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Future oysters CRC-P

Species diversification to provide alternatives for commercial production

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Executive Summary

Pacific Oyster Mortality Syndrome (POMS), the disease caused by OsHV-1 microvariant, results in high and rapid mortality in Pacific Oysters (*Crassostrea gigas*) and has been responsible for significant economic loss to oyster industries in Australia and around the world. The diversification of commercial production into different oyster species (Native Oysters and Rock Oysters), that are not susceptible to POMS, has been proposed as a way to mitigate the risk of POMS in southern Australia. However, the Australian Native Oyster (*Ostrea angasi*) industry is still in its infancy, with knowledge gaps along the production chain. Additionally, there are no wild populations of Rock Oysters (*Saccostrea sp.*) in South Australia. Despite Rock Oyster aquaculture being well established in New South Wales and recently in Western Australia they have never been commercially produced in South Australia and translocation policies to move them around the state are non-existent. This project aimed to improve on-farm production of Native Oysters and determine if Rock Oysters can be safely translocated to South Australia from Western Australia, to help Australian oyster growers to diversify into these species.

The first project objective "To develop Native Oyster on farm growing methods that maximise survival and growth in South Australia and Tasmania" was achieved. On farm trials were run separately in South Australia (Kangaroo Island and Gulf St Vincent) and southern Tasmania (Blackman's Bay) to develop grow-out methods that maximise survival and growth of juvenile Native Oysters using the equipment and infrastructures recommended and contributed by the local oyster growers. The effect of farm location, site (high and low energy; intertidal and subtidal) and growing height on Native Oyster performance was evaluated in each state respectively.

The results of the Native Oyster farm trials were different between South Australia and Tasmania, however in both cases growing height had a significant effect on Native Oyster growth. In South Australia, deepest subtidal treatments had the slowest growth, whereas in Tasmania, Native Oysters grown at the highest intertidal height had the least growth. Except for one South Australian treatment, Native Oyster survival was high (> 97%) across the on-farm trials in both South Australia and Tasmania.

The second and third objectives "To compare the performance between Pacific Oysters and Native Oysters in South Australia" and "To establish a Native Oyster farmers network to share new techniques and knowledge" were achieved. The high survival and growth rates (less but comparable to Pacific Oysters in South Australia) observed in the on farm Native Oyster trials indicate that diversification into this species could be a viable option to mitigate the risk of POMS, however the results indicate that there is not a 'one size fits all' model for growing Native Oysters. The findings from this project will help to advance the Native Oyster industry in Australia. Additionally, this project established a Native Oyster farmers' network for the sharing of knowledge and methodologies related to Native Oyster Aquaculture. Preliminary results from the on-farm trials have been disseminated to this farmers' network through three industry workshops.

The fourth objective "To assess if Rock Oysters can be translocated to South Australia safely" was achieved. Rock Oysters have been proposed as an alternative species to facilitate industry diversification. However, they are considered exotic to South Australia. The introduction of Rock Oysters for aquaculture includes risks of establishment of the exotic bivalve, organisms that may be transferred with introduced stock and pathogens that may cause diseases to other organisms. A cohabitation study of Rock Oysters with Pacific Oysters and Native Oysters was run at the South Australia Aquatic Biosecurity Centre (Roseworthy) to assess the risk of bringing Rock Oysters into existing South Australian oyster growing regions. No nationally or internationally notifiable diseases of molluscs were detected during this study but potential complex interactions between species was noted. Translocation risks can be informed by risk assessment and ranked for management to decrease risk to an acceptable level. Management options are established for diseases and co-translocated pests, but residual risk associated with unknown pathogens and pathogens that occur at low prevalence is

difficult to assess. Adequate mitigation against the naturalisation of Rock Oysters is difficult to establish when the amenity consequences of environmental establishment and the economic consequences on other bivalve culture are high. Various types of mitigation exist for exotic species but generally have low success. The risk of exotic species to ecosystems and the bivalve farming industries are great, and overall, the risk associated with introduction of Rock Oysters was assessed as unable to be adequately mitigated, hence further investigation required in the fifth objective – "Trial Rock Oysters in the field in South Australia to assess their performance viability of a potential industry if agreed by industry and regulators" - was not pursued.

Keywords

***Ostrea angasi, Saccostrea* sp., South Australia, Tasmania, translocation, husbandry**

1 Introduction

Within Australia, edible oysters are the third most valuable aquaculture species group with an estimated farm gate value of $112 million (Mobsby, 2018). There are three types of edible oysters farmed in Australia, Pacific Oyster (*Crassostrea gigas)*, Rock Oyster (*Saccostrea sp*.) and Native Oyster (*Ostrea angasi*). In South Australia (SA) and Tasmania, the Pacific Oyster is the dominant species farmed with an estimated farm gate value of $40 million and $26 million per state respectively (Mobsby, 2018). Within these states, oyster farming is socially and economically important for regional coastal communities.

Pacific Oyster Mortality Syndrome (POMS) is a disease cause by ostreid herpesvirus - 1 microvariants (OsHV-1) that affect Pacific Oysters with a high and rapid mortality rate (e.g., up to 100% mortality within days of initial detection). POMS was detected for the first time in Australia in NSW in November 2010. In February 2016, POMS was detected in south-east Tasmania, with mortalities of up to 87% reported (De Kantzow et al., 2017). In February 2018, the disease was found in wild Pacific Oysters in the Port River, Port Adelaide, SA, however, to date, it has not yet spread to any of the state's oyster growing regions. Currently, substantial prevention and preparedness strategies have been undertaken in POMS free farming regions in Australia to combat the potential occurrence of this disease. The key strategies recommended to manage business risk have included the development of disease resistant Pacific Oysters and the development of alternate species for farming (Roberts et al., 2013).

The Native Oyster has been identified as one of the candidates for species diversification in SA and Tasmania. This southern Australian endemic flat oyster species is closely related to and morphologically similar to the European Oyster (*O. edulis)*, a species that demands a higher price at market compared to other farmed oysters (twice that of Pacific Oysters). In Australia, the Native Oyster industry is still in its infancy, hence there is limited understanding of the best methods for on farm growing of this species. In 2016, an Oysters Australia Industry Partnership Agreement (IPA) project workshop – 'Identifying knowledge gaps for development of the Native Oyster aquaculture industry in South Australia' (FRDC project 2015/229) brought together oyster farmers, hatchery operators and scientists from across Australia to share their knowledge and experience with Native Oyster aquaculture and help to identify the key knowledge gaps in the production chain. At this workshop, the establishment of good husbandry practices was identified as one of the most important research and development needs for the future of the industry (Li and Miller-Ezzy, 2017).

The Pacific Oyster is an intertidal species that is widely considered to be fast growing and environmentally tolerant. In Australia, this species is farmed on intertidal leases, with growing heights varied for the period of daily air and wave exposure required to achieve optimal performances at different farming stages. The Native Oyster, on the other hand, is a subtidal species and likely performs better in a subtidal culture, however, hydrodynamic effects in the intertidal zone (such as wave and tidal action), may help to 'chip back' the frilly Native Oyster shell to create a more desirable shaped oyster (Brake et al., 2003). Currently, Pacific Oyster farmers looking to diversify into Native Oysters to mitigate their POMS risk, want the capacity to produce both species simultaneously, without necessarily investing in new leases/infrastructure for a species that does not yet have an established market. Hence, there is a need to trial Native Oyster performance at sites and growing heights already established for Pacific Oysters.

The Native Oyster experiments in South Australia and Tasmania were designed and conducted separately for the following reasons. In South Australia, there have been previous trials (non-published data) on Native Oysters by Pacific Oyster growers using spat provided by commercial or R&D bivalve hatcheries. The current study aims to build on those trials run by South Australian Industry partners and further refine the methods they have tried over the last few years. In Tasmania, previous grow-out trials have used Native Oyster spat collected from the wild, with catch rates variable between years. Hence a combination of wild caught and hatchery produced spat are used in this study. In addition, it

was agreed the Tasmania component would be conducted as part of a postgraduate project. The two experiments are reported respectively in Sub-chapter 3.1. "Determination of on-farm growing methods that maximise survival and growth of Native Oysters in South Australia" and Sub-chapter 3.2. "Determination of on-farm growing methods that maximise survival and growth of Native Oysters in Tasmania" of Chapter 3. "Native Oyster as an alternative for commercial production". This chapter is to address the first three project objectives: 1. To develop Native Oyster on-farm growing methods that maximise survival and growth in South Australia and Tasmania, 2. To compare the performance between Pacific Oysters and Native Oysters in South Australia and 3. To establish a Native Oyster farmers network to share new techniques and knowledge. Reporting on the establishment of a Native Oyster farmer's network and knowledge sharing can be found in Sub-chapter 3.3.

Rock Oysters (*Saccostrea* sp.) are also proposed as an alternative species to facilitate SA oyster industry diversification. Rock Oysters, like Native Oysters, are not susceptible to POMS. Rock Oysters are an intertidal species, have well established growing techniques and markets, and are primarily grown in New South Wales (Sydney Rock Oysters, *Saccostrea glomerata*) with some commercial production occurring in Western Australia (Western Rock Oysters). It is not currently known if the Western Rock Oyster is a separate species to the Sydney Rock Oyster. Rock Oyster introduction to South Australia was unsuccessfully attempted in the 1950s (Nell, 2001) and they are now considered exotic to the state.

The introduction of exotic bivalves for aquaculture purposes can lead to them becoming established in the new environment. Furthermore, unwanted micro-organisms may be translocated with imported stock and pathogens can establish in the new environment, potentially causing substantial negative environmental, economic, and social effects. The risk can be defined by risk analysis and management measures can be recommended to decrease risk to an acceptable level. This is an established approach for assessing disease risks, but residual risk associated with unknown pathogens and pathogens that occur in source populations at low prevalence is difficult to assess. Controlled co-habitation trials within a biosecure system can help to elucidate if translocated exotic animals are likely to spread disease to native/naturalised or farmed species already present in the environment and vice versa. This information is important for biosecurity risk assessments. The acceptable level of risk for establishment of exotic species is very low, and the contributing factors remain poorly understood and unpredictable, and mitigation for exotic species generally has low success (McKindsey et al. 2007). It was determined that it should be assessed if Rock Oysters could be safely translocated to South Australia prior to any field grow-out trials.

The Rock Oyster investigations are reported in Sub-chapter 4.1. "Cohabitation of Rock Oysters with Pacific Oysters and Native Oysters" and Sub-chapter 4.2. "Biosecurity risk assessment of Rock Oyster introduction to South Australia" of Chapter 4. "Rock Oyster (*Saccostrea* sp.) as an alternative for commercial production". This Chapter is to address the project objectives 4. To assess if Rock Oysters can be translocated to South Australia safely and 5. Trial Rock Oysters in the field in South Australia to assess their performance and viability of a potential industry if agreed by industry and regulators.

2 Objectives

1. To develop Native Oyster on-farm growing methods that maximise survival and growth in South Australia and Tasmania
2. To compare the performance between Pacific Oysters and Native Oysters in South Australia
3. To establish a Native Oyster farmers network to share new techniques and knowledge
4. To assess if Rock Oysters can be translocated to South Australia safely
5. Trial Rock Oysters in the field in South Australia to assess their performance and viability of a potential industry if agreed by industry and regulators

3 Native Oyster as an alternative for commercial production

3.1 Determination of on-farm growing methods that maximise survival and growth of Native Oysters in South Australia

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Method

Farm locations

Three South Australian farm locations were used in this study, all located in Gulf St Vincent. Farm 1 and Farm 2 were located in Port Vincent, adjacent to Stansbury, whereas Farm 3 was located adjacent to American River, Kangaroo Island (Figure 3.1).

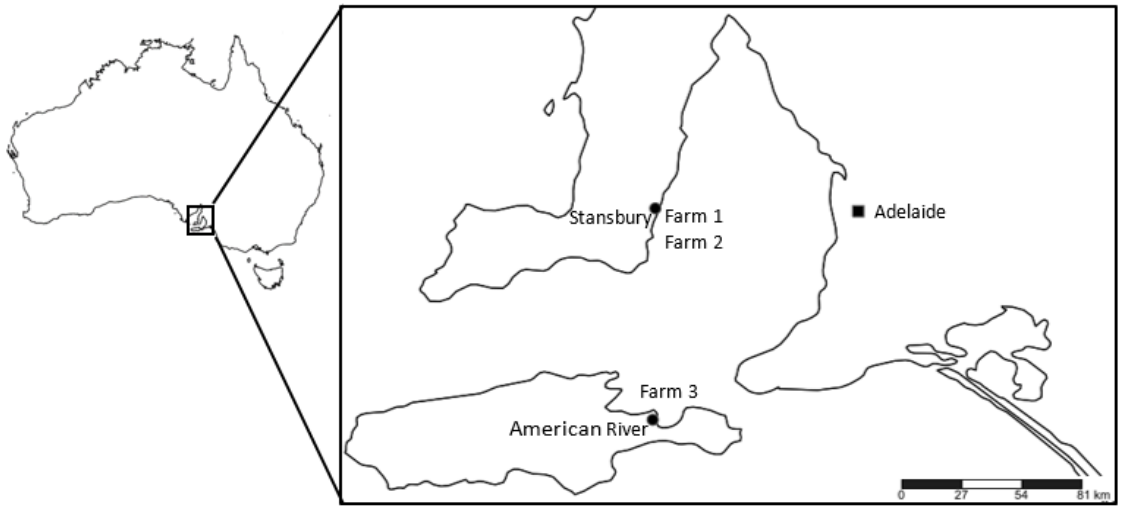


Figure 3.1 Approximate farm locations for Native Oyster (*Ostrea angasi*) on farm trials in Gulf St Vincent, South Australia, Australia.

Native Oyster spat production, transportation, and acclimatisation

Native Oyster spat was produced at the South Australian Research and Development Institute Aquatic Sciences Centre (SARDI-ASC) over the 2017 / 2018 spawning season. The Native Oyster larvae were spawned from farmed broodstock from Coffin Bay, South Australia and reared with the method currently applied at SARDI-ASC for this species. Native Oyster spat were sent to the experimental farm locations on the 14th of August 2018, at an initial shell length of 13.1 ± 4.6 mm and initial live

weight of 0.25 ± 0.24 g using the transportation procedures described by Helm et al. (2004). Native Oyster spat were acclimatised in 3 mm mesh size, 0.6 m^2 trays (SEAPA, Edwardstown, Australia; Farm 1 and Farm 2) or in 3 mm mesh size soft sock (BST Oyster Supplies, Cowell, South Australia, Australia) within 6 mm mesh size, 15 L, oyster baskets (SEAPA, Edwardstown, Australia; Farm 3) at mean low water (MLW) tidal height (Figure 3.2). The on-farm experiments started on August 27th, 2018.

Figure 3.2 Native Oyster (*Ostrea angasi*) spat acclimatisation set up in 3 mm mesh size, 0.6 m^2 trays at Farm 1 and Farm 2 (left) and in 3 mm mesh size sock within 6 mm mesh size, 15 L, oyster baskets at Farms 1 and 2 (cross-control only) and Farm 3 (right) at mean low water (MLW) tidal height.

Initial experimental design for each farm location

At each farm site, the leaseholder informed which tidal heights they wanted to trial growing the Native Oysters at based on their farming knowledge with both Pacific and Native Oysters and existing infrastructure. A single treatment initially grown in 3 mm mesh size sock within 6 mm mesh size, 15 L, oyster baskets (SEAPA, Edwardstown, Australia) and then in 6 mm mesh size, 15 L, oyster baskets was used as a cross-farm control between farm locations. The cross-farm control baskets were stocked at a density of 700 Native Oyster spat per basket with three or four replicates per farm and placed at the sheltered intertidal site at low tide water height at each participating farm (Figures 3.3, 3.4 and 3.5).

Farm 1

At Farm 1, the Native Oyster spat were grown in 3 mm mesh size, 0.6 m^2 trays at an initial stocking density of 950 Native Oyster spat per tray. Three different growing sites were used: sheltered intertidal, exposed intertidal and subtidal (Figure 3.3). At the sheltered intertidal and exposed intertidal sites, three different growing heights were used: + 0.15 m above MLW tidal height (long exposure period), 0.00 m MLW tidal height (short exposure period) and - 0.15 m below MLW tidal height (limited exposure period). At the subtidal site, one growing height was used: - 0.30 m below MLW tidal height (no exposure period). Each treatment had four replicate trays.

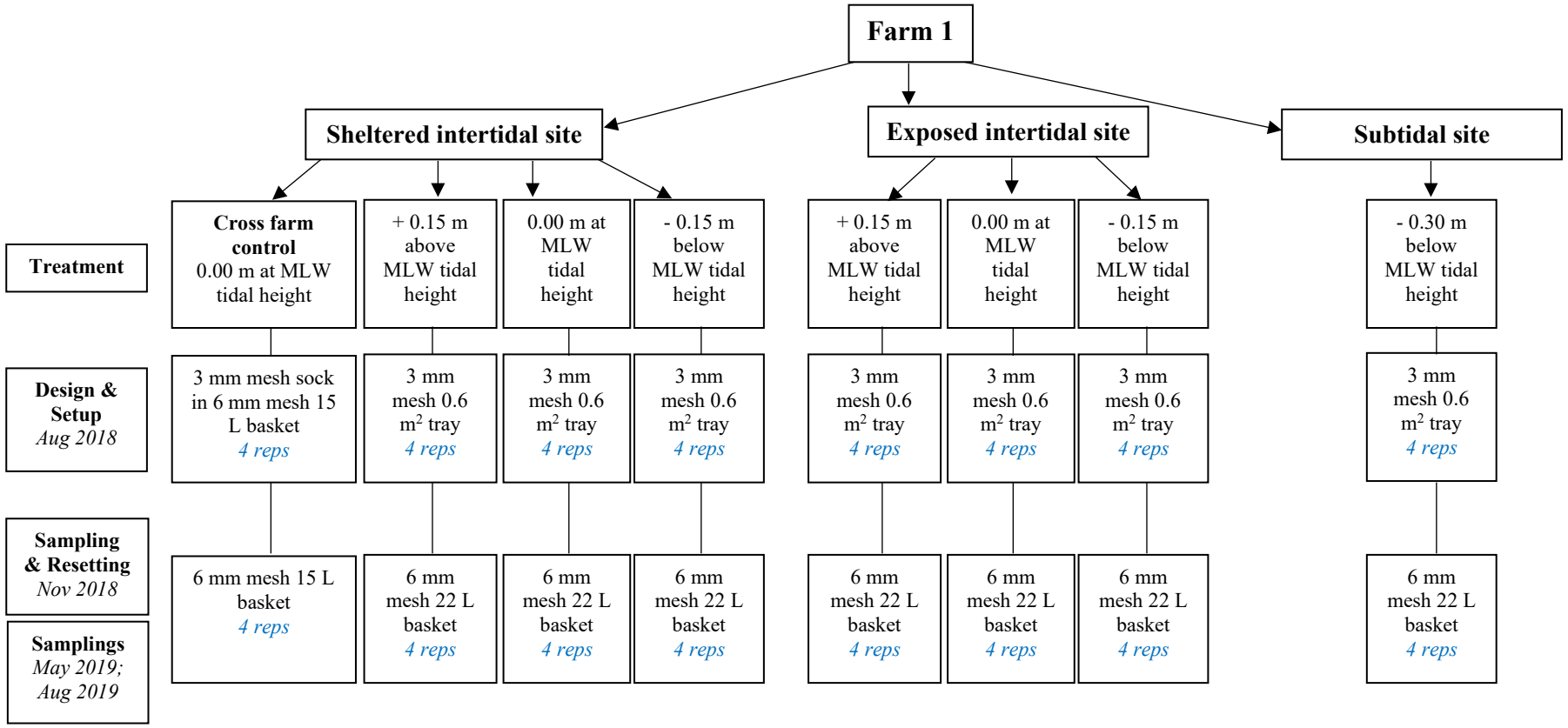


Figure 3.3 Experimental design and setups for Farm 1, Port Vincent, Gulf St Vincent, Australia. Native Oysters (*Ostrea angasi*) were stocked in oyster baskets at a density of 700 Native Oysters per basket (irrespective of basket size) or trays at a density of 950 Native Oysters per tray at three sites at varying growing heights. MLW = mean low water.

Farm 2

At Farm 2, the Native Oyster spat were grown in 3 mm mesh size, 0.6 m^2 trays at an initial stocking density of 950 Native Oyster spat per tray. Three different growing sites were used based on the Farm 2 owner's knowledge of the lease and existing infrastructure: sheltered intertidal, exposed intertidal and subtidal (Figure 3.4). At the sheltered intertidal and exposed intertidal sites, three different growing heights were used: + 0.30 m above MLW tidal height (long exposure period), + 0.18 m above MLW tidal height (medium exposure period) and 0.00 m at MLW tidal height (short exposure period). At the subtidal site, three different growing heights were used: 0.00 m at MLW tidal height (short exposure period), - 0.15 m below MLW tidal height (limited exposure period) and - 0.30 m below MLW tidal height (no exposure period).

Farm 3

At Farm 3, the Native Oyster spat were grown in 3 mm mesh size soft sock (BST Oyster Supplies, Cowell, South Australia, Australia) within 6 mm mesh size, 15 L, oyster baskets (SEAPA, Edwardstown, Australia), at a stocking density of 700 Native Oysters per basket. Three different growing sites were used: sheltered intertidal, exposed intertidal and subtidal (Figure 3.5). At the sheltered intertidal and exposed intertidal sites, three different growing heights were used: + 0.15 m above MLW tidal height (long exposure period), 0.00 m at MLW tidal height (short exposure period) and - 0.15 m below MLW tidal height (limited exposure period). At the subtidal site, floatation devices were applied to the baskets allowing them to float just below the water surface regardless of tidal height (no exposure period).

The 0.00 m at MLW tidal height treatment at the sheltered intertidal site was also used as the cross-farm control.

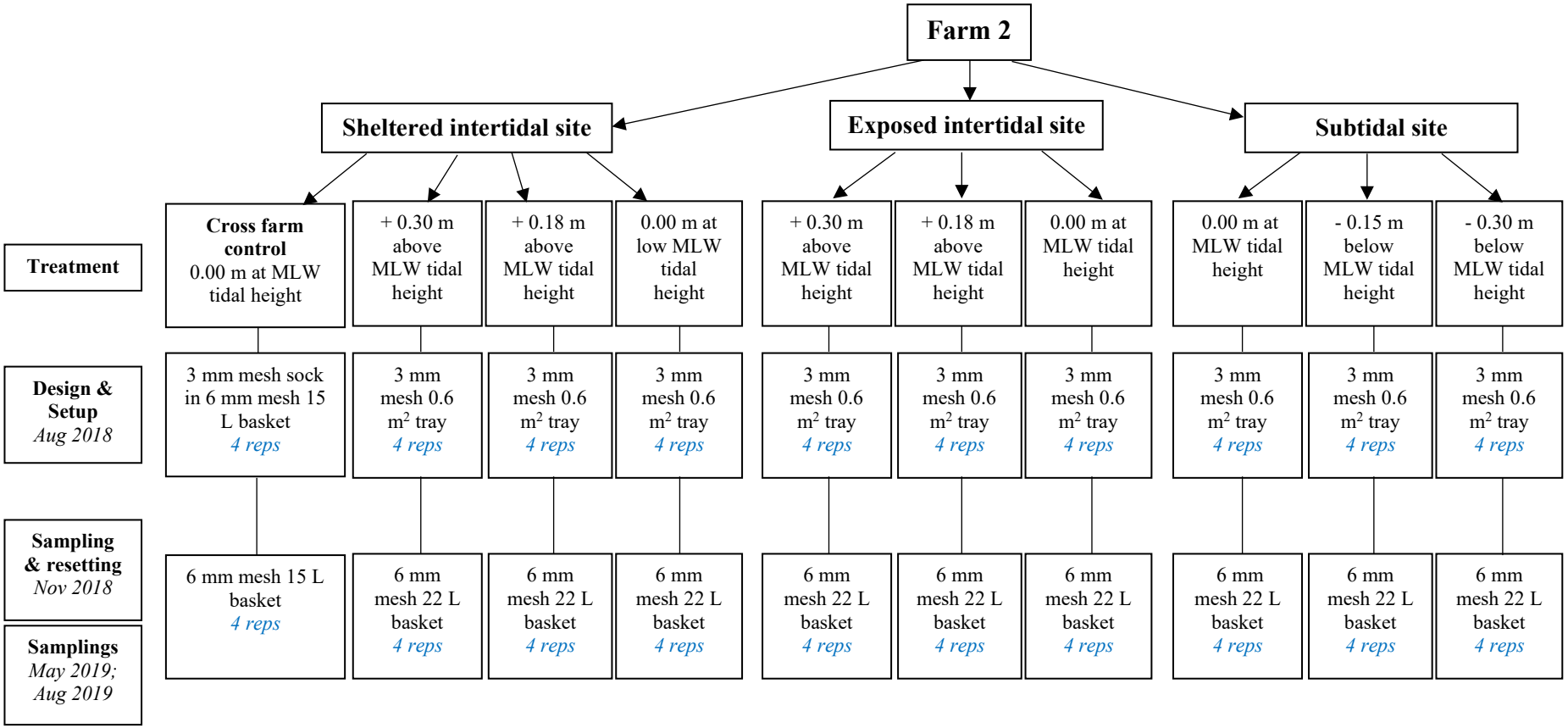


Figure 3.4 Experimental design and setups for Farm 2, Port Vincent, Gulf St Vincent, Australia. Native Oysters (*Ostrea angasi*) were stocked in oyster baskets at a density of 700 Native Oysters per basket (irrespective of basket size) or trays at a density of 950 Native Oysters per tray at three sites at varying growing heights. MLW = mean low water.

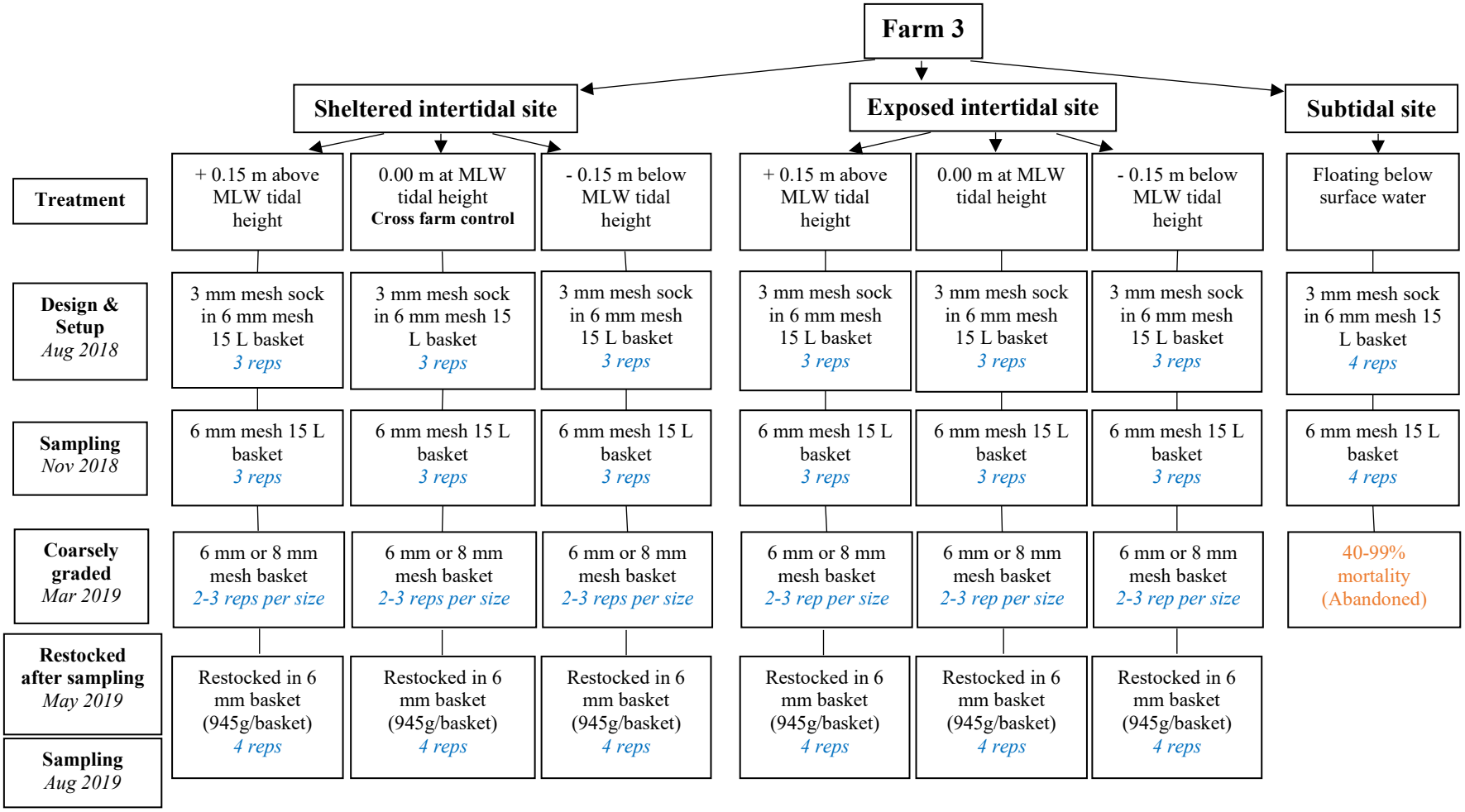


Figure 3.5 Experimental design and setups for Farm 3, American River, Gulf St Vincent, Australia. Native Oysters (*Ostrea angasi*) were initially stocked in oyster baskets at a density of 700 Native Oysters per basket at three sites at varying growing heights. The treatment of 0.00 m low tide water height at the sheltered intertidal site was also used as the cross-farm control. MLW = mean low water.

Native Oyster measurements

In this study, the shell dimensions were measured using digital callipers. Shell length was measured as dorsal-ventral length. At the final sampling event, final shell depth (measured as the thickness between right and left shells) and shell width (anterior-posterior width) were measured to calculate shape score.

Sampling events

There were four sampling events over the 13-month experiment period - August 2018 (initial), November 2018, May 2019 and August 2019, respectively.

Initial shell lengths and live weights were determined by measuring a random subsample of 150 Native Oysters.

November 2018 (Figures 3.3, 3.4 and 3.5)

At the November 2018 sampling event, a random subsample of 30 Native Oysters per replicate (basket or tray) were measured and weighed. Survival was recorded for each replicate.

At Farm 1 and Farm 2, the Native Oysters were transferred from the trays and stocked into 6 mm mesh size, 22 L, oyster baskets at a stocking density of 700 oysters per basket and returned to their correct treatment growing height. In the cross-farm controls the Native Oysters were removed from the 3 mm mesh socks and stocked in their 6 mm mesh size, 15 L, oyster baskets directly.

At Farm 3, the Native Oysters were removed from the 3 mm mesh socks and restocked in 6 mm mesh size, 15 L, oyster baskets at a stocking density of 700 Native Oysters per basket.

At Farm 3, it was observed that an error was made with the experimental set-up that meant only three replicates per treatment were placed at the sheltered intertidal and the exposed intertidal sites.

May 2019 (Figures 3.3, 3.4 and 3.5)

At the May 2019 sampling event, for Farm 1 and Farm 2, a sub-sample of 60 Native Oysters from each replicate were measured and weighed. Survival was recorded for each replicate.

At Farm 3, between the November 2018 sampling event and the May 2019 sampling event, the Native Oysters were coarsely graded on March 26th, 2019 by farm staff into two size classes and returned in 5 baskets of two different mesh sizes per treatment [two to three replicates in 6 mm baskets and two to three replicates in 8mm baskets (Hexcyl Systems Pty Ltd; Denial Bay, Australia)] at uneven stocking densities at the intertidal sites. Given this, for each treatment, oysters from both size grades were combined and randomly divided into three new replicates, before a sub-sample of 60 Native Oysters were measured and weighed.

On March 26th, 2019, high mortality (40 – 99%) was recorded in the subtidal site treatments and they were subsequently abandoned.

Given the high variability in Native Oyster size and stocking density per basket at Farm 3 at the May 2019 sampling event, oysters in each treatment were mixed together and restocked based on biomass as opposed to number. Baskets were stocked at 945 g total biomass per basket and four replicate baskets were placed at the growing heights previously described for Farm 3 (Figure 3.5).

August 2019 (Figures 3.3, 3.4 and 3.5)

At the August 2019 sampling event three replicates per treatment were measured as opposed to the four replicates measured at previous sampling events. For each replicate, a sub-sample of 40 Native Oysters were measured and weighed. Additionally, depths and widths of 10 Native Oysters per

replicate were also measured for shell shape score calculation. Survival was recorded for each replicate.

Comparisons between farm locations

Data from cross-farm control treatments (sheltered intertidal; 0.00 m at MLW growing height) were used to compare Native Oyster performance between the farm locations (Farm 1, Farm 2 and Farm 3).

Statistical analysis

The following Native Oyster performance parameters were calculated as:

- Survival (%) = [1- (number of Native Oysters found dead / initial number of Native Oysters stocked)] × 100
- Daily growth rate for shell length (DGRSL; μm / d) = (final shell length – initial shell length) / days.
- Daily growth rate for weight (DGRW; mg / d) = (final live weight – initial live weight) / days.
- Shape score = (final shell length / final shell width) / (final shell depth / final shell length).

The equation for shape score was used by Rankin et al. (2018) in Sydney Rock Oysters. A lower shape score gives a more commercially desirable oyster shape (i.e., deeper and rounder)

Data is presented as mean ± standard deviation (SD). SPSS v.24 (IBM, Armonk, New York, USA) was used for all statistical analysis. Survival data were arc-sin transformed before analysis. Data was pre-analysed for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test). To meet the ANOVA assumptions, each sampling event was analysed independently. Multifactor ANOVA and Tukey's HSD post hoc test were used to assess significant ($P < 0.05$) differences between treatment means. A simple effects test was used to compare significant interactions when present. Where ANOVA assumptions were not met, a non-parametric independent Kruskal-Wallis Test with pairwise comparisons or Mann-Whitney U Test was used. Data from cross farm controls were analysed to reveal the effects of farming locations. Growth rate data (DGRSL and DGRW) were further analysed to assess differences between sampling events within a farm or between farms within a sampling event. For within farm comparisons, the two intertidal sites were analysed with two factors: site and growing height. For Farm 1, the subtidal site was analysed separately and then compared with the intertidal -0.15 m growing height (one factor = site). For Farm 2, the subtidal treatments were analysed separately with growing height as the factor. As there was no significant difference between sites at the 0.00 growing height at the final sampling event, the intertidal and subtidal data were then analysed together to compare growing height effect.

Comparisons were made at Farm 1 and Farm 2 between oysters grown in baskets for the entire experiment and those grown in trays for the August 2018 – November 2018 period only, after which the oysters were moved to baskets. Data from the cross-farm control and those from the same treatment (0.00 m growing height at the sheltered intertidal sites) at Farm 1 and Farm 2 were used to compare oyster performances between baskets vs trays. This analysis thus has two factors: Treatment (tray vs basket) and Farm (1 vs 2)

Results

3.1.2.1 Comparisons of farm locations

Performances of cross-farm control Native Oysters across the 13 months post stocking are summarised in Table 3.1

Table 3.1 Native Oyster (*Ostrea angasi*) performance (mean ± SD) of cross-farm control treatment at three farm locations in Gulf St Vincent, South Australia, 13 months post stocking[1]

	Farm 1	**Farm 2**	**Farm 3**
Survival (%)	99.18 ± 0.60[a]	100.00 ± 0.00[a]	98.73 ± 1.42[a]
Final shell length (mm)	50.68 ± 1.45[a]	45.14 ± 2.28[ab]	39.77 ± 3.32[b]
Overall DGRSL[2] (μm / d)	102.67 ± 3.97[a]	87.78 ± 6.24[ab]	71.49 ± 8.91[b]
Final live weight (g)	15.05 ± 1.48[a]	10.30 ± 1.20[b]	5.34 ± 0.57[c]
Overall DGRW[3] (mg / d)	40.45 ± 4.03[a]	27.55 ± 3.30[b]	13.66 ± 1.52[c]
Shape score	6.70 ± 0.64[b]	5.31 ± 0.19[a]	7.39 ± 0.19[b]

[1.] Different superscripts in the same row indicate significant differences ($P < 0.05$), n = 3. Initial shell length = 13.1 ± 4.6 mm and initial live weight = 0.25 ± 0.24 g.
[2.] DGRSL = daily growth rate for shell length
[3.] DGRW = daily growth rate for weight.

Survival

Survival over the experiment was close to 100%, with no significant difference in survival between farms ($F_{(2,6)} = 2.52$, $P = 0.160$; one way ANOVA; Table 3.1).

Shell length and DGRSL

There were significant differences in shell length between farms at the November 2018 ($F_{(2,8)} = 7.12$, $P = 0.017$, one way ANOVA), May 2019 ($F_{(2,8)} = 290.98$, $P < 0.001$) and August 2019 sampling events ($F_{(2,6)} = 14.61$, $P = 0.005$, Figure 3.6). At the November 2019 sampling event, there was a significant difference between shell length at Farm 2 and Farm 3 ($P = 0.016$), with no other significant differences between farms ($P > 0.05$, Tukey HSD). At the May 2019 sampling event, all farms had significantly different shell lengths ($P < 0.05$) and at the August 2019 sampling event, Farm 1 and Farm 3 had significantly different shell lengths ($P = 0.004$), with no other significant differences between farms ($P > 0.05$). Farm 1 had the greatest final shell length (50.68 ± 1.45 mm) followed by Farm 2 (45.14 ± 2.28 mm) and Farm 3 (39.77 ± 3.32 mm, Table 3.1).

There were significant differences in DGRSL between sampling events at Farm 1 ($H_{(2)} = 8.91$, $P = 0.012$, Kruskal-Wallis), Farm 2 ($H_{(2)} = 8.91$, $P = 0.012$) and Farm 3 ($H_{(2)} = 7.20$, $P = 0.027$, Figure 3.6). At Farm 1 and Farm 2, between November 2018 – May 2019 had a significantly higher DGRSL than between May 2019 – August 2019 ($P < 0.05$, pairwise comparisons), with no other significant differences between sampling events ($P > 0.05$). At Farm 3, however, between November 2018 – May 2019 had a significantly lower DGRSL than between May 2019 – August 2019 ($P = 0.007$), with no other significant differences between sampling events ($P > 0.05$). The DGRSL over the entire

experiment was significantly different between farms ($F_{(2,6)} = 16.33$, $P = 0.004$, one way ANOVA), with a significant difference observed between Farm 1 and Farm 3 ($P = 0.003$) and no significant difference between Farm 2 and Farm 1 or 3 ($P > 0.05$, Tukey HSD). DGRSL for Farm 1, Farm 2 and Farm 3 were 102.67 ± 3.97 µm / d, 87.78 ± 6.24 µm / d and 71.49 ± 8.91 µm / d respectively (Table 3.1).

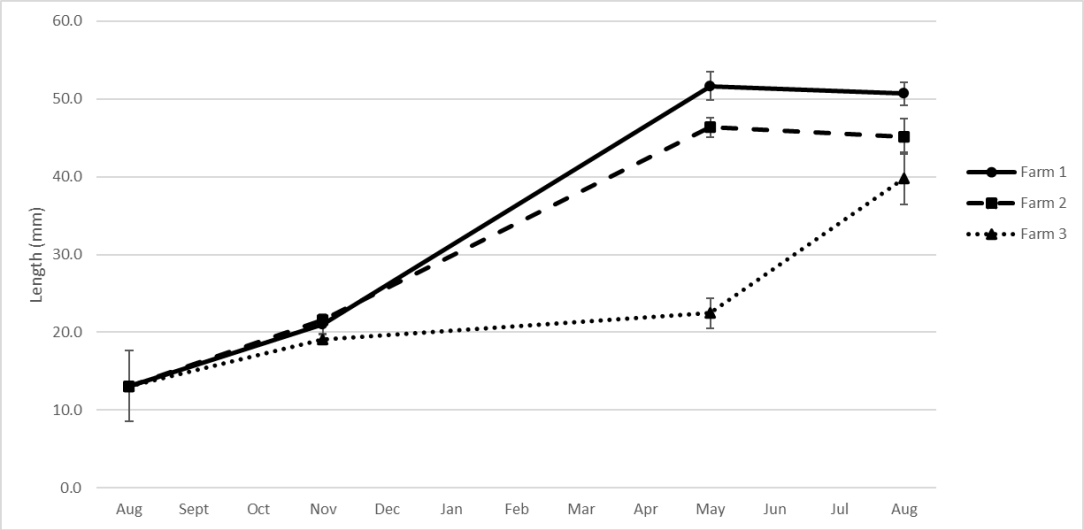


Figure 3.6 Shell length (mean ± SD; n = 4 except for Farm 3 and the second August sampling event where n = 3) of Native Oyster (*Ostrea angasi*) grown at 0.00 m mean low tide growing height over a 13-month sampling period between three farms in South Australia.

Live weight and DGRW

There were significant differences in live weight between farms at the November 2018 ($F_{(2,8)} = 31.89$, $P < 0.001$), May 2019 ($F_{(2,8)} = 101.35$, $P < 0.001$) and August 2019 sampling events ($F_{(2,6)} = 53.70$, $P < 0.001$, one way ANOVA, Figure 3.7). At the May 2019 and August 2019 sampling events, live weight was significantly different between all farms ($P < 0.05$, Tukey HSD). At the November 2018 sampling event, there was no significant difference in live weight between Farm 1 and Farm 2 ($P = 0.080$), but a significant difference between these farms and Farm 3 ($P < 0.05$). Farm 1 had the highest final live weight (15.05 ± 1.48 g) compared to Farm 2 (10.30 ± 1.20 g) and Farm 3 (5.34 ± 0.57, Table 3.1).

There were significant differences in DGRW between sampling events at Farm 1 ($H_{(2)} = 7.21$, $P = 0.027$, Kruskal-Wallis), Farm 2 ($H_{(2)} = 7.21$, $P = 0.027$) and Farm 3 ($H_{(2)} = 7.20$, $P = 0.027$, Figure 3.7). At Farm 1 and Farm 2, between November 2018 – May 2019 had a significantly higher DGRW than between May 2019 – August 2019 ($P < 0.05$, pairwise comparisons) and between August 2018 – November 2018 ($P < 0.05$) with no significant differences in DGRW between August 2018 – November 2018 and between May 2019 – August 2019 ($P > 0.05$). At Farm 3, between August 2018 - November 2018 had a significantly lower DGRW than between May 2019 – August 2019 ($P = 0.007$), with no significant differences between other sampling events ($P > 0.05$). The DGRW over the entire experiment was significantly different between all farms ($F_{(2,6)} = 54.83$, $P < 0.001$, one way ANOVA). DGRW for Farm 1, Farm 2 and Farm 3 was 40.45 ± 4.03 mg / d, 27.55 ± 3.30 mg / d and 13.66 ± 1.52 mg / d respectively (Table 3.1).

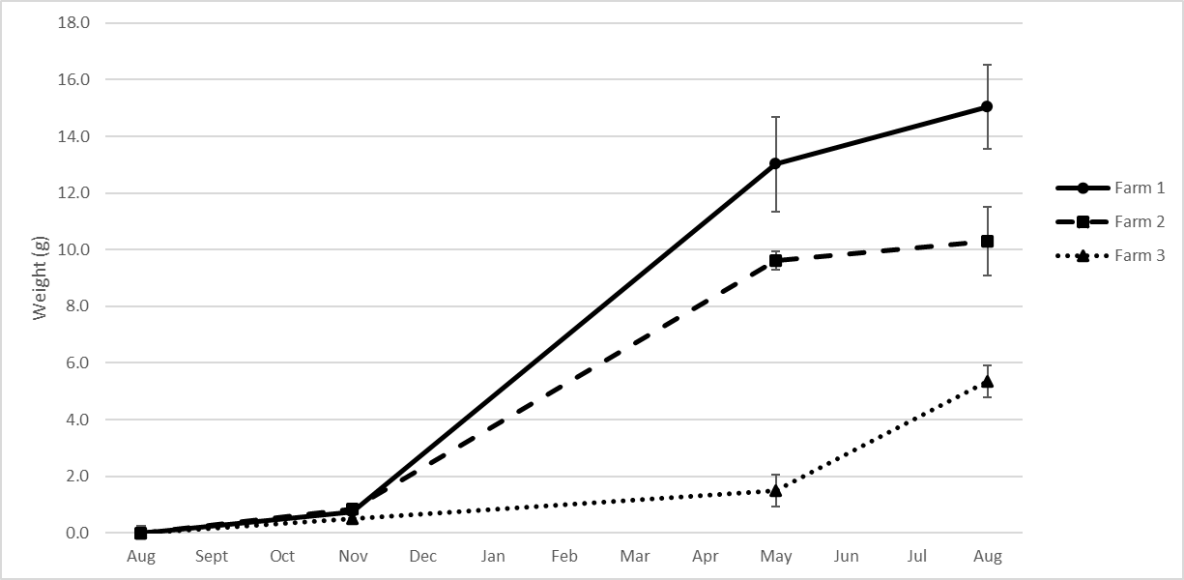


Figure 3.7 Live weight (mean ± SD; n = 4 except for Farm 3 and the second August sampling event where n = 3) of Native Oyster (*Ostrea angasi*) grown at 0.00 m mean low tide growing height over a 13-month sampling period between three farms in South Australia.

Shape Score

There was a significant difference in shape score between farm locations ($F_{(2,6)} = 20.78$, $P = 0.002$; one way ANOVA; Table 3.1). Farm 2 had the lowest shape score (5.31 ± 0.19) and was significantly different to Farm 1 (6.70 ± 0.64; $P = 0.013$) and Farm 3 (7.39 ± 0.19; $P = 0.002$, Tukey HSD). There was no significant difference in shape score between Farm 2 and Farm 3 ($P = 0.172$).

3.1.2.2 Results from Farm 1

Survival

Survival was high (> 99%) for all treatments at Farm 1 (Table 3.2). There was no significant difference in final survival between the sheltered intertidal or exposed intertidal sites ($U = 27.00$, $P = 0.258$, Mann-Whitney U Test) and no significant difference in survival between growing heights ($H_{(2)} = 0.028$, $P = 0.986$; Kruskal-Wallis Test). Survival at both the -0.15 m growing height intertidal sites and at the subtidal site was 100%

Shell length

At all sampling events, shell length at the two intertidal sites at Farm 1 was significantly different between sites ($P < 0.05$) and growing heights ($P < 0.05$) with no significant interaction between these two factors at the May 2019 ($F_{(5,18)} = 7.96$, $P = 0.223$) and August 2019 ($F_{(5,12)} = 13.28$, $P = 0.619$) sampling events, but a significant interaction at the November 2018 sampling event ($F_{(5,18)} = 40.52$, $P = 0.021$, two way ANOVA, Table 3.2; Figure 3.8). The significant interaction at the November sampling event can be explained by no significant difference between sites for the +0.15 m and 0.00 growing heights ($P > 0.05$), but a significant difference between these two sites at the -0.15 m growing height ($P = 0.003$, simple effects test), with the sheltered site at the -0.15 m growing height having a greater shell length (23.81 ± 1.20 mm) than the exposed site at the -0.15 m growing height (21.10 ± 80 mm). At all sampling events, the exposed site had a lower shell length compared to the sheltered site with final average shell lengths of 45.96 ± 3.62 mm and 47.99 ± 4.25 mm respectively. At the November sampling event, all growing heights were significantly different to each other ($P < 0.05$, Tukey HSD) with shell length increasing with deceasing growing heights. At the May sampling event, there was a significant difference in shell length between the +0.15 m and -0.15 m growing heights ($P < 0.001$), but not between the remaining growing heights ($P > 0.05$). At the August sampling event,

shell length was significantly lower at the +0.15 m growing height compared to the remaining growing heights ($P < 0.05$) and there was no significant difference in shell length between the 0.00 m and -0.15 m growing heights ($P = 0.117$). Final average shell length was 42. 39 ± 1.99 mm at the + 0.15 m growing height, 48.11 ± 1.13 mm at the 0.00 m growing height and 50.42 ± 2.78 mm at the – 0.15 m growing height.

Comparisons of shell length between the subtidal -0.30 m site with the lowest (-0.15 m) growing heights at both the exposed and sheltered sites was significant at the November 2018 ($F_{(2,9)} = 8.29$, $P = 0.009$), May 2019 ($F_{(2,9)} = 29.57$, $P < 0.001$) and August 2019 sampling events ($F_{(2,6)} = 19.71$, $P = 0.002$, one way ANOVA, Table 3.2; Figure 3.8). At both the May 2019 and August 2019 sampling events, shell length was significantly lower at the subtidal -0.30 m growing height compared to the -0.15 m growing height at either intertidal site ($P < 0.05$, Tukey HSD). At the November sampling event, there was no significant difference in shell length between the subtidal -0.30 m growing height and the -0.15 m growing height at the exposed ($P = 0.439$) or sheltered intertidal sites ($P = 0.057$). The significant ANOVA for the November 2018 sampling event is due to differences between intertidal sites. The subtidal -0.30 m growing height had the lowest final shell length (38.94 ± 3.15 mm) across all growing heights at all sites at Farm 1.

Live weight

Significant differences in live weight between growing heights at Farm 1 were observed at intertidal sites at all sampling events ($P < 0.05$), with no significant difference between sites ($P > 0.05$) or interactions between the two factors ($P > 0.05$) observed at the November 2018 ($F_{(5,18)} = 14.31$) and the August 2019 sampling events ($F_{(5,12)} = 11.14$, two way ANOVA, Table 3.2; Figure 3.9). However, a significant difference between site at the May 2019 sampling event ($P = 0.005$) was observed, but again there was no significant interaction between the two factors ($F_{(5,18)} = 9.35$, $P = 0.412$). At the May 2019 sampling event, the sheltered intertidal site had a higher live weight (10.81 ± 2.58 g) than the exposed site (8.91 ± 1.89 g). At all sampling events, all growing heights were significantly different from each other for live weight, with heaviest oysters observed at the -0.15 m growing height, followed by the 0.00 m growing height and the lightest at the +0.15 m growing height ($P < 0.05$, Tukey HSD). Final live weights were 14.62 ± 2.31 g, 11.83 ± 1.05 g and 8.77 ± 1.10 g for the -0.15 m, 0.00 m and +0.15 m heights respectively.

At the November 2018 sampling event, there was no significant difference in live weight between the subtidal -0.30 m and the intertidal -0.15 m growing heights ($F_{(2,9)} = 3.89$, $P = 0.061$, one way ANOVA, Figure 3.9). There was, however, significant differences in live weight at both the May 2019 ($F_{(2,9)} = 38.81$, $P < 0.001$) and the August 2018 ($F_{(2,6)} = 29.55$, $P = 0.001$) sampling events with the subtidal -0.30 m growing height being significantly lower than the -0.15 m growing height at both intertidal sites ($P < 0.05$, Tukey HSD). The subtidal -0.30 m growing height had the lowest final live weight (5.87 ± 1.25 g) across all growing heights at all sites at Farm 1.

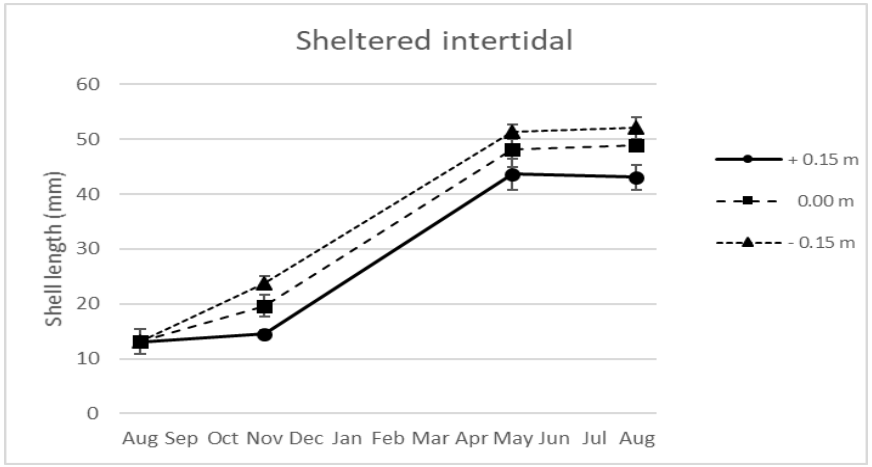
Shape score

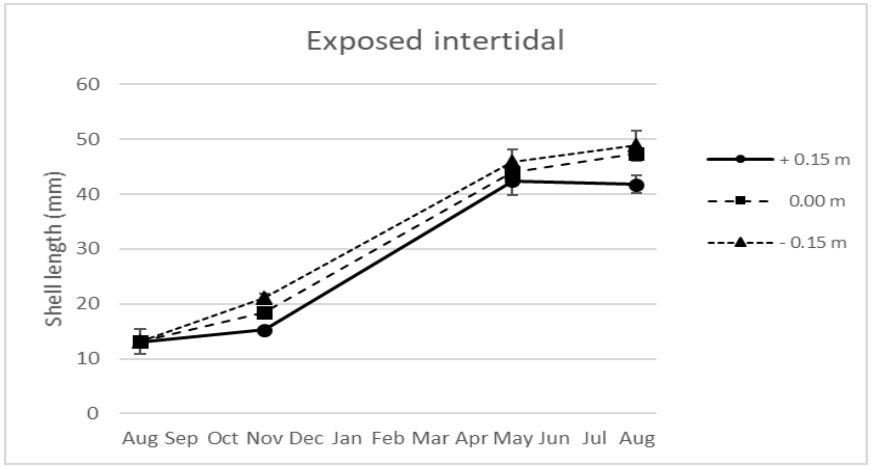
Shape score between the two intertidal heights at Farm 1 was not significantly different between sites ($P = 0.091$) but was between growing heights ($P < 0.001$), with no significant interaction between these two factors ($F_{(5,12)} = 9.46$, $P = 0.692$, two way ANOVA, Table 3.2). All growing heights had significantly different shape scores ($P < 0.05$, Tukey HSD) which were 5.87 ± 0.21, 6.61 ± 0.51 and 5.27 ± 0.31 for the -0.15 m, 0.00 m and +0.15 m growing heights respectively. Shape score was not significantly different between the subtidal -0.30 m growing height and the -0.15 m growing heights at the intertidal sites ($F_{(2,6)} = 4.35$, $P = 0.068$, one way ANOVA)

Table 3.2 Farm 1. Native Oyster (*Ostrea angasi*) final performance (mean ± SD) in terms of growing site and growing height in Gulf St Vincent, South Australia, 13 months post stocking[1]

Site	Growing Height[2]	Survival (%)	Shell length (mm)	Live weight (g)	Shape score[3]
Sheltered intertidal	+ 0.15 m	99.52 ± 0.83	43.05 ± 2.23^{b}	8.83 ± 1.38^{c}	5.53 ± 0.09^{b}
	0.00 m	99.71 ± 0.50	48.87 ± 0.97^{a}	12.03 ± 1.03^{b}	6.70 ± 0.64^{a}
	- 0.15 m	99.76 ± 0.41	52.05 ± 2.00^{a}	16.08 ± 0.58^{a}	5.99 ± 0.16^{c}
Exposed intertidal	+ 0.15 m	100.00 ± 0.00	41.74 ± 1.62^{b}	8.70 ± 1.05^{c}	5.02 ± 0.20^{b}
	0.00 m	100.00 ± 0.00	47.35 ± 0.75^{a}	11.63 ± 1.25^{b}	6.53 ± 0.47^{a}
	- 0.15 m	100.00 ± 0.00	48.80 ± 2.71^{a}	13.15 ± 2.55^{a}	5.76 ± 0.20^{c}
Subtidal	- 0.30 m	100.00 ± 0.00	38.94 ± 3.15	5.87 ± 1.25	6.79 ± 0.64

1. Different superscripts in the same column indicate significant differences ($P < 0.05$), n = 4. Initial shell length = 13.1 ± 4.6 mm and initial live weight = 0.25 ± 0.24 g.
2. Relative to the low MLW tide height.
3. According to Rankin et al. (2018) a lower shape score gives a more commercially desirable oyster shape.





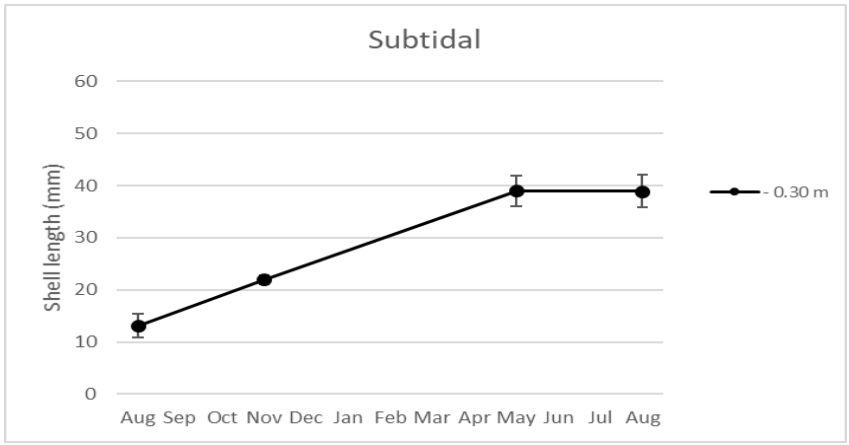
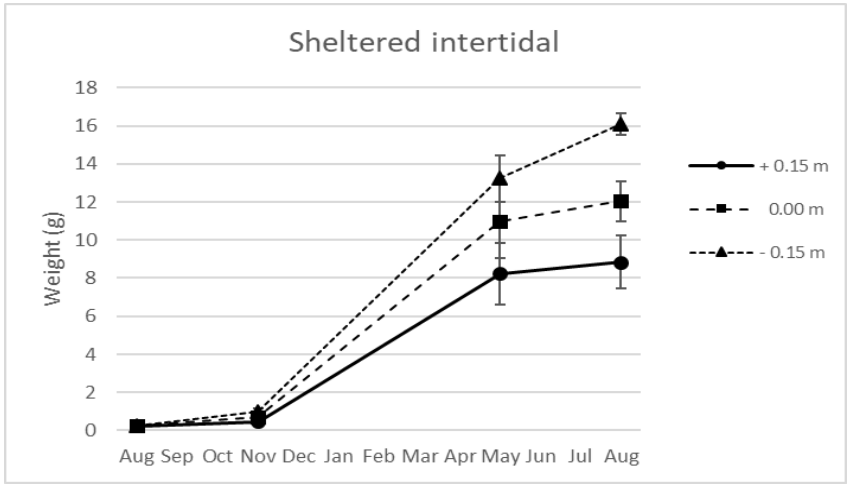
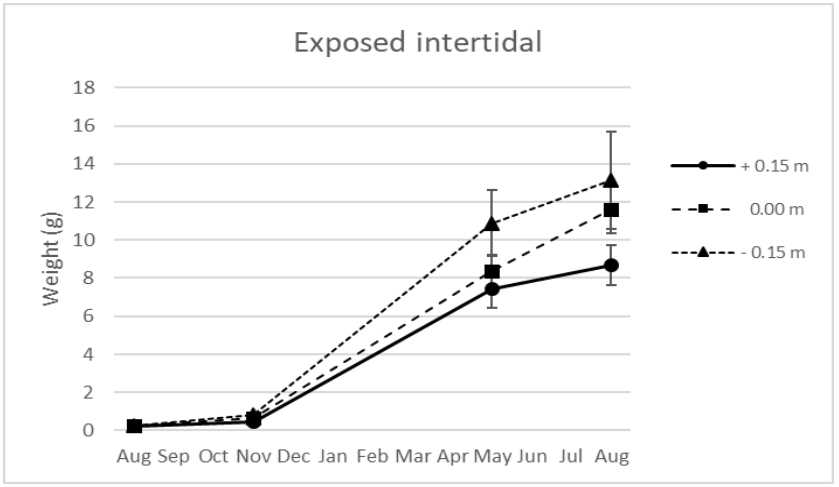


Figure 3.8 Farm 1. Shell length (mean ± SD; n = 4 except for the second August sampling event where n = 3) of Native Oyster (*Ostrea angasi*) over a 13 month sampling period, in terms of growing site (sheltered intertidal, exposed intertidal and subtidal) and growing height (relative to the MLW tide height) in Gulf St Vincent, South Australia. At stocking in August 2018, the initial shell length was 13.1 ± 4.6 mm.





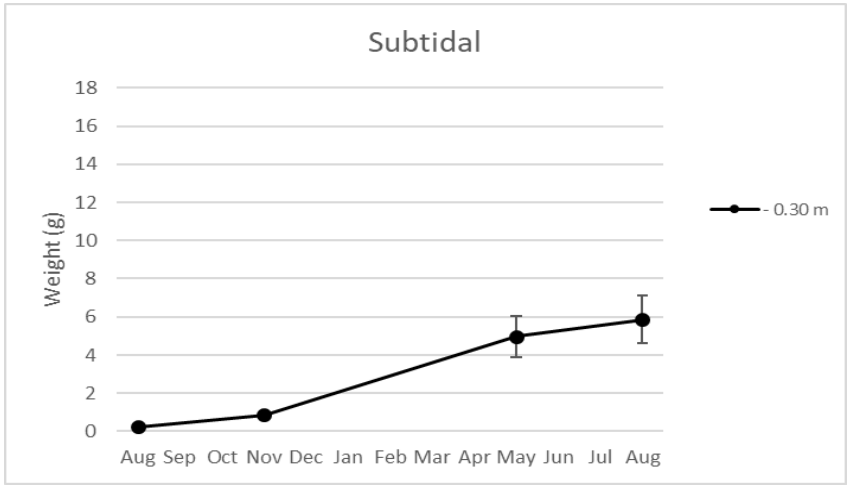


Figure 3.9 Farm 1. Live weight (mean ± SD; n = 4 except for the second August sampling event where n = 3) of Native Oyster (*Ostrea angasi*) over a 13 month sampling period, in terms of growing site (sheltered intertidal, exposed intertidal and subtidal) and growing height (relative to the MLW tide height) in Gulf St Vincent, South Australia. At stocking in August 2018, the initial live weight was 0.25 ± 0.24 g.

3.1.2.3 Results from Farm 2

Survival

Survival at Farm 2 was > 99% for all treatments (Table 3.3). Survival was 100% in all treatments except for one replicate at the + 0.30 m height that was 99%.

Shell length

Shell length at Farm 2 was not significantly different ($P > 0.05$) between the two intertidal sites at any sampling event, however, growing height was significant at the November 2018 sampling event ($F_{(5,18)} = 37.93$, $P < 0.001$) and the August 2019 sampling event ($F_{(5,12)} = 4.01$, $P = 0.005$) but not the May 2019 sampling event ($F_{(5,18)} = 2.48$, $P = 0.076$) and there were no significant interactions between these factors at any sampling event ($P > 0.05$, two way ANOVA, Table 3.3; Figure 3.10). At the November 2018 sampling event, all growing heights were significantly different ($P < 0.05$, Tukey HSD) with the + 0.30 m growing height having the greatest shell length and the + 0.18 m growing height having the least. At the August 2019 sampling event, the + 0.18 m growing height had significantly less shell length than the + 0.30 m growing height ($P = 0.003$), with no significant difference between the other growing heights. Final average shell lengths across the intertidal sites at Farm 2 were 45.80 ± 3.18 mm at + 0.30 m growing height, 43.47 ± 1.90 mm at + 0.18 m growing height and 45.61 ± 1.44 mm at 0.00 m growing height.

At the subtidal site at Farm 2, there was no significant difference in shell length between growing heights at the November 2018 sampling event ($F_{(2,9)} = 1.26$, $P = 0.329$), but there was a significant difference at both the May 2019 ($F_{(2,9)} = 26.66$, $P < 0.001$) and the August 2019 sampling events ($F_{(2,6)} = 8.59$, $P = 0.017$, one way ANOVA, Table 3.3; Figure 3.10). At the May 2019 sampling event, shell length at all growing heights was significantly different ($P < 0.05$, Tukey HSD), with the - 0.30 m growing height having the smallest shell length and the 0.00 m growing height having the greatest. At the August 2019 sampling event, shell length at the - 0.30 growing height was significantly lower than the - 0.15 m ($P = 0.037$) and 0.00 m ($P = 0.021$) growing heights, with no significant difference between the - 0.15 m and 0.00 m growing heights ($P = 0.869$). Final shell lengths at the subtidal site at Farm 2 were 39.74 ± 3.15 mm at - 0.30 m growing height, 45.04 ± 0.61 mm at - 0.15 m growing height and 45.86 ± 1.12 mm at 0.00 m growing height.

Comparison of shell length between the 0.00 m growing heights at all sites (sheltered intertidal, exposed intertidal and subtidal) at Farm 2 showed significant differences between sites at the November 2018 ($F_{(2,9)} = 25.63$, $P < 0.001$) and May 2019 ($F_{(2,9)} = 20.49$, $P < 0.001$) sampling events, but no significant difference between sites at the final August 2019 sampling event ($F_{(2,6)} = 0.17$, $P = 0.845$, one way ANOVA, Table 3.3; Figure 3.10). Given the lack of significant difference in shell length at the 0.00 m growing height between sites in the final August 2019 sampling event, growing heights across the three sites were compared. A significant difference between growing heights was observed ($F_{(4,22)} = 13.09$, $P < 0.001$, one way ANOVA) with shell length at the - 0.30 m growing height being significantly smaller than all other growing heights ($P < 0.05$, Tukey HSD) and the + 0.18 m growing height being smaller than the + 0.30 m growing height ($P = 0.009$, Table 3.3).

Live weight

Live weight at Farm 2 was not significantly different between the two intertidal sites at the May 2019 ($F_{(5,18)} = 2.48$, $P = 0.870$) and August 2019 ($F_{(5,12)} = 4.67$, $P = 0.098$) sampling events, however it was significantly different at the November 2018 sampling event ($F_{(5,18)} = 19.49$, $P = 0.037$), whereas live weight between growing heights was significantly different at all sampling events ($P < 0.05$, two way ANOVA, Table 3.3; Figure 3.11). There were no significant interactions between these two factors at any sampling event ($P > 0.05$). At the November sampling event, the exposed intertidal site had a greater live weight (0.82 ± 0.19 g) compared to the sheltered site (0.75 ± 0.16 g). At both the November 2018 and May 2019 sampling events, the + 0.18 growing height had a significantly lower live weight compared to both the 0.00 m ($P < 0.001$) and + 0.30 m growing heights ($P < 0.001$, Tukey

HSD). The +0.30 m and 0.00 m growing heights were not significantly different for live weight ($P > 0.05$). At the August sampling event, the +0.18 m growing height had a significantly lower live weight than the +0.30 m growing height ($P = 0.003$), with no significant differences between the other growing heights for live weight ($P > 0.05$). Final average live weight across the intertidal sites at Farm 2 were 11.97 ± 1.41 g at +0.30 m growing height, 9.44 ± 0.50 g at +0.18 m growing height and 10.82 ± 1.08 g at 0.00 m growing height.

At the subtidal site at Farm 2, there was a significant difference in live weight between growing heights at the May 2019 ($F_{(2,9)} = 24.71$, $P < 0.001$) and August 2019 ($F_{(2,6)} = 37.11$, $P < 0.001$) sampling events, but not at the November 2018 sampling event ($F_{(2,9)} = 0.24$, $P = 0.792$, one way ANOVA, Table 3.3; Figure 3.11). At the May 2019 sampling event all growing heights had significantly different live weights ($P < 0.05$, Tukey HSD) with the – 0.30 m growing height having the smallest live weight and the 0.00 m growing height having the highest. At the August 2019 sampling event, live weight at the -0.30 m growing height was significantly smaller than at the - 0.15 m ($P = 0.001$) and the 0.00 m growing height ($P < 0.001$). There was no significant difference between the - 0.15 m and 0.00 m growing heights ($P = 0.439$). Final live weight at the subtidal site at Farm 2 was 11.45 ± 0.18 g at the 0.00 m growing height, 10.69 ± 0.57 g at the - 0.15 m growing height and 6.81 ± 1.07 m at the - 0.30 m growing height.

Comparison of live weight between the 0.00 m growing heights at all sites (sheltered intertidal, exposed intertidal and subtidal) at Farm 2 showed significant differences between sites at the November 2018 ($F_{(2,9)} = 17.23$, $P = 0.001$) and May 2019 ($F_{(2,9)} = 28.41$, $P < 0.001$) sampling events, but no significant difference between sites at the final August 2019 sampling event ($F_{(2,6)} = 1.65$, $P = 0.268$, one way ANOVA, Table 3.3; Figure 3.11). At both the November 2018 and May 2019 sampling events, the subtidal site had a significantly greater live weight than the intertidal sites ($P < 0.05$) and there was no significant difference between the intertidal sites ($P > 0.05$, Tukey HSD). Given the lack of significant difference in live weight at the 0.00 m growing height between sites in the final August 2019 sampling event, growing heights across the three sites were compared. A significant difference between growing heights was observed ($F_{(4,22)} = 16.50$, $P < 0.001$, one way ANOVA) with live weight at the - 0.30 m growing height being significantly smaller than all other growing heights ($P < 0.05$, Tukey HSD) and the + 0.18 m growing height being smaller than the + 0.30 m growing height ($P = 0.002$) and the 0.00 m growing height ($P = 0.039$, Table 3.3).

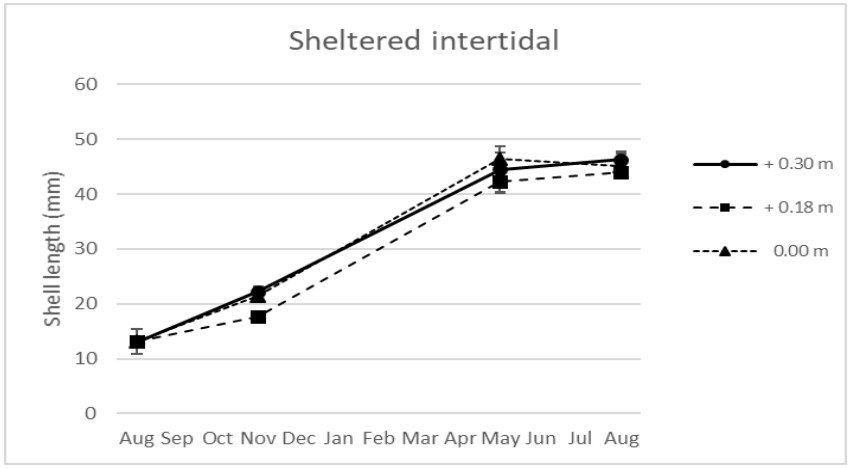
Shape score

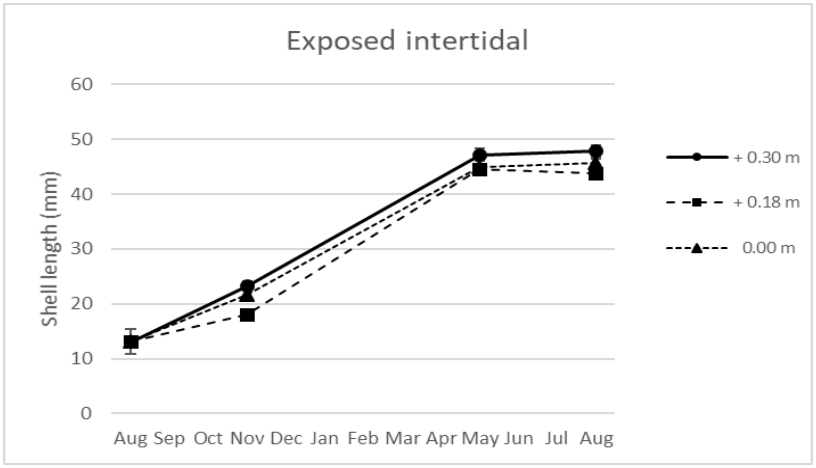
Shape score at Farm 2 was not significantly different between intertidal sites ($P = 0.511$) or growing heights ($P = 0.581$) and there was no significant interaction between the two factors ($F_{(5,12)} = 0.69$, $P = 0.425$, two way ANOVA, Table 3.3) The overall shape score for the intertidal sites was 5.53 ± 0.52. At the subtidal sites, however, there was a significant difference in shape score between growing heights ($H_{(2)} = 6.49$, $P = 0.039$, Kruskal-Wallis Test), with the -0.30 m growing height having a significantly greater shape score (7.94 ± 2.18) than the 0.00 m growing height (5.49 ± 0.67). There was no significant difference in shape score observed between these growing heights and the - 0.15 m growing height (6.27 ± 0.06, $P > 0.05$). There was no significant difference in shape score between the 0.00 m growing heights at all sites (sheltered intertidal, exposed intertidal and subtidal) at Farm 2 ($F_{(2,6)} = 0.13$, $P = 0.882$, one way ANOVA).

Table 3.3 Farm 2. Native Oyster (*Ostrea angasi*) final performance (mean ± SD) in terms of growing site and growing height in Gulf St Vincent, South Australia, 13 months post stocking[1]

Site	Growing height[2]	Survival (%)	Shell length (mm)	Live weight (g)	Shape score[3]
Sheltered intertidal	+ 0.30 m	99.67 ± 0.58	46.46 ± 1.52^{a}	11.24 ± 1.59^{a}	5.35 ± 0.38^{b}
	+ 0.18 m	100.00 ± 0.00	43.89 ± 1.14^{b}	9.40 ± 0.76^{b}	5.68 ± 0.69^{b}
	0.00 m	100.00 ± 0.00	45.14 ± 2.28^{ab}	10.30 ± 1.20^{a}	5.31 ± 0.19^{b}
Exposed intertidal	+ 0.30 m	100.00 ± 0.00	47.94 ± 1.04^{a}	12.70 ± 0.93^{a}	6.00 ± 0.95^{b}
	+ 0.18 m	100.00 ± 0.00	43.78 ± 0.77^{b}	9.49 ± 0.18^{b}	5.48 ± 0.36^{b}
	0.00 m	100.00 ± 0.00	45.63 ± 0.71^{ab}	11.33 ± 0.83^{a}	5.38 ± 0.36^{b}
Subtidal	0.00 m	100.00 ± 0.00	45.86 ± 1.12^{ab}	11.45 ± 0.18^{a}	5.45 ± 0.67^{b}
	- 0.15 m	100.00 ± 0.00	45.04 ± 0.61^{ab}	10.69 ± 0.57^{ab}	6.27 ± 0.06^{b}
	- 0.30 m	100.00 ± 0.00	39.74 ± 3.15^{c}	6.81 ± 1.07^{c}	7.94 ± 2.18^{a}

1. Different superscripts in the same column indicate significant differences ($P < 0.05$), n = 4. Initial shell length = 13.1 ± 4.6 mm and initial live weight = 0.25 ± 0.24 g.
2. Relative to the MLW tidal height.
3. According to Rankin et al. (2018) a lower shape score gives a more commercially desirable oyster shape.





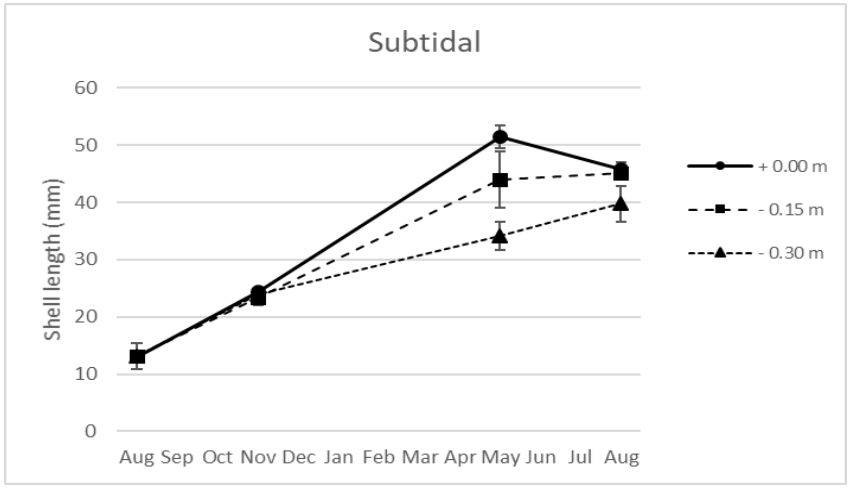
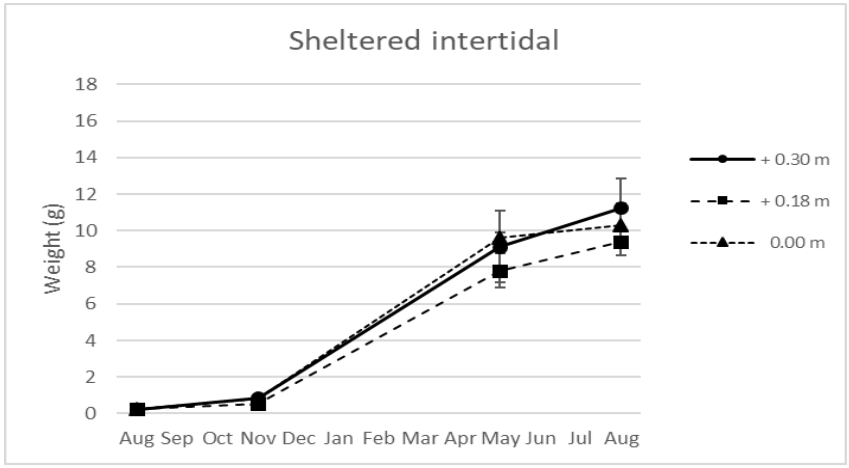
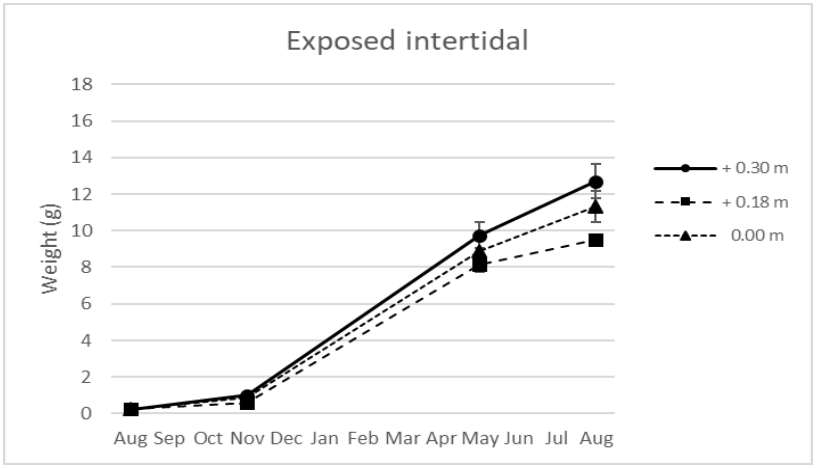


Figure 3.10 Farm 2. Shell length (mean ± SD; n = 4 except for the second August sampling event where n = 3) of Native Oyster (*Ostrea angasi*) over a 13 month sampling period, in terms of growing site (sheltered intertidal, exposed intertidal and subtidal) and growing height (relative to the MLW tidal height) in Gulf St Vincent, South Australia. At stocking in August 2018, the initial shell length was 13.1 ± 4.6 mm.





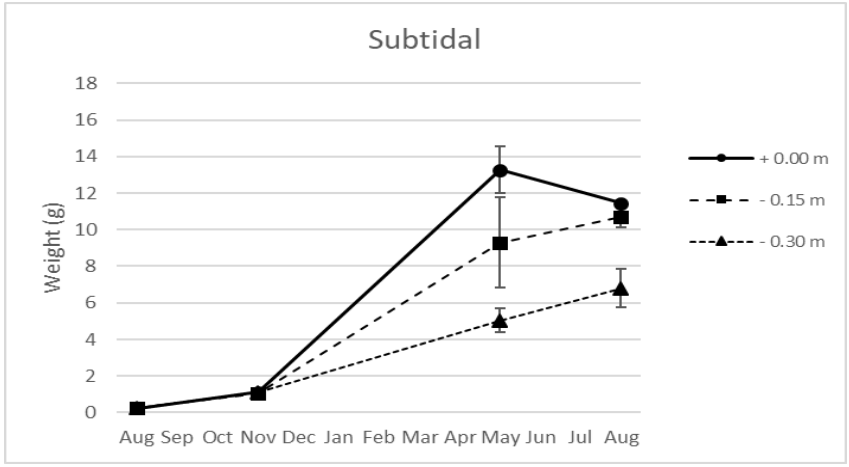


Figure 3.11 Farm 2. Live weight (mean ± SD; n = 4 except for the second August sampling event where n = 3) of Native Oyster (*Ostrea angasi*) over a 13 month sampling period, in terms of growing site (sheltered intertidal, exposed intertidal and subtidal) and growing height (relative to the MLW tidal height) in Gulf St Vincent, South Australia. At stocking in August 2018, the initial live weight was 0.25 ± 0.24 g.

3.1.2.4 Results from Farm 3

Survival

On the 26th of March 2019, high mortalities (average 75.33 ± 25.46%, up to 99% in one replicate) were recorded in the subtidal treatments by the staff at Farm 3. The dead oysters were disposed of by the farm staff and the treatments were removed from the experiment. Data from this treatment is not used in the analyses below. For the two intertidal sites, survival was high (> 97%; Table 3.4). The two intertidal sites at Farm 3 had significantly different survival (U = 10.00, $P = 0.06$, Mann-Whitney U Test), with the exposed site having higher survival (99.99 ± 0.03%) than the sheltered site (98.19 ± 2.15%). There was no significant difference in survival between growing height ($H_{(2)} = 0.36$, $P = 0.834$; Kruskal-Wallis Test)

Shell length

At Farm 3, shell length was not significantly different between intertidal sites at the November 2018 ($F_{(1,4)} = 0.77$, $P = 0.429$) and May 2019 sampling events ($F_{(1,2)} = 13.02$, $P = 0.069$, one way ANOVA, Table 3.4; Figure 3.12). At the August 2019, there was also no significant difference between intertidal sites ($P = 0.687$), but there was a significant difference in shell length between growing heights ($P = 0.025$) and no significant interaction between the two factors ($F_{(5,12)} = 2.31$, $P = 0.550$, two way ANOVA). At the August 2019 sampling event, the + 0.15 m growing height had significantly smaller shell length (36.23 ± 0.68 mm) than the + 0.00 m growing height (39.52 ± 2.39 mm, $P = 0.036$, Tukey HSD). The - 0.15 m growing height (39.29 ± 2.13 mm) was not significantly different to any other growing height ($P > 0.05$).

Live weight

Live weight at Farm 3 was not significantly different between intertidal sites at the November 2018 ($F_{(1,4)} = 0.15$, $P = 0.722$) or May 2019 sampling events ($F_{(1,2)} = 3.04$, $P = 0.223$, one way ANOVA, Table 3.1.4; Figure 3.13). Live weight at the August 2019 sampling event was also not significantly different between sites ($P = 0.752$) or between growing heights ($P = 0.309$) and there was no significant interaction between these two factors ($F_{(5, 12)} = 1.04$, $P = 0.323$, two way ANOVA).

Shape score

Shape score at Farm 3 was significantly different between intertidal sites ($P = 0.011$) and growing heights ($P < 0.001$) with no significant interaction between these two factors ($F_{(5, 12)} = 10.72$, $P = 0.351$, two way ANOVA, Table 3.4). The exposed intertidal site had a higher shape score (7.36 ± 0.46) than the sheltered intertidal site (6.96 ± 0.58). The + 0.15 m low tide growing height had the lowest shape score (6.56 ± 0.39) and was significantly different to the 0.00 m at low tide growing height (7.47 ± 0.24; $P < 0.001$) and the – 0.15 m low tide growing height (7.45 ± 0.39; $P < 0.001$). The 0.00 m at low tide growing height was not significantly different to the – 0.15 m low tide growing height ($P = 0.991$).

Table 3.4 Farm 3. Native Oyster (*Ostrea angasi*) final performance (mean ± SD) in terms of growing site and growing height in Gulf St Vincent, South Australia, 13 months post stocking[1]

Site	Growing height[2]	Survival (%)	Shell length (mm)	Live weight (g)	Shape score[3]
Sheltered intertidal	+ 0.15 m	98.30 ± 2.14	36.33 ± 0.75[b]	5.38 ± 0.28	6.23 ± 0.15[b]
	0.00 m	97.20 ± 3.34	39.77 ± 3.32[a]	5.34 ± 0.57	7.39 ± 0.19[a]
	- 0.15 m	99.07 ± 0.21	38.35 ± 2.68[a]	5.41 ± 1.23	7.27 ± 0.29[a]
Exposed intertidal	+ 0.15 m	100.00 ± 0.00	36.12 ± 0.74[b]	4.73 ± 0.86	6.88 ± 0.19[b]
	0.00 m	100.00 ± 0.00	39.27 ± 1.77[a]	5.67 ± 0.53	7.55 ± 0.29[a]
	- 0.15 m	99.97 ± 0.06	40.23 ± 1.23[a]	6.04 ± 0.59	7.16 ± 0.54[a]
Subtidal[4]	Floating	24.7 ± 25.46[5]	N/A	N/A	N/A

1. Different superscripts in the same column indicate significant differences ($P \leq 0.025$), n = 3. Initial shell length = 13.1 ± 4.6 mm and initial live weight = 0.25 ± 0.24 g.
2. Relative to the MLW tidal height.
3. According to Rankin et al. (2018) a lower shape score gives a more commercially desirable oyster shape.
4. N/A: final length and live weight measurements were not measured due to high mortality within these treatments.
5. The data were collected on March 26th, 2019.

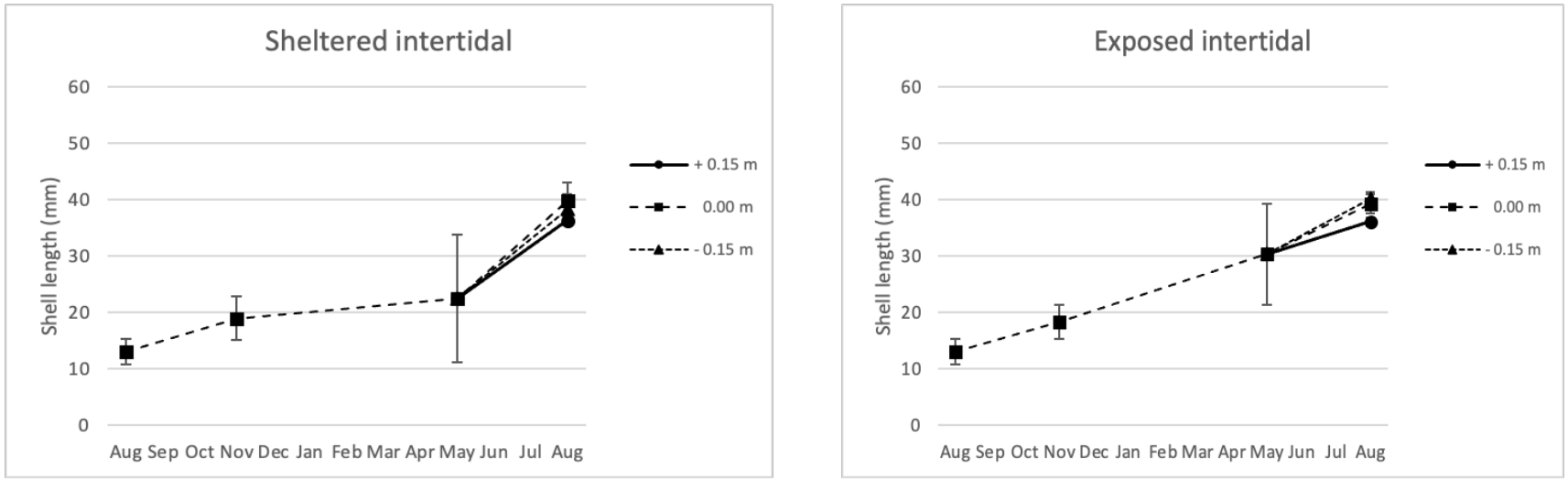


Figure 3.12 Farm 3. Shell length (mean ± SD; n = 3) of Native Oyster (*Ostrea angasi*) over a 13 month sampling period, in terms of growing site (sheltered intertidal and exposed intertidal) and growing height (relative to MLW tidal height) in Gulf St Vincent, South Australia. At stocking in August 2018, the initial shell length was 13.1 ± 4.6 mm.

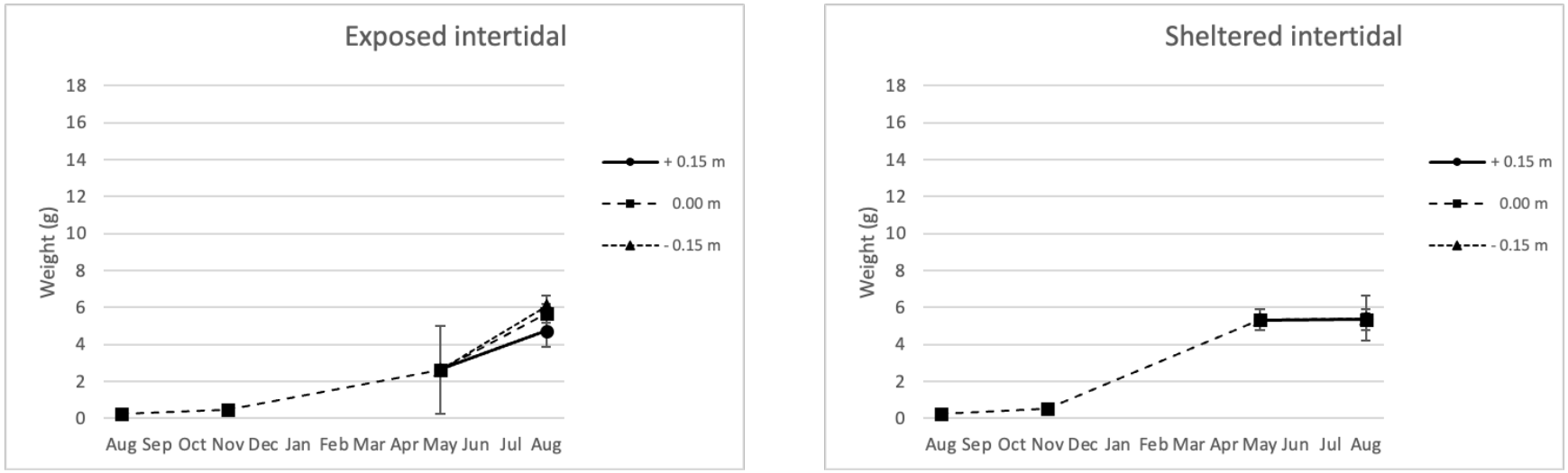


Figure 3.13 Farm 3. Live weight (mean ± SD; n = 3) of Native Oyster (*Ostrea angasi*) over a 13 month sampling period, in terms of growing site (sheltered intertidal and exposed intertidal) and growing height (relative to MLW tidal height) in Gulf St Vincent, South Australia. At stocking in August 2018, the initial live weight was 0.25 ± 0.24 g.

3.1.2.5 Oyster baskets vs trays

Comparisons were made between oysters at Farm 1 and Farm 2 that were grown in baskets for the entire experiment (control baskets, sheltered, intertidal site, 0.00 m growing height) and those grown in trays for the August 2018 – November 2018 period only (experimental trays/baskets, sheltered intertidal site, 0.00 m growing height), after which the oysters were moved to baskets. Results showed a significant difference in shell length between farms at all sampling events ($P < 0.05$), but no significant difference between treatments (tray vs basket, $P > 0.05$) and no significant interactions between the two factors at the November 2018 ($F_{(3,12)} = 2.06$, $P = 0.306$), May 2019 ($F_{(3,12)} = 6.03$, $P = 0.099$) and August 2019 ($F_{(3,8)} = 6.88$, $P = 0.415$, two way ANOVA). Furthermore, live weight was significantly different between farms at the May 2019 ($F_{(3,12)} = 6.10$, $P = 0.003$) and August 2019 ($F_{(3,8)} = 9.79$, $P = 0.002$) sampling events, but not at the November 2018 sampling event ($F_{(3,12)} = 1.55$, $P = 0.054$), and again there was no significant difference between treatments ($P > 0.05$) and no significant interactions between the two factors ($P > 0.05$) at any sampling event. Shape score showed the same pattern. It was significantly different between farms ($P = 0.001$), but not between treatments ($P = 0.124$) with no significant interaction between the two factors ($F_{(3,8)} = 10.40$, $P = 0.124$).

Discussion

Except for the subtidal treatments at Farm 3, survival over this experiment was high (> 97%) and generally not significantly different between farm locations, sites, or growing heights. The mortality observed in this study is less than that reported (6.43 - 22.95%) for wild *O. angasi* over eight months in Georges Bay, Tasmania (Mitchell et al., 2000) or the up to 20% mortality reported for Native Oysters grown in farm trials in Victoria, Australia over 16 months (Hickman and O'Meley, 1988). In the current study, the subtidal treatment at Farm 3 experienced high mortality (~75%) in March 2019. The cause of this mortality is unknown. The mortality was detected by the farm staff and the dead oysters were disposed of, hence histopathology samples for disease analysis could not be collected. It is possible that the mortality was caused by the protozoan parasite *Bonamia spp.*, which has previously been detected in South Australian oyster farms (Buss et al., 2019). *Bonamia spp.* has been responsible for mass mortality in flat oyster species (such as *O. edulis* and *O. chilensis*) worldwide (Engelsma et al., 2014). It has been reported that in South Australia Native Oysters are infected with *Bonamia spp.* at a high prevalence (>50% at some farms), but low intensity (Buss et al., 2019). *Bonamia spp.* prevalence and intensity can vary seasonally, with intensity being linked to increased environmental stress (Hine, 1991; Hine et al., 2002). To date, the *Bonamia spp.* prevalence at Farm 3 is unknown, however mortality was low for this farm at the intertidal sites, suggesting that if *Bonamia spp.* was responsible for the mortality, it was likely linked with environmental stress. A different possible explanation for the sudden mortality observed in the present study at Farm 3 could also be an increase of biofouling at the subtidal site compared to the intertidal. Biofouling has been shown to reduce shellfish fitness and increase mortality (Sievers et al., 2017). The subtidal treatment at Farm 3 was not subjected to any exposure period and was likely more sheltered from wave action than the intertidal treatments, which might have increased biofouling of the baskets and may have led to the mortality observed.

Farm 1 and Farm 2 showed a similar pattern in DGRSL and DGRW across the three sampling events. At these farms, daily growth rates were highest over the warm water temperature period (summer and autumn), between the November 2018 and May 2019 sampling events, likely due to an increase in primary productivity and energy input during these seasons. This concurs with Laing et al. (2005) who reported temperature to be the most important factor limiting growth in *O. edulis* in the United Kingdom. In the current study, for Farms 1 and 2, the daily increase in shell length over the warm water temperature period (144.91 ± 26.17 µm / d for Farm 1 and 128.08 ± 31.18 µm / d) was substantially different from the May 2019 sampling event to the final August 2019 sampling event (4.96 ± 18.57 µm / d for Farm 1 and 2.31 ± 20.84 µm / d for Farm 2). This difference was less

pronounced with the daily growth rate for live weight (from 47.71 ± 15.71 mg / d to 9.21 ± 11.33 mg / d for Farm 1 and from 45.98 ± 13.21 mg / d to 7.31 ± 9.94 mg / d for Farm 2). The reason behind the greater difference in DGRSL compared to DGRW over this period, is likely due to hydrodynamic factors, such as stronger wave action over the winter period, damaging the shells of the Native Oysters. Many of the sampled Native Oysters decreased in shell length over the low temperature period from May to August 2019. A similar pattern was observed in wild Native Oyster in George's Bay, Tasmania, Australia, where little increase in shell length was observed between May to September and some Native Oysters decreased in length, which was attributed to the fragility of Native Oyster shells (Mitchell et al., 2000). Interestingly, in the present study, Farm 3 showed a different pattern in growth rate over the experimental period compared to Farms 1 and 2. At Farm 3, the greatest DGRSL and DGRW was observed between May 2019 and August 2019, however, growth rates were relatively consistent over the study period. This suggests that the Farm 3 location may be more sheltered (i.e., less damage to shells) and experience less temperature variation than Farm 1 or Farm 2. Future studies should assess the impact of temperature and hydrodynamic factors (e.g., wave action, turbidity, tidal flow etc.) on Native Oyster performance to determine the best locations to grow Native Oysters at particular times of the year.

The Native Oyster final live weight was significantly different between farm locations. At harvest, Native Oysters in the control treatments at Farm 1 were ~46% heavier than Farm 2 and ~180% heavier than Farm 3. Additionally, the Native Oysters at Farm 2 were ~ 90% heavier than those at Farm 3. The difference between farms could be environmental. Whilst temperature is often considered to be the main factor influencing oyster performance (Laing et al., 2005), other factors such as chlorophyll a concentrations (i.e., food availability), salinity and turbidity have been shown to impact oyster performance (Celik et al., 2015; Freites et al., 2017). Future studies should aim to measure environmental parameters to determine what variables significantly influence Native Oyster growth. The difference in Native Oyster growth between the farms located at Stansbury (Farm 1 and Farm 2) and Kangaroo Island (Farm 3) observed in this study could also be related to husbandry. On March 26th, 2019, the Native Oysters at Farm 3 only were graded using a commercial grader. Grading could result in damage to oyster shells.

In terms of Native Oyster growth, there was generally no significant difference between final shell length and final live weight of the oysters grown at the sheltered intertidal, exposed intertidal and subtidal sites at Farm 2 and at the first two sites at Farm 3. In the current study, sheltered and exposed intertidal sites were located relatively close (~ 700m) to each other, hence it is probable that they had similar exposure gradients and the difference in energy between the two sites was not extreme enough to equate to differences in oyster performance. At Farm 1, oysters grown at the exposed intertidal site were slightly, but significantly, smaller and lighter than those grown at the sheltered intertidal site. These results are in contrast to Steffani and Branch (2003) who observed faster growth rates on mussels (*Mytilus galloprovincialis*) at exposed sites compared to sheltered sites, which they attributed to higher food availability due to greater water flow. Surprisingly, given that Native Oysters are a subtidal species, at Farm 1 the subtidal site had the poorest performance. Given that there was not a replicate subtidal site and that only one subtidal growing height (- 0.30 m) was analysed, it is not possible to determine if this poor performance is due to site or growing height. However, at Farm 2, the - 0.30 m growing height performed significantly worse than the other subtidal heights, hence it could be that this depth is not optimal for Native Oyster production. A reason for this could be that the - 0.30 m low tide water height was closer to the substrate and possibly experienced greater turbidity. An increase in sediment may decrease the filtration efficiency of bivalves and hence reduce growth rate (Celik et al., 2015). Additionally, subtidal growing heights might have a lower average water temperature than the surface water which may have also impacted on growth. Lee et al. (2017) observed a significant difference in growth in oysters (*Crassostrea gigas*) grown in the surface layer compared to the bottom layer of various sites of the south-east coast of Korea.

Growing height had a significant impact on final shell length and final live weight. Growth rates at different growing heights at the intertidal sites showed different patterns dependant on which farm the Native Oysters were grown. At Farm 1, the – 0.15 m growing height performed the best for final shell

length and final live weight, followed by the 0.00 m growing height, with the + 0.15 m growing height showing the least growth. This result is not surprising, given that Native Oysters are a subtidal species and concurs with La Peyre et al. (2018) who observed a decrease in growth in Eastern Oysters (*Crassostrea virginica*) with daily aerial exposure and suggests that the longer the Native Oysters are submerged in the water, the more opportunity they have to feed, hence the better the growth rate. However, in the current study, at the intertidal sites at Farms 2 and 3, there was little difference in final shell length and final live weight at harvest between growing heights, which is in contrast to Littlewood (1988) who observed better growth in the mangrove oyster (*Crassostrea rhizophorae*) at mid intertidal heights, which was attributed to high air temperatures assisting in energy supplementation through increased assimilation efficiency. Interestingly, at Farm 2, the middle (+ 0.18 m) growing height had the poorest performance for both shell length and weight at both intertidal sites. More research is needed to understand why this occurred; however, it is likely due to a combination of factors including food availability, temperatures (both water and aerial) and hydrodynamic factors.

The results of this study indicate that the optimum growing height for Native Oysters is farm dependant. At Farm 2, the difference in shell length and live weight between the growing heights at the subtidal site was more pronounced at the May 2019 sampling event compared to the final August 2019 sampling event. It is probable that rough weather conditions between May and August did more damage to shells at the highest growing height (0.00 m low tide growing height), which would explain why the Native Oysters within this treatment weighed less at the final August 2019 sampling event compared to the previous May 2019 sampling event. Studies using dry tissue weight might provide further insight into Native Oyster performance at varying growing heights.

The shape scores reported in this study are higher than the 4.5 score that is considered to be the optimal shape for a Sydney Rock Oyster at harvest (Rankin et al., 2018). This, however, is expected as the shape score was developed for cupped oysters not flat oysters, such as the Native Oyster, which are generally wider and not as deep. Although consumer's preference to this index in Native Oysters is not available, the results from this study suggest that the shell shape of Native Oysters could be affected by farming locations. When comparing the Native Oysters grown in control treatments, those at Farm 2 had a lower shape score than those at Farm 1 and at Farm 3. Rankin et al. (2018) observed a significant difference in the shell shape of Sydney Rock Oysters (*Saccostrea glomerata*) between NSW estuaries and concluded that environmental conditions can influence morphometric qualities. There was generally no difference in shape score between intertidal sites. In general, the subtidal treatments (- 0.30 m low tide growing height at Farm 1 and - 0.15 m and – 0.30 m low tide growing height at Farm 2) had the highest shape scores, which equates to a less desirable shape in cupped oysters in Australia. This is likely related to the poorer growth rates observed in these treatments and less water energy at these growing heights (Brake et al., 2003). At Farms 1 and 3, the highest growing height (+ 0.15 m) had the lowest shape score whereas at Farm 2 intertidal growing heights were not significantly different for shape score. Water movement, particularly wave disturbance in the surface waters, is considered an important factor in shaping oysters (Brake et al., 2003; Mizuta and Wikfors, 2019). It is possible that the growing heights used at Farm 2 in this present study are not different enough in terms of water energy and more extreme growing heights should be tested in future studies. However, cautions should be taken as Native Oysters are a subtidal species. Additionally, there is a need to develop a shape score specific for flat oysters, such as the Native Oyster. The optimal 4.5 shape score developed for cupped oysters (Rankin et al., 2018) is likely not achievable in Native Oysters, which are naturally not as deep. Shell appearance and shape is an important factor in defining oyster quality and, ultimately, influences market price (Mizuta and Wikfors, 2019), hence, a shape score to define and compare flat oyster species would be valuable.

The equipment used to grow oysters has been shown to have a significant impact on growth and shape (Rankin et al., 2018; Thomas et al., 2019). In this experiment, there was no difference in shell length, live weight or shape score between the Native Oysters initially grown in trays compared to those grown in baskets for the entire experiment. This indicates that the initial equipment used to grow Native Oysters in this study did not impact the final size or shape of the animals.

One of the limitations of this study was that the Native Oysters were not graded over the experimental period in line with commercial practice, except for the unintentional grade at Farm 3. This may have impacted on the growth results, as the larger Native Oysters in the treatments may have started to out-compete the smaller ones. This also led to a high variation in size. At Farm 1 and Farm 2, the length of Native Oyster in the treatments at growing height equal or higher than – 0.15 m MLW tidal height changed marginally over the period from May to August 2019. This is possibly due to both low water temperatures reducing growth rates and rough weather conditions/ hydrodynamic factors, such as wave action, potentially breaking or "chipping back" the fragile Native Oyster shell. The "chipping back" of the Native Oyster shell frills is possibly beneficial for improving Native Oyster shape. It has been reported that oysters subjected to frequent disturbance tend to grow deeper in Pacific Oysters (Brake et al., 2003; Kube et al., 2011). In the present study, treatments at Farm 1 and Farm 2 recorded better shape scores than Farm 3, which had increased the shell length over the period from May to August 2019.

Early delays to this project, primarily the unavailability of spat, limited the time frame of the field trial. Ideally, future studies should assess survival and growth of Native Oysters from deployment at the farm until they reach harvest size. *Bonamia* infections have been shown to increase in intensity with oyster age, which may impact survival (Arzul et al., 2011). Additionally, Native Oyster mortality has been linked with maturation (Celik et al., 2015). Spawning events have been shown to significantly impact the immune capability of oysters (Li et al., 2009). Given that sexual maturation can also affect growth rate and condition of oysters (Li et al., 2009), it would be valuable to determine the average age / size that Native Oysters reach maturation in South Australia.

Future field trials with Native Oysters should consider a rotation system between sites and / or growing heights, as is common practice in Pacific Oyster farming, to take advantage of different seasonal environmental conditions to maximise growth and / or improve shape score. Given the significant differences observed between farm locations in this present study, any rotation study would likely be site specific, hence recording environmental parameters (e.g., *Bonamia* infection, chlorophyll a concentration, temperature, wave action, tidal speed, turbidity, etc.) would provide useful information for best growing conditions for Native Oysters in South Australia.

Differences in performance between Pacific Oysters and Native Oysters in South Australia

Except for the Farm 3 subtidal site, survival in this study was high and comparable to Pacific Oysters grown in Gulf St Vincent, South Australia (Li et al., 2009). In South Australia, Pacific Oysters take 1 – 2 years to reach market size. There is currently no standard market size for Native Oysters in Australia. The overall growth rates in terms of shell length for Native Oysters observed in this current study are comparable to the von Bertalanffy growth curve developed for *O. anagsi* by Mitchell et al. (2000), indicating that Native Oysters would take 3-4 years to reach market size in South Australia. However, in the present study, the Native Oysters were not graded as per commercial practice. Grading can improve overall growth rates as it allows for the removal of the slowest growing individuals and reduces inter-oyster competition between large and small oysters (Rubio, 2010).

To get a better estimate of the commercial growth rate of Native Oysters, at the final August 2019 sampling event, the stock from the control treatment (sheltered intertidal site at 0.00 m low tide growing height) were coarsely graded into large and small size classes based on the mean shell length (Farm 1 ~ 50 mm shell length; Farm 2 ~ 45 mm shell length; Farm 3 ~ 40 mm shell length). From each of the three replicate baskets, 45 Native Oysters from each size class were weighed and measured. The results for the large size class are presented in Table 3.5.

The shell length of the large size class oysters (~50% of the control treatment) at Farm 1 and Farm 2 was greater than the ~ 48 mm shell length the von Bertalanffy growth curve model for wild Native Oysters (Mitchell et al., 2000) predicts for 12 months growth in Tasmania, Australia, which indicates that, at these South Australian locations, Native Oyster growth is faster and the time to reach market size would be less than the 3 – 4 year estimate. Farm 3 shell length was comparable to that of the

growth curve (Mitchell et al., 2000) highlighting the importance of farm location for maximising growth in Native Oysters. Over the 13 month trial period, the large size class at Farm 1 and Farm 2 reached a comparable shell length as that required for a bistro Pacific Oyster (50–60 mm, Nell, 2001), but not comparable live weights (~33 g). Native Oysters are naturally flatter than Pacific Oysters and unlikely to reach similar shell heights. Regarding final live weight, it is expected that DGRW would reduce as the Native Oysters age. If the DGRW was to halve over the following 12 months, then Native Oysters at Farm 1 would reach a market weight of + 30 g (comparable to a bistro Pacific Oyster) after two years on farm. Farm 2 and Farm 3 would take longer to reach market size. In addition, the Native Oysters might grow better if they are farmed at the most productive Pacific Oyster farming locations (e.g., Coffin Bay) in South Australia.

Table 3.5 Native Oyster (*Ostrea angasi*) large size class (Farm 1 > 50 mm shell length; Farm 2 > 45 mm shell length; Farm 3 > 40 mm shell length) performance (mean ± SD; n = 3) in the cross-farm control treatment at three farm locations (sheltered intertidal; at 0.00 m low tide growing height) in Gulf St Vincent, South Australia, 13 months post stocking*.

	Farm 1	Farm 2	Farm 3
Final shell length (mm)	59.32 ± 1.76	51.41 ± 2.25	48.35 ± 1.97
Overall DGRSL (µm / d)	126.29 ± 4.80	104.94 ± 6.15	94.49 ± 5.29
Final live weight (g)	20.47 ± 1.44	14.00 ± 1.78	8.45 ± 0.80
Overall DGRW (mg / d)	55.25 ± 3.93	37.68 ± 4.84	21.99 ± 2.16

* Initial shell length = 13.1 ± 4.6 mm and initial live weight = 0.25 ± 0.24 g. DGRSL = daily growth rate for shell length and DGRW = daily growth rate for weight.

3.2 Determination of on-farm growing methods that maximise survival and growth of Native Oysters in Tasmania

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Methods

Collection of wild Native Oyster spat.

Zapco spat collectors (Zapco Aquaculture, Perth, Western Australia) were deployed at Blackman Bay, Dunalley and Cloudy Bay, Bruny Island, Tasmania in November 2017. These bays were known to produce substantial quantities of wild Native Oyster spat and Pacific Oyster farmers at these locations have shown interest in farming Native Oysters. The collectors were placed in the low intertidal and subtidal at both locations. Spat were removed from the collectors in Blackman Bay in March and Cloudy Bay in April 2018.

The spat collected in Blackman Bay were estimated to be 5-10 mm in length. They were placed in the intertidal sheltered area on the middle clip (approximately 0.30 m from MLW tidal height) in 3mm SEAPA baskets and not handled until June 2018 when doubles were split and oysters were rehoused, within 61 baskets in intertidal waters and 25 baskets in subtidal waters. Oysters from this cohort of intertidal oysters were used for the grow-out trial that commenced in November 2018.

Hatchery production of Native Oyster spat.

In late spring 2018, we worked with Spring Bay Seafoods to produce Native Oyster spat from their hatchery at Triabunna, south-eastern Tasmania. They had not previously reared *O. angasi* larvae.

Two spawning trials were conducted using broodstock collected from Georges Bay, St Helens, Tasmania on two occasions by IMAS divers. On November 7th, 2018, 80 Native Oysters > 90 mm were collected at ambient water temperature (13.8 °C). They were held in a biosecure system at Spring Bay Seafoods at 13.5° C until November 20th, 2018, when the broodstock were anaesthetised using 5% $MgCl_2$, and larvae were gently rinsed from the gills.

On December 5th, 180 Native Oyster broodstock were collected from Georges Bay, at water temperature 14.7 °C, anaesthetised with $MgCl_2$, and any larvae present in the gills were flushed out.

Resultant larvae were fed a diet of *Chaetoceros meulleri* and *Pavlova lutheri* at a rate of 50,000 to 110,000 cells per larva per feed and reared at 23°C. Larvae were set when eyed using epinephrine bitatrate at $1x10^{-3}$ M for 1 hour. Once spat had settled they were placed in downwellers for two weeks (Figure 3.14), then upwellers at a water temperature of 21°C and fed a diet of mainly *Chaetoceros meulleri* until deployed to field sites.

Figure 3.14 Native Oyster (*Ostrea angasi*) spat production during the downweller nursery phase at Spring Bay Seafoods, Triabunna, Tasmania, Australia.

Phase one: Native Oyster spat grow out trials

Hypotheses for development of Native Oyster on-farm growing methods were:

1. The largely subtidal species *O. angasi* would grow faster with greater survival in subtidal compared to intertidal locations,

2. Native Oysters are fragile, therefore growth and survival would be greater in calmer and more sheltered areas compared to more exposed or rougher areas.

3. Intertidal areas would positively influence the shape of *O. angasi*.

Hypotheses 1-3 were tested at Blackman Bay, Dunalley, Tasmania (Figures 3.15 & 3.16), utilising four growing heights: three intertidal heights on a rack and line system with suspended baskets and one subtidal (Figure 3.17). Intertidal clip heights were determined as top clip (TC) 0.45 m, middle clip (MC) 0.30 m, and bottom clip (BC) 0.15 m above MLW mark on existing oyster basket lines. Subtidal baskets were hung in a ladder formation from a floating line in groups of six at approximately 1 m below the water surface. All Native Oysters were housed in SEAPA baskets with 20 mm mesh to allow comparable mesh size to be used for the subtidal and intertidal systems.

These growing heights were tested at both sites (Site 1 = exposed and Site 2 = sheltered, Figures 3.15 & 3.16). Site 1 experienced strong prevailing winds from the west-northwest, whereas Site 2 was at the back of the bay and protected from wind and waves by land and other racks (Figure 3.17). Tidal range during the trial was 0.1 m low to 1.4 m high.

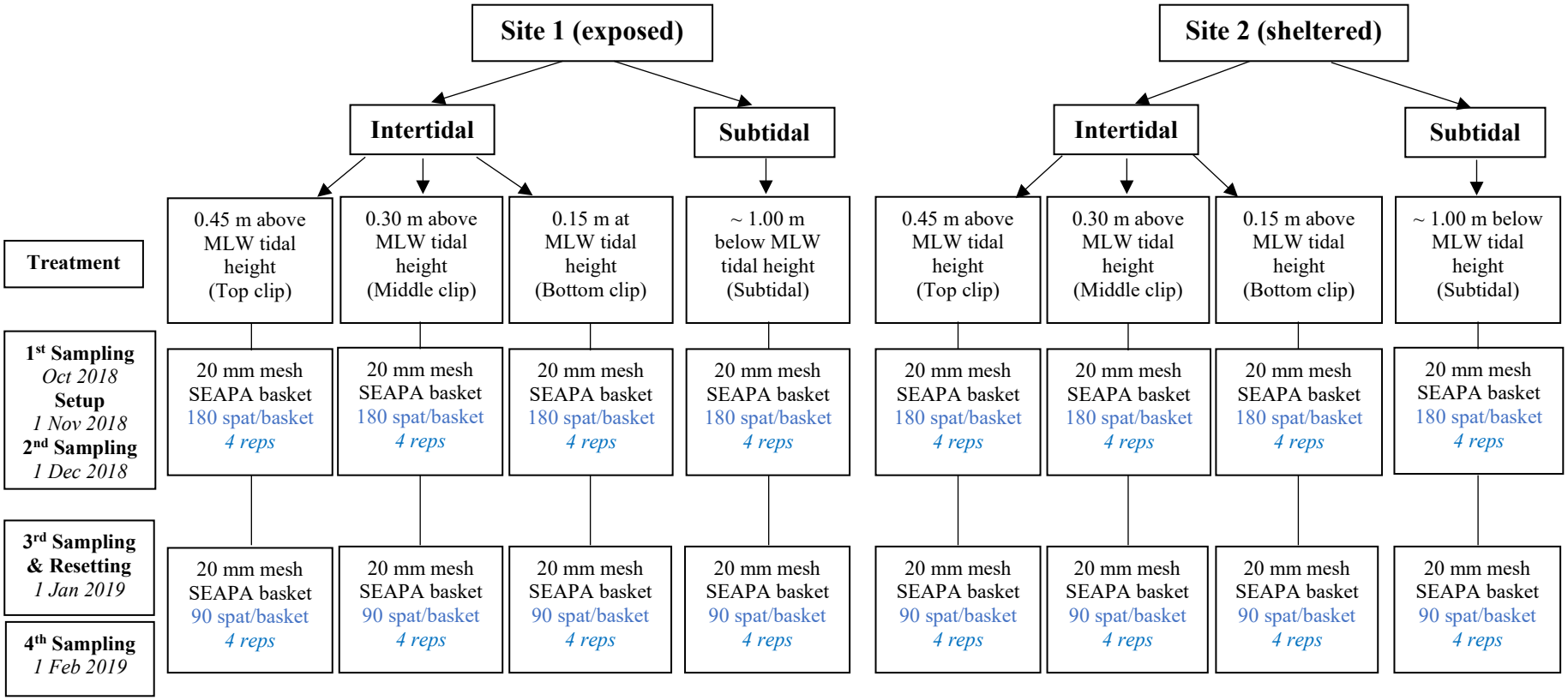


Figure 3.15 Native Oyster (*Ostrea angasi*) grow-out experimental design and setups at Blackman Bay, Dunalley, Tasmania, Australia. MLW = mean low water.

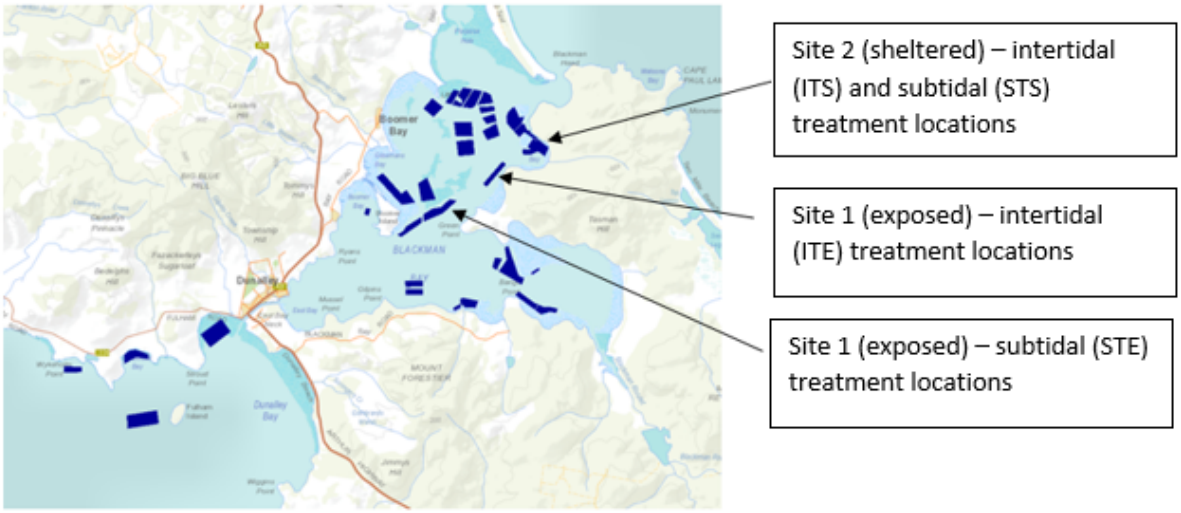


Figure 3.16 Location of Native Oyster (*Ostrea angasi)* grow-out trial sites in Blackman Bay, Dunalley, Tasmania, Australia. Dark blue polygons indicate commercial oyster lease sites.

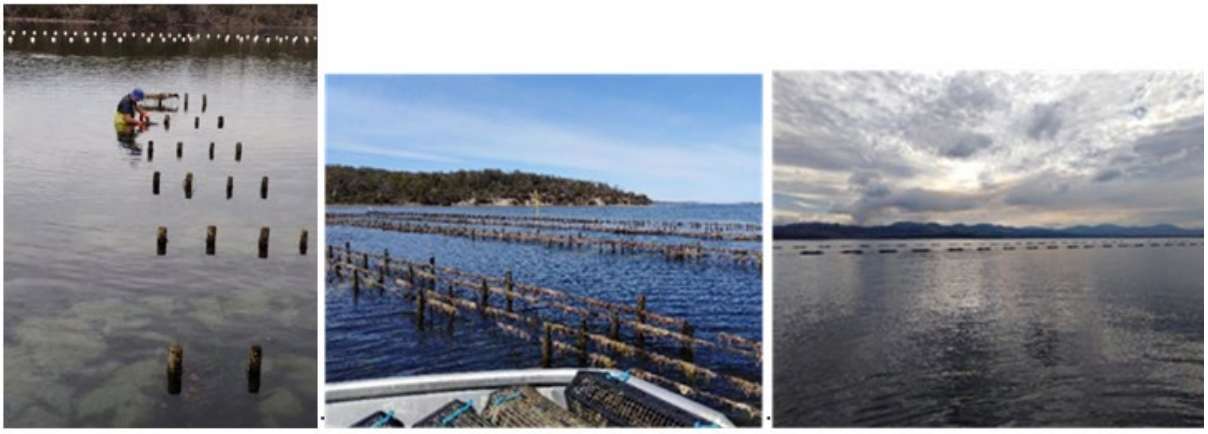


Figure 3.17 Images of grow-out trial sites for Native Oysters (*Ostrea angasi*) in Blackman Bay, Dunalley, Tasmania, Australia. Intertidal sheltered (foreground) and subtidal sheltered (white buoy line; left), intertidal exposed (middle) and subtidal exposed longlines (right).

In October 2018, 61 SEAPA baskets containing approximately 31,000 *O. angasi* spat that were wild caught over the summer of 2017/18 were graded using a mechanical custom-built rotating barrel grader. Middle grade spat, 35 mm diameter hole section were selected (Figure 3.18). The majority of the cohort were this grade size.

Figure 3.18 Rotating barrel grader selecting for 35 mm hole size grade (largest pile in photo) Native Oysters (*Ostrea angasi*) for grow-out trials in Blackman Bay, Dunalley, Tasmania, Australia.

Native oyster densities in the baskets were based on having a single layer of Native Oysters on the bottom of the basket. The volumetric density determined in this manner was equivalent to 180 Native Oysters per basket for the grade (35 mm) obtained.

A random sample of Native Oysters (n = 70) was measured for shell length (dorso-ventral measure [DVM]), width (anterio-posterio measure [APM]) and depth (Figure 3.19) using digital callipers. From this initial sample, the minimum sample size to measure in subsequent observations was determined as 26 Native Oysters per basket to obtain a mean within 5% of the population's average. Initial average size shell length of the Native Oysters was 42.9 ± 6.6 mm.

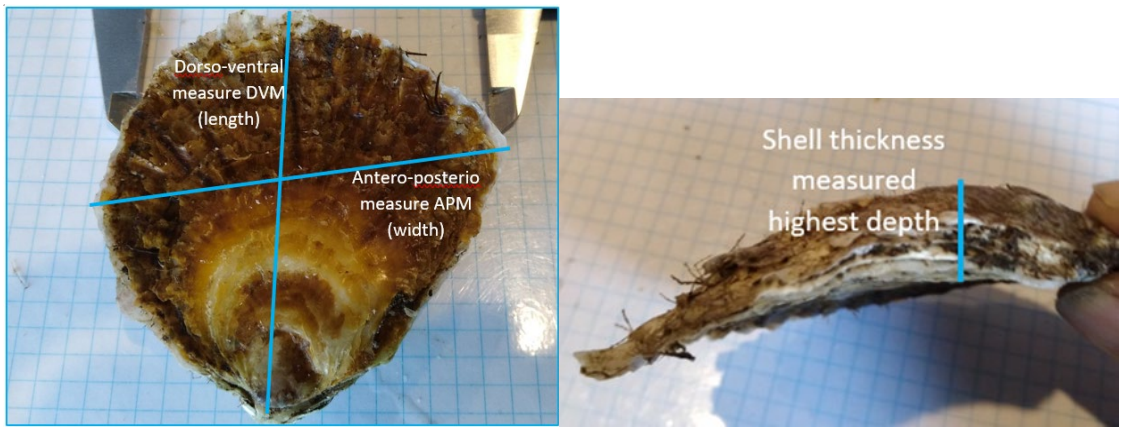


Figure 3.19 Shell length (DVM), width (APM) and depth measurements taken for Native Oysters (*Ostrea angasi*) in the grow-out trials.

All Native Oysters were deployed on the farm at an intertidal sheltered location at middle clip height for a two-week recovery period following grading and handling and before commencing the trials.

Trials commenced on November 1st, 2018, with four replicate baskets per treatment (Figure 3.20). The total biomass of Native Oysters in each basket was weighed before deployment. One Hobo® UA-001-08 pendant temperature datalogger (Onset Computer Corporation, Bourne, Massachusetts, USA) was placed in one basket from each treatment (top clip, middle clip and bottom clip) at the intertidal sheltered and intertidal exposed sites to estimate the duration of exposure to air based on temperature differences at each basket clip height. A SONDE data logger (YSI Exo2, YSI Incorporated, Yellow Springs, Ohio, USA) deployed in the sheltered lower intertidal recorded temperature and salinity at half hourly intervals. Outlier data were excluded from analysis when the SONDE data logger was

exposed to air. Data from the SONDE data loggers are not available from the end of January 2019, three weeks prior to the end of the trial, because of problems with the equipment. Temperature profiles for February 2019 were captured by Hobo ® pendant data loggers.

Figure 3.20 SEAPA baskets containing Native Oysters (*Ostrea angasi*) ready for deployment as intertidal baskets (left) and in ladder formation for subtidal treatments (right).

Native Oysters were sampled every month and total biomass of each basket and any mortalities in each replicate were recorded. To account for the changes in growing density that occurred during the trial (from observation 2 - 3), survival was calculated for each basket as the average of survival over the three sample periods, weighted for the duration of each observation period. Twenty six randomly selected Native Oysters from each replicate were measured for shell length, shell width and shell depth. A shell shape score of (shell length / shell width) / (shell depth / shell length) was calculated for each measurement (Rankin et al., 2018).

At the January 2019 sampling event, the density in each basket was halved (90 per basket) to reflect farm practice and maintain growth.

At the end of the experiment and after all Native Oysters had been measured, they were placed in the sheltered middle clip area for four weeks prior to commencement of Phase two – market conditioning.

Whole Native Oyster samples were randomly chosen for cellular energy analysis (CEA), with approximately 16 Native Oysters from each treatment (i.e. 4 per replicate) used. Three replicates were pooled for each treatment due to low meat weights (2 g fresh wet weight) as freeze drying required a minimum 0.5 -1g dry weight (Jill Bartlett pers. comm). These Native Oysters were freeze dried, then crushed to a fine powder and scanned in duplicate per sample using a portable near infrared spectrophotometer NIRS Labspec® 5000 fitted with ASD pro reflectance probe (Analytical Spectral Devices Inc., Boulder, Colorado, USA) at CSIRO Marine Laboratories, Hobart, Tasmania. Spectral data analysis was performed using Grams AI and Grams IQ software (v. 9.0; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). Chemical composition data determined by the reference (calibration) sample sets were loaded into the software and models developed by Bartlett et al. (2018) using near-infrared spectroscopy (NIRS) quantitative model generation. Total available energy stores, total glycogen, protein and lipid content and the energy consumption (E_c) were estimated using models developed by Bartlett et al. (2018).

Water samples were taken monthly for nutrients, nitrates and phosphates, total dissolved (TDS) and total suspended solids (TSS). These samples were vacuum filtered on Whatmans G/F filters and frozen (- 20 ºC) for later analysis.

Data analysis

Data were analysed using three-way ANOVA with independent factors of height (four clip heights), site (Site 1 and Site 2) and time (three sampling events). In instances when site did not have a significant effect, data were pooled to compare the effect of clip height using one-way ANOVA. The Least Significance Difference (LSD) post hoc test was used to analyse for differences between treatments. T-test pair wise comparisons were used as required. Data were tested for normality with Shapiro-Wilk and homogeneity of variance with Leven's using SPSS v. 26 statistical package (IBM, Armonk, New York, USA). The significant level was set at $P < 0.05$.

Ongoing Research

The following trials were started when this chapter was written, but they were not completed due to the departure of key project staff.

Phase two: Market conditioning of Native Oysters trial

This trial was designed to investigate the effects of Native Oyster density, position in the water column (subtidal/intertidal) and a rotational movement between water depths, on Native Oyster growth, survival, and condition.

Treatments were: subtidal/mixed - in subtidal ladders for 4 or 10 weeks, then intertidal for 2 weeks, then back to subtidal ladders for 4 or 10 weeks; and intertidal - bottom clip intertidal for 4 or 10 weeks, then top clip for 2 weeks, then back to bottom clip for 4 or 10 weeks. Each treatment has six replicates with 40 or 60 oysters in 20 mm mesh SEAPA baskets.

Water quality parameters were monitored using temperature HOBO® pendant data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA), SONDE data loggers (YSI Incorporated, Yellow Springs, Ohio, USA) and sampling for chlorophyll a, TSS, TDS and nutrients continued as in Phase One. In addition, motion sensor pendant loggers HOBO® UA-004-64 (Onset Computer Corporation, Bourne, Massachusetts, USA) were deployed to compare water movement.

Hatchery Native Oyster spat growth trials at three locations

The objective of this field trial was to monitor the growth and survival of hatchery produced Native Oyster spat at subtidal and intertidal locations at farms at Dunalley, Coles Bay and Bruny Island, Tasmania using different spat grow out technology. The choice of gear was determined by the farmers based on what they use on their farm and what they thought would be most suitable for *O. angasi* spat. A cost benefit analysis was planned to provide a comparative measure of efficiency of different systems throughout the spat growth phases.

Hatchery produced *O. angasi* spat (n = 44,000) from Spring Bay Seafoods were deployed at Dunalley in April 2019 into SEAPA baskets at farmer determined densities. On May 1st, 2019, a second batch of hatchery produced spat were deployed at Coles Bay in the sheltered intertidal area of the farm below low tide height. The Native Oysters were evenly distributed into six SEAPA 20 mm mesh baskets with 1 mm SEAPA mesh inserts at approximately 8,000 spat per basket. In August 2019 the remaining (approximately 42,000) spat were deployed in SEAPA baskets in the intertidal area at Simpsons Bay, Bruny Island, Tasmania.

Results

Collection of wild spat

Native Oyster spat were removed from the collectors in Blackman Bay, Dunalley in March 2018, with 44,000 spat taken from approximately 300 collectors. In Cloudy Bay, Bruny Island, approximately 5,000 spat were collected in April 2018. The collectors and spat at Blackman Bay were relatively free of biofouling, whereas those at Cloudy Bay had a heavy coating of cunjevoi (*Pyura* sp.).

In April 2019, approximately 9,000 Native Oyster spat of length 10-20 mm were collected from 276 slats in Blackman Bay. There was biofouling (red and brown algae, barnacles and ascidians) on collectors and the top collectors caught very little due to smothering with sediment.

Hatchery production of Native Oyster spat

For the first spawning with 80 Native Oyster broodstock, only 7% had 'white sic' larvae; the other 93% of broodstock had already spawned. Approximately 8 million larvae were collected with a recovery of 37.8%, resulting in 3.8 million larvae for rearing.

Of the 180 Native Oyster broodstock used for the second spawning, 96.6% were spent. 7.7 million larvae at 'grey sic' stage and 1.5 million larvae at 'white sic' stage were recovered.

The number of broodstock contributing to each of the spawning events was low 3.5-7% (n = 6) due to the variability of reproductive stage between individuals and because spawning had already commenced at water temperatures between 12-13°C.

The total number and screen size of Native Oyster spat deployed to field trial locations is listed in Table 3.6. A total of 105,917 *O. angasi* spat were produced in the size range of 3 mm – 8 mm screen i.e., 4 mm to 10 mm Native Oysters.

Table 3.6 Total number per screen size of Native Oyster (*Ostrea angasi*) spat produced at Spring Bay Seafoods, Triabunna, Tasmania, Australia and the location and date of deployment to grow-out trial sites.

Spat size (screen)	Number	Location	Deployment date
3mm	10936	Dunalley	3/4/2019
4mm	23428	Dunalley	3/4/2019
6mm	9755	Dunalley	3/4/2019
3mm	48327	Coles Bay	1/5/2019
4mm	29329	Bruny Island	16/8/2019
6mm	7800	Bruny Island	16/8/2019
8mm	5671	Bruny Island	16/8/2019

Phase one: Native Oyster spat grow out trials

Native Oyster survival

Native Oyster survival was consistently high 99.73 ± 0.11% to 100.00 ± 0.00% throughout the trial. One replicate was lost from the Site 1 treatment on intertidal top clip due to rough weather.

Native Oyster Growth

Shell length (DVM) increased over the duration of the experiment and was not influenced by site (P = 0.567; ANOVA; Figure 3.21; Appendix 1 – Table A1.1). There was a significant effect of clip height, which was consistent throughout the experiment (time x height; P = 0.079), and after four months oysters in the top clip treatment were significantly smaller in length than those at the lower heights (P < 0.001; Figure 3.22).

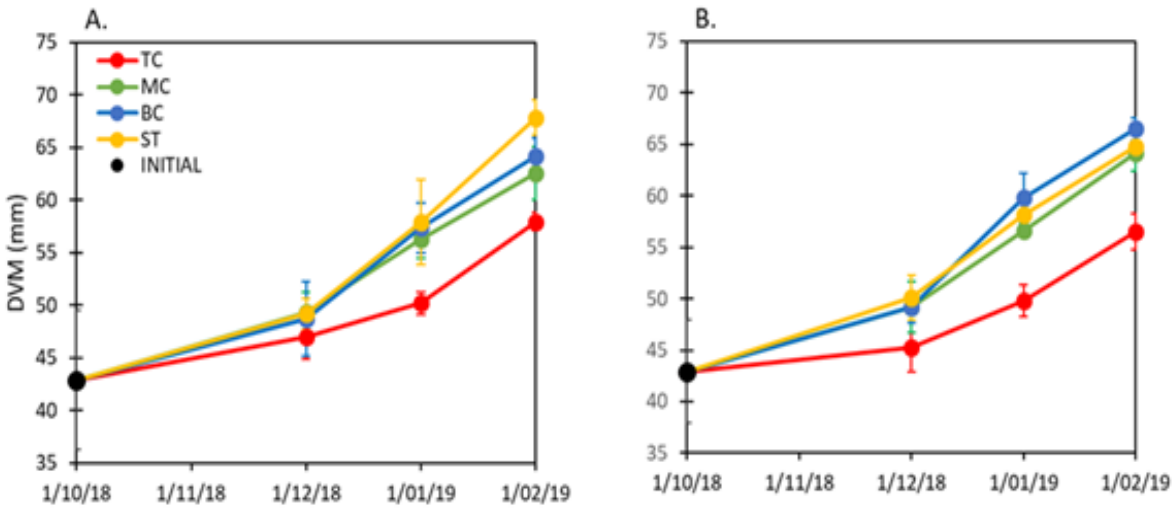


Figure 3.21 Shell length (DVM; mean ± SD) of Native Oyster (*Ostrea angasi*) grown at four clip height treatments. Observations from Site 1 (A) and Site 2 (B). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface. Initial DVM was 42.9 ± 6.6 mm.

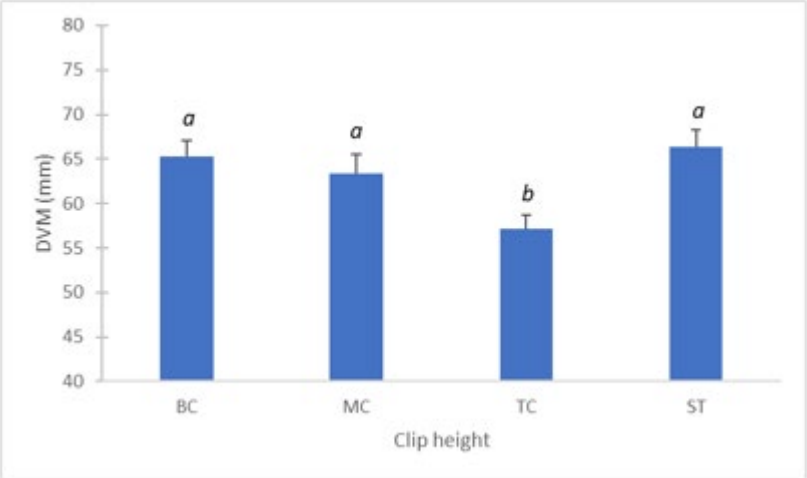


Figure 3.22 Final shell length (DVM; mean ± SD) of Native Oyster (*Ostrea angasi*) at both sites combined. Clip heights with different superscript letters were significantly different ($P < 0.001$). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

Native Oyster shell width (APM) increased significantly during the experiment ($P < 0.001$; ANOVA; Figure 3.23; Appendix 1 – Table 10.2), and there was a significant effect of site ($P < 0.01$) and clip height ($P < 0.001$; Figure 3.24). The effect of site was consistent throughout the experiment (time x site; $P = 0.119$), whereas the effect of clip height changed during the experiment, becoming more pronounced with time (time x height; $P < 0.001$; Figure 3.23). Width was significantly greater in Native Oysters grown at Site 2 ($P < 0.01$) and significantly less in top clip Native Oysters compared to all other treatments ($P < 0.001$). Bottom clip intertidal Native Oysters were significantly wider than subtidal Native Oysters ($P < 0.01$; Figure 3.24). Even though the Native Oysters at Site 1 were smaller than those grown at Site 2 ($P < 0.01$), the effect of clip height was the same across site (site x height; $P = 0.082$).

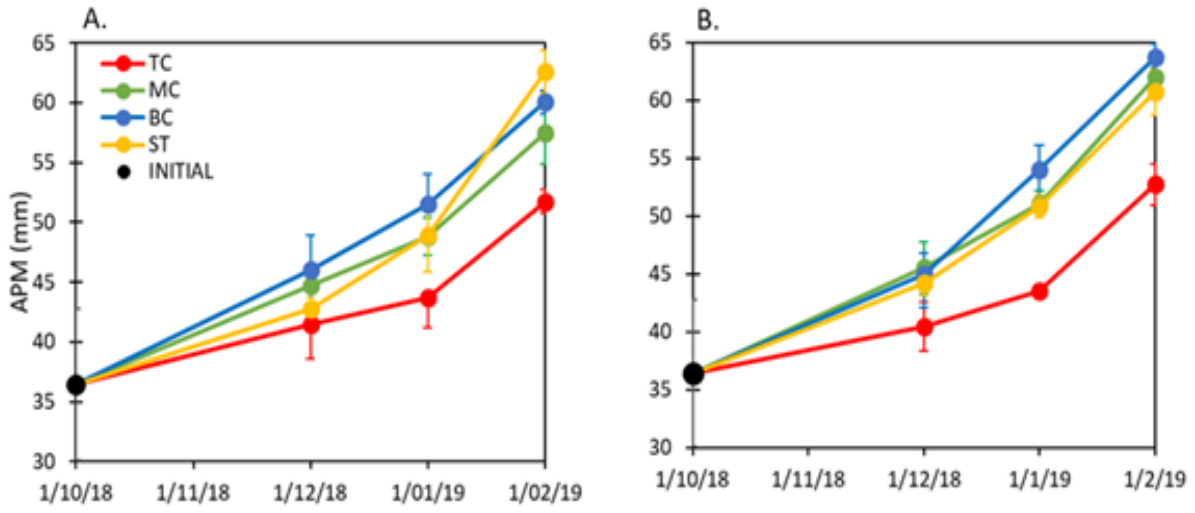


Figure 3.23 Shell width (APM; mean ± SD) of Native Oyster (*Ostrea angasi*) at four clip heights. Observations from Site 1 (A) and Site 2 (B). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

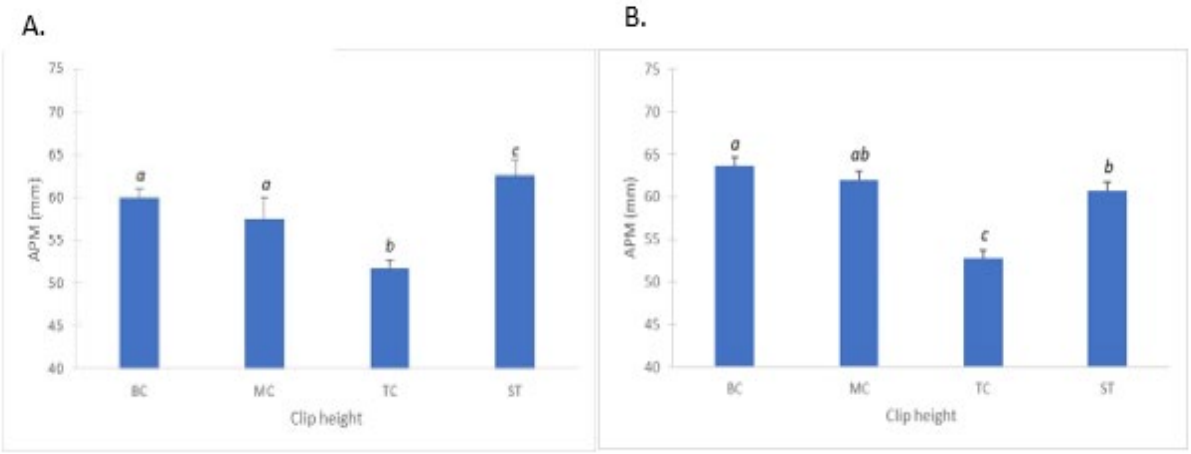


Figure 3.24 Final shell width (APM; mm; mean ± SD) of Native Oysters (*Ostrea angasi)* at four clip heights at Site 1 (A) and Site 2 (B). Clip heights with different superscript letters were significantly different ($P < 0.01$). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

Shape score declined significantly during the experiment ($P < 0.001$; ANOVA; Figure 3.25; Appendix 1 – Table 10.3). There was no effect of site ($P = 0.236$), but clip height was significant ($P < 0.01$), with subtidal Native Oysters having a higher shape score than middle and top clip Native Oysters (Figure 3.26). The effect of site and height were consistent throughout the experiment (time x site; $P = 0.298$; time x height; $P = 0.411$).

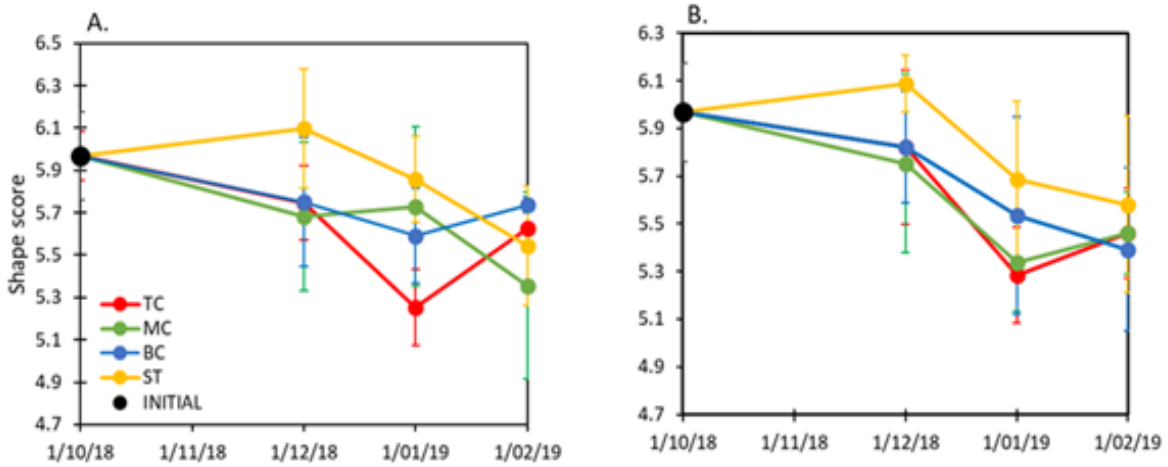


Figure 3.25 Shape score (mean ± SD) of Native Oyster (*Ostrea angasi*) at four clip heights. Observations from Site 1 (A) and Site 2 (B). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

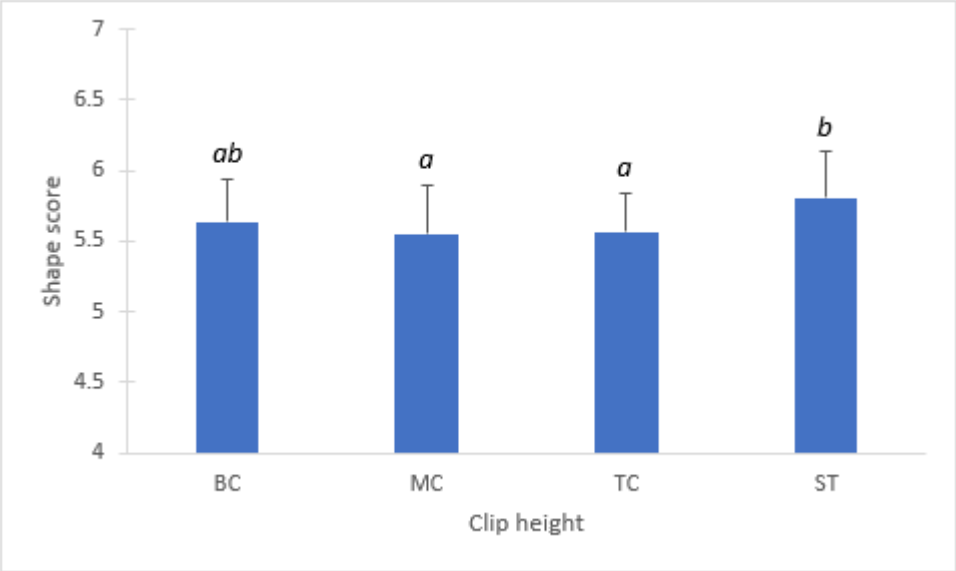


Figure 3.26 Overall shape score for combined sites (3 observations made at each site; mean ± SD) for Native Oysters (*Ostrea angasi*) grown at different clips heights. Clip heights with different superscript letters were significantly different ($P < 0.01$). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

Live weight of Native Oysters increased significantly throughout the experiment and there was no overall effect of site ($P = 0.105$; ANOVA; Appendix 1 – Table 10.4). Clip height had a significant influence on live weight ($P < 0.001$); however, the clip height effect was different between two sites (site x height; $P < 0.001$; Figures 3.27 & 3.28). By the end of the experiment this differential effect of clip height with site was observed in bottom and middle clip Native Oysters, where Native Oysters at Site 1 were lighter than those at Site 2 ($P < 0.05$; t test). By contrast, the subtidal and top clip treatments were heavier at Site 1 compared to Site 2 ($P < 0.05$; Figure 3.29).

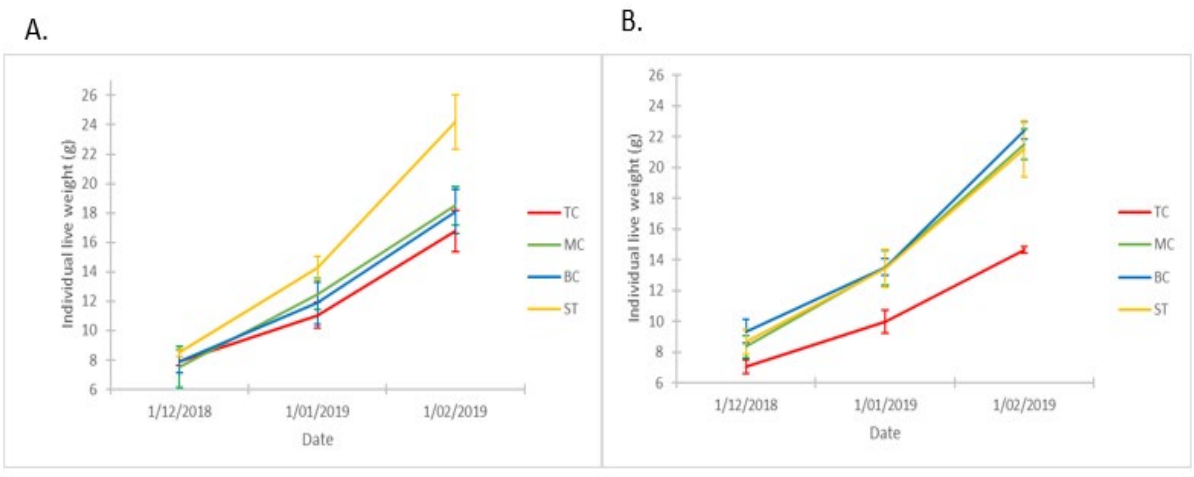


Figure 3.27 Individual live weights (mean ± SD) of Native Oysters (*Ostrea angasi)* grown at four clip height treatments. Observations from Site 1 (A) and Site 2 (B). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

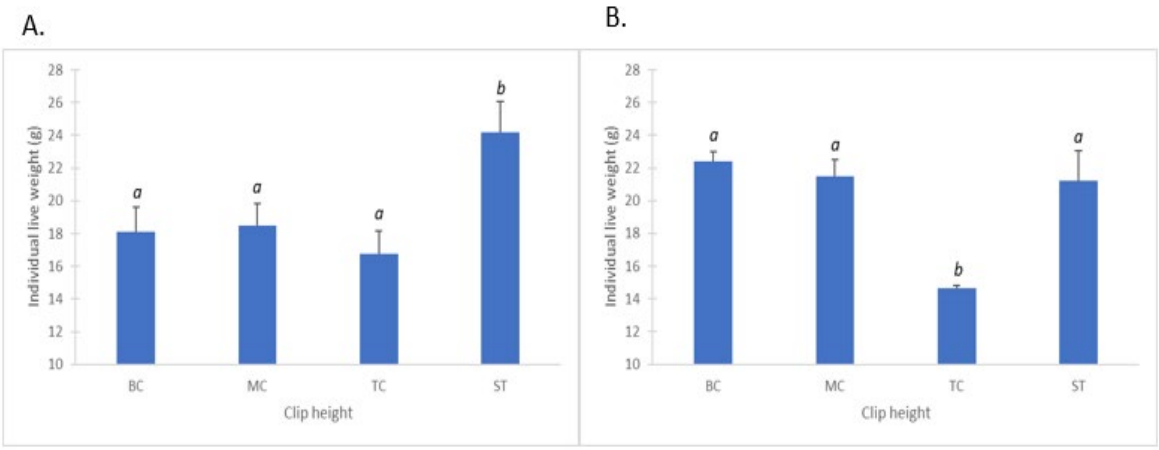


Figure 3.28 Individual final live weight (mean ± SD) of Native Oysters (*Ostrea angasi*) grown at four clip heights at Site 1 (A) and Site 2 (B). Clip heights with different superscript letters were significantly different ($P < 0.05$). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

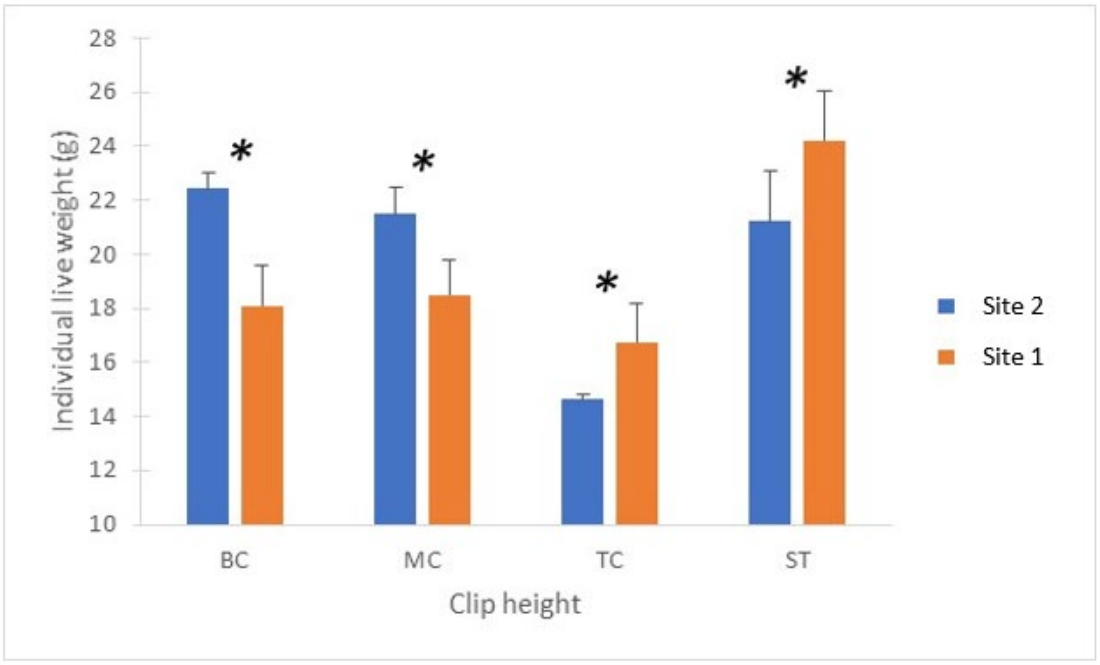
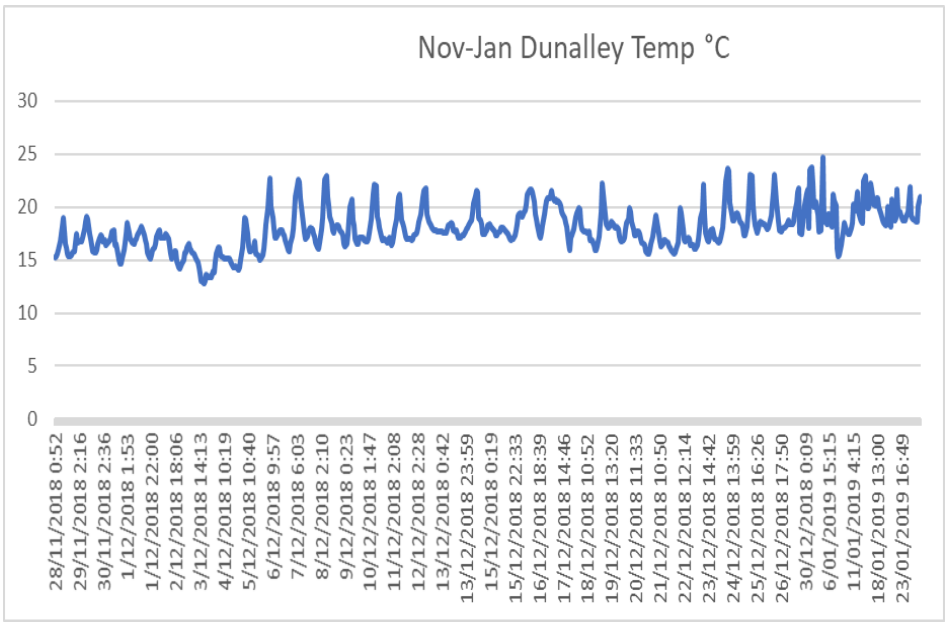


Figure 3.29 Comparison of the differential effect of site on the final live weight (mean ± SD) of Native Oyster (*Ostrea angasi*) grown at different clip heights. Asterisks designate clip heights where site had a significant effect on individual weight ($P < 0.05$; t-test). TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

Water quality

Temperature ranged from 12.08 to 26.1 °C during the trial and salinity ranged from 24.7 to 34.7 ppt (Figure 3.30).

(A)



(B)

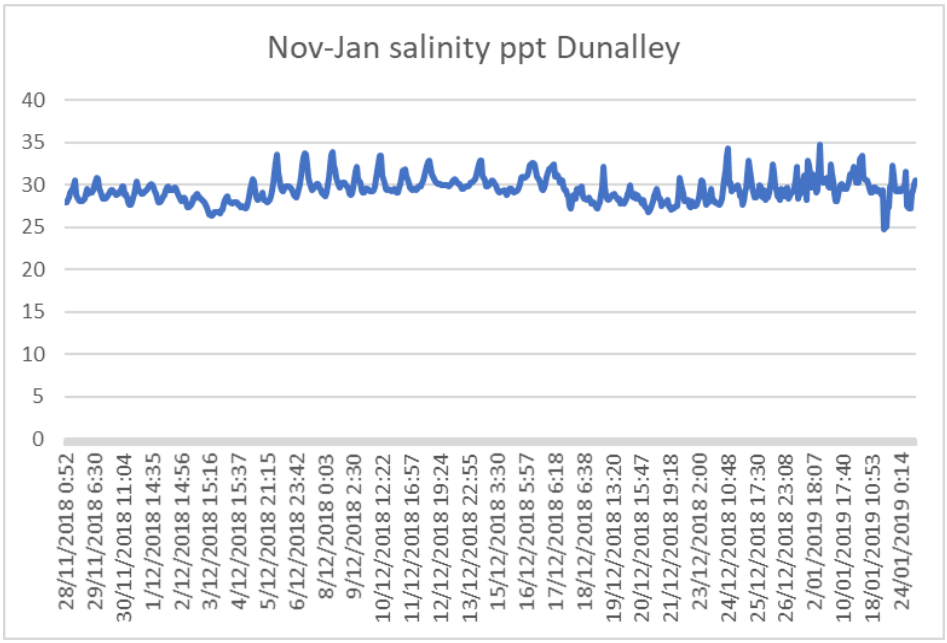


Figure 3.30 Temperature (°C; A) and salinity (ppt; B) profile recorded by SONDE data loggers (YSI Exo2) from November 2018 to January 2019.

Cellular energy allocation

Cellular energy and proximate composition for glycogen, protein and lipid showed that all treatments had a positive cellular energy. Composition was similar to market sized *Ostrea angasi* from Taranna, Tasmania sampled in September 2018, where CEA was 543 (glycogen 295.29 mg/g, protein 206.46 mg/g and lipid 19.11 mg/g; unpublished data). Positive values for CEA represent good oyster condition and values obtained in this study reflect healthy oysters with no obvious treatment

differences for proximate composition. There was no significant treatment effect ($P > 0.05$) for site or height on any of the factors listed in Table 3.7.

Table 3.7 Treatment results for cellular energy allocation and chemical proximate composition (mg / g; mean ± SD) in Native Oysters (*Ostrea angasi*) at two sites (Site 1 and Site 2) and four clip heights.

Treatment*	Mean CEA	±SD	Glycogen (mg/g)	±SD	Protein (mg/g)	±SD	Lipid (mg/g)	±SD
ITE-TC	993.99	164.29	263.72	9.82	223.82	8.50	17.33	0.87
ITE-MC	1001.10	137.73	272.10	6.21	224.04	7.73	17.07	0.55
ITE-BC	864.70	181.37	264.02	10.47	213.18	2.66	17.54	0.23
STE-ST	678.87	131.43	262.83	4.24	210.69	9.69	17.16	0.62
ITS-TC	1099.96	379.15	268.51	5.68	216.27	4.41	17.90	0.35
ITS-MC	868.74	125.31	263.29	0.28	215.10	0.62	17.66	0.25
ITS-BC	873.50	216.13	262.55	8.14	222.28	5.32	16.99	0.25
STS-ST	683.14	199.99	259.58	4.23	216.89	3.61	17.69	0.31

* ITE = Site 1 intertidal, STE = Site 1 subtidal, ITS = Site 2 intertidal, STS = Site 2 subtidal, TC = top clip - 0.45 m above MLW tidal height, MC = middle clip - 0.30 m above MLW tidal height, BC = bottom clip - 0.15 m above MLW tidal height, ST = subtidal - ~1 m below the water surface.

Discussion

The successful provision of Native Oyster progenies from both wild spat collection and hatchery production trials indicate that both these methods can provide significant quantities of Native Oyster spat. The numbers collected should also increase with further trials and increased knowledge and experience in timing of deployment and location for spat collectors and collection of broodstock, and methods of Native Oyster larval rearing.

Of significance from the grow-out trials was the high survival rate of Native Oysters, despite being mechanically rumbled and left onshore overnight on occasions (never during hot dry conditions). Many farmers have commented that Native Oysters are more difficult to grow than Pacific Oysters and mortalities are higher, especially in mature Native Oysters (Crawford, 2016). The high survival at Blackman Bay suggests that this is a good environment for growing Native Oysters, but further trials are required at more sites to better understand the environmental requirements and tolerances of Native Oysters.

The results of the grow-out trial show that the growing (clip) height had a significant effect on Native Oyster shell length, shell width, and live weight. As expected, the top clip treatment resulted in lowest values for the parameters tested and the subtidal and bottom clip oysters the highest (Figure 3.31). The interaction between clip height and site on the farm gave some unexpected results, especially in the subtidal and top clip treatments. It was expected that the sheltered deep water gutter area (i.e., the subtidal Site 2 treatment) would provide a more suitable growing location for Native Oysters than the exposed subtidal location (i.e., the subtidal Site 1 treatment) in open water in the middle of the bay. Juvenile Pacific Oysters (*C. gigas*) grow well in this location (P. Glover pers. comm.). The subtidal

exposed location (Site 1) was thought to be potentially too rough for the more 'fragile' Native Oysters. However, after three months the Native Oysters in the subtidal exposed (Site 1) treatment weighed significantly more and were significantly wider, but not significantly longer, than Native oysters in the subtidal sheltered treatment (Site 2). Visual observations of oysters indicated greater biofouling at the sheltered subtidal site (Site 2) suggesting that *O. angasi* grow best in subtidal exposed location where biofouling is reduced and current flow is high. Further study with greater replication of sites (and hence exposure) is needed to confirm this finding, however, it is in accords with the only known remaining Native Oyster reef habitat occurring in a narrow channel area in mid Georges Bay, Tasmania, Australia, where the currents are strong and the site is relatively exposed. Additionally, faster growth rates have been observed on mussels (*Mytilus galloprovincialis*) at exposed sites compared to sheltered sites and attributed to higher food availability due to greater water flow (Steffani and Branch, 2003).

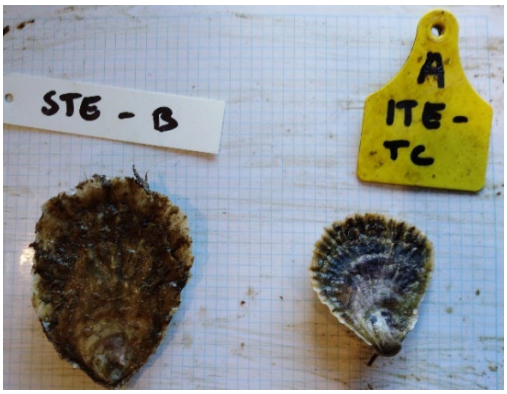


Figure 3.3131 Size comparison between Native Oysters (*Ostrea angasi*) grown at Site 1 in the subtidal treatment (left) and intertidal at the highest (top clip) growing height.

The better performance of Native Oysters at the higher (top clip) Site 1 (exposed) location compared to the top clip Site 2 (sheltered) location was also not expected because of their purported fragile nature compared with Pacific Oysters. Whilst top clip treatments resulted in the lowest overall growth of all the treatments, the Site 1 top clip Native Oysters were significantly heavier in weight compared to the Site 2 one. This could be explained by a greater hardening of the shell because of the constant chipping that occurs due to water movements in more exposed locations, a practice used by farmers to promote physiological responses such as increased meat condition and shell hardness in Pacific Oysters (Kube et al., 2011; Rankin et al., 2018). The other intertidal treatments (middle and bottom clip) showed the opposite effect to site, with Native Oysters at Site 2 performing significantly better for weight.

Oyster shape is an important market consideration for growers with standards applied for Pacific Oysters and Sydney Rock Oysters (*Saccostrea glomerata*; Ryan 2008, Kube et al., 2011). Farmers influence shape characteristics during the grow out phase by moving oysters between and around growing areas to promote shell growth and shape as well as market condition (Robert et al., 1993; Rankin et al., 2018). However, optimal market shape characteristics are yet to be defined for *O. angasi* due to the current low production of this species. Growers try to optimise a cup shape that has depth and provides a greater meat yield (Brake et al., 2003; Ryan, 2008), which is indicated by a lower shape score (Rankin et al., 2018). The results of the present study show the shape of the Native Oysters was influenced by growing height, with the higher clip heights resulting in significantly lower shape score compared to subtidal oysters.

The cell energy allocation (CEA) results in this study, whilst not significantly different between sites or growing heights, showed some variations between treatments. One explanation for the observed differences in CEA could be a potential energetic shift in those Native Oysters exposed to air for greater periods of time on the top and middle clips. Greater air exposure results in a reduction of

metabolic processes such as feeding and respiration, enhancing energy conservation for many oyster species (Bayne, 2002).

In conclusion, these results indicate that intertidal conditions are suitable for growing Native Oyster juveniles, and growth and shape can be influenced by growing height in a similar fashion to Pacific Oysters. It is likely that a trade-off will be required between faster growth in the lower intertidal to subtidal and an increased cupped shell shape in the higher intertidal (Figure 3.31). This agrees with the common practice amongst Pacific Oyster farmers to move their stock around their farms to maximise growth or shape depending on the environmental conditions around their lease, as well as strengthening the adductor muscle when out of the water. Further grow-out trials from spat to harvest at a range of sites are required to better understand growth and survival of Native Oysters under a range of environmental conditions and farming practices.

Establishment of a Native Oyster farmers network

The Native Oyster farmers' network began with the workshop – Identifying Knowledge Gaps for Development of the Native Oyster Aquaculture Industry in South Australia (FRDC Oysters Australia IPA 2015/229 Project), where the need for a communication network to share information about Native Oyster aquaculture in Australia was highlighted (Li and Miller-Ezzy, 2017). Participants of the workshop and other potentially interested parties, ~ 30 people in total, were sent an email about the establishment of a Native Oyster farmer's network and asked if they would be interested in participating. Fourteen positive responses (12 from industry) were received.

Three *Ostrea Angasi* workshops have been held since the establishment of the Native Oyster farmers' network, where preliminary results from this study were presented (Appendix 2). The first workshop was part of the NSW Oyster Industry Conference (NSW *angasi* Workshop, August 8th, 2019) and included presentations on preliminary results of this study, updates of *Ostrea angasi* health status, current situation in NSW regarding Native Oyster farming and a discussion on where to now for *Ostrea angasi* culture. The second workshop was held in Tasmania as part of the Shellfish Futures Conference (August 17th, 2019) and included presentations from a number of projects linked to the Future Oysters CRC -P (FRDC project 2017-233) including this current study. The final workshop was held in Streaky Bay, SA on the 21st of August with the theme "A successful flat oyster growing industry, how do we get there from here?" and included presentation of preliminary data from this study and discussions on the current status of Native Oyster farming in SA.

4 Rock Oyster (*Saccostrea* sp.) as an alternative for commercial production

4.1 Cohabitation of Rock Oysters with Pacific Oysters and Native Oysters.

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Methods

Cohabitation and disease assessment

Experimental animals

Juvenile *C. gigas* (weight: 0.11 ± 0.05 g, shell length: 9.72 ± 1.14 mm, mean ± SD; Pacific Oysters) and *O. angasi* (weight: 1.6 ± 0.55 g, shell length: 12.6 ± 3.54 mm; Native Oysters) were sourced from the South Australian Research and Development Institute (SARDI) Aquatic Sciences Centre Mollusc Hatchery (West Beach, Adelaide, SA). Juvenile oysters were tested (30 pools of five oysters, n = 150 oysters per species) using a *Bonamia* sp. quantitative PCR (qPCR) (Corbeil et al., 2006) following Buss et al. (2019) and no *Bonamia* was detected. *Saccostrea* sp. (weight: 0.08 ± 0.06 g, shell length: 6.95 ± 1.40 mm; Rock Oysters) were obtained from Athair Aquaculture Pty. Ltd. (Albany, WA). Oysters were maintained separately under quarantine conditions at the South Australian Aquatic Biosecurity Centre (SAABC), Roseworthy Campus, SA, with each species in separate 500 L fibreglass tanks supplied with continuous aeration.

Experimental system

Nine experimental 50L tanks were set up at SAABC containing aerated seawater. Three control tanks each contained 500 Pacific (Tank 7), Rock (Tank 8) or Native (Tank 9) Oysters, three tanks (Tanks 10-12) contained 500 Pacific, 500 Rock and 300 Native Oysters and three tanks (Tanks 13-15) contained 500 Rock and 500 Pacific Oysters.

The water was exchanged three times per week. Oysters were fed 1.25 L (2.0×10^6 cells/mL) of a mixed culture of *Chaetoceros muelleri, Skeletonema costatum* and *Pavlova lutheri* three times weekly following water exchange, supplemented with 20-25mL of Instant Algae® Shellfish 1800® algal concentrate daily. Tank placement, maintenance and operation of the system were designed to prevent cross-contamination. Water quality was normal in all tanks for the duration of the experiment: water

temperature was maintained at 16.83 ± 2.27 °C (mean ± SD), salinity was maintained at 36‰ and dissolved oxygen was 97.88 ± 1.53 % saturation; 7.74 ± 0.40 mg/L (mean ± SD).

Tanks in the cohabitation trial were checked for mortalities three times per week. The experiment was maintained for 90 days. Mortalities were sampled for pathology where material was in adequate condition. Oysters that died or were sampled were replaced with oysters in labelled mesh pouches separate to the experimental animals to maintain tank biomass. 50 oysters from each control and treatment were sampled on day 90.

Histology and health analysis

From each sampled oyster, a diagonal 3-5 mm tissue section was taken ensuring each sample included mantle, gills, digestive gland and gonad. This section was placed in a histology cassette and fixed in 10% buffered neutral formalin with filtered seawater for 48 h, then transferred to 70% ethanol for storage. Samples were embedded in paraffin wax, sectioned at 7 µm and stained with haematoxlyin and eosin. Histology slides were examined with a compound light microscope (Brightfield Olympus BX53 Upright Microscope).

Molecular analyses

A pool of mantle and gill and a piece of digestive gland from 30 oysters showing hypertrophied nuclei with condensed, marginated chromatin was tested using the Martenot and Jenkins OsHV-1 qPCR assays following Deveney and Wiltshire (2020).

Statistical analyses

To visualize results Kaplan-Meier survival curves were generated using the *survival* package in *R.* A Cox proportional hazards model was run to determine the effect of treatment on survival. This model was run using Integrated Nested Laplacian Approximation (INLA) with Treatment as a fixed effect and Tank as a random effect (= frailty in the context of survival analysis), and assuming a Weibull baseline distribution. INLA was chosen as it can accommodate the interval censored data in addition to random effects.

Results

Cohabitation experiment

One Rock Oyster in the control tank died. In cohabitation tanks with Pacific Oysters (Tanks 13-15), few mortalities occurred, with two of these treatment tanks having 5 mortalities each and the last having a single mortality. In the cohabitation treatment tanks with both Pacific and Native Oysters (Tanks 10-12), there were 55, 54 and 66 rock oyster mortalities over 90 days. All tanks initially contained 500 Rock Oyster spat. The majority of mortalities occurred after > 60 days. The resulting Kaplan-Meier survival curves are shown in Figure 4.1.

Histopathology

No notifiable parasitic disease agents of molluscs listed by the World Organisation for Animal Health (formerly Office International des Epizooties, OIE) or on Australia's National List of Reportable Diseases of Aquatic Animals (*Bonamia* spp, *Haplosporidium* spp, *Perkinsus* spp., *Mikrocytos* spp., *Marteilia* spp., *Marteilioides* spp.) were observed or confirmed to occur in these samples. Relatively high prevalences of necrotic epithelial cells containing slightly hypertrophied nuclei with condensed, marginated chromatin were observed at moderate intensities in necrotic digestive gland tubule lesions in Pacific Oysters in the control tank (15.6% prevalence), Tank 10 (67.5% prevalence), Tank 11

(84.4% prevalence) and Tank 12 (59.4% prevalence) (Figure 4.2). Similar lesions were observed at lower intensities in smaller numbers of Native Oysters in the control tank (33.3% prevalence), and in Tank 10 (43.75% prevalence), Tank 11 (37.5% prevalence) and Tank 12 (18.75% prevalence). These non-specific and non-pathognomic digestive gland lesions are reminiscent of infections with a herpes-like virus which is reported from *O. angasi* in Australia (Hine and Thorne, 1997). Given the likely relatedness of these viruses with OsHV-1 var and OsHV-1 µvar (microvariant) responsible for POMS, further testing of these samples with molecular diagnostic tools was indicated. The qPCR assays for OsHV-1 provided not detected results for all samples.

Pacific Oysters and Rock Oysters in all tanks had relatively high prevalences of atrophy of digestive gland tubule epithelia, suggesting that rearing conditions and/or feed provided may not have been optimal for either of these species. The Rock Oysters from Tanks 10 and 12 had relatively high prevalence of bacterial enteritis and necrosis, which again may suggest that feed and/or rearing conditions may have been suboptimal for this species (Figure 4.3). The only other notable disease agents found included rickettsia-like organisms (RLO) which were present at moderate prevalences and intensities in Native Oysters from the control (33.3% prevalence), Tank 10 (37.5% prevalence), Tank 11 (53.1% prevalence) and Tank 12 (40.6% prevalence) (Figure 4.4). Symbionts found occasionally at low prevalence (< 3.1%) in all tanks included *Ancistrocoma*-like ciliates in the gills or lumen of the digestive tubules.

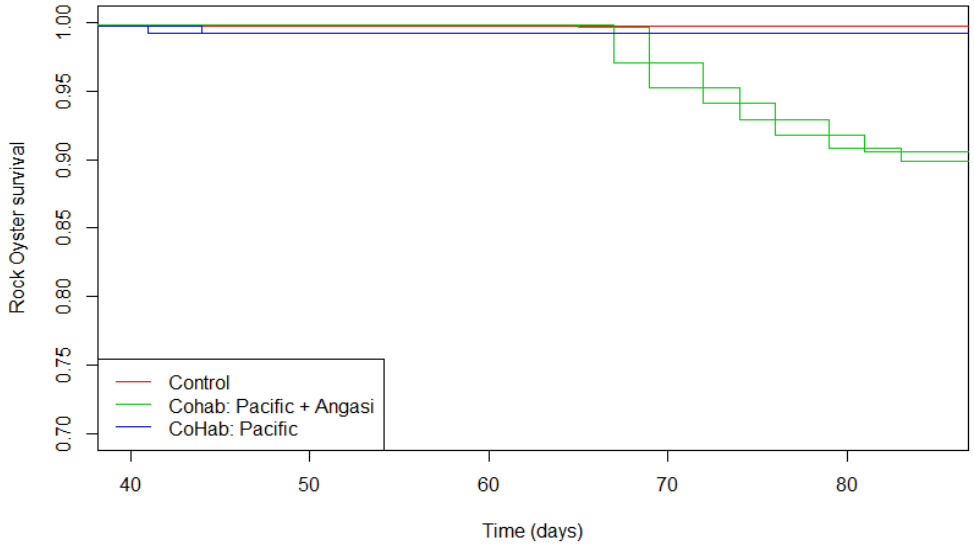

Figure 4.1 Kaplan-Meier survival curves for Rock Oysters over the cohabitation experiment with Pacific or Pacific and Native Oysters.

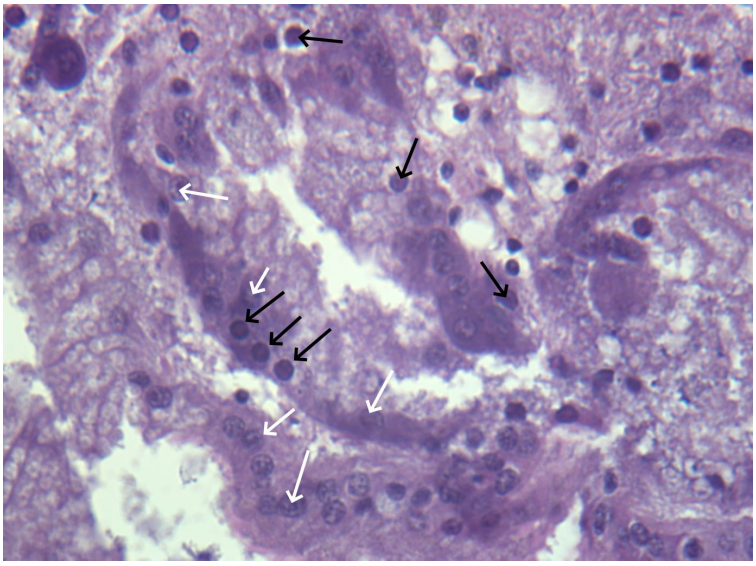

Figure 4.2 Digestive gland tubule epithelium of Pacific oyster #8 from Tank 10. Note the slightly hypertrophied nuclei with condensed, marginated chromatin (black arrows) in areas of tubule cell necrosis, whereas the nuclei of normal cells (white arrows) are normal. 500x magnification.

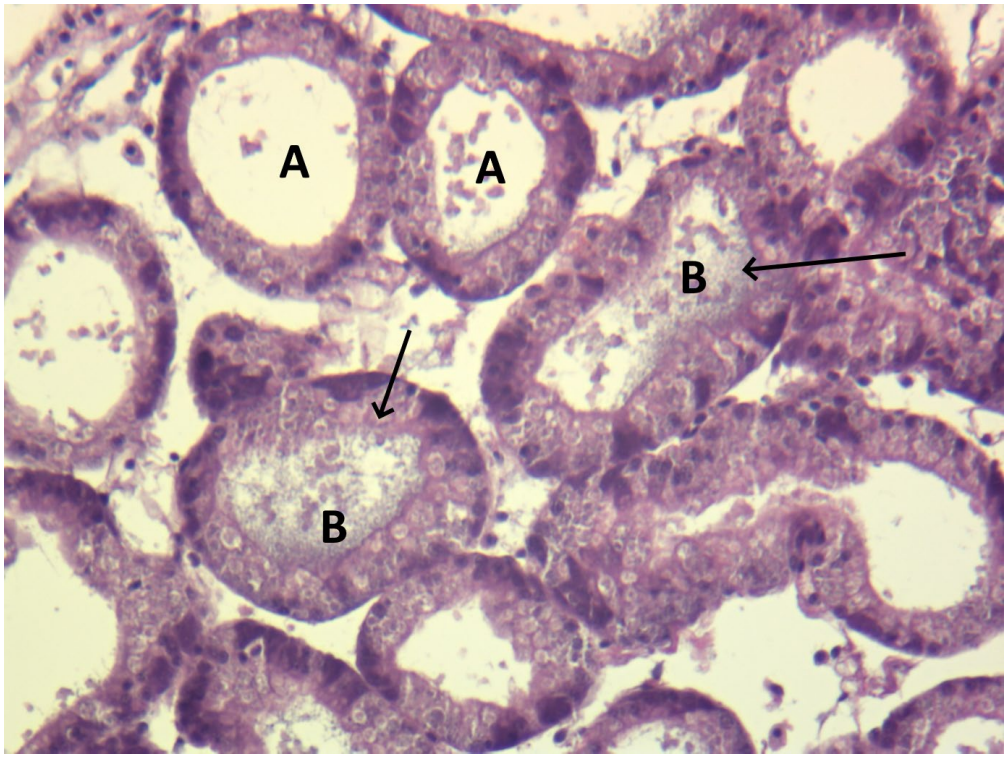

Figure 4.3 Bacterial enteritis causing atrophy (A) of digestive gland tubules in Rock Oyster #20 from Tank 10. Arrows point to tubules with active bacterial (B) infection. 200x magnification.

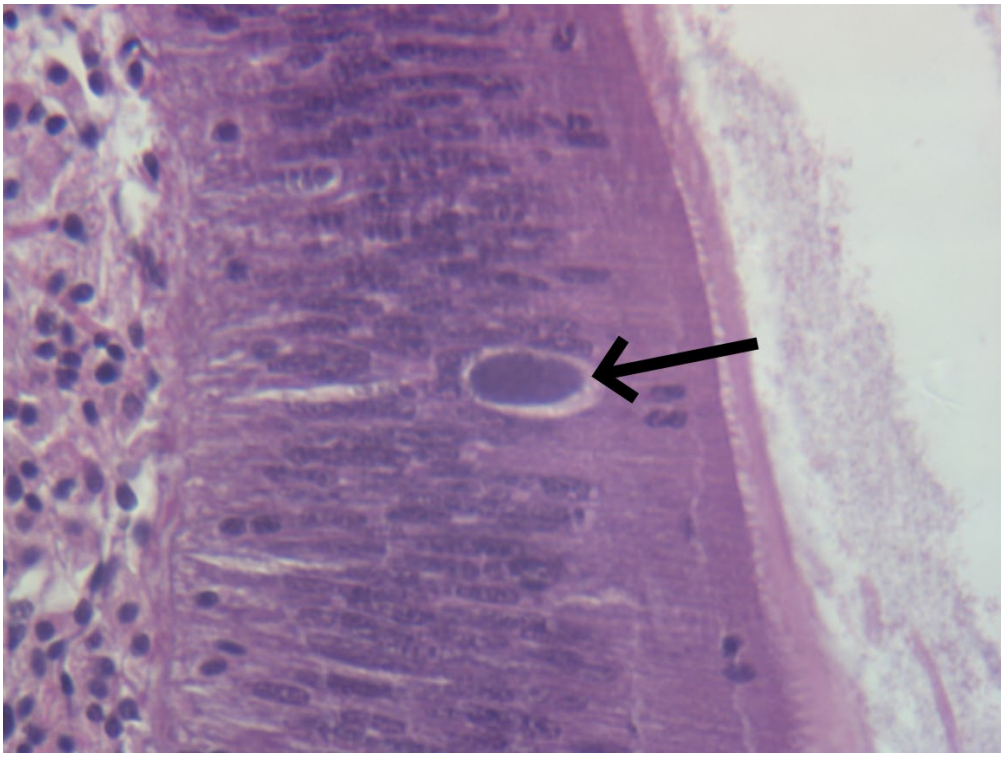

Figure 4.4 An inclusion of rickettsia-like organisms (RLOs, arrow) in the gut epithelium of Native Oyster #28 from Tank 9. 200 x magnification.

Discussion

No nationally or internationally notifiable diseases of molluscs were detected in these oysters. The hypertrophied nuclei with condensed marginated chromatin observed in the digestive gland tissues of Pacific and Native Oysters are non-specific lesions which often occurred in the absence of bacterial infection. The lesions observed here were similar to lesions described in hatchery reared Pacific Oysters in New Zealand and Europe, hatchery reared Native Oysters from Albany in WA, and hatchery reared Rock Oysters from NSW which were infected by herpes-like viruses (Hine and Thorne, 1997; Renault et al., 2000; 2001; Jenkins et al., 2013). These oysters tested negative, however, for OsHV-1.

The digestive tract of several oysters with these lesions appeared to be in relatively poor condition with focal or multifocal necrosis of digestive gland epithelia, sometimes with bacterial necrosis which may represent possible secondary infection. The patterns of mortality and pathology suggest that close cohabitation between Rock, Native and Pacific Oysters could be poor practice and may result in mortalities, although the mechanism by which the oysters died is unclear.

The digestive tubule atrophy in some Rock and Pacific Oysters, mainly those also cohabited with Rock Oysters, indicates that the feed provided may not have been adequate. The low incidence of haemocytosis and diapedesis (haemocyte migration across the intestinal epithelium) in all species of oysters from all tanks suggests the oysters were not exposed to parasites or toxins such as heavy metals (McGladdery et al., 1993). The rarity of haemocytosis observed may have been due to metabolic processes, such as phagocytosis of tissue or uptake of nutrients from the gut, rather than responses to parasitic infections. In contrast, several individual oysters of all three species exhibited enteritis associated with bacteria within the digestive tubule lumen and adjacent tubule epithelium where they were associated with focal areas of digestive tubule necrosis. It is possible that these

bacterial enteric infections were symptomatic of bacterial communities of different oysters being incompatible when cohabited (Lokmer and Wegner, 2015).

The RLOs were present at moderate prevalence (33.3-53.1%) and intensity in Native Oysters from all tanks. RLOs and other related intracytoplasmic bacteria are probably ubiquitous in marine bivalves (Hine and Diggles, 2002). Usually they occur at low intensities, as in the present study, and are not associated with disease. If the host becomes stressed due to factors which may include unfavorable environmental conditions or metabolic imbalances, the RLOs can proliferate and may cause disease (Hine and Diggles, 2002).

Overall, the cohabitation trial shows that mixing oysters creates complex interactions not observed in monocultures that may be associated with competition for food and/or differences in microbiomes. The elevated mortality observed in the mixed Pacific, Native and Rock oyster cohabitation trials certainly suggests that care should be taken when introducing new species to otherwise stable, healthy culture systems.

4.2 Biosecurity risk assessment of Rock Oyster introduction to South Australia

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Methods

In this analysis, risk estimations were made on a semi-quantitative basis because, as is the case with most aquatic animal risk assessments, there are insufficient scientific data to take a completely quantitative approach. The methodology used in this analysis was based on: Fletcher, 2005 and Biosecurity Australia (e.g. AQIS, 1999; Biosecurity Australia, 2007) and is consistent with ISO 31000: Risk (International Standards Organisation, 2019).

Risk analyses for establishment of disease and pest potential comprise two components (Tables 4.1 and 4.2):

1. ***likelihood of introduction, spread and establishment assessment***: the likelihood that the animal (genetics) or disease agent is introduced, spreads and establishes, and
2. ***consequence assessment***: the severity of impacts (ie. long term vs short term) resulting from that establishment.

All likelihoods or probabilities of an event occurring are assessed semi-quantitatively using the following descriptors (modified from AQIS, 1999):

5 - *Likely*:	Event would be expected to occur
4 - *Occasional*:	There is less than an even chance of the event occurring
3 - *Possible*:	Event would occur uncommonly, in less than a decadal frequency
2 - *Unlikely*:	Event would occur rarely, less than once a century
1 - *Remote*:	Chance of event occurring so small it can be ignored in practical terms

The likelihood of establishment considers release and exposure. Taking a precautionary approach, the likelihood of establishment is taken to be the lower of the two values for the release and exposure assessments.

The following range of terms are used to describe the significance or severity of likely consequences:

***4 - Extreme*:** associated with the establishment of disease / pest that would be expected to significantly harm economic performance at a State level over a long term. Would have a significant economic impact (i.e., employment) on regional areas. Alternatively, or in addition, they may cause serious, irreversible harm to the environment (e.g. to wild endemic stocks).

***3- High*:** associated with the establishment of disease / pest that would have serious biological consequences (e.g., high mortality, low production). Such effects would normally be felt for a prolonged period (greater than or equal to a normal production cycle) and would not be amenable to control or eradication. This would be expected to significantly harm economic performance at a 'whole' industry level. Alternatively, or in addition, they may cause serious harm to the environment (e.g., to wild endemic stocks).

***2 - Moderate*:** associated with the establishment of disease / pest that have less pronounced biological consequences. This may harm economic performance significantly at an enterprise / regional level, but they would not have a significant economic effect at the 'whole industry' level. This may be amenable to control or eradication at a significant cost, or their effects may be temporary. They may affect the environment (e.g., wild endemic stocks), but such harm would not be serious or may be reversible.

***1 - Low*:** associated with the establishment of disease / pest that would have mild biological consequences and would normally be amenable to control or eradication. This would be expected to harm economic performance at the enterprise or regional level but to have negligible significance at the industry level. Minor or temporary effects on the environment (e.g., wild endemic stocks).

***0 - Negligible*:** associated with the establishment of disease / pest that would have no significant biological consequences, may be transient and/or are readily amenable to control or eradication. The economic effects would be expected to be low to moderate at an individual enterprise level and insignificant at a regional level. Effects on the environment would be negligible.

Table 4.1 Risk Matrix – numbers in cells indicate risk value, the colours / shades indicate risk rankings. Note that the risk level is calculated by multiplying the likelihood value by the consequence value. Blue – negligible risk. Green – low risk. Yellow – moderate risk. Orange – high risk. Red - extreme risk. Consequence is shown in column headings and likelihood in row headings.

		Consequence				
		Negligible	**Low**	**Moderate**	**High**	**Extreme**
Likelihood		0	1	2	3	4
Remote	1	0	1	2	3	4
Unlikely	2	0	2	4	6	8
Possible	3	0	3	6	9	12
Occasional	4	0	4	8	12	16
Likely	5	0	5	10	15	20

Table 4.2 Hazard identification for biosecurity risk assessment for Rock Oysters

Pathogen	**Present in Rock Oysters**	**Present in SA**	**Main host**	**OIE/NACA listed**	**Official control in Australia**	**Further consideration?**
OsHV-1	No	Yes	*C. gigas*	Yes	Yes	Yes
Malacoherpesvirus	Yes	Uncertain	*O. angasi*	No	No	Yes
Bonamia exitiosa	Yes	Yes	*O. angasi*	Yes	No	No
Haplosporidium spp. and *Minchinia* spp.	Yes	No	Various	Yes	Yes	Yes
Marteilia sydneyi	Yes	No	*S. glomerata*	No	Yes	Yes
Marteilia spp. and *Marteilioides* spp.	Yes	No	Various	Yes	Yes	Yes
Perkinsus olseni and *Perkinsus* spp.	Yes	Yes	Various	Yes	Yes	No
Oyster oedema disease*	Yes	No	*Pinctada maxima*	No	Yes	Yes
Winter mortality*	No	No	*S. glomerata*	No	Yes	No

* Diseases of unknown aetiology.

Acceptable level of risk

In this analysis risk is assessed in a partially controlled fashion, i.e., without additional specific biosecurity mitigations considered but with standard PIRSA Fisheries and Aquaculture controls and normal industry management included as mitigating factors in the assessment. Generally, risks ranked as "Negligible" or "Low" are regarded as acceptable and the current regulatory framework is considered adequate, while risks of "Moderate" or higher require consideration of further management steps to mitigate risk. This approach is consistent with PIRSA's pre-existing management framework and the methodology used in Import Risk Analysis for translocations and Disease Risk Analysis used to prioritise health risks to aquaculture by PIRSA and the National Appropriate Level of Protection (ALOP). In pest biosecurity, establishment of non-native species is undesirable and any risks that exceed the ALOP and cannot be mitigated adequately indicate that the activity or proposed translocation should not proceed.

For this risk assessment, hazards with a score of > 6 (includes Moderate, High, Extreme) will require further mitigation strategies to reduce the overall level of risk.

The main aim of the risk assessment was to determine if the translocation of Rock Oysters to South Australia poses unacceptable disease or pest biosecurity risks and, if those risks are unacceptable, if those risks can be managed to make them acceptable.

Results

Disease risk assessment

OsHV-1

Ostreid Herpesvirus 1 microvariant (OsHV-1 microvariant), a double stranded DNA virus classified in the genus Ostreavirus in the family Malacoherpesviridae in the order Herpesvirales (Davison et al, 2005; 2009) causes POMS. OsHV-1 microvariant is a genetic variant of the reference strain of OsHV-1 defined in part on the basis of partial sequence data exhibiting a systematic deletion of 13 bp in a microsatellite zone (C region) of the Open Reading Frame 4 (ORF4) of the genome (Segarra et al., 2010). OsHV-1 is not OIE listed but is reportable and listed as regionally significant by NACA.

OsHV-1 microvariant was first recorded in Australia in Pacific Oysters in Woolooware Bay, Georges River estuary in Botany Bay in late November 2010 (Jenkins et al., 2013). The disease was also detected in *C. gigas* in the Paramatta River, Sydney Harbour in January 2011 (Jenkins et al., 2013), and then spread 50 km north to the Hawkesbury River in January 2013 causing severe mortalities (Paul-Pont et al., 2014). In late January 2016 OsHV-1 microvariant caused mass mortalities of farmed *C. gigas* in Tasmania (de Kantzow et al., 2017) and was detected in the Port River, South Australia in feral oysters in February 2018. The South Australian Pacific oyster farming industry remains free of the virus and POMS as of August 2022 OsHV-1 microvariant has not been recorded from Western Australia but the source hatchery holds *S. glomerata* stock from NSW where the virus is recorded.

There is growing evidence that OsHV-1 microvariant infections are not limited to *C. gigas* hosts (O'Reilly et al., 2017; Bookelaar et al., 2018) and there is substantial evidence that healthy adult molluscs can be asymptomatic carriers of herpes-like viruses and in these cases infected oysters may display no clinical or pathological signs of disease (Hine and Thorne 1997; Arzul et al., 2002). Healthy juvenile oysters may also be infected with OsHV-1 microvariant in the carrier state with no clinical or pathological signs (Dundon et al., 2011; Evans et al., 2017).

OsHV-1 causes severe disease and has had substantial impacts where it occurs (AusVet, 2011). South Australia has a large, established Pacific Oyster industry which provides strong regional employment and economic activity. The SA industry was substantially affected by controls on spat translocation from Tasmania following the 2016 Tasmanian outbreak of OsHV-1 and spat supply was not re-established until the second half of 2019.

Overall, the likelihood of rock oysters from WA releasing OsHV-1 microvariant in SA is unlikely, but the consequences of establishment of the virus would be extreme.

Other malacoherpesviruses

This section examines malacoherpesviruses other than OsHV-1 microvariant and AbHV-1 in abalone. These agents are not OIE or NACA listed. Malacoherpesviruses have been recorded from bivalves in Australia including hatchery reared Native Oysters (*Ostrea angasi*) from near Albany in WA (Hine and Thorne, 1997), and Sydney Rock Oysters (SRO; *S. glomerata*) in Port Stephens, NSW (cited in Jenkins et al., 2013). There is unpublished evidence that a Malacoherpesvirus occurs in *O. angasi* in South Australia (Tim Green, unpublished data). Herpesviruses and herpes-like viruses have been reported from a wide variety of bivalve molluscs in many parts of the world.

Herpesviruses were visualised using TEM in hatchery reared SRO larvae experiencing mortalities in Port Stephens, NSW in the 1990s (cited in Jenkins et al., 2013), but it was unclear if the viruses were responsible for the mortality. Malacoherpesviruses have also been reported from Native Oysters in WA, and these viruses are considered to be naturally present in WA waters (Hine and Thorne, 1997), however whether the viruses in NSW and WA are the same or unique remains to be determined. Malacoherpesviruses are unlikely to survive free in seawater outside the host for longer than around 2

days at 20°C, but in contrast the virus remained viable in wet or dry, non-living oyster tissues for at least 7 days at 20°C (Hick et al., 2016). Herpesviruses can persist in their hosts in asymptomatic latent infections, with virus expression and active replication being associated with exposure of carrier hosts to stressful conditions (Eide et al., 2011). Control of herpesviruses in hatcheries is well understood (Rodgers et al., 2019). PCR and sequencing of *O. angasi* from SA has indicated that a malacoherpesvirus may be present (Tim Green, unpublished data). There are no clear links between disease in oysters and malacoherpesviruses other than OsHV-1.

Taking this information into account, the risk of release is possible. The lack of clear links to disease makes the consequence low.

Haplosporidium *spp. and* Minchinia *spp*.

Haplosporidium spp. and *Minchinia* spp. protozoans in the phylum Cercozoa, Order Haplosporida. *Haplosporidium* spp. infect mainly connective tissues and epithelia of bivalves, gastropods, abalone and other invertebrates including tunicates, annelids and crustaceans (Burreson and Ford, 2004). Most *Haplosporidium* species develop spores 3-12 x 2-5 µm with an apically hinged operculum and other ornaments (Azevedo and Hine, 2016). Those that infect molluscs have an unknown, probably indirect life cycle, possibly requiring intermediate (possibly planktonic) hosts (Haskin and Andrews, 1988). Members of the genus *Minchinia* infect mainly oysters or crabs and develop spores 2-12 µm in greatest dimension without ornamentation and the epispore cytoplasm is never attached to the spore wall (Azevedo and Hine, 2016). Those that infect molluscs appear to have an indirect life cycle, requiring intermediate (possibly non-molluscan) hosts (Powell et al., 1999).

Haplosporidium spp. are not OIE or NACA listed. Haplosporidosis of pearl oysters is reported in WA where *Pinctada maxima* spat can be infected with *Haplosporidium hinei* (Hine and Thorne, 1998). A *Haplosporidium* sp. reported from *Saccostrea cucullata* in WA by Hine and Thorne (2002) was described as *M. occulta* Bearham et al. (2008). Infection of molluscs by *Haplosporidium* spp. has resulted in economically and ecologically significant mortalities in many parts of the world (Burreson and Ford, 2004). *Haplosporidium* has established following translocations (Burreson et al., 2000), despite the probable requirement for an intermediate host(s) for transmission (Ford et al., 2001). Modelling suggests that the infective stage of *H. nelsoni* is waterborne and most likely acquired by feeding (Haskin and Andrews, 1988). Movement of alternative or reservoir hosts by ballast water or shipping could also have been the mechanism of spread of *Haplosporidium* to new locations (Burreson et al., 2000).

In Australia, haplosporidians of the genus *Haplosporidium* and *Minchinia* have caused sporadic but heavy mortalities in hatchery reared pearl oysters (*Pinctada maxima*) and wild Rock Oysters (*Saccostrea cucullata*) in Western Australia (Hine and Thorne, 1998). The *Haplosporidium* sp. parasite in pearl oysters was first found in 6 out of 106 *P. maxima* spat of 5-10 mm in shell height from a hatchery at Oyster Creek, Canarvon in northern WA in the early 1990s (Hine and Thorne, 1998). By the time the presence of the infection was detected, however, the remaining spat had been moved to a grow-out area, where they apparently all died (Hine and Thorne, 1998). Subsequent studies found it difficult to detect the parasite again, until a second occurrence in December 1995 found the *Haplosporidium* sp. at a prevalence of 4.6% in a sample of 150 spat taken 6 weeks after their deployment to a nursery area at Cascade Bay in King Sound north of Broome (Jones and Creeper, 2006). By the time the oysters were destroyed 15 days later, the prevalence had increased to 10% (Jones and Creeper, 2006). The parasite was described as *Haplosporidium hinei*, a pathogen that is considered to represent a serious risk to the pearl industry (Bearham et al., 2008). The second parasite originally observed by Hine and Thorne (2000), in samples of diseased *S. cucullata* from northern WA in 1993-94 has been associated with mortalities of up to 80% in wild Rock Oysters around Exmouth Island (Hine and Thorne, 2000) and was eventually described as *Minchinia occulta* (Bearham et al., 2008). Mixed infections of *M. occulta* and *H. hinei* have also been recorded in hatchery reared *P. maxima* with *M. occulta* occurring at prevalences up to 26.7% during disease outbreaks (Bearham et al., 2009).

The likelihood of *Haplosporidium* spp. and/or *Minchinia* spp. being translocated with Rock Oysters from Western Australia and establishing in South Australia is possible.

The probable consequence of establishment of *Haplosporidium* spp. and/or *Minchinia* spp. in SA is moderate.

QX disease/ Marteilia sydneyi

Marteilia sydneyi, a protozoan parasite classified in the Order Paramyxida within the phylum Cercozoa causes QX disease in *Saccostrea glomerata*. Members of the Paramyxida are parasites of invertebrates, mainly oysters, polychaete worms and crustaceans (Ward et al., 2016) which are characterised by their distinctive "cell within cell" development (Carrasco et al., 2015). *Marteilia sydneyi* infects Sydney Rock Oysters *S. glomerata* (Perkins and Wolf, 1976) and some polychaete annelids, including *Nephtys australiensis* (Adlard and Nolan, 2015).

Marteilia sydneyi has been detected in association with heavy mortalities in wild and cultured *S. glomerata* in many areas of QLD and NSW, and at low prevalence in northern WA (Hine and Thorne, 2000). The parasite is also known to infect polychaetes within the Family Nephtyidae, and many other species of filter feeding invertebrates in estuaries are likely to temporarily accumulate *Marteilia* spores in their digestive tract (Audemard et al., 2002), and hence could act as mechanical vectors. Broodstock *S. glomerata* from some NSW estuaries are infected by *M. sydneyi* at high prevalence. *Marteilia sydneyi* is present in the waters of northern WA at low prevalence (Hine and Thorne, 2000; Jones and Creeper, 2006).

Paramyxean parasites of the genus *Marteilia* have caused significant disease and economic impacts on oyster culture in several regions of the world (Berthe et al., 2004). *Marteilia refringens* devastated the flat oyster (*Ostrea edulis*) industry in France beginning in the late 1970's (Grizel et al., 1974; Grizel, 1985), and this parasite also infects other bivalves including mussels (Robeldo and Figueras, 1995; Longshaw et al., 2001), and razor clams (Lopez Flores et al., 2008). *Marteilia sydneyi* is responsible for QX disease that has caused massive losses (up to 98% mortality) in wild and cultured Sydney Rock Oyster along the east coast of Australia from Great Sandy Straits in QLD to the NSW / Victoria border since the late 1960's (Wilkie et al., 2013). It appears that this parasite was first problematic in Southeast Queensland then later emerged in rivers further to the south (Wolf, 1979) or was translocated into more southern estuaries via movements of infected oysters (Kleeman et al., 2004). Then in the early 1990's an identical parasite was found in *S. glomerata* near King Bay in the Dampier Archipelago, northern WA at very low prevalence (1 out of 933) in surveys undertaken between 1992 and 1994 (Hine and Thorne, 2000), as well as in subsequent surveys (1 of 411 *S. glomerata*) undertaken in 1995 between Carnarvon and the Dampier Archipelago (Jones and Creeper, 2006).

The likelihood of *Marteilia sydneyi* being translocated with Rock Oysters and establishing in South Australia is possible. The probable consequence of establishment of *Marteilia sydneyi* in SA is high.

Marteilia *spp. and* Marteilioides *spp*.

Marteilia spp. and *Marteilioides* spp. are protozoan parasites classified in the Order Paramyxida within the phylum Cercozoa. Members of the Paramyxida are parasites of invertebrates, mainly oysters, polychaete worms and crustaceans (Ward et al., 2016) which are characterised by their distinctive "cell within cell" development (Carrasco et al., 2015). This section includes *Marteilia* spp. other than *Marteilia sydneyi* that causes QX disease in Sydney Rock Oysters.

A paramyxid resembling *Marteilia lengehi* was found in *Saccostrea cucullata* from near Exmouth in northern WA at low prevalence (Hine and Thorne, 2000). In southern NSW an unidentified *Marteilia*-like protozoan was observed in histological sections of *O. angasi* from Bermagui and Narooma at ~1% prevalence (Heasman et al., 2004). Species of *Marteilioides* are described in the ovary of *Saccostrea echinata* from the NT and the gills of *S. glomerata* from northern NSW (Anderson and Lester, 1992).

Marteilioides branchialis and an unidentified *Marteilia* spp. occur in NSW oysters, and the occasional detection of new paramyxids in Australian oysters at low prevalences demonstrates that the full range of paramyxid infections present in the various species of Australian molluscs remains to be determined. The risk that *Marteilioides branchialis* and/or an undescribed *Marteilia* or *Marteilioides* spp. may occur in apparently healthy *S. glomerata* broodstock and be transported into the hatchery in Western Australia remains non-negligible. Translocation of infected oysters from areas where these parasites are enzootic into the hatchery and subsequent translocation of spat could transport these parasites to new regions. Infection and establishment of these parasites in new hosts would occur only if sufficient viable infective stages were introduced into areas where susceptible intermediate hosts and oyster final hosts were present under suitable environmental conditions for transmission. At this time the lifecycles of these parasites are unknown, however for *Marteilia sydneyi* and *M. refringens*, some of their alternative hosts include crustaceans and polychaetes in the Family Nephtyidae (Audemard et al., 2002; Ward et al., 2016). Both host groups are ubiquitous in the Australian environment (Bennett, 1987), meaning that the indirect lifecycle of these parasites may not be a barrier to their wider dissemination through movements of oysters or other molluscs.

Although paramyxids besides *M. sydneyi* occur in populations of wild molluscs in some regions of Australia, other regions appear free of infection at this time. The intermediate hosts for paramyxids include invertebrate taxa that are widespread, and therefore the presumed indirect lifecycle may not restrict dispersal of these parasites. There is, however, little evidence that other paramyxid parasites cause major disease outbreaks or significant impacts on populations of wild or cultured molluscs in Australia. They are not listed by the OIE or NACA, or reportable in any State, however any new species of *Marteilia* must be referred to the relevant competent authority for identification given that *Marteilia refringens* is an OIE listed disease agent that is a reportable disease in all states. The spread of at least some paramyxids into new areas is therefore likely to adversely impact trade. Considering all of these factors, establishment of *Marteilia* or *Marteilioides* spp. in new areas would have some biological consequences and could cause significant economic harm for mollusc aquaculture industries, together with potentially significant and irreversible environmental effects for ecosystems (where oysters act as ecosystem engineers providing habitat and important bentho-pelagic coupling services) and wild mollusc fisheries.

The likelihood of *Marteilia* and *Marteilioides* spp. being translocated with Rock Oysters from Western Australia and establishing in South Australia is possible. The probable consequence of establishment of *Marteilia sydneyi* in SA is moderate.

Oyster oedema disease

A mass mortality occurred in farmed pearl oysters (*Pinctada maxima*) in Exmouth Gulf, WA in October 2006 (Jones et al., 2010). Around 2.8 million *P. maxima* of all class sizes (up to 120 mm shell height) died, including approximately 60% of recently seeded oysters, with mortalities up to 90% or higher in smaller oysters with an apparent spread of the disease to all lease sites in Exmouth Gulf (Madin, 2007). Gross signs included severe mantle retraction, muscle weakness, mild oedema of the mantle tissue and palps, and mortality (Jones et al. 2010). Only *P. maxima* were affected, with other bivalves including black pearl oysters (*P. margartifera*) and Shark Bay pearl oysters (*P. albina*) remaining apparently healthy (Jones et al., 2010). Histopathology revealed focal loss of epithelial cells along the mantle margins but otherwise no haemocyte inflammatory processes or known pathogens were present (Jones et al., 2010). Based on the oedematous tissue changes which occurred in affected oysters (Jones et al., 2010), the syndrome was called oyster oedema disease (OOD). Since 2006, OOD has continued to effect *P. maxima* farming in some locations in WA (Goncalves et al., 2017).

The 2006 disease outbreak appeared to follow movements of personnel and was consistent with a propagating infectious process. Water samples showed no known toxic algal species and water quality parameters were normal (Jones et al., 2010). Attempts to transmit the disease under experimental conditions, however, were equivocal and did not point to an infectious cause (Humphrey and Barton, 2009). The high susceptibility of juvenile pearl oysters to infection suggested the immunocompetence of the host is important in the condition (Bearham et al., 2009). The aetiology of the oedematous

lesions associated with OOD is perplexing because oysters are osmoconformers and generation of oedema in the absence of osmotic gradients is unclear (Jones et al., 2010). The OOD lesions could therefore be due to a pathogenic agent affecting the epithelial cells directly, a variety of insults (including pathogens) that provoke a generalized oedematous response either at the cellular level or through a neuroendocrine-mediated response, or the outcome of a flaccid hydraulic system, perhaps indicating a loss of muscular control (Jones et al., 2010). The movement of the disease with personnel suggests it can be translocated, but the disease is associated only with *P. maxima* and not *Saccostrea* spp.

The likelihood of OOD being translocated with Rock Oysters from WA and establishing in South Australia is negligible. The probable consequence of establishment of OOD in SA is negligible.

Pest risk assessment

Rock Oysters as an environmental pest

Rock Oysters are primarily estuarine (Wilkie et al., 2013) and in hatcheries are stimulated to spawn by exposure to 24°C dry air followed by exposure to water of increasing temperature (24°C raised to 30°C) and reduced salinity (from 35 PSU to 24 PSU) (O'Connor et al., 2008). There are, however, populations of *S. glomerata* in oceanic conditions (Scanes et al., 2016). Pacific Oysters have, however, shown good recruitment at temperatures and salinity outside the ranges considered optimal for this species (Wiltshire, 2008) and assessment of the likelihood of establishment for introduced molluscs is difficult (Deveney et al., 2013). While the salinities are likely to be too high and the temperatures too low for optimal recruitment of Rock Oysters in SA, if Rock Oysters survive and grow adequately to support an industry in SA, at least some recruitment is likely to occur.

Rock Oysters grow densely in the intertidal zone (Wilkie et al., 2013) are sharp and can occur abundantly (Scanes et al., 2016). Additional loads of oysters on natural substrates could decrease amenity and community safety in areas used for recreation and commercial activities such as scalefish fishing. The likelihood of Rock Oysters establishing in South Australia is occasional. The probable consequence of establishment of rock oysters in SA is high.

Industry fouling/overcatch establishment

The likelihood of Rock Oysters from WA becoming fouling organisms on Pacific Oyster infrastructure is occasional. Overcatch is when oyster spat set on commercial oyster stocks. Rock Oysters are more resistant to dessication than Pacific Oysters (Scanes et al., 2016) and mechanical removal is required where overcatch occurs in NSW (as opposed to managing Pacific Oyster overcatch on Rock Oysters which is managed by dessication which kills Pacific Oysters without impacting Rock Oysters). The probable industry consequences of Rock Oyster overcatch and fouling are high.

The risk assessment matrices are detailed in Table 4.3.

Table 4.3 Risk assessment for Rock Oysters

Specific Risk	**Likelihood**	**Consequence**	**Risk Ranking**	**Comments**	**Further mitigation required?**
OsHV-1	Unlikely	Extreme	**Moderate**	Moderate risk but the extreme consequences dictate risk mitigation.	**Y**
Other Malacoherpesviruses	Possible	Low	**Low**	Release of these viruses may occur but they are not known to be pathogenic.	**N**
Haplosporidium spp. *Minchinia* spp.	Possible	Moderate	**Moderate**	These parasites are known to occur in the source region, can be translocated with stock and are known to be pathogenic.	**Y**
Marteilia sydneyi	Possible	High	**Moderate**	*M. sydneyi* occurs in the source region, can be translocated with stock and are known to be pathogenic.	**Y**
Marteilia spp. and *Marteilioides* spp.	Possible	Moderate	**Moderate**	These parasites are known to occur in the source region, can be translocated with stock and are known to be pathogenic.	**Y**
Oyster oedema disease	Negligible	Negligible	**Low**	OED affects only *P. maxima*.	**N**
Environmental pest establishment	Occasional	High	**High**	Understanding the likelihood of establishment is difficult. If Rock Oysters do establish impacts on amenity and the environment will occur.	**Y**
Oyster industry fouling and overcatch	Occasional	High	**High**	Understanding the likelihood of settlement on Pacific oyster infrastructure is difficult. If Rock Oysters do establish there will be additional management costs for industry but there are established methods for managing these impacts.	**Y**

Discussion

There are two biosecurity categories that require examination to assess if mitigation can make risks acceptable: disease and pest biosecurity risks. Risk mitigation methods are well established for oyster farming and are outlined in the National Biosecurity Plan Guidelines for Australian Oyster Hatcheries (Spark et al., 2018) and by Deveney et al. (2013). These were used to form the basis of biosecurity controls to reduce risks of translocating pathogens of concern to acceptable levels and for managing pest risk.

The source hatcheries should be accredited to the standards outlined in the National Biosecurity Plan Guidelines for Australian Oyster Hatcheries, including all relevant input, output and internal controls. The Biosecurity Plan and the hatchery must be third party audited. Given that many potential source facilities hold multiple species of molluscs from different sources, all stock must be considered in the plan.

For the pathogens of translocation concern that require risk mitigation (OsHV-1, *Haplosporidium* and *Minchinia* spp., *Marteilia sydneyi*, *Marteilia* and *Marteilioides* spp.) testing could be undertaken to provide evidence of freedom. For OsHV-1 best practice involves qPCR testing to OIE standards as outlined in OIE (2019). For the other pathogens, histology should be undertaken as both a health screen and to provide evidence of freedom from translocation relevant diseases. Broodstock should be tested and stock for translocation should be batch tested. Consideration could be made of permitting only an initial or series of initial translocations, with the aim of establishing broodstock in South Australia that could be bred locally following importation. The high likelihood of establishment of unknown pathogens and low prevalence of known pathogens, however, are well understood (Gaughan, 2001) and it is likely that pathogens could become established despite translocation management.

Managing established pests is difficult and bivalves in intertidal areas pose some particular challenges (Deveney et al., 2013). The risks associated with establishment of Rock Oysters in South Australia could be mitigated using a staged approach including a range of controls that operate at different scales. A trial zone could be established as agreed between PIRSA and industry to spatially limit the initial risk. After establishment of farming, an assessment of the area would clarify if any Rock Oysters settle in the environment. If settlement is occurring those animals should be controlled. It is noteworthy, however, that any establishment would both represent an exceedance of PIRSA's ALOP and be difficult or impossible to eradicate.

In aquaculture, establishment is typically managed by stocking infertile animals such as triploids. There are triploid Rock Oysters produced by chemical induction, but ~5% of individuals remain fertile, leaving a substantial reproductive capacity in a population of farmed oysters comprising millions of individuals. The NSW oyster industry members who grow Pacific and Rock Oysters in the same growing regions experience substantial problems with overcatch (Cox et al., 2012). This is managed in different ways but a cold shock treatment is relatively inexpensive ($0.04/dozen) albeit with a relatively high start up cost ($12,000 in 2012) and shown to be effective for the oyster industry (Cox et al., 2012). Mussel growers, however, who were one of the primary proponents for growing Rock Oysters in SA do not have an obvious mechanism to manage Rock Oyster overcatch.

Overall, the issue of overcatch is vexing and unlikely to be adequately mitigated. Campbell and Hewett (2008) note that risk assessment is useful but McKindsey et al. (2007) point out for bivalves that mitigation indicated by these processes is often not very successful.

5 Conclusion

Whilst this report found that diversification of Pacific Oyster leases with Rock Oysters would be too much of a risk for both the oyster industry and the local ecosystems, the option to diversify with Native Oysters is promising. Higher than expected growth rates were observed, particularly at the intertidal sites in South Australia. With further research, the Native Oyster could be produced at leases alongside Pacific Oysters to mitigate the risk of POMS.

Except for one subtidal treatment in South Australia, juvenile Native Oysters showed excellent survival in the on-farm field trials. In both states, farm location was an important factor influencing the growth and shape. In South Australia, Native Oysters grown in the low subtidal had poorer growth and shape than those in the intertidal. This is in contrast to the grow-out trials in Tasmania, where the intertidal top clip height (longest exposure period) had the slowest growth (please note this extreme height treatment was not included in the trials in South Australia). Native Oysters would likely benefit from a rotation system, similar to what is used in Pacific Oyster farming, where they are periodically moved between productive growing locations / heights that promote growth to exposed sites that chip back shell and promote shape. Longer trials are needed to assess Native Oyster growth rates to market size and if there is a link between maturity / age and mortality. Additionally, it would be valuable to determine a standard shape score and market size specific to Native Oysters. Trials to compare the performances between Native Oysters growing in intertidal and subtidal floating systems recently developed in Tasmania would be needed for the potential expansion into subtidal leases in Australia.

The risks associated with diseases and their mitigation are well described and Australian mollusc aquaculture biosecurity is among the most advanced worldwide. Introduction of a new species such as Rock Oysters being farmed in South Australia carries substantial risk, and while Rock Oyster pathogens are well described, the range of environments in which Rock Oysters can reproduce, particularly from large, introduced farmed populations, is not well understood. The environmental and industry risks associated with establishment are substantial and their mitigations are expensive and are unlikely to be 100% successful. Overall, the risk associated with translocating Rock Oysters to South Australia is greater than the expected benefit and is not recommended.

6 Implications

The results of this report will provide information for Pacific Oyster farmers looking to mitigate their POMS risk by diversifying into alternative species. Whilst Rock Oysters were not found to be a suitable alternative, Native Oysters showed potential to be grown at existing Pacific Oyster leases, with growth rates greater than previously reported for this species.

The information derived from this project will have positive implications on the future of Native Oyster farming in South Australia and Tasmania. The project has shown strong survival and growth rates in Native Oysters across lease sites in both states. The results indicate that optimal growing height and location is site specific and strongly driven by local hydrodynamic features, indicating that there may not be a 'one size fits all' model for growing Native Oysters.

Rock Oysters are unsuitable for translocation to South Australia. Although this may decrease the available choices for diversification, avoiding overcatch, fouling and the community and environmental effects of establishment of Rock Oysters will be of substantial benefit to the oyster industry.

7 Recommendations

This project recommends that longer term studies on Native Oyster performance in southern Australian waters should be conducted to determine survival and growth rates to harvest size. The rationale behind this recommendation is that both the South Australian and Tasmanian grow-out trials in this project resulted in strong survival rates, however farmers have previously reported high mortality in this species as they reach maturity. Additionally, it would be beneficial to trial a rotation system between exposed and sheltered conditions over annual cycles to determine the impact of seasonal dynamics and how these can be exploited to improve both growth and shell shape.

The following recommendations are put forward for designing future Native Oyster performance studies:

- Spat can be deployed in either trays or baskets, as initial growing infrastructure had no significant effect on Native Oyster final performance or shape in this study.
- Studies should put a greater emphasis on growing height in the water column compared to on farm site selection, as this had a greater influence on oyster performance.
- Future sites for growing Native Oysters should be assessed based on their local hydrodynamic conditions.
- Native Oysters should be graded in line with commercial practice to reduce competition and gain more accurate time to market size estimates.
- Subtidal trials will be needed to determine if Native Oysters can be farmed in the subtidal leases.

It is recommended that Rock Oysters are not translocated to South Australian oyster growing regions.

Further development

Future Native Oyster performance studies would benefit from further research, development and optimisation of the following:

- Longer temporal studies to determine survival and time to market size.
- The use of a site/growing height rotational system to further optimise growth and shell shape over seasonal cycles.
- Further research into the relationships between Native Oyster growth and condition and the abiotic and biotic environment.
- Performances of Native Oysters in subtidal leases with different grow-out technologies (e.g., floating cages developed overseas).
- Determination of the correlation between Native Oyster shape scores and marketability.
- Establishment of triploid Rock Oysters for aquaculture derived from tetraploid parents could provide the South Australian oyster industry with an increased opportunity for diversification.

8 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 related state agencies (e.g., PIRSA Fisheries and Aquaculture), interested South Australian and Tasmanian oyster farmers and the general public through this final report.

The information has also been extended to industry members in oral presentations at the three state Native Oyster workshops described in section 3.3.

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

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10 Appendices

Appendix 1

ANOVA tables for Chapter 3.2

Table A1.1 Effect of sampling time, site, and growing height on the shell length (DVM) of Native Oyster (*Ostrea angasi*)

Length	SS	df	F	*P*
Time	3386.54	2	277.44	< 0.001
Site	2.02	1	0.33	0.567
Height	469.58	3	25.65	0.000
Time x Site	2.45	2	0.20	0.819
Time x Height	72.93	6	1.99	0.079
Site x Height	36.75	3	2.01	0.121
Time x Site x Height	37.67	6	1.03	0.414
Error	415.02	68		

Table A1.2 Effect of sampling time, site, and growing height on the shell width (APM) of Native Oyster (*Ostrea angasi*)

Width	SS	df	F	*P*
Time	3662.34	2	545.80	< 0.001
Site	29.99	1	8.94	0.004
Height	713.80	3	70.92	< 0.001
Time x Site	14.71	2	2.19	0.119
Time x Height	112.27	6	5.58	< 0.001
Site x Height	23.48	3	2.33	0.082
Time x Site x Height	44.45	6	2.21	0.052
Error	231.50	69		

Table A1.3 Effect of sampling time, site, and growing height on the shell shape of Native Oyster (*Ostrea angasi*)

Shape	SS	df	F	*P*
Time	2.04	2	12.82	< 0.001
Site	0.11	1	1.43	0.236
Height	1.03	3	4.31	0.008
Time x Site	0.20	2	1.23	0.298
Time x Height	0.49	6	1.04	0.411
Site x Height	0.01	3	0.06	0.980
Time x Site x Height	0.38	6	0.80	0.573
Error	5.50	69		

Table A1.4 Effect of sampling time, site, and growing height on the live weight of Native Oyster (*Ostrea angasi*)

Weight	**SS**	**df**	**F**	***P***
Time	2007.19	2	871.90	< 0.001
Site	3.12	1	2.70	0.105
Height	149.44	3	43.28	< 0.001
Time x Site	0.49	2	0.21	0.809
Time x Height	63.92	6	9.26	< 0.001
Site x Height	63.45	3	18.37	< 0.001
Time x Site x Height	27.25	6	3.95	0.002
Error	77.120	67		

Appendix 2

Presentation given at Ostrea angasi workshops

Species diversification to provide alternatives for commercial production

SARDI

FRDC 2016-807

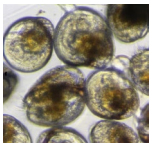
Australian Government Department of Industry, Innovation and Science | Business Cooperative Research Centres Program

Presenter Name
August 2019

FRDC | SOUTH AUSTRALIA | Government of South Australia Primary Industries and Regions SA | SARDI Research and Development

Project Objectives:

1. To develop Native Oyster on-farm growing methods that maximise survival and growth in South Australia and Tasmania
2. To compare the performance between Pacific Oysters and Native Oysters in South Australia
3. To establish a Native Oyster farmers network to share new techniques and knowledge
4. To develop translocation protocols for the safe translocation of Western Rock Oysters to South Australia
5. Trial Western Rock Oysters in the field in South Australia to assess their performance and viability of a potential industry if agreed by industry and regulators



Develop Native Oyster on-farm growing methods that maximise survival and growth in South Australia

- Xiaoxu Li, Mark Gluis and Penny Ezzy

Oyster Australia IPA workshop in 2016 identified key R&D needs

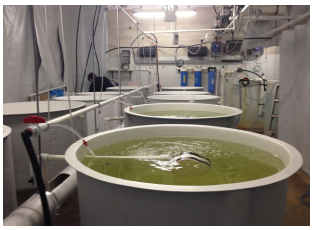
- Having a constant and reliable spat supply
- Development of a selective breeding program
- Establishment of good husbandry practices
- Increase the shelf life/improve packaging/develop processing methods

Experiments	Priority
Investigation into key factors/parameters that affect shelf life of *angasi* oysters	1
Comparison between different farm methods/equipment that have been trialled by growers in Australia	2
Development of techniques to extend the shelf life of *angasi* oysters	3
Comparison between angasi performances farmed at different heights	4
Investigation into seasonal effects on *angasi* performance and quality	5
Comparison between angasi performances farmed at subtidal and intertidal leases	6
Optimization of stocking densities for different size/grade *angasi* oysters	7
Investigation into handling effects on *angasi* performance	8
Comparison between different grading methods/equipment (if available)	9
Comparison of performance between Pacific and *angasi* oysters with methods optimised in this project.	10

Delayed due to unavailability of spat

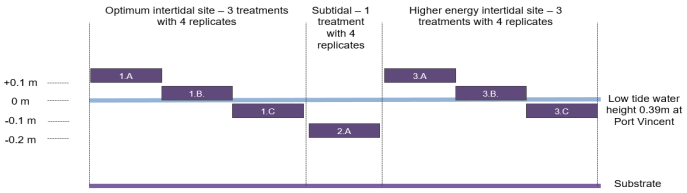
SARDI delivered approximately 100,000 Native Oyster spat to three farms

- Farm 1 – Stansbury
- Farm 2 – Stansbury
- Farm 3 – Kangaroo Island

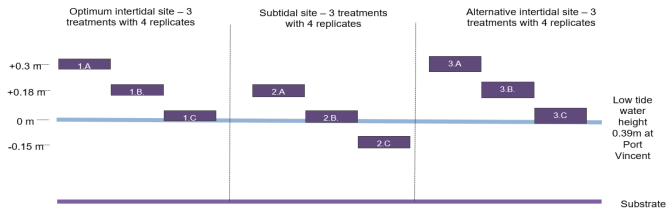


Set up

Farm 1

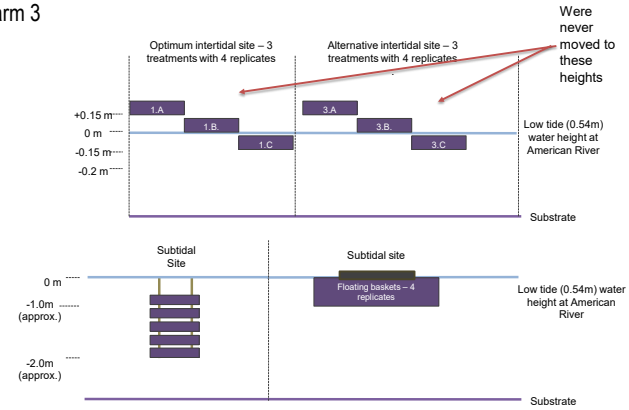


Farm 2



Set up

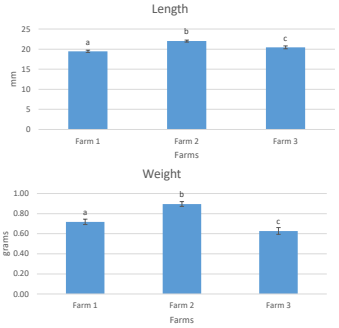
Farm 3



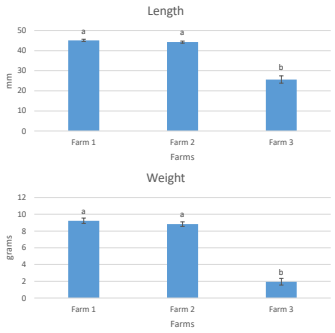
Subtidal sites were abandoned in May due to high mortality

Results so far

November 2018



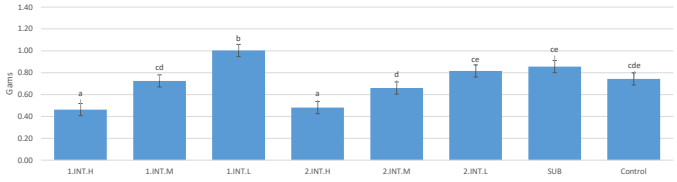
May 2019



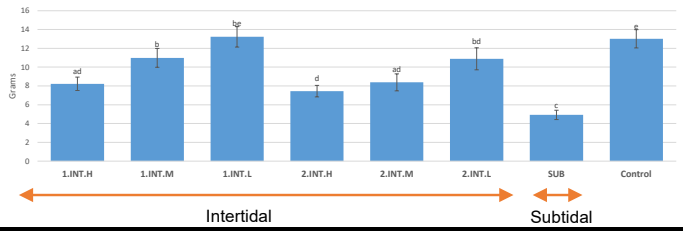
Mean length and weight of Native Oysters collected across all treatments at three farms. Error bars represent 2x standard error.

Results so far – Farm 1

November 2018



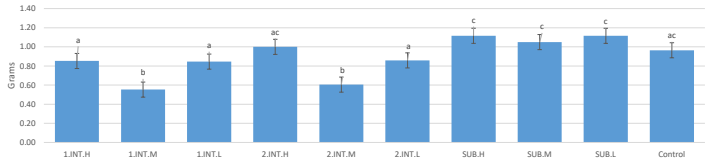
May 2019



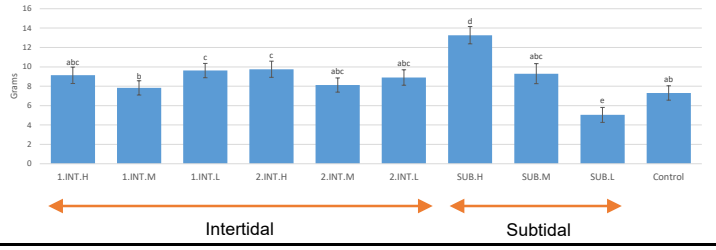
Mean weight (g) at Farm 1
Different lower case letters represent significant differences (p=.05).
Error bars represent 2x standard error.

Results so far – Farm 2

November 2018



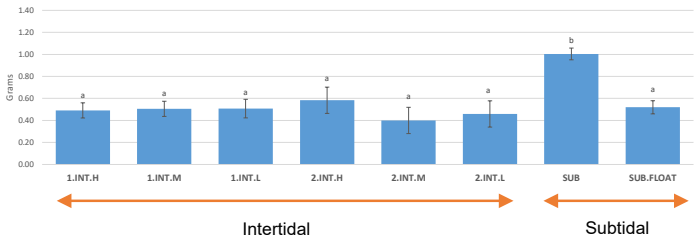
May 2019



Mean weight (g) at Farm 2. Different lower case letters represent significant differences (p=.05). Error bars represent 2x standard error.

Results so far – Farm 3

November 2018



Mean weight (g) at Farm 3. Different lower case letters represent significant differences (p=.05). Error bars represent 2x standard error.

May 2019

High mortality in the subtidal treatments meant this part of the experiment had to be abandoned.

Due to factors outside of our control, no meaningful data on the difference between intertidal treatments could be obtained for this period.

Conclusions so far

- With the exception of Kangaroo Island subtidal treatments, mortality has been minimal across the experiment.
- The intertidal treatments at Farm 1 are preforming better than the subtidal, with the low height intertidal preforming the best.
- At Farm 2, the high subtidal treatment is preforming the best and the low subtidal treatment has the least growth, with little difference between intertidal treatments.
- The results suggest that both site and height in the water column can have an impact on Native Oyster growth.
- The final field measurements (winter) will be completed in August 2019.

Cohabitation experiment of Rock Oysters (Saccostrea sp.) from Western Australia with Pacific Oysters (Crassostrea gigas) and Native Oysters (Ostrea angasi)

- Marty Deveney, Jessica Buss and Kathryn Wiltshire

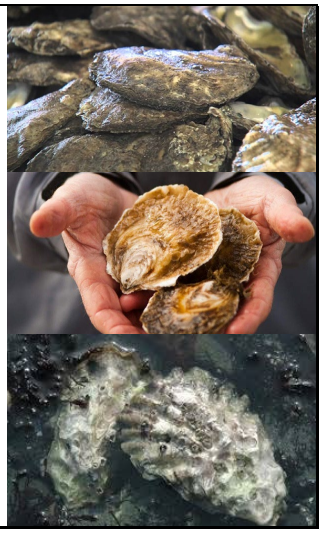
Assess if WA Rock Oysters carried any pathogens which may pose a risk to existing industry, and to assess if WA Rock Oysters are likely to survive cohabitation with oysters grown by the existing industry.

Rock oyster assessment background

- Rock oysters assessed to import to SA
- Species diversification in SA to mitigate POMS risk
- Risk assessment + cohabitation trial + risk management measures development
- Hazards identified for management:
  - OsHV-1/POMS
  - *Haplosporidium* sp. / *Minchinia occulta*
  - *Marteilia sydneyi* / *Martelioides* sp.
- Unknown risks remain, manage using basic biosecurity

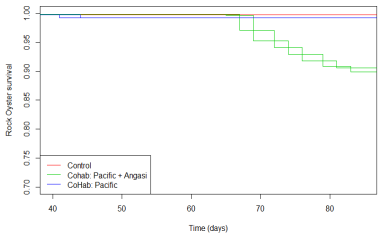
Rock oyster cohabitation

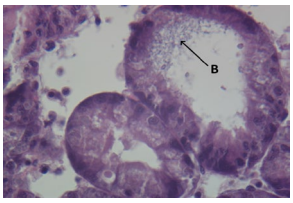
- Cohabited Rock, Pacific and Native Oysters
- 90d fed algae
- Assess transfer of unknown pathogens frm Rock Oysters to SA Native and Pacific Oyster industry species

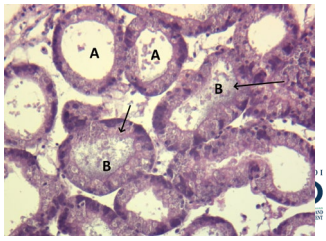


Rock oyster cohabitation

- No unusual mortality in Pacific, Native oysters
- Elevated mortality in Rock oysters
- Bacterial infection observed in digestive tubules







Proposed controls

- Translocation likely to meet appropriate level of risk if:
  - Basic biosecurity:
    - Biosecurity plan that meets national guidelines
    - 3rd party audited
  - Specific controls
    - Demonstrated hatchery freedom from translocation relevant diseases
  - Receiving zone controls and communication
    - Industry agreement on importation
    - Identification of trial zone
    - Assessment of initial farming in SA
- Proposed controls now with regulator