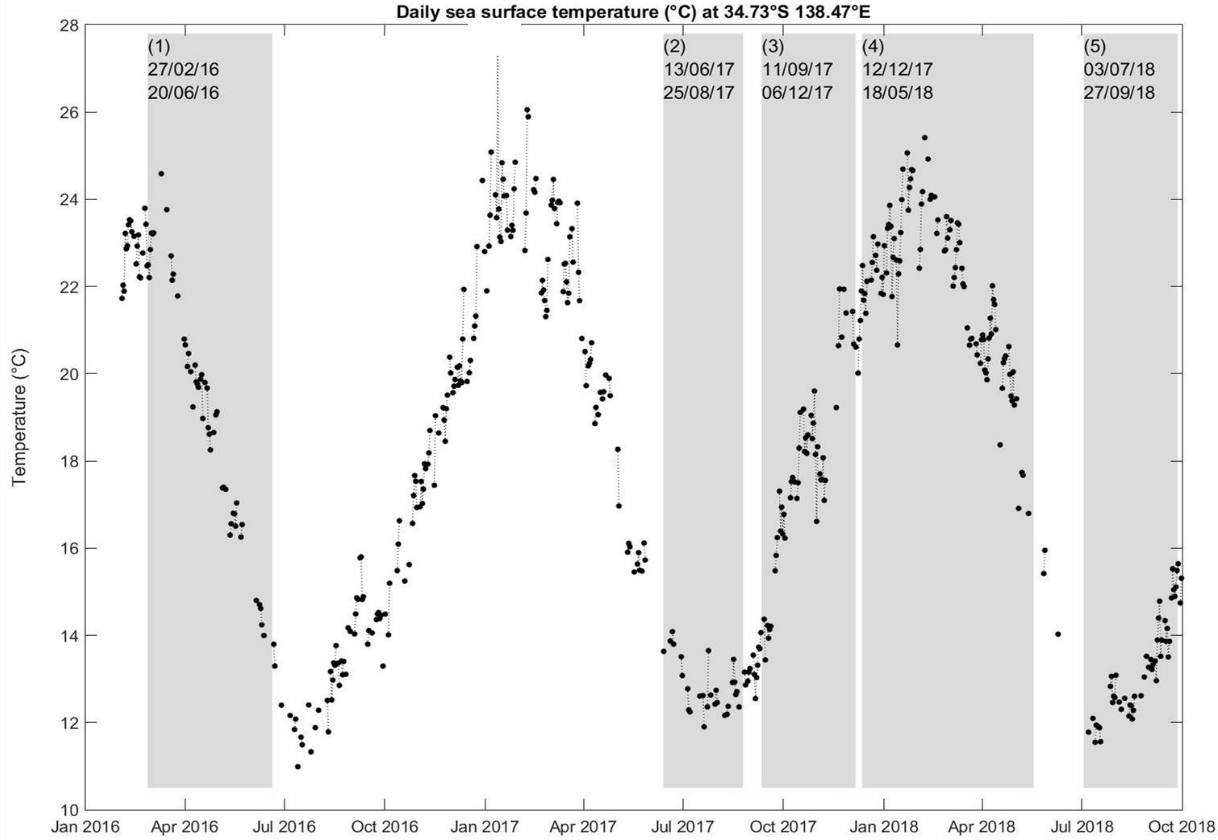


Figure 7: Daily satellite sea surface temperature data at 0.02° resolution (IMOS, 2020) extracted at the closest possible location to the target area (34.73°S 138.48°E; Figure 2). Areas highlighted in grey are the field trial periods. (1) Re-seeding trial autumn 2016; (2) Re-seeding trial winter 2017; (3) Re-seeding trial spring 2017; (4) Re-seeding trial summer 2017/2018; and (5) Re-seeding trial winter 2018.

2.1.1 Apparent survival

It was observed during the field experiment set-up that the hatchery-produced Mud Cockle spat were slow to bury into the substrate once seeded at low tide and, subsequently, some animals were lost from the experimental plots by tidal movement when the water level increased. This would partially contribute to the overall low apparent survival of re-seeded Mud Cockles, with an average of 8.1% for the high stocking density treatments and 11.1% for the low stocking density treatments.

Apparent survival was significantly different between sites ($P < 0.001$; Kruskal-Wallis). Site A had higher apparent survival (12.5 ± 1.2%) than Site B (6.7 ± 1.1 %). There was no significant difference in apparent survival between size classes ($P = 0.962$; Kruskal-Wallis) or stocking densities ($P = 0.444$). With regard to predator protection method, there was a significant difference in apparent survival between treatments ($P < 0.001$; Kruskal-Wallis). The closed tray treatment showed significantly better apparent survival (16.6 ± 1.7%) compared to the open tray (8.8 ± 1.2%; $P = 0.003$; Kruskal-Wallis with pairwise comparisons) and tray frame treatments (3.3 ± 0.6%; $P < 0.001$; Kruskal-Wallis with pairwise comparisons), the latter two were also significantly different from each other ($P < 0.001$; Kruskal-Wallis with pairwise comparisons).

2.1.2 Final shell length and DGRSL

No Mud Cockles were recovered from the low stocking density, large and small size class Mud Cockles in the tray frame treatment at Site B, hence no final shell length data for this treatment could be included in the statistical analysis.

For final shell length, a significant difference was observed between sites ($P < 0.001$; Kruskal-Wallis), with Mud Cockles at Site B (13.19 ± 0.25 mm) growing larger than those at Site A (12.11 mm ± 0.19 mm). The

DGRSL for Sites A and B were 0.061 mm / day and 0.069 mm / day, respectively. There was a significant effect of size class on final shell length (*P* < 0.001; Kruskal-Wallis). At harvest, the average final shell length of the large size class was 13.00 ± 0.23 mm, whereas the final shell length of the small size class was 12.07 ± 0.19 mm. The DGRSL for the large and small size classes were 0.060 mm / day and 0.072 mm / day, respectively. There was no significant effect of stocking density (*P* = 0.619; Kruskal-Wallis) or predator protection method (*P* = 0.510; Kruskal-Wallis) on final shell length at harvest.

The overall DGRSL for Mud Cockles in this trial, which was completed in autumn 2016 water temperatures (20.1 ± 3.0 °C; Figure 7), was 0.066 mm / day.

2.2 Trial 2. Re-seeding trial winter 2017 (13th June - 25th August)

Results from Trial 2 are summarised in Table 5

Table 5: Results (mean ± SE) from Trial 2 for 5 mm screen size hatchery produced Mud Cockles (*K. scalarina*) re-seeded at the Section Bank in winter 2017. High density = 1000 individuals / m^2; Low density = 200 individuals / m^2

	Site A				**Site B**			
	High Density		**Low Density**		**High Density**		**Low Density**	
	Closed Tray	**Tray Frame**	**Closed Tray**	**Tray Frame**	**Closed Tray**	**Tray Frame**	**Closed Tray**	**Tray Frame**
Recovery (%)	68.27 ± 4.07	70.37 ± 3.29	64.88 ± 4.42	63.77 ± 4.18	73.72 ± 2.42	67.19 ± 5.12	66.27 ± 7.10	62.23 ± 5.78
Apparent survival (%)	63.75 ± 3.46	66.90 ± 3.34	55.30 ± 4.87	61.80 ± 4.45	70.02 ± 2.91	64.85 ± 5.00	59.54 ± 7.91	54.44 ± 5.99
Apparent mortality (%)	4.52 ± 1.14	3.47 ± 0.86	9.58 ± 2.57	1.97 ± 0.95	3.71 ± 1.07	2.34 ± 0.98	6.74 ± 0.95	7.79 ± 4.19
Whelk predation (%)	26.00 ± 9.90	0.50 ± 0.50	16.67 ± 10.54	0.00 ± 0.00	28.17 ± 9.66	0.50 ± 0.50	16.67 ± 10.54	0.00 ± 0.00
Final shell length (mm)	11.39 ± 0.06	11.84 ± 0.06	11.73 ± 0.22	11.38 ± 0.06	11.68 ± 0.04	11.93 ± 0.11	11.67 ± 0.20	12.02 ± 0.15
Final weight (g)	0.36 ± 0.01	0.38 ± 0.00	0.39 ± 0.02	0.37 ± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.40 ± 0.02	0.42 ± 0.02

2.2.1 Recovery

The overall recovery for this trial, which was run during winter 2017 water temperatures (14.2 ± 0.7 °C; Figure 7), was 67.1 ± 1.6%.

There were no significant interactions between treatments (*P* > 0.050; three-way ANOVA). At harvest, there was no significant difference in recovery of Mud Cockles between Sites A and B (*P* = 0.875; three-way ANOVA), high and low stocking densities (*P* = 0.103; three-way ANOVA) or predator protection method (*P* = 0.480; three-way ANOVA).

2.2.2 Apparent survival

The overall apparent survival for this trial, which was run during winter 2017 water temperatures (14.2 ± 0.7 °C; Figure 7), was 62.1 ± 1.8%.

For apparent survival, there were no significant interactions between treatments (*P* > 0.050; three-way ANOVA). There was no significant difference in apparent survival between Site A and Site B (*P* = 0.938; three-way ANOVA). In regards to stocking density, a significant difference was found in apparent survival between treatments (*P* = 0.019; three-way ANOVA). The high stocking density treatments had higher apparent survival (66.4 ± 1.8%) than the low stocking density treatments (57.8 ± 2.8%). There was no significant difference in apparent survival between predator protection method (*P* = 0.956; three-way ANOVA).

2.2.3 Apparent mortality

The overall apparent mortality for this trial, which was run during winter 2017 water temperatures (14.2 ± 0.7 °C; Figure 7), was 5.0 ± 0.7%.

There were no significant interactions between treatments for apparent mortality (*P* > 0.050; three-way ANOVA). There was no significant difference in apparent mortality between sites (*P* = 0.859; three-way ANOVA) or stocking densities (*P* = 0.279; three-way ANOVA). In contrast, there was a significant difference in apparent mortality between predator protection methods (*P* = 0.020; three-way ANOVA). The closed tray treatment had significantly higher apparent mortality (6.1 ± 0.8%) than the tray frame treatment (3.9 ± 1.1%).

2.2.4 Whelk predation

Overall, 11.1 ± 2.9% of the dead shell recovered had evidence of whelk predation (i.e. drill holes).

There was no significant difference in whelk predation between site (*P* = 0.959; Kruskal-Wallis) or stocking density (*P* = 0.135; Kruskal-Wallis). There was a significant difference in snail predation between predator protection methods (*P* = 0.001; Kruskal-Wallis), with the Mud Cockles in the closed tray treatment having significantly higher snail predation (21.9 ± 4.8%) than the tray frame treatment (0.2 ± 0.2%).

2.2.5 Final shell length and DGRSL

There was a significant difference in final shell length at harvest between site (*P* = 0.011; three-way ANOVA), with Mud Cockles at Site B (11.83 ± 0.07 mm) growing larger than those at Site A (11.58 ± 0.07 mm). The DGRSL for Sites A and B were 0.023mm / day and 0.026 mm / day, respectively. There was no significant difference in final shell length between stocking density (*P* = 0.910; three-way ANOVA) or predator protection method (*P* = 0.063; three-way ANOVA). There were no significant interactions between the main factors (*P* > 0.050; three-way ANOVA).

The overall DGRSL for Mud Cockles in this trial, which was run during winter 2017 water temperatures (14.2 ± 0.7 °C; Figure 7), was 0.024 mm / day.

2.2.6 Final weight and DGRW

For final weight at harvest, there were no significant interactions between treatments ($P > 0.050$; three-way ANOVA). There was a significant difference in final weight between site ($P = 0.009$; three-way ANOVA), with Mud Cockles at Site B (0.402 ± 0.007 g) being heavier than those at Site A (0.377 ± 0.06 g). The DGRW for Sites A and B were 0.001 g / day and 0.002 g / day, respectively. There was no significant difference in final weight between stocking density ($P = 0.329$; three-way ANOVA) or predator protection method ($P = 0.704$).

The overall DGRW for Mud Cockles in this trial, which was run during winter 2017 water temperatures (14.2 ± 0.7 °C; Figure 7), was 0.002 g / day.

2.3 Trial 3. Re-seeding trial spring 2017 (11th September - 6th December)

The results from Trial 3 are summarised in Table 6. The small size class was excluded from analyses given the low (6.0%) recovery of Mud Cockles for this trial within this treatment.

Table 6: Results (mean ± SE) from Trial 3 for hatchery produced Mud Cockles (*K. scalarina*) re-seeded at the Section Bank in spring 2017 at a density of 1000 individuals / m^2. Small size class = 3mm screen size spat; Large size class = 5 mm screen size spat

	Site A				Site B			
	Large size class		Small size class		Large size class		Small size class	
	Closed Tray	Tray Frame	Closed Tray	Tray Frame	Closed Tray	Tray Frame	Closed Tray	Tray Frame
Recovery (%)	26.06 ± 5.71	14.35 ± 2.18	10.19 ± 8.70	3.94 ± 2.15	59.25 ± 1.61	30.94 ± 4.61	6.02 ± 1.70	3.52 ± 0.96
Apparent survival (%)	13.3 ± 43.34	5.59 ± 2.06	6.39 ± 6.18	3.12 ± 2.21	46.2 ± 04.99	24.8 ± 24.14	4.24 ± 1.73	2.61 ± 0.75
Apparent mortality (%)	12.72 ± 4.06	8.76 ± 1.93	3.80 ± 2.54	0.82 ± 0.36	13.05 ± 4.23	6.12 ± 2.23	1.77 ± 0.76	0.91 ± 0.33
Whelk predation (%)	11.53 ± 5.00	7.73 ± 3.65	1.33 ± 1.33	21.67 ± 10.14	5.17 ± 5.17	26.33 ± 5.72	3.51 ± 3.51	37.70 ± 15.19
Final shell length (mm)	11.11 ± 0.59	11.52 ± 0.15	11.72 ± 0.25	8.00 ± 0.24	12.23 ± 0.10	12.05 ± 0.13	8.33 ± 0.09	8.57 ± 0.15
Final weight (g)	0.35 ± 0.04	0.38 ± 0.02	0.42 ± 0.02	0.13 ± 0.01	0.43 ± 0.01	0.41 ± 0.01	0.14 ± 0.00	0.15 ± 0.01

2.3.1 Recovery of large size class

The overall recovery of the large size class Mud Cockles for this trial, which was run during spring 2017 water temperatures (18.5 ± 2.1 °C; Figure 7), was 32.7 ± 3.9%.

For recovery, there was a significant difference between sites ($P < 0.001$; two-way ANOVA) and a significant difference between predator protection methods ($P < 0.001$; two-way ANOVA). There was a significant interaction between the site and predator protection method ($P = 0.046$; two-way ANOVA). The significant interaction may be explained by the tray frame treatment at Site B being not significantly different to the closed tray treatment at Site A ($P = 0.814$; two-way ANOVA with simple effects test). At Site A, there was no significant difference between predator protection method ($P = 0.181$; one-way ANOVA with Tukey HSD post hoc test). At Site B, however, the closed tray treatment had significantly higher recovery (59.3 ± 1.6%) than the tray frame treatment (30.9 ± 4.6%; $P < 0.001$; one-way ANOVA with Tukey HSD post hoc test). At Site B, both the tray frame treatment ($P = 0.033$; one-way ANOVA with Tukey HSD post hoc test) and closed tray treatments ($P < 0.001$; one-way ANOVA with Tukey HSD post hoc test) had significantly higher recovery than their corresponding treatments at Site A (tray frame treatment at Site A = 14.3 ± 2.2%; closed tray treatment at Site A = 26.1 ± 5.7%).

2.3.2 Apparent survival of large size class

The overall apparent survival of the large size class for this trial, which was run during spring 2017 water temperatures (18.5 ± 2.1 °C; Figure 7), was 22.4 ± 3.6%.

There were no significant interactions between treatments ($P = 0.646$; two-way ANOVA). A significant difference in apparent survival between sites was observed ($P < 0.001$; two-way ANOVA), with more Mud Cockles at Site B surviving (35.5 ± 4.5%) than at Site A (9.5 ± 2.2%). In regards to predator protection method, a significant difference was found in apparent survival between treatments ($P = 0.002$; two-way ANOVA). The closed tray had greater apparent survival (29.7 ± 5.7%) than the tray frame treatment (15.2 ± 3.6%).

2.3.3 Apparent mortality of large size class

The overall apparent mortality of the large size class for this trial, which was run during spring 2017 water temperatures (18.5 ± 2.1 °C; Figure 7), was 10.2 ± 1.6%.

There were no significant interactions between treatments ($P = 0.655$; two-way ANOVA). There was no significant difference in apparent mortality between site ($P = 0.587$; two-way ANOVA) or predator protection method ($P = 0.133$; two-way ANOVA).

2.3.4 Whelk predation of large size class

Overall, for this trial 12.7 ± 2.9% of the dead shells recovered in the large size class had evidence of whelk predation (i.e. drill holes).

There was no significant difference in whelk predation between site ($P = 0.491$; Kruskal-Wallis) or predator protection method ($P = 0.082$; Kruskal-Wallis).

2.3.5 Final shell length and DGRSL of large size class

There were no significant interactions between treatments ($P = 0.355$; two-way ANOVA). There was a significant difference in final shell length at harvest between site ($P = 0.015$; two-way ANOVA), where Mud Cockles grew significantly larger at Site B (12.15 ± 0.08 mm) than at Site A (11.31 ± 0.30 mm). The DGRSL for Sites A and B were 0.020 mm / day and 0.030 mm / day, respectively. There was no significant difference in final shell length at harvest between predator protection method ($P = 0.722$; two-way ANOVA).

The overall DGRSL for the large size class Mud Cockles in this trial, which was run during spring 2017 water temperatures (18.5 ± 2.1 °C; Figure 7), was 0.025 mm / day.

2.3.6 Final weight and DGRW of large size class

For final weight at harvest, there were no significant interactions between treatments (P = 0.222; two-way ANOVA). There was a significant difference in final weight at harvest between site (P = 0.036; two-way ANOVA), where Mud Cockles were significantly heavier at Site B (0.419 ± 0.009 g) compared to Site A (0.366 ± 0.021 g). The final DGRW for Sites A and B were 0.001 g / day and 0.002 g / day, respectively. There was no significant difference in final weight at harvest between predator protection method (P = 0.894; two-way ANOVA).

The overall DGWR for the large size class Mud Cockles in this trial, which was run during spring 2017 water temperatures (18.5 ± 2.1 °C; Figure 7), was 0.002 g / day.

2.4 Trial 4. Re-seeding trial summer 2017 / 2018 (12th December - 18th May)

For this trial, the short window of available sampling time at low tide meant Mud Cockles from 43 of the 48 plots were recovered. Three plots remained unrecovered from Site A (one tray frame without small mesh, one closed tray without small mesh and one closed tray with small mesh treatment) and two plots from Site B (two tray frames without small mesh treatments). This resulted in uneven sample sizes for subsequent statistical analysis. The results from Trial 4 are summarised in Table 7

Table 7: Results (mean ± SE) from Trial 4 for 5 mm screen size, hatchery produced Mud Cockles (*K. scalarina*) re-seeded at the Section Bank in summer 2017-2018 at a density of 1000 individuals / m^2.

	Site A				Site B			
	Closed Tray with small mesh	**Tray Frame with small mesh**	**Closed Tray without small mesh**	**Tray Frame without small mesh**	**Closed Tray with small mesh**	**Tray Frame with small mesh**	**Closed Tray without small mesh**	**Tray Frame without small mesh**
Recovery (%)	74.72 ± 8.93	53.37 ± 7.57	18.13 ± 3.21	10.26 ± 3.47	66.06 ± 11.15	68.91 ± 5.10	18.05 ± 1.11	3.24 ± 0.93
Apparent survival (%)	6.84 ± 5.58	0.09 ± 0.09	0.31 ± 0.25	0.00 ± 0.00	14.94 ± 11.04	0.00 ± 0.00	0.52 ± 0.27	0.00 ± 0.00
Apparent mortality (%)	67.88 ± 10.32	53.28 ± 7.54	17.82 ± 3.26	10.26 ± 3.47	51.12 ± 9.72	68.91 ± 5.10	17.53 ± 1.11	3.24 ± 0.93
Whelk predation (%)	1.56 ± 1.04	15.67 ± 5.25	2.22 ± 1.11	9.34 ± 6.99	2.71 ± 2.71	3.94 ± 1.68	5.63 ± 4.62	21.11 ± 12.70
Final shell length (mm)	18.90 ± 0.19	13	18.33 ± 0.88	NA	16.37 ± 0.34	NA	16.50 ± 0.76	NA
Final weight (g)	1.88 ± 0.06	0.73	1.75 ± 0.25	NA	1.33 ± 0.07	NA	1.35 ± 0.16	NA

2.4.1 Recovery

The overall recovery for this trial, which was run from 12th December 2017 to 18th May 2018 (average water temperature 22.6 ± 1.8 °C; Figure 7), was 39.1 ± 4.5%.

For recovery, there were no significant interactions between factors (*P* = 0.103; two-way ANOVA). There was no significant difference in recovery between site (*P* = 0.542; two-way ANOVA). Mud Cockle recovery was significantly different between predator protection method (*P* < 0.001; two-way ANOVA). The treatments with the small mesh (tray frame with small mesh = 61.1 ± 4.9% and closed tray with small mesh = 70.4 ± 6.9%) were not significantly different to each other (*P* = 0.736; two-way ANOVA with Tukey HSD post hoc test), but were significantly different to the treatments without the small mesh (tray frame with no small mesh = 6.7 ± 2.0% and closed tray with no small mesh = 18.1 ± 1.6%; *P* < 0.001). The closed tray with no small mesh had significantly higher recovery than the tray frame with no small mesh (*P* = 0.002; two-way ANOVA with Tukey HSD post hoc test).

2.4.2 Apparent survival

The overall apparent survival for this trial, which was run during summer 2017/2018 and early autumn 2018 water temperatures (22.6 ± 1.8 °C; Figure 7), was 2.8 ± 1.6%.

Only three plots had apparent survival higher than 5% at harvest. The three plots included two from the closed tray small mesh treatment at Site B (68.9% and 15.0% survival) and one plot from the closed tray small mesh treatment at Site A (43.2%). Of the remaining treatment plots, 33 of the 43 (77%) had 0% apparent survival and the remaining 10 plots had less than 5% apparent survival. Given the low apparent survival observed for this trial, the data was not statistically analysed for apparent survival.

2.4.3 Apparent mortality

The overall apparent mortality for this trial, which was run during summer 2017/2018 and early autumn 2018 water temperatures (22.6 ± 1.8 °C; Figure 7), was 36.3 ± 4.2%.

No significant interactions between factors were present (*P* = 0.081; two-way ANOVA) There was no significant difference in apparent mortality between site (*P* = 0.319; two-way ANOVA). There was a significant difference between predator protection method (*P* < 0.001; two-way ANOVA). The treatments with the small mesh (tray frame with small mesh = 61.1 ± 4.9% and closed tray with small mesh = 59.5 ± 7.2%) were not significantly different from each other (*P* = 0.976; two-way ANOVA with Tukey HSD post hoc test), but did have significantly higher apparent mortality than both the treatments without the small mesh (tray frame without small mesh = 6.7 ± 2.0% and closed tray without small mesh = 17.7 ± 1.6%; *P* < 0.001; two-way ANOVA with Tukey HSD post hoc test). The closed tray without small mesh had significantly higher apparent mortality than the tray frame without small mesh (*P* = 0.002; two-way ANOVA with Tukey HSD post hoc test).

2.4.4 Whelk predation

Overall, 7.8 ± 2.1% of the dead shell recovered had evidence of whelk predation (i.e. drill holes).

There was no significant difference in whelk predation between site (*P* = 0.664; Kruskal-Wallis) or predator protection method (*P* = 0.120; Kruskal-Wallis).

Final shell length, shell growth rate, final weight and DGRW

Given only 110 Mud Cockles were recovered alive (from 10 experimental plots) within this trial, final shell length and final weight at harvest data was not statistically analysed. Visual observation of the dead Mud Cockles indicated that they increased in shell length prior to death.

The overall DGRSL for Mud Cockles in this trial, which was run during summer 2017/2018 and early autumn 2018 water temperatures (22.6 ± 1.8 °C; Figure 7), was 0.045 mm / day and the DGRW was 0.008 g / day.

2.5 Trial 5. Re-seeding trial winter 2018 (3rd July - 27th September)

Trial 5A

The results from Trial 5A are summarised in Table 8

Table 8: Results (mean ± SE) from Trial 5A for 5 mm screen size, hatchery produced Mud Cockles (*K. scalarina*) re-seeded at the Section Bank in winter 2018 at a density of 1000 individuals / m^2.

	Site A				Site B			
	Closed Tray with small mesh	**Tray Frame with small mesh**	**Closed Tray without small mesh**	**Tray Frame without small mesh**	**Closed Tray with small mesh**	**Tray Frame with small mesh**	**Closed Tray without small mesh**	**Tray Frame without small mesh**
Recovery (%)	93.26 ± 1.62	73.75 ± 6.75	48.70 ± 6.16	47.06 ± 6.95	92.57 ± 1.58	72.11 ± 7.82	61.40 ± 3.73	40.59 ± 2.85
Apparent survival (%)	33.33 ± 16.23	30.92 ± 8.88	43.78 ± 5.85	43.52 ± 6.85	82.38 ± 4.68	38.86 ± 9.33	58.20 ± 3.39	32.73 ± 2.00
Apparent mortality (%)	59.93 ± 16.79	42.83 ± 6.83	4.92 ± 1.30	3.54 ± 1.41	10.19 ± 3.72	33.25 ± 10.90	3.20 ± 0.65	7.86 ± 1.79
Whelk predation (%)	0.00 ± 0.00	1.66 ± 1.10	22.78 ± 15.16	25.24 ± 12.82	0.00 ± 0.00	7.18 ± 4.34	16.67 ± 16.67	84.19 ± 8.19
Final shell length (mm)	9.58 ± 0.30	9.44 ± 0.07	10.56 ± 0.10	10.89 ± 0.21	10.53 ± 0.19	9.81 ± 0.14	10.96 ± 0.11	10.93 ± 0.14
Final weight (g)	0.29 ± 0.02	0.30 ± 0.01	0.36 ± 0.01	0.39 ± 0.00	0.28 ± 0.01	0.36 ± 0.01	0.38 ± 0.00	0.38 ± 0.01
Sediment composition (%):								
Fine	2.75 ± 0.67	2.78 ± 0.86	2.34 ± 0.74	1.37 ± 0.08	2.72 ± 0.42	2.80 ± 0.43	1.92 ± 0.14	1.34 ± 0.11
Medium	93.77 ± 1.13	94.15 ± 1.22	93.45 ± 1.28	95.08 ± 0.22	94.32 ± 0.27	92.28 ± 0.83	93.40 ± 0.69	93.69 ± 0.71
Coarse	3.49 ± 0.48	3.07 ± 0.38	4.21 ± 0.81	3.55 ± 0.23	2.95 ± 0.30	4.93 ± 0.81	4.68 ± 0.67	4.98 ± 0.61
Depth of anoxic layer (mm)	12.50 ± 3.67	15.83 ± 4.67	20.83 ± 4.96	33.33 ± 7.53	6.67 ± 1.49	6.94 ± 1.85	10.28 ± 1.90	16.39 ± 1.25

2.5A.1 Recovery

The overall recovery for this trial, which was run during winter and early spring 2018 water temperatures (13.9 ± 0.8 °C; Figure 7), was 66.2 ± 3.2%.

For recovery, there was no significant interaction between site and predator protection method (P = 0.312; two-way ANOVA). No significant difference in recovery between site was observed (P = 0.795; two-way ANOVA). In regards to predator protection method, there was a significant difference in recovery (P < 0.001; two-way ANOVA). All predator protection method treatments were significantly different from each other (P < 0.05; two-way ANOVA with Tukey HSD post hoc test), with the exception of the tray frame without small mesh (43.8 ± 3.7 %) and the closed tray without small mesh (55.1 ± 3.9%) treatments that were not significantly different from one another (P = 0.158; two-way ANOVA with Tukey HSD post hoc test). The treatments with the small mesh (tray frame with small mesh = 72.9 ± 4.9%; closed tray with small mesh = 92.9 ± 1.1%) had higher significantly recovery than the treatments without the small mesh (P < 0.050; two-way ANOVA with Tukey HSD post hoc test).

2.5A.2 Apparent survival

The overall apparent survival for this trial, which was run during winter and early spring 2018 water temperatures (13.9 ± 0.8 °C; Figure 7), was 45.5 ± 3.6%.

For this trial, there was a significant difference in apparent survival between site (P = 0.013; two-way ANOVA), predator protection method (P = 0.027; two-way ANOVA) and a significant interaction between the two factors (P = 0.008; two-way ANOVA). The significant interaction may be explained by the significant response observed between the closed tray treatments between Site A and Site B (P < 0.001; two-way ANOVA with simple effects test). The closed tray with small mesh treatment at Site B had the highest apparent survival (82.4 ± 4.7%). Whereas, the remaining predator protection method treatments did not significantly differ between sites (P > 0.050; two-way ANOVA with simple effects test). At Site A, there was no significant difference between any of the predator protection method treatments for apparent survival (P > 0.050; one-way ANOVA), whereas, at Site B the closed tray without the small mesh had significantly higher apparent survival (58.2 ± 3.4%) than the tray frame without the small mesh (32.7 ± 2.0%). All other treatments at Site B were not significantly different from each other (P > 0.050; one-way ANOVA).

2.5A.3 Apparent mortality

The overall apparent mortality for this trial, which was run during winter and early spring 2018 water temperatures (13.9 ± 0.8 °C; Figure 7), was 20.7 ± 3.9%.

For this trial, there was a significant difference in apparent mortality between site (P = 0.012; two-way ANOVA), predator protection method (P < 0.001; two-way ANOVA) and a significant interaction between the two factors (P = 0.005; two-way ANOVA). The interaction may be explained by the closed tray with the small mesh being significantly different between site (P < 0.001; two-way ANOVA with simple effects test), with significantly higher apparent mortality within this treatment at Site A (59.9 ± 16.8%) compared to Site B (10.2 ± 3.7%). The other predator protection method treatments were not significantly different for apparent mortality between site (P > 0.050; two-way ANOVA with simple effects test).

At Site A, the treatments with the small mesh had significantly higher apparent mortality (tray frame with small mesh = 42.8 ± 6.8%; closed tray with small mesh = 59.9 ± 16.8%) than the treatments without the small mesh (tray frame without small mesh = 3.5 ± 1.4%; closed tray without small mesh = 4.9 ± 1.3%; P < 0.050; one-way ANOVA with Tukey's HSD post hoc test). The treatments with the small mesh (tray frame with small mesh; closed tray with small mesh) were not significantly different from each other (P = 0.557; one-way ANOVA with Tukey's HSD post hoc test) and the treatments without the small mesh (tray frame without small mesh; closed tray without small mesh) were not significantly different from each other (P = 1.000; one-way ANOVA with Tukey's HSD post hoc test).

At Site B, the tray frame with the small mesh treatment had the highest apparent mortality (33.2 ± 10.9%) and was significantly different to the tray frame without small mesh (7.9 ± 1.8%; P = 0.028; one-way

ANOVA with Tukey's HSD post hoc test) and the closed tray without small mesh treatments (3.2 ± 0.6%; P = 0.008; one-way ANOVA with Tukey's HSD post hoc test). The closed tray with small mesh treatment (10.2 ± 3.7%) was not significantly different to any of the other predator protection method treatments at Site B (P > 0.050; one-way ANOVA with Tukey's HSD post hoc test). Furthermore, the treatments without the small mesh (tray frame without small mesh; closed tray without small mesh) were not significantly different from each other for apparent mortality (P = 0.941; one-way ANOVA with Tukey's HSD post hoc test).

2.5A.4 Whelk predation

Overall, 19.7 ± 5.0% of the dead shell recovered had evidence of whelk predation (i.e. drill holes).

There was no significant difference in whelk predation between site (P = 0.302; Kruskal-Wallis). However, there was a significant difference in whelk predation between predator protection method (P < 0.001; Kruskal-Wallis). The tray frame without small mesh treatment had significantly higher whelk predation (54.7 ± 11.4 %) than the other predator protection method treatments (P < 0.050; Kruskal-Wallis with pairwise comparisons). The remaining treatments (tray frame with small mesh = 4.4 ± 2.3%; closed tray with small mesh = 0.0 ± 0.0%; closed tray without small mesh = 19.7 ± 10.7%) were not significantly different to each other (P > 0.050; Kruskal-Wallis with pairwise comparisons).

2.5A.5 Final shell length and DGRSL

For final shell length, there was no significant interaction between site and predator protection method (P = 0.081; two-way ANOVA). There was a significant difference between site (P < 0.001; ANOVA), with Mud Cockles at Site B growing larger (10. 59 ± 0.04 mm) than those at Site A (10.21 ± 0.05 mm). The DGRSL for Sites A and B were 0.007 mm / day and 0.012 mm / day, respectively. In regards to predator protection method, there was a significant difference for final shell length at harvest between treatments (P < 0.001; two-way ANOVA). The treatments without the small mesh (tray frame without small mesh = 10.91 ± 0.12 mm; closed tray without small mesh = 10.76 ± 0.09 mm) were not significantly different to each other (P = 0.815; two-way ANOVA with Tukey's HSD post hoc test), but were significantly larger than the treatments with the small mesh (tray frame with small mesh = 9.62 ± 0.09 mm; closed tray with small mesh = 10.05 ± 0.22 mm; P < 0.050; two-way ANOVA with Tukey's HSD post hoc test). The latter two treatments were not significantly different to each other for final shell length at harvest (P = 0.075; two-way ANOVA with Tukey's HSD post hoc test).

The overall DGRSL for Mud Cockles in this trial, which was run during winter and early spring 2018 water temperatures (13.9 ± 0.8 °C; Figure 7), was 0.010 mm / day.

2.5A.6 Final weight and DGRW

For this trial, there was a significant difference in final weight between site (P = 0.044; two-way ANOVA), predator protection method (P < 0.001; two-way ANOVA) and a significant interaction between the two factors (P = 0.002; two-way ANOVA). The interaction could be explained by the closed tray with the small mesh treatment being significantly different between site (P < 0.001; two-way ANOVA with simple effects test), with significantly greater final weight within this treatment at Site B (0.355 ± 0.012 g) compared to Site A (0.291 ± 0.018 g). The other predator protection method treatments were not significantly different between site (P > 0.050; one-way ANOVA with simple effects test).

At Site A, the Mud Cockles in the treatments without the small mesh had significantly greater final weight (tray frame without small mesh = 0.392 ± 0.005 g; closed tray without small mesh = 0.359 ± 0.007 g) than the treatments with the small mesh (tray frame with small mesh = 0.295 ± 0.007 g; closed tray with small mesh = 0.291 ± 0.018 g; P < 0.050; one-way ANOVA with Tukey's HSD post hoc test). The treatments with the small mesh (tray frame with small mesh; closed tray with small mesh) were not significantly different from each other (P = 0.991; one-way ANOVA with Tukey's HSD post hoc test) and the treatments without the small mesh (tray frame without small mesh; closed tray without small mesh) were not significantly different from each other (P = 0.147; one-way ANOVA with Tukey's HSD post hoc test).

At Site B, the Mud Cockles in the tray frame with the small mesh treatment had a significantly lower final weight (0.285 ± 0.012 g) than all the other predator protection treatments (*P* < 0.050; one-way ANOVA with Tukey's HSD post hoc test). The remaining predator protection method treatments (closed tray with small mesh = 0.355 ± 0.012 g; tray frame without small mesh = 0.381 ± 0.011 g; closed tray without small mesh = 0.378 ± 0.005 g) were not significantly different to each other at Site B (*P* > 0.050; one-way ANOVA with Tukey's HSD post hoc test).

The overall DGRW for Mud Cockles in this trial, which was run during winter and early spring 2018 water temperatures (13.9 ± 0.8 °C; Figure 7), was 0.001 g / day.

2.5A.7 Mud Cockle movement

The number of Mud Cockles recovered within each tray frame treatment (43.8 ± 3.7%), within quadrant 1 (11.3 ± 1.3%; Figure 5; section 1.2.5) and within quadrant 2 (7.0 ± 1.3%; Figure 5; section 1.2.5) was not significantly different between site (*P* > 0.050; one-way ANOVA; Figure 8).

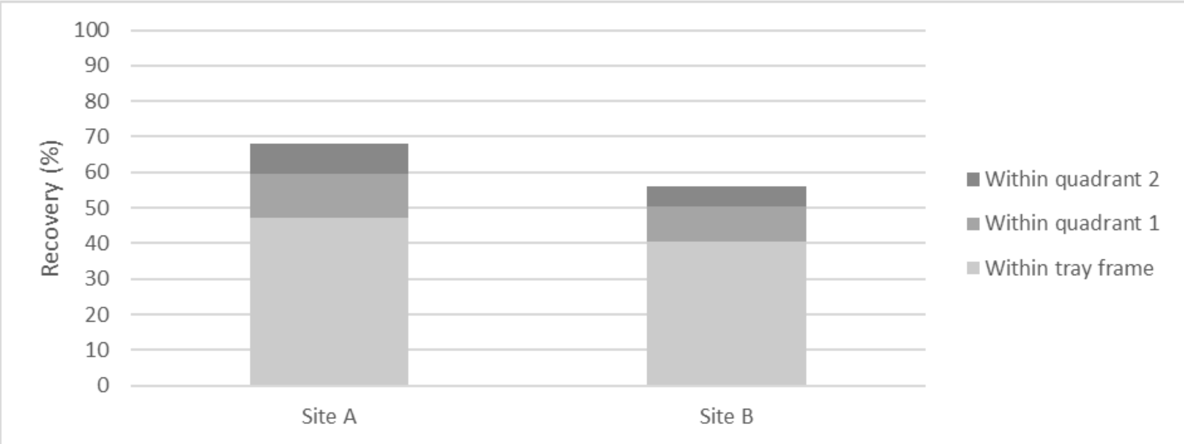


Figure 8: Mud Cockle movement as signified by recovery (mean ± SE; n = 6) of hatchery produced Mud Cockles (*K. scalarina*) within each tray frame treatment, within quadrant 1 and within quadrant 2, 86 days post seeding at the Section Bank in Trial 5A.

2.5A.8 Sediment analysis

Sediment size

There was no significant difference in sediment composition between Site A and Site B for the fine (*P* = 0.433; Kruskal-Wallis), medium (*P* = 0.141; Kruskal-Wallis) or coarse (*P* = 0.070; Kruskal-Wallis) sediment size classes. Additionally, there was no significant difference between each site and their corresponding control samples (*P* > 0.050; Kruskal-Wallis; S1-8, Figure 5; section 2.6). Overall, the medium sediment size class (250-1000 µm) made up the majority of each sample, with an average proportion of 93.4 ± 0.3% medium sediment size class, 4.1 ± 0.2% coarse sediment size class (> 1000 µm) and 2.5 ± 0.2% fine sediment size class (< 250 µm).

Anoxic layer

The depth of the anoxic layer (sand depth before it turned grey) within the treatment plots at Site A (20.63 ± 3.01 mm) were not significantly different from Site A control measurements (27.50 ± 5.20; S1-4, Figure 6; section 1.2.5; *P* = 0.559; one-way ANOVA). At Site B however, measurements taken from within the treatment plots were significantly different to the Site B control measurements (S4-8, Figure 6; section 1.2.5;

P = 0.031; one-way ANOVA). The measurements from within the treatments at Site B had a shallower anoxic layer (10.07 ± 1.12 mm) compared to the control measurements for Site B (26.25 ± 5.54 mm).

For the depth of the anoxic layer, there was no significant interaction between site and predator protection method (P = 0.615; two-way ANOVA). There was a significant difference in the depth of the anoxic layer between site (P = 0.003; two-way ANOVA), with Site A having a significantly deeper anoxic layer (20.62 ± 3.00 mm) than Site B (10.07 ± 1.12 mm; P < 0.001; two-way ANOVA). In regards to predator protection method, there was no significant difference in the depth of the anoxic layer between treatments (P = 0.210; two-way ANOVA).

Linear regression found no significant relationship between depth of anoxic layer and apparent survival (P = 0.195; r^2 = 0.036; Figure 9A), apparent mortality (P = 0.106; r^2 = 0.056; Figure 9B) or final shell length (P = 0.083; r^2 = 0.064, Figure 9C). There was a significant positive relationship between depth of anoxic layer and final weight (P = 0.012; r^2 = 0.130, Figure 9D)

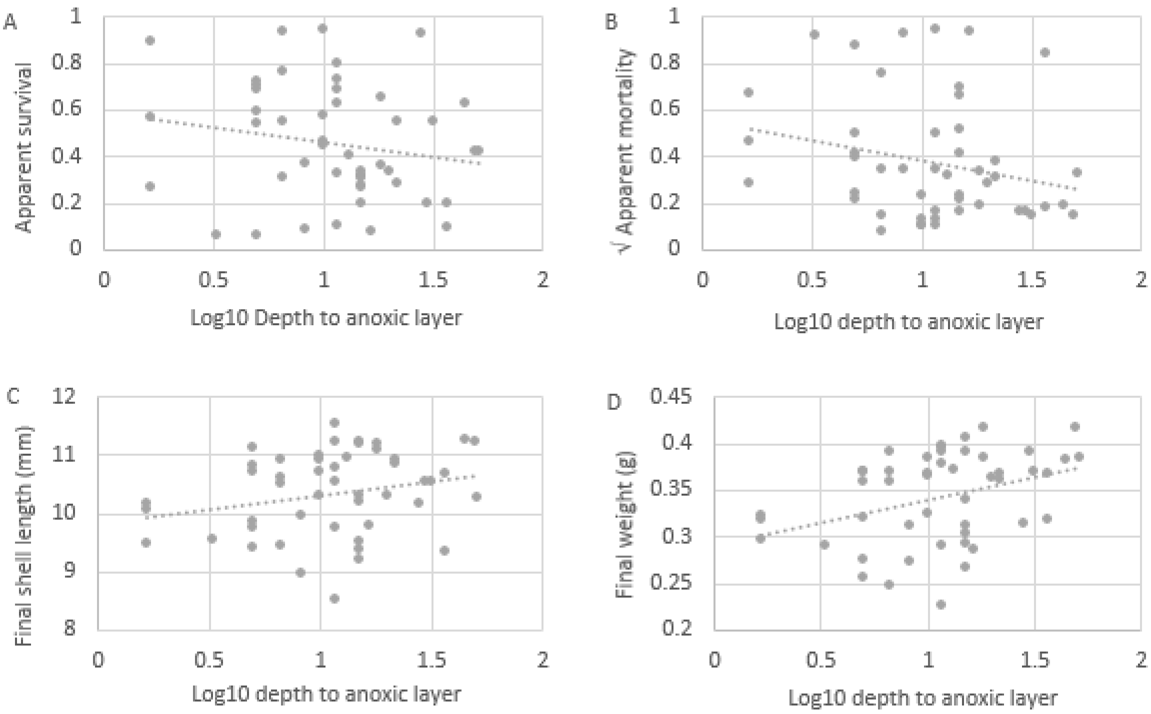


Figure 9 Relationship between the depth of the anoxic layer (Log_{10} transformed) and apparent survival (A; P = 0.195; r^2 = 0.036) apparent mortality (B; square root transformed; P = 0.106; r^2 = 0.056), final shell length (P = 0.083; r^2 = 0.064) and final weight (P = 0.012; r^2 = 0.130; linear regression) for hatchery produced Mud Cockles (K. scalarina) 86 days post seeding at the Section Bank in Trial 5A.

<u>Trial 5B</u>

Results from Trial 5B are summarised in Table 9.

Table 9: Results (mean ± SE) from Trial 5B for 5 mm screen size, hatchery produced Mud Cockles (*K. scalarina*) re-seeded at the Section Bank in winter 2018 at a density of 1000 individuals / m^2.

	Original Site B		New Site B	
	Closed Tray with small mesh	**Closed Tray without small mesh**	**Closed Tray with small mesh**	**Closed Tray without small mesh**
Recovery (%)	86.44 ± 3.09	84.37 ± 4.46	92.57 ± 1.58	61.40 ± 3.73
Apparent survival (%)	79.02 ± 4.64	78.32 ± 3.71	82.38 ± 4.68	58.20 ± 3.39
Apparent mortality (%)	7.43 ± 1.69	6.04 ± 1.69	10.19 ± 3.72	3.20 ± 0.65
Final shell length (mm)	10.52 ± 0.26	11.16 ± 0.09	10.53 ± 0.19	10.96 ± 0.11
Final weight (g)	0.36 ± 0.02	0.40 ± 0.00	0.36 ± 0.01	0.38 ± 0.00
Sediment composition (%):				
Fine	3.09 ± 0.26	4.12 ± 0.54	2.72 ± 0.42	1.92 ± 0.14
Medium	92.94 ± 0.25	90.59 ± 1.30	94.32 ± 0.27	93.40 ± 0.69
Coarse	3.97 ± 0.47	5.29 ± 0.82	2.95 ± 0.30	4.68 ± 0.67
Depth of anoxic layer (mm)	14.72 ± 1.00	13.89 ± 1.96	6.67 ± 1.49	10.28 ± 1.90

2.5B.1 Recovery, apparent survival, apparent mortality and growth.

When comparing the plot sites used in Trials 2, 3 and 4 (Sections 1.2.2, 1.2.3 and 1.2.4; Original Site B; Figure 6) to the corresponding plot sites (closed tray with small mesh and closed tray without small mesh treatments; New Site B, Figure 6) used in the current trial (Trial 5A), there was a significant difference in recovery (P = 0.032; one-way ANOVA). More Mud Cockles were recovered at the plot sites used in the current trial (Trial 5A; 87.3 ± 2.5%) compared to the previous trials (Trial 2, 3 and 4; 75.0 ± 4.8%). There was no significant difference in apparent survival (74.5 ± 2.8%; P = 0.133; one-way ANOVA) or apparent mortality (6.7 ± 1.2%; P = 0.628; one-way ANOVA) between the locations used in Trials 2, 3 and 4 compared to the location used in Trial 5A. Additionally, there was no significant difference in final shell length (P = 0.402) or final weight (P = 0.053, Kruskal-Wallis) observed.

2.5B.2 Sediment analysis.

Sediment Size

The sediment size compositions for the plot sites used in Trials 2, 3 and 4 (Sections 1.2.2, 1.2.3 and 1.2.4; Original Site B; Figure 6) were significantly different from the corresponding control samples (S9-10, Figure 6; section 1.2.5) for the fine sediment size class (control = 1.4 ± 0.1%; trial plot sites = 3.6 ± 0.3%; P = 0.020; one-way ANOVA), but was not significant for the medium sediment size class (control = 92.0 ± 1.6%; trial plot sites = 91.8 ± 0.7%; P = 0.911; one-way ANOVA) or the coarse sediment size class (control = 6.6 ± 1.6%; trial plot sites = 4.6 ± 0.5%; P = 0.161; one-way ANOVA).

There was a significant difference in the sediment size composition between the plot sites used in Trials 2, 3 and 4 (Sections 1.2.2, 1.2.3 and 1.2.4; Original Site B; Figure 6) and the corresponding plot sites (closed tray with small mesh and closed tray without small mesh treatments; New Site B, Figure 6) used in the current trial for the fine sediment size class (P = 0.005; one-way ANOVA) and the medium sediment size class (P = 0.018; one-way ANOVA), but not the coarse sediment size class (4.2 ± 0.3%; P = 0.232; one-way ANOVA). The plot sites used in Trials 2, 3 and 4 (Sections 2.3, 2.4 and 2.5) had a higher proportion of fine sediment (3.6 ± 0.3%) and a lower proportion of medium sediment (91.8 ± 0.7%) compared to the corresponding plots (closed tray with small mesh and closed tray without small mesh treatments) used in the current trial (Trial 5A; fine sediment size class = 2.3 ± 0.2%; medium sediment size class = 93.9 ± 0.4%).

Anoxic layer

The mean depth of the anoxic layer (sand depth before it turned grey) within the treatment plots at the site used in Trials 2, 3 and 4 (14.31 ± 1.06 mm; Sections 1.2.2, 1.2.3 and 1.2.4; Original Site B; Figure 6) was not significantly different from control samples taken from the same site (22.50 ± 7.50 mm; S9-10, Figure 6; section 1.2.5; P = 0.067; one-way ANOVA). There was a significant difference in the depth of the anoxic layer between site (P = 0.002; one-way ANOVA). The treatment plots at the site used in Trials 2, 3 and 4 (Sections 1.2.2, 1.2.3 and 1.2.4; Original Site B; Figure 6) had a significantly deeper anoxic layer (14.31 ± 1.06 mm) than the corresponding plot sites (closed tray with small mesh and closed tray without small mesh treatments; New Site B, Figure 6) used in the current trial (Trial 5A; 8.47 ± 1.27 mm). There was no significant difference in the depth of the anoxic layer for predator protection method (P = 0.405; ANOVA one-way ANOVA), and no significant interaction between factors (P = 0.188; one-way ANOVA) were observed.

2.6 Seasonal analysis

Results of the stepwise linear regression are detailed in Table 10.

Table 10: Stepwise linear regression of field trials (Trials 1-5A) results. Significant predictors (factor*)* of hatchery produced Mud Cockle (*K. scalarina;* 5 mm screen size; stocking density = 1000 individuals / m^2) recovery, apparent survival, apparent mortality and growth (daily growth rate length - DGRL and daily growth rate weight – DGRW) rates at the Section Bank. Df = degrees of freedom. Significance = * $P \leq 0.050$ ** $P \leq 0.010$ *** $P \leq 0.001$.

Dependent variable	**r^2**	**Df**	**F**	**Factor**	**Δ r^2**	**β**
Recovery rate	0.740	1-91	83.57***	Water temperature	0.691***	-0.838
				Predator protection	0.036***	-0.187
				Site	0.013*	-0.114
Apparent survival rate	0.819	1-114	167.05***	Water temperature	0.767***	-0.881
				Site	0.026***	-0.161
				Predator protection	0.023***	-0.152
Apparent mortality rate	0.125	1-91	6.34**	Predator protection	0.069*	0.254
				Water temperature	0.056*	0.237
DGRL	0.510	1-96	49.47***	Water temperature	0.485***	0.690
				Site	0.026*	-0.160
DGRW	0.486	1-72	67.25***	Water temperature	0.486***	0.697

Recovery rate

The results of the regression indicated that the model explained 74.0% of the variation observed in recovery rate and was significant ($F_{(1, 90)} = 83.57$; $P \leq 0.001$; stepwise linear regression, Table 10). Water temperature, predator protection and site were all significant predictors of recovery ($P \leq 0.001$). Water temperature had the strongest influence on recovery rate accounting for 69.1% of the variation observed ($\Delta F_{(1, 90)} = 201.63$; $P \leq 0.001$). Predator protection method accounted for 3.6% of the variation ($\Delta F_{(1, 89)} = 11.70$; $P \leq 0.001$) and site accounted for 1.3% of the variation ($\Delta F_{(1, 88)} = 4.39$; $P = 0.039$). There were no significant interactions between factors ($\Delta r^2 = 0.015$; $P = 0.291$).

Apparent survival rate

The model explained 81.6% of the variance of the apparent survival rate data and was significant, ($F_{(1,114)} = 166.02$; $P \leq 0.001$; stepwise linear regression, Table 10), with temperature, site and predator protection method all being significant predictors for apparent survival rate ($P \leq 0.001$). Water temperature had the strongest influence on apparent survival rate accounting for 76.7% of the variation observed in the data ($\Delta F_{(1, 114)} = 375.58$, $P \leq 0.001$) whereas site and predator protection method accounted for 2.6% and 2.3% of the variation observed respectively ($\Delta F_{(1, 113)} = 14.16$ and $\Delta F_{(1, 112)} = 14.24$; $P \leq 0.001$). There were no significant interactions between factors ($\Delta r^2 = 0.008$; $P = 0.323$).

Apparent mortality rate

For apparent mortality rate, the model was significant and explained 12.5% of the variation observed in the data ($F_{(1, 90)} = 6.34$; $P = 0.003$; stepwise linear regression, Table 10). Predator protection method accounted for 6.9% of the variation observed ($\Delta F_{(1, 90)} = 6.66$; $P = 0.011$), whereas water temperature accounted for 5.6% of the variation ($\Delta F_{(1, 89)} = 5.68$; $P = 0.019$; linear regression). Site was not significant ($P = 0.436$). There was a significant interaction between water temperature and predator protection method for apparent mortality rate ($P = 0.038$, stepwise linear regression). This interaction can be explained by the apparent mortality rates between the tray frame and closed tray treatments not being significantly different in the cool water temperatures (winter trials - Trial 2 = 14.2 ± 07 °C and Trial 5 = 13.9 ± 0.8 °C; $P > 0.05$), but higher mortalities observed in the closed tray over the warmer trials (Spring [Trial 3; 18.5 ± 2.1 °C; $P = 0.031$] and Summer [Trial 4; 22.6 ± 1.8 °C; $P = 0.001$]; two-way ANOVA with simple effects test; Figure 10). When this interaction was included in the linear regression analysis, the model explained 18.7% of the variation, a significant increase of 6.2% ($\Delta F_{(1, 88)} = 6.74$; $P = 0.011$; stepwise linear regression).

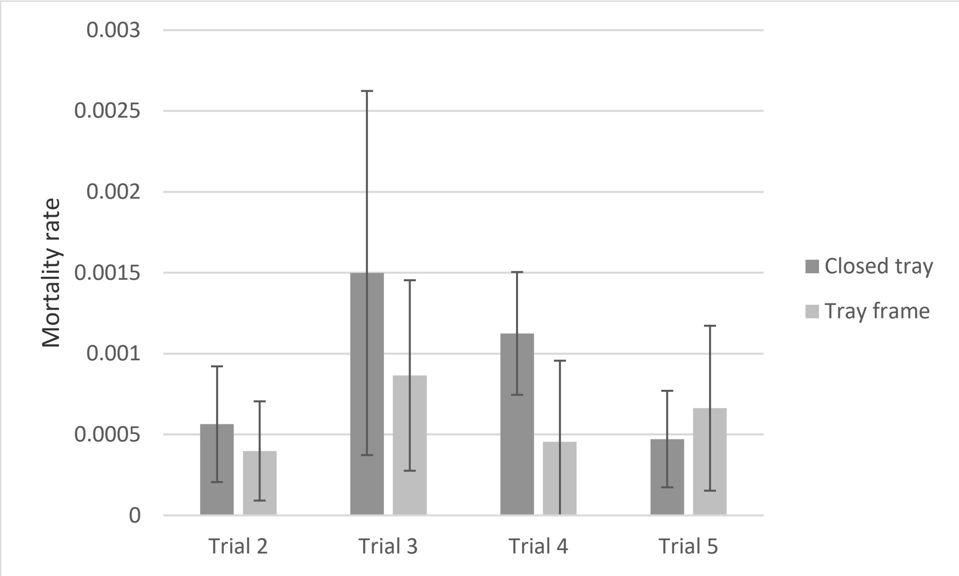


Figure 10: Mortality rates (mean ± SE) across Trials 2-5 for hatchery produced Mud Cockles (*K. scalarina;* 5 mm screen size; stocking density = 1000 individuals / m^2) at the Section Bank.

DGRSL

For DGRSL, the model explained 51.0% of the variation and was significant ($F_{(1, 96)} = 49.47$; $P \leq 0.001$; stepwise linear regression). Water temperature was a significant predictor of DGRSL, accounting for 48.5% of the variation observed ($\Delta F_{(1, 96)} = 90.24$; $P \leq 0.001$). Site was also a significant predictor of DGRSL, accounting for 2.6% of the variation ($\Delta F_{(1, 95)} = 4.97$; $P = 0.028$). Predator protection method was not significant ($P = 0.716$). There were no significant interactions between factors ($\Delta r^2 = 0.029$; $P = 0.235$).

DGRW

Water temperature was the only significant predictor of DGRW and explained 48.6% of the variation in the data ($F_{(1, 71)} = 67.25$; $P \leq 0.001$; stepwise linear regression). Site and predator protection method were not significant ($P = 0.188$ and $P = 0.567$ respectively) and there were no significant interactions between factors ($\Delta r^2 = 0.048$; $P = 0.156$).

2.6 Trial 6. Re-seeding - summer 2018

As proposed in the application, completion of this trial and future monitoring of the re-seeded Mud Cockles will be undertaken through the SARDI / PIRSA Mud Cockle stock assessment program. In the next SARDI / PIRSA Mud Cockle stock assessment program, the experimental and control plots marked out in Trial 6 will be sampled. This will involve sampling of five randomly placed 0.5 m^2 quadrants within each of the six 10 m^2 plots per site. Sampling will be performed as per the rest of the assessment program. The results of the assessment will indicate if re-seeding as per the methods in the current study are effective long term.

2.7 Benefit cost evaluation

In order to calculate a benefit cost ratio, an estimated survival to harvest rate is required. The field trials in this study were unable to conclusively determine survival to harvest because a) they were not run for the duration it would take the Mud Cockles to reach market size of 30 mm; the ~85 weeks (about three years from fertilisation) were estimated according to the average spat growth rate in this study while the ~137 weeks (about four years from fertilisation) were based on the published survey results in Mud Cockles (Cantin, 2010; Tarbath and Gardner, 2015), and b) confounding factors between trials as methodologies were optimised to increase recovery in this study. The closed tray treatment at Site B was selected for use in benefit cost modelling due to its greater performance. Survival data was standardised for the number of days of each trial, season was calculated as 90 days. No reliable survival data was obtained for autumn (Trial 1) and summer (Trial 4) field trials, hence the spring survival data was used as an estimate for these seasons. For winter survival, an average of the apparent survival in the closed tray treatment at Site B for Trial 2 and Trial 5 was used. The Mud Cockle movement study in Trial 5A suggested ~18% of Mud Cockles moved away from the plot. Mud Cockle movement was not assessed in conjunction with the closed tray treatment, hence a conservative movement rate of 10% was used to estimate survival to harvest. No data was available for survival of large (>10mm shell length) Mud Cockles. Other bivalve species have been shown to have increased survival with growth and time in the field (Marelli and Arnold, 1996; Nakaoka, 1996; Cigarria and Fernandez, 2000). A four year study on *K. scalarina* in Princess Royal Harbour, Western Australia (similar longitude to the Section Bank) found an annual survival rate of 78.2% ± 2.7% for caged wild Mud Cockles greater than one year old (Peterson et al., 1994). Additonally, Bellchambers (1998) reported an annual mortality of less than 10% in wild 20-25 mm shell length *K. scalarina*. Based on this data, the current study assumes a conservative annual survival rate of 60% of Mud Cockles one year post seeding, which equates to a 10% mortality rate per season. After taking into account these assumptions, the estimated survival over the 90 weeks (seven seasons) from planting to harvest of Mud Cockles would be 9.1% (Table 11). It is also anticipated, based on *K. scalarina* mortality rates from Peterson et al., (1994), that 95% of the Mud Cockles would survive the one additional year if 137 weeks (about four years from fertilisation) would be required for them to grow to the market size of 30 mm, resulting in the final survival rate of 7.3%.

Table 11: Calculation of survival to harvest rate of Mud Cockles (*K. scalarina*) re-seeded at the Section Bank over 90 weeks. Survival data was obtained from Trials 2, 3 and 5A and standardised for season (90 days). Movement assumption = % Mud Cockles expected to be found within 1 meter at each side from experiment plot.

	Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Survival to harvest**
Survival (%)	70.2	43.7	43.7	43.7	90	90	90	
Movement assumption (%)	10.0	10.0	10.0	10.0				
Total (%)	80.2	53.7	53.7	53.7	90	90	90	**9.1%**

Using the assumption of survival to harvest rate of 9.1% over the 90 weeks re-seeding period (Table 11), in order to reach the previous TACC of 11.3 t for 2010, 9 million spat (5 mm screen size) would need to be re-seeded annually.

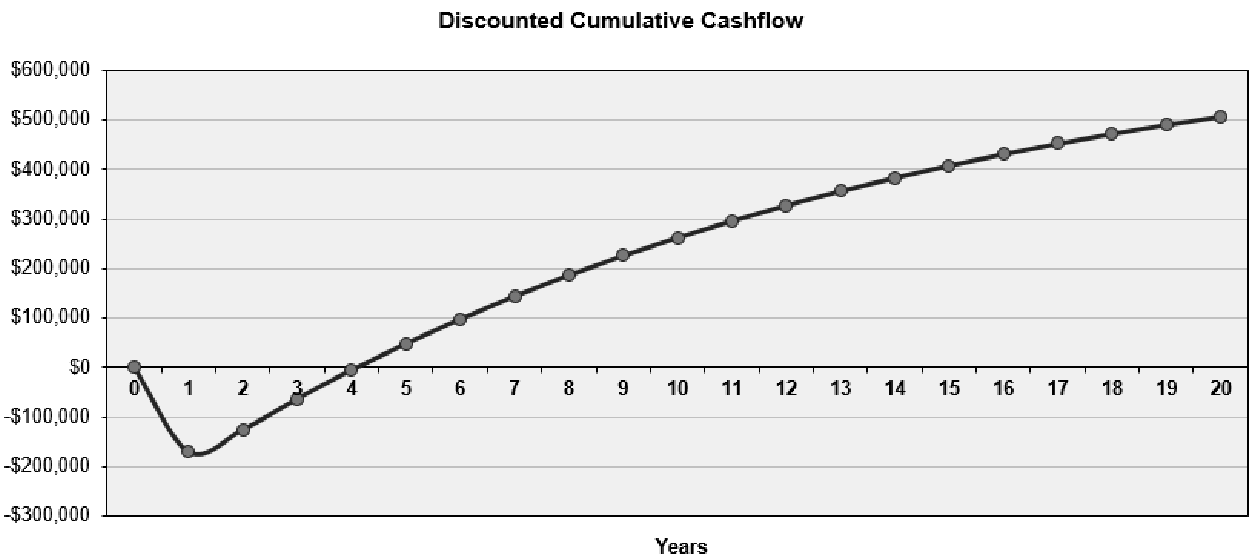
Results in Table 12 details the difference in the benefit cost ratio with varying market prices. At the current market price of $25 / kg, assuming a survival to harvest rate of 9.1%, the benefit cost ratio is 1.28:1. If the market price was to drop to $20 / kg, the benefit cost ratio would reach breakeven 1.02:1. If survival to harvest was to increase slightly to 12%, which results from Trial 5A indicate is possible, the breakeven market price would reduce to less than $15 / kg (Table 12).

Table 12: Benefit cost ratios of varying survival rates and market prices of Mud Cockles (*K. scalarina*) re-seeded at the Section Bank. Calculated using the model developed by Phelps et al. (2009) with the assumptions of 9.5 mm initial shell length spat size, $0.004 per mm spat cost, 30 mm size at harvest and 90 weeks re-seeding period (Table 3; Section 1.3).

	Market price		
Survival to harvest	**$25 / kg (current price)**	**$20 / kg (expected average)**	**$15 / kg (expected minimum)**
9.1%	1.28:1	1.02:1	0.78:1
12.0%	1.68:1	1.34:1	1.01:1

The discounted cumulative cash flow model calculated on the current market price of $25.00 and a survival to harvest rate of 9.1% indicates that harvesting an 11.3 t TACC would provide an annual return of AUD $51, 716 after 20 years with an internal rate of return of 38.74% and a payback period of four years (Figure 11A). If survival to harvest was increased to 12% (benefit cost ratio = 1.68:1), harvesting an 11.3 t TACC would provide an annual return of AUD $95, 272 after 20 years with an internal rate of return of 78.83% and a payback period of two and half years (Figure 11B).

A.



B.

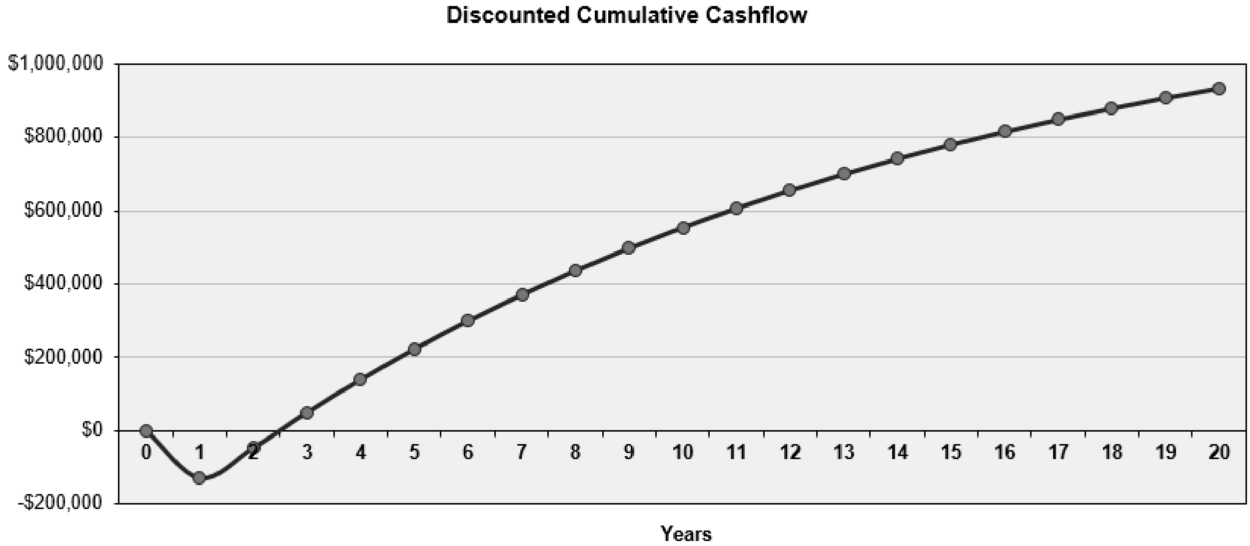


Figure 11: Discounted cumulative cashflow model (AUD $) of Mud Cockle (*K. scalarina*) re-seeding at the Section Bank, calculated using the model described in Phelps et al. (2009) with the assumption of 90 weeks until harvest size. A = survival to harvest of 9.1% and B = survival to harvest of 12%.

Using the assumptions for 137 weeks re-seeding period and survival to harvest rate of 7.3%, at the current market price of $25 / kg, the benefit cost ratio is 1.02:1. In order to reach the previous TACC of 11.3 t for 2010, 11 million spat (5 mm screen size) would need to be re-seeded annually.

The discounted cumulative cash flow model calculated on the current market price of $25.00 and a survival to harvest rate of 7.3% indicates that harvesting an 11.3 t TACC would provide an annual return of $5, 269 after 20 years with an internal rate of return of 10.97% and a payback period of about 13 years (Figure 12).

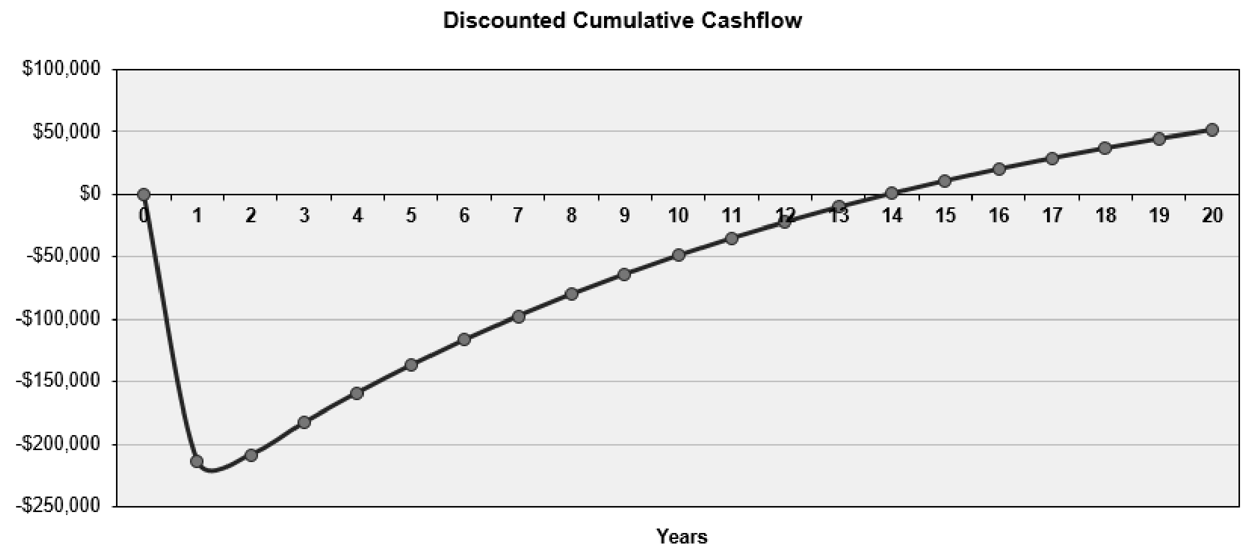


Figure 12: Discounted cumulative cashflow model (AUD $) of Mud Cockle (*K. scalarina*) re-seeding at the Section Bank, calculated using the model described in Phelps et al. (2009) with the assumption of 137 weeks until harvest size and survival to harvest of 7.3%

The benefit cost analysis assumes a 90% harvest efficiency of re-seeded Mud Cockles that have survived to harvest. There is currently no information on how harvesting at this rate would affect the wild population or stock restoration efforts. A 90% harvesting efficiency was selected because the collapse of the fishery suggests a high fishing pressure, however, reducing this efficiency to 80% had a -0.01 change on the benefit cost analysis.

Discussion

The primary aim of this study was to develop re-seeding methods that optimise growth and survival of re-seeded Mud Cockles at the Section Bank, at the confluence of the Port River and Gulf St Vincent, South Australia. Results from the study were positive and suggested that stock enhancement of Mud Cockles in this area could be marginally to modestly profitable and a potentially viable option. The Mud Cockle population at the Section Bank has shown no signs of recovery since its closure in 2011, suggesting that it may have dropped below the critical “sterile” level where natural recruitment alone is not enough to restore the population and intervention through a stock restoration program is needed (Stoner and Ray-Culp, 2000). The current project has provided valuable information for the design of such a program, however due consideration needs be given to the following biotic and abiotic factors: season, site location, size at stocking, stocking density, predator protection methods, recruitment rates and genetic population structure. The discussion will outline each of these variables in light of maximising the performance of future Mud Cockle re-seeding.

Benefit cost analysis

The secondary aim of this project was to evaluate the benefit cost ratio of a re-seeding program from a commercial perspective. The model used for this analysis assumed a 90% harvest effort of re-seeded Mud Cockles that have survived to harvest. This figure was chosen based on the strong fishing pressure that Section Bank historically had. There is currently no information on how harvesting at this rate would affect the wild population or stock restoration efforts. Future research on re-seeded Mud Cockle recruitment is needed to determine at what level re-seeded Mud Cockles could be harvested without comprising stock restoration. Reducing the harvesting efficiency to 80% had a -0.01 change on the benefit cost analysis

The benefit cost analysis for re-seeding of Mud Cockles in the current study was marginally to modestly positive and showed that at the current market price of $25.00 / kg and a survival to harvest rate of 7.3% over a 137 weeks re-seeding period or 9.1% over a 90 weeks period, the benefit cost ratio would be 1.02 and 1.28:1, respectively, implying that Mud Cockle re-seeding at the Section Bank may be a profitable venture with further optimisation of re-seeding techniques. The ratio of 1.28:1 is comparative to Peterson et al., (1995) who reported a 1.40-2.33:1 benefit cost ratio from a desktop study for the re-seeding of Hard Clams (*Mercenaria mercenaria*). Phelps et al. (2009) reported a higher benefit cost ratio of 3.34:1 for the re-seeding of Pipis (*Donax deltoids*) in New South Wales and suggested this was a good return on investment; however, this was determined by a desktop study. Given the lack of practical studies examining the benefit cost ratios of re-seeding of Mud Cockles and other molluscs it is difficult to compare between ratios. For example, Phelps et al. (2009) based their survival to harvest value (14.1%) for Pipis (*Donax deltoids*) on figures calculated for scallop re-seeding. Therefore, we suggest future re-seeding studies are carried out in the field to ensure more precise benefit cost analyses.

The benefit cost analysis indicated that re-seeding of Mud Cockles at the Section Bank was sensitive to duration from deployment to harvest, survival to harvest and market prices. For the 90 weeks re-seeding duration, the discounted cumulative cash flow model adopted from Phelps et al. (2009) indicated an annual return of AUD $51,716 after 20 years with an internal rate of return of 38.74% and a payback period of four years. The model showed that increasing the survival to harvest rate marginally to 12% significantly improved the benefit cost ratio. When the re-seeding duration increases by one year to 137 weeks the benefit cost ratio decreased substantially to 1.02:1 although a high survival rate of 95% was assumed in the final year. It is also important to note that re-seeding infrastructure costs (e.g. predator protection methods) and the potential upkeep costs were not included in the model. This is because the predator protection method used in this study was relatively ineffective and a more effective method (e.g. finer mesh) may require regular cleaning to reduce Mud Cockle mortality. Further study would be needed to assess efficiency and cost of such a strategy and how it would change the benefit cost ratio. As an example, a $5,000 annual upkeep cost would reduce the benefit cost analysis by 0.06.

In order to re-establish the Mud Cockle fishery at the Section Bank to the previous TACC of 11.3 t for 2010, with a 90% harvesting effort of re-seeded cockles that have survived to harvest, 9 or 11 million spat of 9.5

mm would need to be re-seeded annually for 85 and 137 weeks re-seeding durations, respectively. However, this number would need to be re-evaluated and adjusted annually in the subsequent years depending on survival, recruitment and its impact on stock restoration efforts. It is practical to produce this number of spat in a commercial hatchery annually, however, while the current study provides useful preliminary survival to harvest data, it is variable and needs more research. It was difficult to ascertain survival in the field trials due to variable recovery and refinement of methodologies with subsequent trials. As a result, survival to harvest rate may be an underestimation or overestimation due to the following factors. First, it is unknown how many unrecovered Mud Cockles remained alive. The results from the second winter trial (Trial 5A) examining Mud Cockle movement show that around 33% of unrecovered Mud Cockles had moved away from the plot and were still alive. Second, the duration of the trials were short and did not run for the time (~85 or 137 weeks) required for the Mud Cockles to reach market size. Third, no reliable apparent survival data were obtained for summer or autumn when high temperatures may increase mortality (Cummings et al., 2007) and forth, little information on the survival rate of different size classes was obtained. It is probable that larger Mud Cockles have a greater survival rate than smaller Mud Cockles (Marelli and Arnold, 1996; Nakaoka, 1996; Cigarria and Fernandez, 2000). Longer term trials and further optimisation of sampling/harvesting and predator protection methods may provide greater insight into survival rates.

Future studies on the recruitment rate of Mud Cockles at re-seeded and control sites within the Section Bank would help to provide a better estimation of how many Mud Cockles would need to be re-seeded annually. The natural recruitment rate for Mud Cockles at the Section Bank is unknown, although it is likely to be highly temporally and spatially variable and also affected by adult densities. Currently, it is suspected that the population at the Section Bank has dropped below the critical sustainable “sterile” level and hence natural recruitment alone may not be enough to restore the population (Stoner and Ray-Culp, 2000). In order to re-establish a sustainable population, sufficient re-seeded Mud Cockles would have to be able to reach maturity and spawn before harvest, thus enabling the population to return to above sterile levels. This would also require protection of re-seeded cockle spats for at least the initial period. These practices need to be incorporated into future management policies. Planktonic dispersal of bivalve larvae is dependent on oceanographic conditions and the ability of spat to settle close to their source population is site dependent (Underwood and Fairweather, 1989; Arnolds et al., 1998; Vassiliev et al., 2010). In the current study, Site A and Site B were located ~ 50 m apart and hence probably experienced similar oceanographic conditions. On site observations indicated that both sites had the same exposure time at low tide, similar vegetation cover and were a similar distance from the shore. In the Peconic Bays of eastern Long Island, New York, USA the Bay Scallop (*Argopecten irradians*) fishery collapsed following algal blooms and showed no sign of recovery for the next 12 years implying recruitment limitation (Tettelbach et al., 2015). An intensive re-seeding program with Bay Scallop was implemented (Tettelbach and Smith, 2009), which resulted in up to a 32-fold increase in recruitment ~5 years after the re-seeding (Tettelbach et al., 2013). In contrast, Tezuka et al. (2012) reported that a collapsed Asari Clam (*Ruditapes philippinarum*) fishery in Nakatsu, Japan was supressed by high mortality post settlement and not recruitment limitation. Advancements of knowledge in recruitments will therefore be critical to the success of any Mud Cockle restoration program at the Section Bank.

The overall shell growth rate of spat observed in this study was 0.035 mm /day indicating that it would take ~85 weeks for Mud Cockles (9.5 mm shell length) to reach market size (30 mm) at the Section Bank or about three years if estimated from fertilisation. This growth rate was faster than that previously reported for *K. scalarina* (~0.012 mm /day) in Tasmania, Australia (Bellchambers, 1998). In Tasmania, Mud Cockles are estimated to take between four to six years to reach a market size of 32 mm (Riley et al., 2005). The present study observed an increase in growth rate over warmer seasons (autumn and summer), suggesting that the growth rates for Mud Cockles will be faster in South Australia, where average water temperatures are higher, compared to Tasmania. Gluis and Li (2014b) reported that the spat growth rate of closely related *K. rhytiphora* in Coffin Bay, South Australia was ~ 0.07 mm / day from February to June 2012, which is comparable to the 0.066 mm / day observed for Mud Cockle spat in autumn in this study. However, further research into the growth rates of Mud Cockles from re-seeding to harvest is required to gain a more accurate estimate of time to market size, as it is probable that growth rate reduces as the Mud Cockles reach maturity (Bellchambers, 1998; Cantin, 2010). This would impact the outcome of the discounted cumulative cashflow model. Additionally, it was observed over Trial 1 (autumn 2016) that small Mud Cockles grew > 10% faster than larger Mud Cockles, indicating a longer study is needed in order to conclusively determine the time it would take the re-seeded Mud Cockles to reach a harvestable size at the Section Bank.

Transportation of Mud Cockles

The commonly used method for transporting oyster spat (Helm et al., 2004) was adapted for this project and was suitable for Mud Cockles. Using this method, Mud Cockles did not show any increase in mortality in pilot laboratory trials and showed good apparent survival and growth in the field following transport and stocking. It is important to note that within the hatchery the Mud Cockles were kept in UV treated water. This may have had an influence on subsequent survival after transport. Studies have shown that depuration of shellfish with either ozone or UV sterilised water can reduce viable bacteria numbers and increase survival in bivalves during culture (Croci et al., 2002; Pan et al., 2018). We would recommend the transportation method used in the current project for future Mud Cockle re-seeding activities.

Recovery of Mud Cockles

Recovery of Mud Cockles was determined at the completion of four trials in this study. Trials 2, 3 and 5A, which all ran for approximately three months, showed almost half (50.7%) of the re-seeded Mud Cockles were recovered within the plots, indicating about half of the re-seeded Mud Cockles were either actively or passively moved from the plots. Bellchambers (1998) reported that *K. scalarina* is capable of significant migration, however, under natural conditions is mostly sedentary. In the current study, it appears that restocked Mud Cockles were active and capable of movement from their plots. Results from the movement experiment run in the winter 2018 (Trial 5A), indicated that ~18% of the re-seeded Mud Cockles (33% of those unrecovered) were found within 1 meter at each side of the tray frame treatment plots, which suggests that the majority of unrecovered Mud Cockles were likely moved further afield by tide or current, or removed by larger predators (birds, crabs, rays, octopus etc.). Tidal movement in this area is significant, with a mean tide ranging from 2.83 m to 0.04 m (BOM, 2019). Tag shedding was insignificant in the pilot laboratory trials, and was unlikely to have contributed to recovery results in the field trials in the current study. The Rosario Pink paint treatment used in the field trials was effective for identifying experimental Mud Cockles. In order to define the optimum plot size for future re-seeding studies, it would be valuable to determine the maximum daily movement rate of Mud Cockles to ensure that all individuals that actively travelled could be recovered. Stocking density may also have an effect of Mud Cockle movement. Cummings et al. (2007) observed a transplant density effect on movement in the New Zealand Cockle (*Austrovenus stutchburyi*), wherby cockles stocked at higher densities were more likely to move away from the plot. In the present study Mud Cockle movement was not assessed in conjuction with stocking density, hence it would be useful in future studies to see if the movement patterns change with varying stocking densities. It would also be valuable to assess different predator protections methods that also stop Mud Cockles from leaving the experimental plots. The current study tried to do this with the addition of a small mesh in Trials 4 and 5, however this significantly increased mortality, most likely caused by biofouling of the mesh. It would be useful to test different mesh sizes and/or the practicality and cost of monthly cleaning/replacement of predator protection methods.

Recovery may also be affected by the burrowing speed of Mud Cockles at stocking. Slow burrowing speed may leave the animals prone to several factors including increased predation, impairment of feeding or being washed away. In the present study, a low recovery in Trial 1 (autumn 2016), prompted the use of a small (1.7 mm^2) mesh size screen to cover all the plots for 24 hours to allow time for the Mud Cockles to burrow into the sand; this improved methodology increased recovery from ~10% in Trial 1 to ~50% in subsequent trials. Burrowing speed is species dependent and predator protection methods do not always increase recovery. A 15-36% recovery rate after 12 months was observed in the New Zealand Cockle (*Austrovenus stutchburyi*; Cummings et al., 2007) where protection method (caged or uncaged) did not significantly affect recovery. Additionally, Joaquim et al. (2008) reported a 45% recovery in transplanted White Clam (*Spinsula solida*) after 12 months with no protective treatment being applied. Whilst the use of a small (1.7 mm^2) mesh size screen for 24 hours improved Mud Cockle recovery in the present study, the use of the same sized mesh for the entirety of the trial period (approximately 3 months) resulted in increased mortality. In Trial 5A (winter 2018), the addition of a small mesh over the tray frame and closed tray treatments for the entirety of the trial increased recovery to ~83%, but also potentially resulted in a higher mortality, dependent on if the unrecovered Mud Cockles in the treatments without small mesh were dead or alive. Approximately three times the number of Mud Cockles recovered in the treatments with small mesh for the entirety of the trial were dead compared to the treatments without the small mesh, hence the addition of a small mesh for long periods is probably not suitable for re-seeding applications. It would be useful for future re-seeding programs to determine the recovery of Mud Cockles planted at the Section Bank for longer periods (> 3 months) with more frequent monitoring (e.g. monthly sampling) to determine if more are being lost at the start of the trial due to early mortality from the change in environment from hatchery conditions to wild conditions compared to later on when they have acclimatised.

Seasonal variation

Seasonal variation is an important factor influencing survival and growth of re-seeded bivalves. The linear regression analysis in the current study suggests that water temperature was the primary driver behind differences in apparent survival, recovery and growth, accounting for 48.5 -76.7% of the variation in the data. A negative relationship was observed between water temperature and apparent survival and recovery, whilst a positive relationship was observed between water temperature and growth. Other studies have reported strong effects of seasonal variation of environmental parameters on survival and growth. A small-scale re-seeding study on the New Zealand Cockle (*Austrovenus stutchburyi*) reported a strong correlation between weather conditions and survival, with higher mortality observed during seasons of low rainfall and large temperature variations (Cummings et al., 2007). Additionally, season was shown to influence survival in transplanted Hard Clams (*Mercenaria mercenaria*), where higher survival was recorded in autumn/winter compared to late summer (Peterson et al., 1995). A study on Mud Cockles in Tasmania, Australia, showed that natural mortality across season was variable between sites (Riley et al., 2005). In the current study, apparent survival in the autumn trial (Trial 1) was low (9.6%), however this is likely a reflection of recovery. Whilst no recovery data was recorded for this trial, it was observed that many of the Mud Cockles were washed away from the plots by the tide, hence the use of the small screen covering in later trials. For the summer 2017 / 2018 trial (Trial 4), the apparent survival of those recovered was the lowest of all the trials (1.3%) and it is probable that the higher water temperatures over summer contributed to this low apparent survival (Cummings et al., 2007). However, this trial ran for twice as long as the other seasonal trials, due to the PIRSA Pacific Oyster Mortality Syndrome closure of the Section Bank and associated restrictions on removal of stock over the trial period. High levels of biofouling were observed at the end of the summer trial across all treatments; this may have contributed to the high apparent mortality. It would be desirable to run trials looking at the effect of biofouling of predator protection infrastructure on Mud Cockle survival and growth. The results of this study indicate that cooler water temperatures increase the apparent survival of Mud Cockles, which suggests that future re-seeding events at the Section Bank should be carried out in winter. This would give the Mud Cockle spat an opportunity to acclimatise to field conditions before the stress of warmer water conditions.

Site selection

The regression analysis indicated that site was a significant predictor of apparent survival, recovery and growth (final shell length). On average, Mud Cockle performance was better at Site B compared to Site A. Cummings et al. (2007) observed a difference in the survival of the New Zealand Cockle (*Austrovenus stutchburyi*) between two sites (East and West), which was attributed to environmental conditions including water temperature. The sites used in the current study were ~50 m apart and likely experienced similar environmental conditions (water temperature, salinity, pH, exposure times etc.). Site B is located closer to a mangrove region, which could impact on faunal communities and organic load (Alfaro, 2010). It is likely that the difference between sites is related to food availability and sediment characteristics. The sediment analysis in the current study revealed no significant difference in sediment size between sites. There was a significant difference in the depth of the anoxic layer between the sites, with Site A on average having a deeper layer than Site B, however, this was not correlated with apparent survival, mortality or final shell length. It was positively correlated with final weight, however there was no significant difference in final weights between sites. This indicates that further investigation into sediment characteristics is needed, in particular assessing the level of organic load within the sediment and food availability in the water column between sites as the proximity to the mangroves may have increased food availability at Site B and contributed to increased growth and survival.

An alternative reason for the increased growth rate observed at Site B could be a positive feedback from the existing higher wild cockle population. Many bivalve species, including Mud Cockles, are known ecosystem engineers who can modify the environment to their own advantage (Jones et al., 1994; Bertness and Leonard, 1997; Jones et al., 1997; Dairain et al 2020; Thomas et al., 2020). Cockle density has been shown to influence sediment stability by creating mucus rich bio-deposits and facilitating the growth of sediment binding diatoms (Ciutat et al., 2007; Donadi et al., 2013). Donadi et al. (2014) reported an increase in juvenile Cockle (*Cerastoderma edule*) abundance in the presence of high density adult Cockle populations, which was attributed to the ability of adult Cockles to increase sediment stability and, hence, Cockle recruitment. In the present study, Site B had a higher wild bivalve population which may have modified the habitat making it a better suited for Mud Cockle re-seeding. The depletion of Mud Cockles from the Section Bank from anthropogenic pressures could have decreased the suitability of the sediment for Mud Cockle habitation, which could have long lasting effects on Mud Cockle recruitment in the future.

It is also important to understand if the re-seeded stock will be self-seeding. Results using a combined hydrodynamic and particle tracking model to predict larval dispersion patterns for the common Cockle *Austrovenus stutchburyi* indicates that the potential of self-seeding varied substantially among release sites in Whangarei Harbour, New Zealand (Lundquist et al., 2009). Timbs et al. (2018) revealed that besides spawning stock biomass, variations in larval transport and spatially different rates of mortality post-settlement are also the primary determinants of recruitment success in the Atlantic Surfclam *Spisula solidissima*. It is recommended that future studies should assess the ecosystem engineering ability of Mud Cockles and the potential positive feedback loops adult populations may play on recruitment. This would provide useful information for selecting sites and densities for future Mud Cockle re-seeding efforts.

Predator protection

Predator protection with cages or mesh may be used to exclude predatory snails, crabs, birds, rays and other carnivorous predators from Mud Cockle production plots. Additionally, such methods may inhibit escape, leading to increased biomass retention. The regression analysis in the current study indicated that predator protection method was a significant predictor of Mud Cockle recovery, apparent survival and apparent mortality and did not inhibit growth.

In general, the methods of predator protection used in the current study did not appear to reduce whelk predation. Whelk predation was highly variable between treatments, but on average low throughout this study, equating to 2.2% of the mortality of the recovered Mud Cockles. This is in contrast to Stewart and Creese (2004), who reported that up to 40% of the mortality observed in aggregations of Clams (*Austrovenus stutchburyi*) could be attributed to predation by one whelk species (*Lepsiella scobina*). Within the current study, in three (Trials 3-5A) of the four trials where whelk predation was assessed, there was no significant difference in whelk predation of Mud Cockles between the different predator protection methods (tray frame and closed tray treatments), hence it is likely that the closed tray treatment provided limited protection from predatory snails. Whelks were observed in both the tray frame and closed tray treatment plots at stocking and harvest. Adding the small mesh to tray frame and closed tray protection methods did not provide any further protection from whelks. The sediment was not sieved prior to filling the closed tray with small mesh treatments with sand, hence whelks were probably present within these plots. In the current study, whelk predation was not statistically assessed in conjunction with Mud Cockle size given the low recovery of small size class Mud Cockles in Trial 3. It is possible that smaller Mud Cockles may be more susceptible to whelk predation. Morton (2005) demonstrated the predatory snail (*Lepsiella paivae*) had a feeding preference for small (5 mm shell length) Mud Cockles. Future re-seeding studies should determine if whelk predation rates change with Mud Cockle size at stocking.

Within the current study, crabs were regularly observed within the tray frame and closed tray predator protection method treatments. Predation by crabs may have increased the number of unrecovered Mud Cockles. Crabs must break the Mud Cockle shell to feed and the resulting fragmented dead shell would not have been recovered when the plots were sieved at harvest. The 6 mm oyster mesh that covered the closed tray treatments in the current study likely did not provide any protection from crab predation. Dethier et al. (2019) reported that small 5-10 mm carapace width shore crabs (*Hemigrapsus* spp.) were able to consume hard shell Manila clams (*Ruditapes philippinarum*) less than 10 mm shell length. Future studies into re-seeding Mud Cockles, particularly of smaller initial shell length than used in the present study, should consider crab predation. To do this, a finer predator protection mesh should be trialled with more frequent monitoring to ensure biofouling does not limit food supply. Additionally, future studies should consider if it is practical to sieve the sediment prior to filling caged treatments to ensure that large crabs and predatory snails are excluded at stocking. The closed tray treatment could have provided protection from bird, fish, octopus and ray predation, which may explain the higher recovery and apparent survival in this treatment compared to the tray frame treatment.

The regression analysis showed no difference in Mud Cockle growth performance between tray frame and closed tray treatments. This concurs with Cummings et al. (2007) who observed no difference in New Zealand Cockle (*Austrovenus stutchburyi*) growth between their caged and uncaged treatments. In contrast, Peterson and Black (1993) found a 50% reduction in the growth of *Katelysia* spp. in roofed cages. In the present study, the 6 mm oyster mesh used to cover the closed tray treatments was large enough for the re-seeded Mud Cockles to pass through, hence this treatment should not be considered roofed. The small mesh predator protection treatment used in Trial 5A, however, was fine enough to contain the Mud Cockles within the treatment, hence it did act as a roof. A 10% reduction in final shell length was observed in the treatments with small mesh in Trial 5A, indcating a reduction in Mud Cockle performace in roofed treatments, probably due to increased biofouling, concuring with Peterson and Black (1993). Future studies are required to determine an optimal predator protection method that reduces predation without comprimising survival or growth.

Stocking density

The effect of Mud Cockle stocking density did not significantly impact growth or apparent mortality within the current study. This supports findings from previous studies of density as a growth and mortality factor. Peterson and Black (1993) observed no difference in mortality due to competition in different stocking densities, 20–320 Mud Cockles (*Katelysia* spp.) per m^2, within all but one of their experiments. Cummings et al. (2007) observed no difference in New Zealand Cockle (*Austrovenus stutchburyi*) growth at densities of 832 and 222 spat per m^2, however they did observe a higher mortality in the high stocking density treatments under extreme environmental conditions(i.e. high maximum daily temperature and maximum rainfall). Additionally, Bellchambers (1998) only observed increased mortality of Mud Cockles stocked at high density (686 Mud Cockles per m^2) when there was also an increase in environmental stress (i.e. increase air exposure period). In the present study, the non-significance in apparent survival and mortality in the high stocking density (1000 spat per m^2) treatments suggest that this density could be below the carrying capacity at the Section Bank and that higher densities should be tested in future trials. This would be of particular importance if high densities of Mud Cockles result in a positive habitat modification, by increasing sediment stability, which may improve recruitment success (Jones et al., 1997).

In the present study, approximately half the Mud Cockles were not recovered from the plots. If this loss occurred early on in the trial, then the impact of reduced stocking density would be affected, as the Mud Cockles would be growing under lower density for the remainder of the trial. More frequent monitoring/sampling could be a method to assess this in future studies. Density was not assessed in conjunction with the movement analysis. It is possible that more Mud Cockles stocked at high density might actively move away from the plots than those stocked at lower densities, or that density effects might have attributed to the higher mortality observed in the small mesh predator protection method treatments (Cummings et al., 2007). Additionally, it would be valuable to assess density over a summer period, when environmental conditions are more extreme (e.g. warmer water temperatures). The current study did not test density over the summer period due to the lack of significance between density treatments in the previous three trials and the greater need to test a different predator protection method (fine mesh) to try to improve recovery rates. It is recommended that density be assessed in conjunction with recruitment in future studies. Peterson and Black (1993) observed an increase in recruitment of Mud Cockles with increasing density treatments, whereas Bellchambers (1998), who used higher density treatments than Peterson and Black (1993), observed a decrease in recruitment within high density (686 Mud Cockles per m^2) treatments. A negative correlation between high density of adult stock and recruitment rates has also been reported in other species such as beaked Clams *Eumarcia paupercula* (Mugabe et al., 2019) and Cockles *Cerastoderma edule* (Flach, 1996; Van Colen et al., 2013). More research is required to determine optimal stocking density of Mud Cockles for re-seeding programs at the Section Bank.

Stocking size

In the present study, there was no significant difference in survival between the large and small size classes of Mud Cockles in Trial 1 (autumn 2016). However, there was a significant difference in recovery between small and large size classes in Trial 3 (spring 2017), where ~6% of the small Mud Cockles were recovered compared to 36.7% of the large Mud Cockles. The reason for this difference could be due to hydrodynamic factors, such as tide or wave action, moving the smaller, lighter Mud Cockles away from the treatment plots.

Alternatively the difference could be related to increased predation of the smaller Mud Cockles by crabs, rays, fish or birds. There was a significant difference in the initial growth rate between size classes, with smaller Mud Cockles growing faster than larger ones following stocking. The difference between shell length in small and large size classes at stocking was ~20%, but was less than 10% at the completion of the trial. Some studies have shown an inverse relationship between size and survival of transplanted bivalves (Peterson et al., 1995; Stewart and Creese, 2002), whereas others have shown no difference (Joaquim et al., 2008). The seasonal effect of size at stocking on growth and survival of re-seeded Mud Cockles was unable to be determined in this study due to the unavailability of suitable sized Mud Cockles for stocking of the trials due to project delays. The delays, associated with permit approvals, led to the majority of Mud Cockles housed at the SARDI hatchery growing too large for use in the trials. This delay could have potentially impacted on the growth results of later trials, as it is likely that slower growing Mud Cockles were indirectly selected for use in the later field trials. The delays to this project may have also impacted on the growth results if the Mud Cockles had reached sexual maturity in the hatchery and started expending energy on reproduction as opposed to growth when stocked in the field trials. In the present study Mud Cockle reproductive condition was not assessed. It would be valuable to determine the optimal stocking size for survival and growth of re-seeded Mud Cockles at the Section Bank, as the ability to re-seed smaller Mud Cockles would reduce hatchery costs and increase the benefit cost ratio.

Sediment analysis

Differences in sediment composition depth of the anoxic layer and particle size may impact growth and survival of re-seeded Mud Cockles (Rhoads and Young, 1970; Jaafar et al., 2018). In this study, the depth of the anoxic layer was not significantly different between predator protection methods and there was no correlation between apparent survival / mortality and the anoxic layer depth. This indicates that anoxic sand was not building up within the closed tray treatment and causing mortality. The relationship between anoxic layer depth and growth was inconclusive, with no significant correlation with final shell length, but a weak positive correlation with final weight.

The depth of the anoxic layer was variable between sites. Site A and the original Site B (Trials 2-4) had significantly deeper anoxic layers than the corresponding new Site B used in Trial 5A; however, the non-significance in apparent survival, mortality and growth between sites suggest that the depth of the anoxic layer has little impact on re-seeding success. With respect to sediment particle size, difference in particle size composition between the two sites was assessed (Trial 5A) and no discernible differences was observed. The medium particle size class (250-1000 µm) made up the majority of the sediment at each site. This supports the findings of Bellchambers (1998), which found no significant effect of sediment particle size, organic content, redox or pH on the shell length of Mud Cockles (*K. scalarina*) housed in cages in the field in Tasmania, Australia for one year. Interestingly, in the current study, the site repetitively used for the trials had a small but significant increase in the proportion of fine and medium sized sediment. It is not known if this was natural variation or if the disturbance effects of the trials impacted on the sediment size. It could also be an artefact of using top level adjacent sand to fill the tray frame treatments prior to seeding. This site also differed from the adjacent control samples (S9-10, Figure 5; section 2.6) for fine sediment. Growth and apparent mortality did not differ between the original site and the new site B (Trial 5A), hence if the project did have some impact on the sediment it was not enough to detect.

Future research and monitoring

To date, there has been limited research on the genetic population structure of Mud Cockles (*K. scalarina*) in South Australia, however, a genetic study on the closely related *K. rhytiphora* in South Australia revealed cryptic speciation and the need for future taxonomy research of *Katelysia spp*. in this region (Li and Rodda, 2015). Determining the population structure of Mud Cockles in South Australia would provide valuable insights into recruitment, which in turn will contribute to the success of future re-seeding efforts at the Section Bank. Additionally, it would be advantageous to develop genetic markers for differentiating between hatchery stock and wild Mud Cockles to assess the fitness of the re-seeded Mud Cockles compared to the wild population.

One of the project objectives was to develop a monitoring program that could be incorporated within the existing Mud Cockle stock assessment program to determine the long-term performance of spat in the 10 m^2

re-seeding trial. As Mud Cockle stock assessment has not been conducted at the Section Bank since December 2018, the data from this trial was not available. Discussions are underway with PIRSA Fisheries and Aquaculture to incorporate the large-scale re-seeding areas (Section 1.2.6) of this project into future Mud Cockle stock assessments. It is planned that the twelve 10 m^2 plots set up in the current study will be sampled (five randomly allocated quadrants per plot) as part of the assessment to determine the long term effect of Mud Cockle re-seeding.

Conclusion

The results of this study indicate that a Mud Cockle re-seeding could potentially be a viable venture for restoring the depleted fishery at the Section Bank from its “sterile” status, however it is likely a long-term prospect. From an economic perspective, cost benefit analysis was marginally to modestly profitable, however it requires further refinement to ensure that harvest rates do not impact stock restoration efforts. Growth performance was excellent throughout the study, with spat shell growth rates higher than that previously recorded for this species. Recovery and apparent survival may be improved with further refinement of re-seeding methods, such as determining the duration that protection is needed. To ensure the success of a future re-seeding program, more research is needed into the effect of season, size at stocking, stocking density and predator protection methods on the performance of re-seeded Mud Cockles. Additionally, an estimation of the recruitment rate of Mud Cockles at the Section Bank and a greater understanding of their genetic population structure would help to optimise a re-seeding program. The results of this project will assist in re-establishing the Mud Cockle fishery at the Section Bank and at other depleted areas. Information from this project, in particular growth and survival estimates, may also be used to inform current and future policy evaluation for the Mud Cockle fishery at the Section Bank.

Implications

The information derived from this project will have positive implications on the future of Mud Cockle stocks and fisheries in South Australia and cockle stocks elsewhere. Results from this project have indicated that Mud Cockle re-seeding at the Section Bank, Port River, South Australia, Australia could potentially be a viable method to restore the depleted population. Historically, the Section Bank Mud Cockle fishery has been an important resource for both commercial and recreational fishing, hence, restoration of the population at Section Bank would have significant social and economic benefits. There would also be environmental benefits, given the ecosystem engineering capability of this species. From an economic perspective, Mud Cockle stock enhancement could be marginally to modestly profitable, with a 1.02:1 or 1.28:1 benefit cost ratio when you assume an average market price of $25.00 / kg and a reseeding period of 137 weeks or 85 weeks, respectively. However, it is likely a long term prospect and more research into the success of re-seeding in different seasons, effects of stocking sizes and densities on growth and survival, and an estimation of the Mud Cockle recruitment rate in wild populations are required to further improve re-seeding efforts at the Section Bank. Information from this project may be used to aid in developing future management decisions for the Mud Cockle fishery in the Port River Zone and elsewhere in South Australia and Australia.

Recommendations

This project recommends that a larger Mud Cockle re-seeding program should be established to assist in the restoration of the wild population. The Mud Cockle population at the Section Bank has shown no signs of recovery since it was closed in 2011, hence it has likely dropped below the critical sterile level and needs human intervention to restore it to the important social and economic resource it once was. Additionally, given that Mud Cockles are ecosystem engineers, there are concerns that further degradation of the natural system will occur until the population is restored.

The following recommendation are put forward for designing a larger Mud Cockle re-seeding program:

- Hatchery techniques described by Gluis and Li (2014a) are used to produce Mud Cockle spat.
- The transportation method used in the current project, adapted from Helm et al. (2004), should be used for future Mud Cockle re-seeding activities.
- Rosario Pink (Montana Colours, Barcelona, Spain) can be used as an effective marker for identifying re-seeded Mud Cockle spat in experimental trials.
- To maximise survival, Mud Cockle re-seeding should occur in winter.
- Initial protection methods (e.g. small mesh cover for at least 24 hours) should be used when seeding to allow the Mud Cockles time to bury into the substrate.
- To facilitate temporal comparisons, Mud Cockles should be re-seeded at the sites used in this project.

Further development

The purposes of stock restoration differ from those of stock enhancement. According to the definition by Rose et al. (2001) and Grant et al. (2017), stock restorations are to replenish a depressed population to a previous level of abundance using progenies produced by the remnant population when natural population growth has failed to increase numbers. Stock restorations could be used to recover the "sterile" population in which a threshold level of abundance is needed for successful reproduction and recruitment.

Stock enhancements, on the other hand, continue indefinitely to boost the production of a self-sustaining population that has not reached carrying capacity with the progenies produced by the local broodstock. Enhancements may be motivated by an attempt to mitigate for habitat deterioration from pollution by coastal industries, or to enhance (augment, replenish, supplement) stocks above present levels of abundance.

As suggested in the Discussion, future Mud Cockle stock restoration and/or enhancement studies would benefit from further research, development and optimisation of the following:

1. General

- Mud Cockle performance over summer and autumn.
- The period of time needed for predator protection methods at seeding.
- The recovery, survival and growth rates of Mud Cockles from re-seeding to harvest (i.e. long term performance).
- Initial mortality after re-seeding due to changes in environments from hatchery to wild conditions.
- Optimal Mud Cockle size for re-seeding according to their survival rate, production costs, growth rates, etc.
- Mud Cockle movements in conjunction with stocking density.

2. Stock Restoration

- Natural recruitment rate and what level of re-seeding is needed to bring the population out of its "sterile" state.
- Site-specific dispersal patterns of Mud Cockle larvae at the Section Bank and adjacent areas.
- Environmental changes Mud Cockles can bring about due to their ecosystem engineering ability and the potential positive feedback loops adult populations may play on recruitment.

3. Stock Enhancement

- The existing carry capacity of the re-stocking regions (Section Bank regions in this study).
- More precise benefit cost analysis according to the new information available.

4. Genetics

- The development of genetic markers and research into the genetic population structure of Mud Cockles in South Australia.

Extension and Adoption

Information derived from this project has been extended rapidly to key stakeholders including interested industry members, government departments and educational institutions.

The results of this project will also be available to and communicated to PIRSA Fisheries and Aquaculture, interested South Australian cockle fishers and the general public through this final report.

The information has also been extended to industry members in an oral presentation entitled "Mud cockle (*Katelysia* spp.) stock enhancement/Restoration at Section Bank: Progresses & key findings" at the Vongole workshop on 16th May 2017. The workshop was organised by PIRSA at Lincoln Marine Sciences Centre, and attended by representatives from PIRSA Fisheries and Aquaculture, SA cockle fishers, the Marine Fishers Association (SA), restaurants and SARDI.

Information from this project may also be prepared and published in manuscript form in an international peer reviewed scientific journal and also presented at future scientific conferences.

Information contained within this report will contribute to future refinement of the Mud Cockle monitoring and assessment program by PIRSA Fisheries and Aquaculture. The information will also be made available to investors interested in being involved with the Mud Cockle fishery or aquaculture in South Australia.

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Appendices

Appendix 1 List of researchers and project staff

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