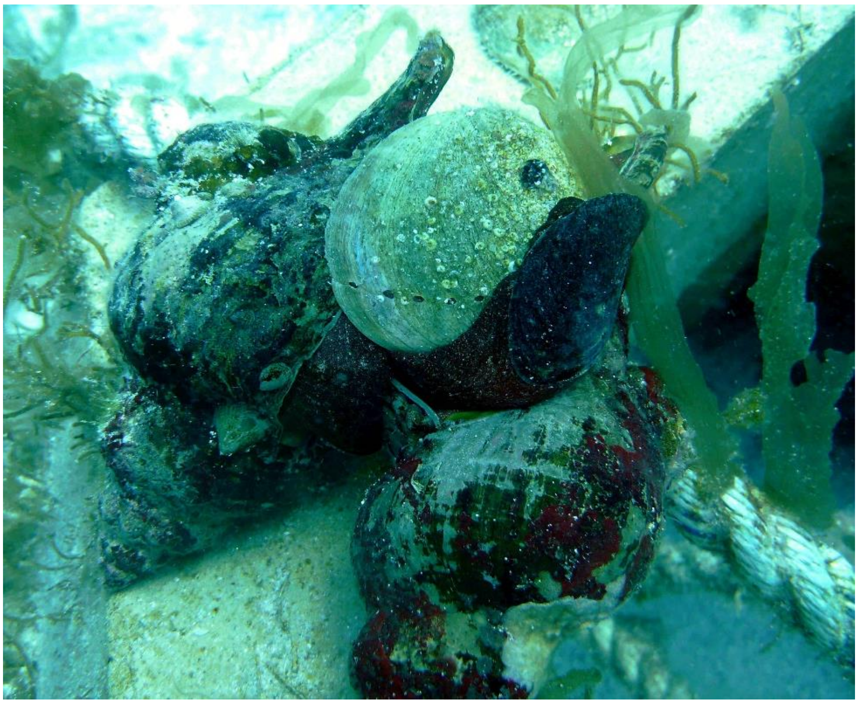
Survival and growth rate of ranched Greenlip Abalone off South Australia



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Cover Photo: Whelks (*Pleuroploca australasiae*) eating Greenlip Abalone (*Haliotis laevigata*); Dr Ben Stobart

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Abbreviations

OGA – Ocean Grown Abalone;

PIRSA – Primary Industries and Regions South Australia;

RFTM – Ray’s Fluid Thioglycolate Medium;

SARDI – South Australian Research and Development Institute.

Executive Summary

The aim of this project was to investigate the viability of Greenlip Abalone (*Haliotis laevigata*) ranching in Thorny Passage, near Port Lincoln, South Australia. Abalone ranching involves stocking and growing juvenile Greenlip Abalone on benthic artificial reefs. This technique has been successfully developed and applied by Ocean Grown Abalone (OGA) in Western Australia. The South Australian Research and Development Institute (SARDI) worked collaboratively with OGA to measure the survival and growth of Greenlip Abalone at four research leases (hereafter referred to as 'sites'). All of the sites assessed in this trial were characterised by low survival and slow growth, relative to equivalent trials in Western Australia. After eight months, survival ranged from 5 - 31% and shell growth from 4 - 14 mm. High levels of predation and low food availability were considered the primary causes of poor survival and growth. A commercial decision was made by OGA to terminate the trial after eight months, which was originally intended to run for 12-months.

Abalone are of substantial importance to Australia's aquaculture and wild catch sectors. Ocean Grown Abalone have successfully developed a commercial Greenlip Abalone sea ranching business in Western Australia, using its patented purpose-built artificial reefs (called "Abitats"). The success of the company has been underpinned by collaborative research and development undertaken prior to establishing new commercial ranching locations. This philosophy was applied in South Australia, because OGA was considering expanding its operations to waters in Thorny Passage near Port Lincoln, South Australia. This location is a logical choice for commercial Greenlip Abalone sea ranching because it already supports abundant natural populations of Greenlip Abalone, is proximal to a major port with easy access to local personnel with suitable marine experience, and existing Greenlip Abalone farms nearby can provide juveniles from local broodstock for stocking the ranches.

The South Australian Research and Development Institute worked collaboratively with OGA to assess the viability of Greenlip Abalone ranching at four sites. The initial project objectives were to 1) measure the survival and growth of Greenlip Abalone over 12-months; 2) quantify the influence of abiotic and biotic conditions (e.g. predators, food availability, temperature, tide, swell) on survival and growth; 3) monitor the prevalence of *Perkinsus olseni* (hereafter *Perkinsus*) in ranched and wild Greenlip Abalone; 4) determine the reproductive state of Greenlip Abalone from the ranches; and 5) compare and contrast these results with replicated studies from Western Australia. Due to low survival rates and slow growth at each of the sites, the intended 12-month research trial was terminated by OGA after eight months, and the project scope was reduced, particularly Objectives 2 and 5.

Ten Abitats were deployed and positioned by OGA within each of the research leases to establish a trial reef (approximately 20 × 2 m in overall size). In September 2017, each site was stocked with 4000 juvenile Greenlip Abalone (mean length 48.8 mm) by OGA. The survival and growth of Greenlip Abalone was measured by SARDI, while OGA monitored the presence of predators, food availability and recorded mortalities on a fortnightly basis. Due to the vicinity of the research trial to wild stocks, risks associated with genetics and disease were also assessed. Ranched and nearby wild populations of Greenlip and Blacklip Abalone were tested by SARDI for infection by the protozoan parasite *Perkinsus* on two occasions. The sexual maturity of Greenlip Abalone was also assessed prior to release and at the conclusion of the trial.

Low Greenlip Abalone survival and growth were observed at all four sites throughout the trial. After eight-months, survival of Greenlip Abalone ranged from 5 - 31%, while growth ranged from 4 - 14 mm shell length. Predation by whelks, crabs and starfish were considered the primary cause of poor Greenlip Abalone survival. Food availability, measured as an index of drift algae, was also low throughout the trial. The highest survival and growth rates were recorded at Thistle 2 (31% and 14 mm after 8 months). This site was the shallowest and most tidally influenced of the four locations, and had the greatest food availability during the trial. Growth was slow at all other sites (approx. 4 mm after 8 months).

Perkinsus prevalence did not differ between the ranched and wild Greenlip Abalone. There was, however, evidence that *Perkinsus* prevalence was higher during summer (*i.e.* December) than late-autumn (*i.e.* May), most likely associated with higher water temperatures. The prevalence and observed intensity of *Perkinsus* in both the ranched and wild Greenlip Abalone was low when compared with wild Blacklip Abalone, which are known to have high prevalence of clinical signs of *Perkinus* infection in the Thorny Passage area. The ranched Greenlip Abalone were not considered to be sexually mature either before or after the trial.

This project identified low survivorship and slow growth rates of ranched Greenlip Abalone in Thorny Passage. Even at the site with the highest survival and growth (Thistle 2), these parameters were approximately 50% lower than equivalent trials in Western Australia. These findings imply that the sites in Thorny Passage are not commercially viable for Greenlip Abalone ranching. This inference is consistent with OGA's commercial decision to terminate the trials in SA eight months into the planned 12-month program.

Keywords

Greenlip Abalone, *Haliotis laevigata*, Ocean Grown Abalone, ranching, Port Lincoln, mortality, growth, *Perkinsus olseni***.**

Introduction

Abalone are sedentary, long-lived and slow growing marine molluscs of importance to Australia's aquaculture and wild catch fishing industries (Mobsby and Koduah 2017). In domestic and international markets, they are renowned as a premium and high value delicacy. To address growing worldwide demand for abalone, production from land-based and sea-cage aquaculture has grown rapidly since the 1980s (Park and Kim 2013, Cook 2016). However, there are substantial costs associated with different aspects of these production techniques, including energy, labour, feed and infrastructure, which can decrease their economic viability (Cloete and Jensson 2009, Cook 2016).

Ocean Grown Abalone (OGA) have successfully developed a commercial Greenlip Abalone sea ranching business in waters of Flinders Bay, Western Australia. Using patented purpose-built artificial reefs (called "Abitats"), OGA was considering expanding its operations to waters near Port Lincoln, South Australia. The success of the company has been underpinned by collaborative research and development, particularly through undertaking feasibility studies prior to establishing new commercial ranching locations. Initial research focussed on survival and growth of Greenlip Abalone to: optimise the benthic structures used for ranching; optimise the size of stock at release; and ensure locations selected were suitable before commencing commercial production (Melville-Smith et al. 2013). In trials from Flinders Bay and Wylie Bay WA, OGA have recorded growth rates of approximately 30 - 40 mm per year and annual survival between 50 - 90% of stocked individuals (Melville-Smith et al. 2013; OGA 2018).

A host of abiotic and biotic conditions that are important for successful Greenlip Abalone ranching have also been monitored in previous studies, such as food availability, predation and hydrological conditions (*i.e.* swell, tide; Melville-Smith et al. 2013). More recent studies have refined this understanding in Flinders Bay, through seasonally resolved hydrological modelling, and testing the quality and type of different algal species available as a food source for ranched Greenlip Abalone (Melville-Smith et al. 2017).

The waters of Thorny Passage near Port Lincoln are a suitable location for testing commercial Greenlip Abalone sea ranching prior to considering commercial production. Setting up a commercial ranching operation requires: access to a major port and large vessels to assist with deployment and retrieval of infrastructure; access to personnel with suitable marine skills and experience; and proximity to existing Greenlip Abalone farms nearby that can provide juveniles from local broodstock. Port Lincoln can supply all of these and, importantly, the area also supports substantial natural populations of both wild Greenlip and Blacklip Abalone, which have been harvested commercially in this region and surrounds since the 1960s (Mayfield et al. 2011, Stobart et al. 2017). Suitable environmental conditions are therefore likely to be available for ranching Greenlip Abalone in this area.

Thorny Passage is near commercially-fished wild populations of Greenlip and Blacklip Abalone, therefore any risks or threats to these populations were considered a key licencing consideration and research objective.

There is potential for the ranched Greenlip Abalone to interact genetically with the wild stocks through either spawning or escape. Greenlip Abalone can reach maturity from lengths of approximately 70 mm (Burnell et al. 2018, Stobart et al. 2018), and despite their sedentary nature have been documented to migrate small distances across sand (Dixon et al. 2006).

Disease risks are also important for the interactions between wild and ranched abalone. *Perkinsus* is a protozoan parasite that is common among many mollusc species worldwide, including abalone (Villalba et al. 2004). The disease can reduce the marketability of abalone due to visible lesions on the foot and mantle, and often results in mortality of the host, particularly when animals are stressed by high water temperatures (Lester and Davis 1981, Goggin and Lester 1995, Villalba et al. 2004). Thorny Passage is a known hotspot for *Perkinsus* in Blacklip Abalone (Lester and Davis 1981) and it has been documented there frequently since the 1970s (Lester and Davis 1981, Goggin and Lester 1995, Lester and Hayward 2005). As a result, commercial fishing for Blacklip Abalone in the area has subsequently declined substantially since the 2000s (Stobart et al. 2017). For Greenlip Abalone, the presence and expression of *Perkinsus* throughout South Australia is generally much lower. *Perkinsus* was, however, implicated in mass mortalities of Greenlip Abalone observed in Gulf St Vincent in the 1980s (O'Donoghue et al. 1991; Goggin and Lester 1995). Due to its presence in Thorny Passage and documented ability to impact populations of Greenlip Abalone, a key research objective was to quantify the prevalence of *Perkinsus* infection in ranched and wild Greenlip Abalone during the OGA trial.

Objectives

The aim of this study was to quantify and document the survival and growth of Greenlip Abalone on sea ranches in Thorny Passage near Port Lincoln, South Australia. This follows successful testing of Greenlip Abalone ranching technology and subsequent transition to commercial production by OGA in Western Australia.

The five objectives of the project were as follows:

1. Quantify rates of survival and growth of Greenlip Abalone at sea ranches in South Australia;
2. Quantify the influence of abiotic (*i.e.* tide, swell, temperature) and biotic (*i.e.* food availability, predator activity) conditions on survival and growth of Greenlip Abalone at sea ranches in South Australia;
3. Monitor the prevalence of the *Perkinsus* infection in Greenlip Abalone at sea ranches and in nearby wild populations;
4. Determine the reproductive state of Greenlip Abalone from sea ranches; and
5. Compare and contrast the survival and growth of Greenlip Abalone from sea ranches in South Australia with those from replicated studies in Western Australia.

In February 2018, approximately six months after the project commenced, OGA informed SARDI they were abandoning the trial due to poor survival and growth of Greenlip Abalone. At this time, the objectives of the project were modified, in accordance with an agreed significant variation in the project scope and costs. Primarily, the final sampling planned for September 2018 was undertaken four months early (*i.e.* during May 2018), and the objectives 2 and 5 were reduced.

Methods

Study site

The study was undertaken at four, 1-hectare research leases located in Thorny Passage near Port Lincoln, South Australia (Figure 1). The research leases were separated by a minimum of 5 km from known commercial populations of Greenlip Abalone. Specific locations of the trial reefs within the research leases (referred to as ‘sites’) were selected by OGA to ensure separation from any significant seagrass or reef habitats. The sites selected within these research leases had depths between 14 - 22m with soft shale sediment.

Site preparation

Prior to stocking, the trial reefs were deployed by OGA and allowed to condition on the sea-bed for four weeks. For a detailed description of the trial reefs (called “Abitats”) see Appendix 1a - c, and Melville-Smith et al. (2013). The Abitats at each site were arranged in a staggered row creating a ~20m long reef in an East-West direction (see Appendix 1a - c). Orienting the reefs perpendicular to the tidal flow maximised the likelihood of capturing drift algae for the abalone to consume. Concrete sleepers were also arranged in a perimeter 3 - 4m away surrounding the Abitat reefs, to record Greenlip Abalone that migrated from the trial reefs (see Appendix 1d).

Stocking of Abitats

From the 25 - 28th September 2017, OGA stocked 16,000 juvenile Greenlip Abalone (mean length ± se = 48.8 ± 0.2 mm, n = 473) at the four trial reefs (*i.e.* 4000 per site) using purpose built release units (see Appendix 1e). Greenlip Abalone were sourced from Yumbah Aquaculture, Port Lincoln. Prior to stocking, translocation approvals were obtained through Primary Industries and Regions SA (PIRSA), Fisheries and Aquaculture division. The stocked juvenile abalone were the progeny of broodstock selected for several generations in land-based systems, and thus were considered domesticated. Abalone were individually removed by-hand at the hatchery prior to transport and stocking. At the beginning and conclusion of the trial, SARDI visually assessed the gonad development of 50 Greenlip Abalone at the Port Lincoln Marine Science Centre to determine if any of the abalone were sexually mature. None of the Greenlip Abalone inspected on either occasion were considered to be sexually mature (Appendix 1f).

Survival, growth, mortality and migration

Survival and growth of Greenlip Abalone were recorded two months post-release, and again after eight months, when the trial was concluded. Collection of the survival and growth data were shared between OGA and SARDI. The number of Greenlip Abalone stocked per site (*i.e.* 4000) and the mean length of the stock prior to release were recorded by OGA. *In situ* counts and length measurements of stock were undertaken two months after release by SARDI divers using submersible digital calipers (ZebraTech, Nelson NZ). At the conclusion of the trial after eight months OGA divers harvested and counted the stock from all sites, and provided samples to SARDI for final length measurements and biological checks (*i.e.* sexual maturity and *Perkinsus*). Growth estimations during the trial were based on changes in the mean length of all abalone measured. Survival was measured as the number of abalone remaining at each site, and is expressed in raw numbers or as a percentage (i.e. (number remaining / 4000)*100).

Mortality and migration were recorded *in situ* by OGA divers on each occasion they visited the trial reefs. Mortality was defined as the number of empty shells found surrounding the trial reefs, and almost certainly underestimates true mortality (Dixon et al. 2006). Migration was defined as the number of individual Greenlip Abalone that were found on the surrounding perimeter of concrete sleepers on each sampling date. Greenlip Abalone that had migrated to the sleepers were removed and returned to the trial reefs during each visit by OGA.

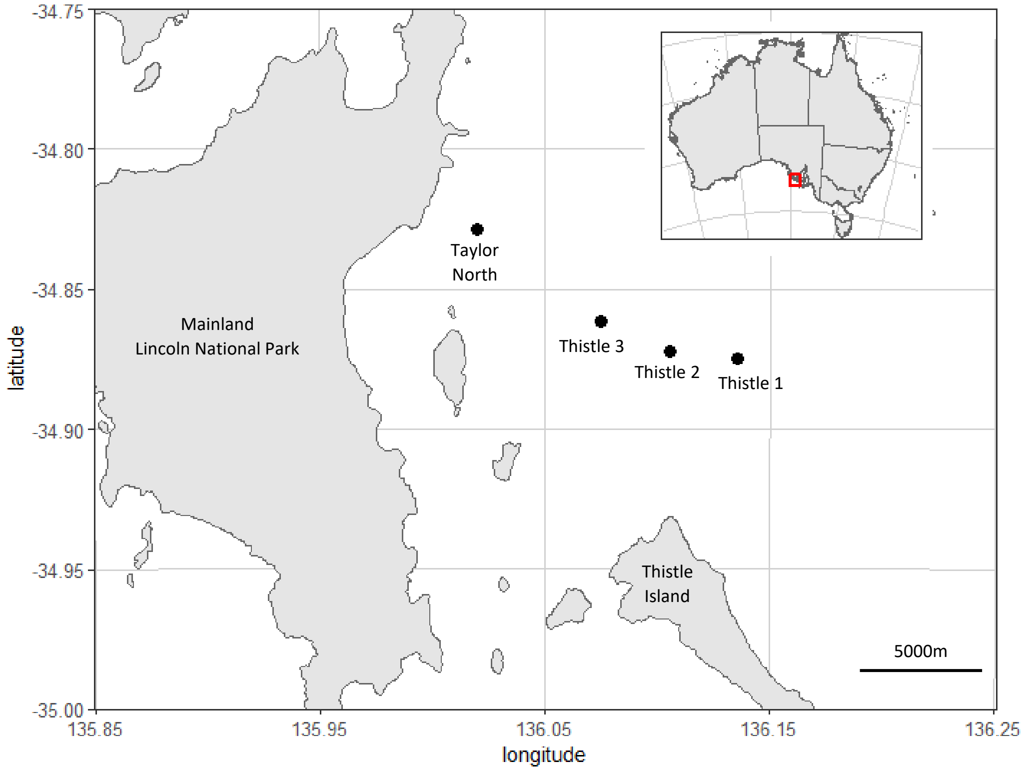


Figure 1. Map of the four OGA research leases near Thorny Passage, South Australia.

Biotic conditions - food availability and predator abundance

Data collection of biotic conditions occurred each time OGA divers visited the sites. Visits were intended to occur on a fortnightly basis, but this was subject to accessibility due to sea conditions, visibility and tides.

Food availability was quantified by the coverage of drift algae (called ‘food index’), scored as an index of coverage ranging from 0 (*i.e.* no algae) to 5 (*i.e.* reefs completely buried by algae).

Invertebrate predators were recorded by OGA divers at each site using a visual search of the trial reefs. Initially, predatory whelks, sea-stars and octopuses were included in the counts. Later, when the significance of crabs as a predator became evident at the Taylor North site, these were also included in the counts at this location only. All predators were relocated to nearby areas, to conform with normal fortnightly operations at OGA’s existing leases in WA.

Abiotic conditions - temperature

Water temperatures were recorded on HOBO® data loggers (Onset, MA, USA) at each of the four sites are reported between December (12/12/2017) and the conclusion of the trial (15/5/2018). Data loggers were programed to take hourly measurements, which were used to calculate mean daily temperature.

Perkinsus *testing*

To estimate the prevalence of *Perkinsus* infection, approximately 20 Greenlip Abalone were collected from each site and two nearby wild populations in December and May. Blacklip Abalone were also collected concurrently from the wild populations to serve as a reference collection.

Testing followed the Ray’s Fluid Thioglycolate Medium (RFTM) procedure described by the European Union Reference Laboratory for Molluscs Diseases (2011), which has been adapted from Ray (1952). Gill material from each abalone was used for the test. After incubation, samples were pipetted on to a slide for inspection under an optical microscope. A selection of samples were stained with Lugol’s iodine to confirm *Perkinsus* infection. Each slide was then classified as either positive, negative or indeterminate for *Perkinsus*. Indeterminates are samples where an organism was detected but could not be confirmed to be *Perkinsus* due to differences from the described criteria for *Perkinsus* in size or staining. These may be *Perkinsus* positives but would require further investigation for confirmation.

A logistic regression using the R package lme4 (Bates et al. 2015; R Core Team 2018) was used to compare the effect of population (*i.e.* wild *vs* ranched) and sampling date (December *vs* May) on the likelihood that an individual Greenlip Abalone was infected with *Perkinsus*. Site was included as a random factor, nested within each of the populations. The model took the form: glmer *(Perkinsus* ~ Population + Date + (1|Population/Site), family=binomial(link='logit')), where Greenlip Abalone infected were binary coded as “1” and those not infected were coded as “0” for the dependent variable *Perkinsus*. Indeterminates and Blacklip Abalone were excluded from the analysis. Likelihood ratio tests were undertaken using the drop1 function from the R package stats (R Core Team 2018) to determine the significance of the fixed effects (i.e. Population and Date) in predicting infection by *Perkinsus*. While no quantitative measure of infection intensity was made during this study, observational differences between the two species are included in the results.

Results

Growth, Survival, Mortality & Migration

The largest increase in mean length was observed at Thistle 2, while at the other three sites growth was slower (Figure 2a). Two months after release, Greenlip Abalone had grown 1 - 3 mm across the four sites, indicating initial growth at all sites was slow (see Appendix 2a-b). When the Greenlip Abalone were harvested at the conclusion of trial, the mean shell length at Thistle 2 was ~63 mm compared with ~53 mm at the other three sites. This equated to a mean increase of 14 mm at Thistle 2 (average growth rate 0.059 $mm.day^{-1}$) over the eight-month trial, compared with ~4 mm at the other three sites (average growth rate ~0.018 $mm.day^{-1}$).

Two months post-release, survival was similar among the four sites, ranging from 54 - 58% (Figure 2b). These post-release survival rates are likely to be an underestimate, because many Greenlip Abalone occupied cryptic habitat underneath the trial reefs, which was created by scouring of the sediment. At the conclusion of the trial after eight-months survival ranged from 5 - 31%. Survival was highest at Thistle 2 (31%) and lowest at Thistle 1 (5%).

Recovered mortalities underestimated total mortality (Figure 2c). Shells from dead Greenlip Abalone are not recovered for various reasons (e.g. predation, tidal movement). Initial mortality rates were high, but generally decreased throughout the trial. The site with the highest survival, Thistle 2, also had the lowest number of recovered mortalities. The remaining three sites all had higher mortality than Thistle 2. Recovered mortalities were not recorded on the final sampling date.

The total number of Greenlip Abalone migrations throughout the trial ranged from 95 - 161 at each site (Figure 2d). There was generally an inverse relationship between survival and migration. The lowest number of migrations (n = 95) were observed at Thistle 2, which had the highest survival rates. While the highest number of migrations occurred at Thistle 1 (n = 165), which had the lowest survival rates. Migrations are not expressed as a percentage of the stock, as Greenlip Abalone were returned to the reefs following migration. Thus, the same Greenlip Abalone could have completed multiple migrations. Migrations were not recorded on the final sampling date.

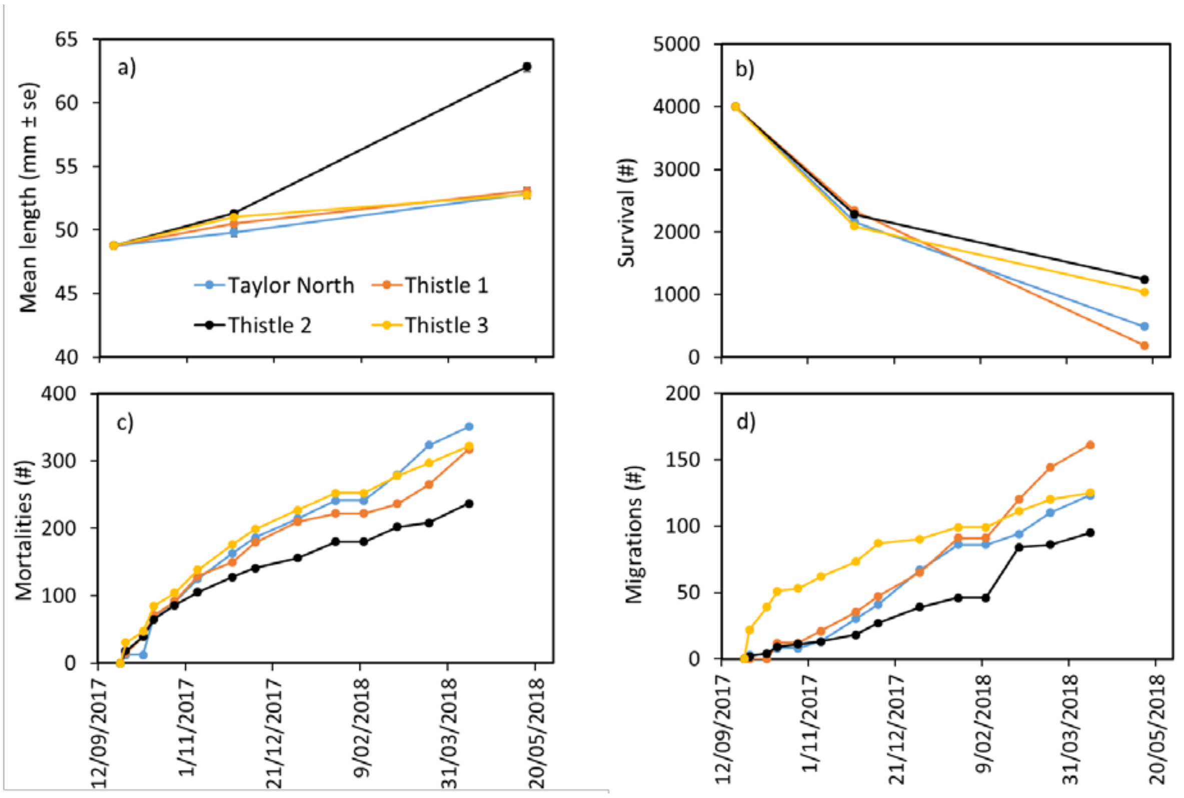


Figure 2. a) mean length (mm ± se), b) survival (number), c) mortalities (cumulative number) and d) migrations (cumulative number) of Greenlip Abalone at the four OGA sites.

Biotic Conditions - food availability and predator abundance

The availability of algal food for the Greenlip Abalone was lower than anticipated across all of the sites during the trial period (Darren Tressider pers. comm.). The mean food index, which was scored from 0 to 5 on each occasion the sites were visited, was highest at Thistle 2 (mean of 2.08), and lower at the other three sites, Thistle 1 (1.25), Thistle 3 (1.17) and Taylor North (0.73; Table 1). Higher availability of food at Thistle 2, therefore coincided with the highest growth and survival, and the lowest rates of mortality and migration. In general, the food index was highest when the Greenlip Abalone were released in September and near the end of the trial, during autumn, but was generally lower throughout the summer months.

The abundance of predators varied over time and among the sites (Table 1, see Appendix 2c, 2d, 2e). Starfish were most abundant during autumn, except for Taylor North. The mean number of starfish was highest at Thistle 2 (1.69 per visit) and lowest at Taylor North (1.08 per visit). The number of predatory whelks was relatively consistent for the sites over time. Thistle 2 consistently had the highest number of whelks (14.5 per visit), whilst the lowest number were recorded at Taylor North (3.75 per visit). The abundance of crabs was not recorded consistently throughout the trial period at each site. Crabs were, however, opportunistically recorded when they were encountered predating on Greenlip Abalone at the Taylor North site. At this site, crabs were observed to be the key predator of Greenlip Abalone. No octopus were observed throughout the trial period.

Table 1. Food index (scored from no algae = 0 to completely buried = 5), starfish (number), whelks (number) and crabs (number) recorded on each sampling occasion and the mean per visit at the four OGA sites. NA indicates no sampling was undertaken.

		Taylor North				Thistle 1				Thistle 2				Thistle 3			
Season	Date	crabs	starfish	whelks	food index	crabs	starfish	whelks	food index	crabs	starfish	whelks	food index	crabs	starfish	whelks	food index
Spring	28/09/2017	NA	1	1	2	NA	0	9	3	NA	2	12	3	NA	1	5	2
	8/10/2017	NA	0	0	NA	NA	1	15	1	NA	1	14	1	NA	2	11	1
	14/10/2017	NA	2	15	1	NA	0	6	2	NA	1	15	1	NA	0	18	1
	26/10/2017	NA	1	3	1	NA	1	1	1	NA	0	18	1	NA	0	4	2
	8/11/2017	NA	2	5	1	NA	0	3	1	NA	2	11	1	NA	0	9	1
	28/11/2017	NA	2	2	1	NA	2	7	1	NA	1	15	1	NA	0	12	1
Summer	11/12/2017	20	1	6	0	NA	1	5	1	NA	2	14	2	NA	2	6	1
	4/01/2018	20	1	1	0	NA	3	9	1	NA	1	21	2	NA	1	4	0
	26/01/2018	30	0	3	0	NA	0	12	1	NA	1	19	2	NA	0	4	0
	11/02/2018	NA	0	0	NA	NA	0	0	NA	NA	0	0	NA	NA	0	0	NA
Autumn	2/03/2018	20	1	5	0	NA	1	8	1	NA	3	7	3	NA	3	7	1
	20/03/2018	20	1	2	1	NA	3	11	1	NA	2	11	4	NA	1	7	2
	12/04/2018	20	1	2	1	NA	5	6	1	NA	3	21	4	NA	2	3	2
	15/05/2018	NA	0	0	NA	NA	2	3	NA	NA	3	11	NA	NA	4	9	NA
	mean (per visit)	**21.67**	**0.93**	**3.21**	**0.73**	**NA**	**1.36**	**6.79**	**1.25**	**NA**	**1.57**	**13.50**	**2.08**	**NA**	**1.14**	**7.07**	**1.17**

Temperature

The recorded mean daily temperature was similar among all four of the sites (Figure 3). The highest temperature of 22.2°C was recorded during February. The lowest temperature of 17.4°C was recorded in May near the conclusion of the trial.

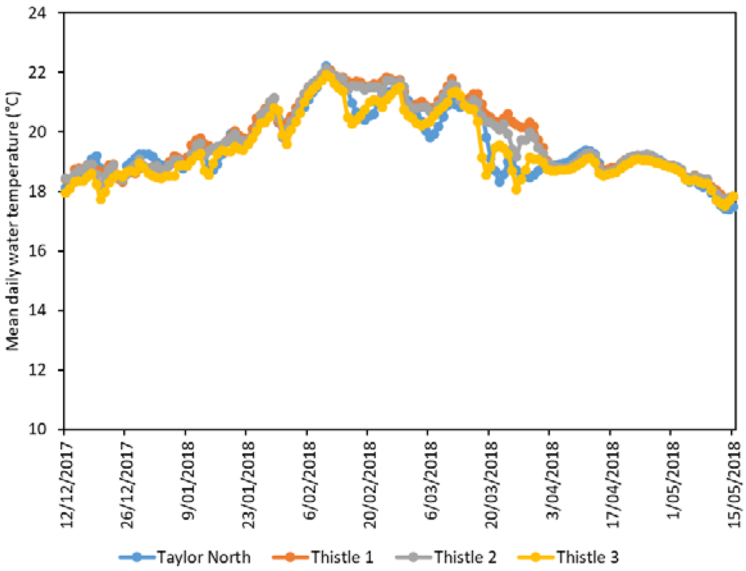


Figure 3. Mean daily water temperature at the four OGA sites throughout the duration of the trial.

Perkinsus

For ranched Greenlip Abalone, the overall prevalence of Perkinsus was 15%, compared with 11% for the wild Greenlip Abalone (Figure 4). The source population (*i.e.* ranched *vs.* wild) was not, however, a significant predictor for *Perkinsus* prevalence in the logistic regression (Table 2, 3). When the source population was dropped from the model as a predictor the AIC decreased, indicating it was not a significant predictor of *Perkinsus* prevalence. In contrast, *Perkinsus* prevalence did vary significantly between the two sampling dates (Table 2, 3). When the sampling date was dropped from the model as a predictor the AIC increased, indicating it was a significant predictor of *Perkinsus* prevalence. In December, *Perkinsus* prevalence for Greenlip Abalone was approx. 18%, but this decreased to 9% in May. For Blacklip Abalone, the *Perkinsus* prevalence in the wild population was higher than for Greenlip Abalone. Blacklip Abalone samples were primarily included as a reference collection to ensure there were positive Perkisus samples available for comparative purposes, thus were not included in the logistic regression.

Observational data indicated the intensity of the *Perkinsus* infection varied markedly between Greenlip and Blacklip Abalone. On visual inspection of the mantle many wild Blacklip Abalone showed evidence of lesions consistent with *Perkinsus* infection (31% in December and 10% in May), whereas there were no visible lesions for either the ranched or wild Greenlip Abalone for either sampling date. For Greenlip Abalone, the observed intensity of the infection was very low. Typically, infections in both the ranched and wild Greenlip Abalone were limited to one or a few pre-spore phase cells (Appendix 3a, 3b). However, there were instances of zoosporangia with well-developed zoospores in both the wild and farmed Greenlip Abalone (Appendix 3c, 3d), but these were rare. In contrast, for Blacklip Abalone, the observed intensity of the infection was generally high for samples that tested positive for *Perkinsus,* with 10 to 100s of well-developed zoosporangia per slide (Appendix 3e, 3f, 3g).

Table 2. *Perkinsus* prevalence in ranched Greenlip Abalone at the four OGA sites and wild Greenlip and Blacklip Abalone from two nearby wild populations from two sampling dates.

Site	Date	Population	Species	*n*	Length (mean ± se)	Visual (% infected)	Positive	Negative	Indeterminate	Prevalence (% infected)
Thistle 1	12/12/2017	Ranched	GL	20	52.80 ± 0.84	0.00	7	11	2	35.00
Thistle 2	12/12/2017	Ranched	GL	20	57.75 ± 1.29	0.00	3	16	1	15.00
Thistle 3	12/12/2017	Ranched	GL	20	56.30 ± 0.99	0.00	1	18	1	5.00
Taylor North	12/12/2017	Ranched	GL	21	53.55 ± 1.01	0.00	5	15	1	23.81
Taylor Island	12/12/2017	Wild	GL	24	108.66 ± 5.25	0.00	1	21	2	4.17
Thistle Island	12/12/2017	Wild	GL	26	130.35 ± 5.08	0.00	6	20	0	23.08
Taylor Island	12/12/2017	Wild	BL	24	134.82 ± 2.94	25.00	17	7	0	70.83
Thistle Island	12/12/2017	Wild	BL	5	138.60 ± 4.17	60.00	3	2	0	60.00
Thistle 1	15/05/2018	Ranched	GL	20	61.14 ± 0.72	0.00	2	16	2	10.00
Thistle 2	15/05/2018	Ranched	GL	20	75.05 ± 0.83	0.00	3	17	0	15.00
Thistle 3	15/05/2018	Ranched	GL	20	62.20 ± 0.50	0.00	1	19	0	5.00
Taylor North	15/05/2018	Ranched	GL	20	60.95 ± 0.53	0.00	2	18	0	10.00
Taylor Island	15/05/2018	Wild	GL	20	142.35 ± 6.51	0.00	3	16	1	15.00
Thistle Island	15/05/2018	Wild	GL	20	148.20 ± 2.01	0.00	0	20	0	0.00
Taylor Island	15/05/2018	Wild	BL	10	139.70 ± 8.41	10.00	8	2	0	80.00
Thistle Island	15/05/2018	Wild	BL	0	NA	0.00	0	0	0	0.00

Table 3. Likelihood ratio test outcomes from the drop1 function for the logistic regression of *Perkinsus* prevalence in Greenlip Abalone.

	Df	AIC	LRT	Pr(Chi)	
<none>		200.87			
Population	1	199.79	0.93	0.34	
Date	1	203.34	4.48	0.03	*

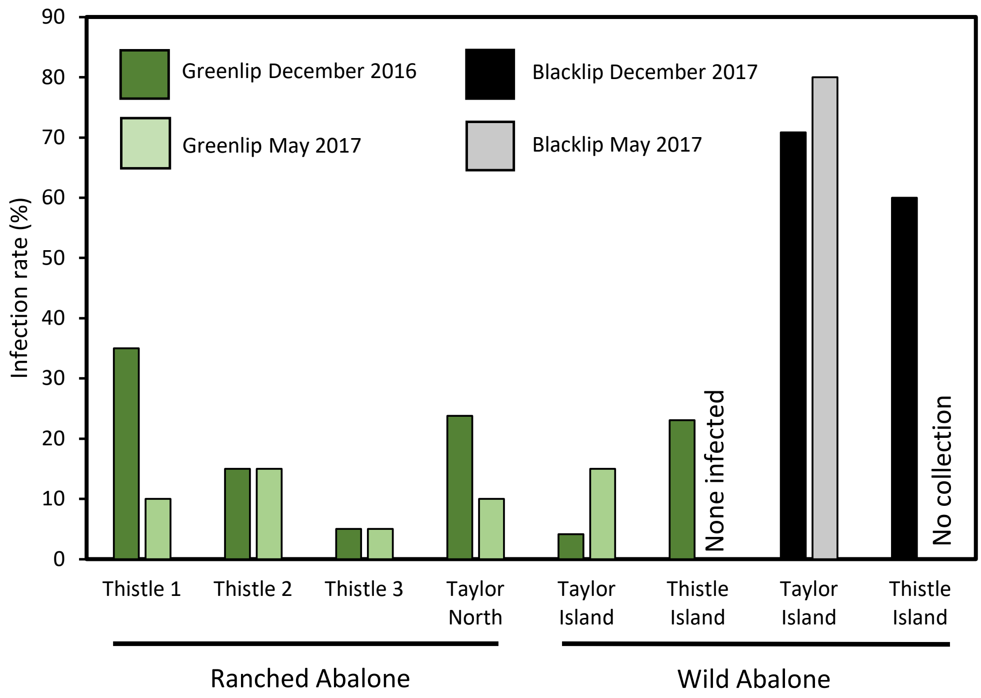


Figure 4. *Perkinsus* prevalence in ranched Greenlip Abalone at the four OGA sites and wild Greenlip and Blacklip Abalone from two nearby populations. Sampling dates are indicated by shading (darker shades, 12/12/2017; lighter shades, 15/05/2018).

Discussion and Conclusion

The low survival rate and poor growth recorded indicate Thorny Passage is unlikely to be a suitable location for Greenlip Abalone ranching. Survival rates were substantially below those for similar length stock released on OGA's Abitats in Flinders Bay and Wylie Bay, Western Australia (Melville-Smith et al. 2013, OGA 2018), where annual survival rates between 50 - 90% have been observed, compared with 5 - 31% over 8-months in this trial.

Stocked farm-reared juvenile abalone are vulnerable to a multitude of biotic and abiotic conditions that govern survivorship, including predation, food availability, disease and other physiological stressors, such as high water temperatures (Dixon et al. 2006, Russell et al. 2012, Hansen and Gosselin 2013). Low survival, comparable to that observed in Thorny Passage has been reported for juvenile Greenlip Abalone at other sites in South Australia (Dixon et al. 2006). The high mortality in this trial was associated with predation by starfish, crabs, and whelks. Dixon *et al.* (2006) also found predation by starfish and crabs was likely to be among the most significant causes of Greenlip Abalone mortality.

High predation was likely compounded by physiological stress from the combination of low food availability and summer water temperatures in Thorny Passage. The availability of drift algae as a food source in this study was substantially lower than previous studies in Western Australia (Melville-Smith et al. 2013). The combination of low food availability and increased physiological demands associated with higher water temperatures are known to limit survival and growth of abalone (Vilchis et al. 2005, Lange et al. 2014, Stone et al. 2014). Occurrences of greater food availability in late-summer and autumn appear to have promoted comparatively higher survival and growth at some sites (e.g. Thistle 2). However, high summer water temperatures alone are unlikely to have limited the success of this trial. The maximum summer water temperature recorded during this study was 22.2°C, which was similar with summer maxima of approx. 21-22°C recorded during successful OGA operations in Flinders Bay, WA (Adams unpublished).

The survival of the stocked hatchery-reared juvenile abalone may also have been dependent upon the background of broodstock used for rearing. Hatchery-reared juvenile abalone are known to respond differently to wild abalone when exposed to natural stressors, such as predation (Lachambre et al. 2017; Hansen and Gosselin 2016), and therefore, selection of broodstock should be a key consideration for any ranching, restocking or restoration trial (Roussel et al. 2019). The juvenile stock used in this trial were considered domesticated, as the broodstock were selected from individuals that had spent multiple generations in land-based systems. Comparable studies in Western Australia used progeny of broodstock collected directly from the wild for stocking OGA's reefs (Melville-Smith et al. 2013). The extent to which this impacted the success of this trial and the high predation rates are unknown.

The growth rate of Greenlip Abalone at Thistle 2 was 3-fold greater than the other sites. Thistle 2 was also observed to have the highest tidal flows (Darren Tressider pers. comm.) and was the shallowest site. The water depth at Thistle 2 was ~15m, whilst at the other sites it ranged from ~18 - 22m. Growth at this site (*i.e.* ~14 mm over eight months) was still slower than annual growth increments from comparable studies in WA (*i.e.* 33 - 36 mm over 12 months; Melville-Smith et al. 2013, OGA 2018).

There are a number of key environmental differences between Thorny Passage and locations from Western Australia where Greenlip Abalone ranching has been successful. The comparatively poor outcome of this trial may have been contingent on a number of these environmental differences. The locations of the research leases in Thorny Passage are tidally-driven, soft-bottom, and seagrass dominated habitats at the southern end of Spencer Gulf, while Augusta in Western Australia is an oceanic and swell-driven, sandy embayment. Accordingly, the two locations are likely subject to different annual hydrological and temperature regimes, and biological differences, such as food quality, food availability and predator assemblages. While an understanding of these conditions is important for optimising Greenlip Abalone ranching (Melville-Smith et al. 2017), further investigation of these conditions were not undertaken due to low survival in Thorny Passage.

During this trial there was no evidence that ranched Greenlip Abalone were more vulnerable to *Perkinsus* infection than wild Greenlip Abalone. In contrast, there was some evidence the prevalence of *Perkinsus* in Greenlip Abalone was higher during the summer sampling period (*i.e.* December), than during late-autumn (*i.e.* May), probably associated with higher water temperatures (Goggin and Lester 1995). As anticipated, there was also evidence of higher *Perkinsus* prevalence in Blacklip Abalone than in ranched and wild Greenlip Abalone, including lower intensity observed in infected Greenlip Abalone samples. In the majority of positive Greenlip Abalone tests, observational information indicated the *Perkinsus* cells were from a pre-spore phase, were poorly developed and/or were dead. In contrast, the Blacklip Abalone positives were characterised by a higher intensity of infection (*i.e.* 10s to 100s of zoosporangia per slide). In interpreting these results it is worth noting the ranched Greenlip Abalone were considerably smaller than the wild Greenlip Abalone used for the Ray's test. Future comparisons between the two species should consider quantifying intensity, rather than just prevalence, and exploring the relationship between infection and abalone size. Our observations suggest the intensity of infection in Blacklip Abalone would far exceed that for Greenlip Abalone.

In conclusion, this project to investigate the viability of Greenlip Abalone ranching in Thorny Passage has identified low survivorship and slow growth for stocked abalone. The inference from these findings is that the research leases in Thorny Passage are unlikely to be commercially viable for Greenlip Abalone ranching operations. This inference is consistent with OGA's commercial decision to terminate the trials in SA eight months into the anticipated 12-month program.

Implications

This project identified the survival and growth of Greenlip Abalone at four sites in Thorny Passage near Port Lincoln were low compared with successful ranching locations in WA. The key implication of this project is that a commercial abalone ranching lease is unlikely to be successful in these locations. This is consistent with OGA's commercial decision to prematurely terminate the research trial.

Recommendations

Future abalone ranching trials could be enhanced through greater research and planning regarding site selection, broodstock and the timing of abalone release. This should include the use of information obtained during this project that suggests attention to, food availability and predator abundance are important factors in determining the suitability of ranching sites. Greater consideration should also be given to the broodstock used to rear abalone for stocking purposes, to help maximise the chances of survival where predation rates are likely to be high.

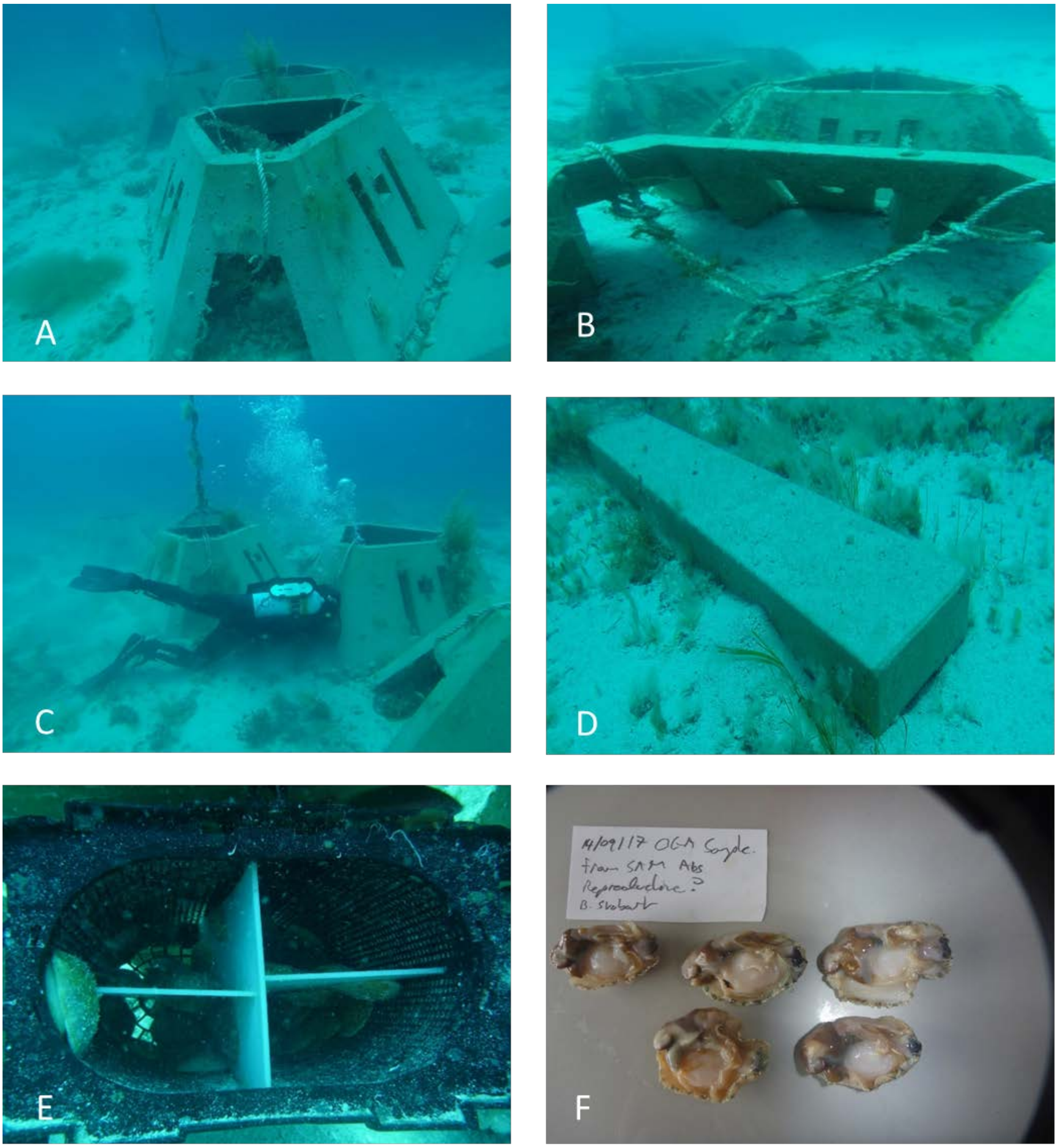
Extension and Adoption

A detailed extension and adoption plan was intended to be developed for this project, however, due to poor Greenlip Abalone survival and OGA's decision to terminate the trial this requirement was removed in accordance with the reduced project scope. Documentation of the results from this trial will help inform and educate future research or commercial ventures in the region.

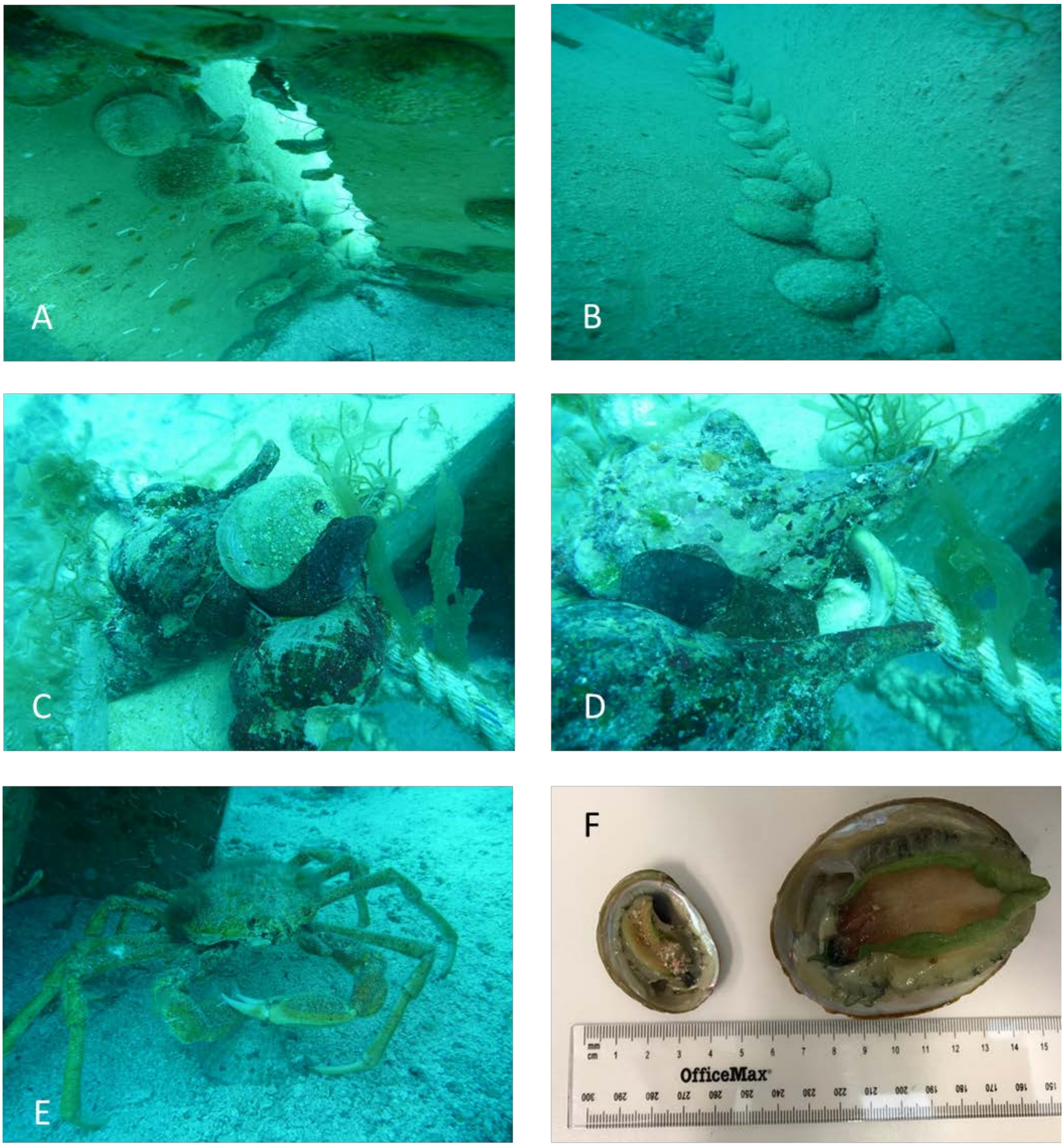
Project materials developed

A scientific manuscript is currently being prepared for an international peer-reviewed journal. Data from the project are archived with the Fisheries Information and Statistics Unit and can be requested by contacting SARDI Aquatic Sciences on 08-8207 5400 or pirsa.sardiaquatics@sa.gov.au

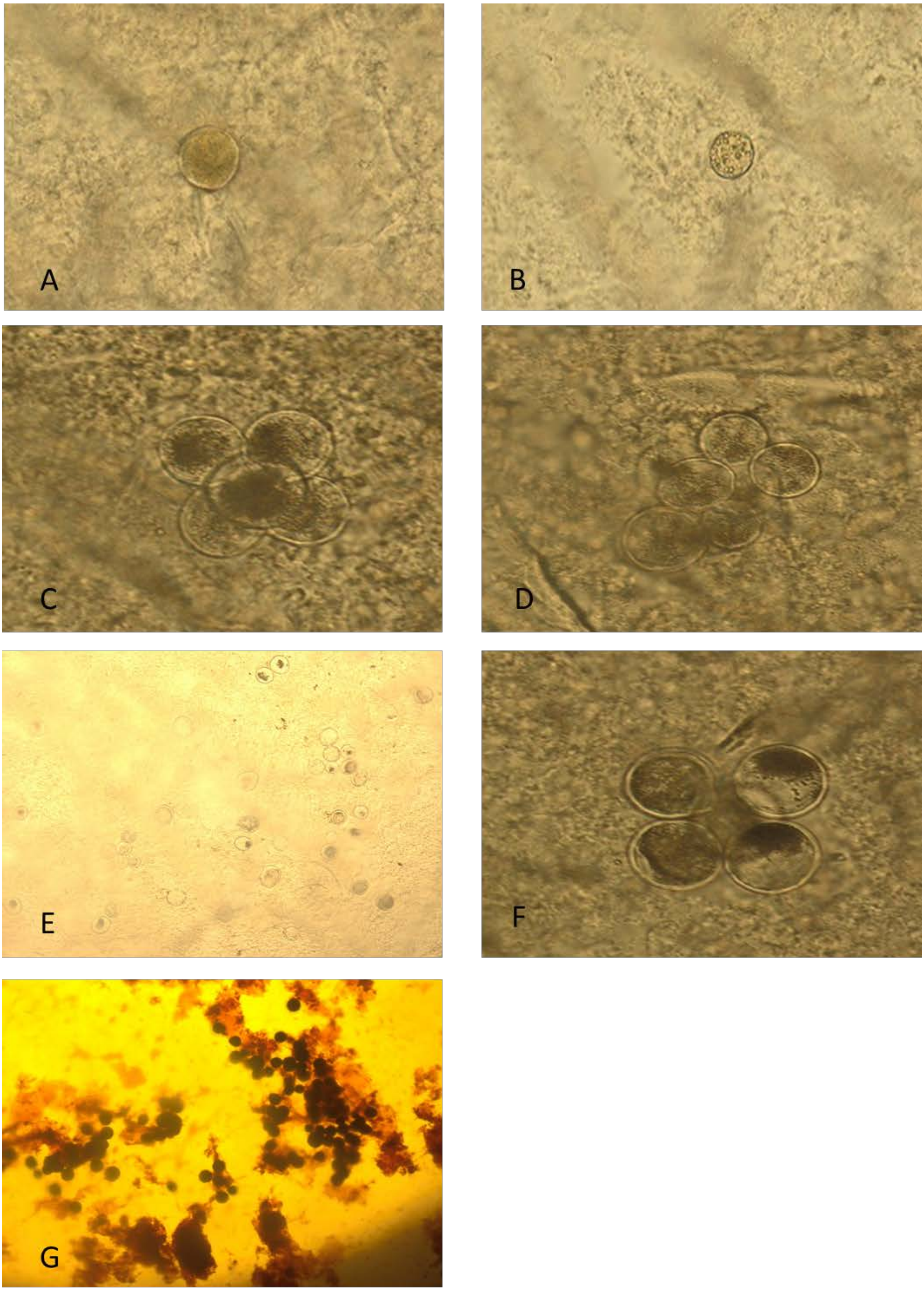
Appendices



Appendix 1. a-c) Overview of OGA's Abitats arranged into the trial reefs on the seafloor, d) concrete sleepers were arranged in a perimeter around each of the trial reefs, e) OGA release units for abalone, and f) juvenile Greenlip Abalone dissected to assess maturity prior to release at the research leases.



Appendix 2. a-b) Greenlip Abalone (*Haliotis laevigata*) on the trial reefs two months post release, c-d) whelks (*Pleuroploca australasiae*) eating abalone, e) spider crab (*Naxia aurita*) and f) size range among individual abalone harvested at the end of the eight month trial period



Appendix 3. a-b) single *Perkinsus* zoosporangia from pre-spore phase in ranched Greenlip Abalone (cell size approx. 40 µm), c-d) multiple zoosporangia with zoospores in wild Greenlip Abalone (cell size approx. 150 µm), e-f) multiple zoosporangia with zoospores in wild Blacklip Abalone (cell size approx. 150 µm), and g) sample of zoosporagia stained blue-black with Lugol's Iodine from a heavily infected wild Blacklip Abalone (cell size approx. 150 µm).

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