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Fisheries Research and Development Corporation

Develop the non-maxima pearl industry at the Abrolhos Islands (*Pinctada imbricata*)

Derek Cropp (Principal Investigator)

Aquatech Australia Pty Ltd

FRDC Project No: 2007/216

FINAL REPORT

June 2011

Abrolhos Pearls

LATITUDE

Develop the non-maxima pearl industry at the Abrolhos Islands (*Pinctada Imbricata*)

June 2011

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Pinctada imbricata – Final Harvest May 2011 - Shell and Akoya Pearls

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4.8 Effect of Environmental Factors on Pearl Oysters and Pearls

NON-TECHNICAL SUMMARY

2007/216 Develop the non-maxima pearl industry at the Abrolhos Islands (*Pinctada imbricata)*

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OBJECTIVES

1. Develop broodstock selection protocols for Abrolhos pearl oysters both on farm and in the hatchery to identify desirable broodstock (*P. imbricata* = *P. fucata* = *P. martensii*) with the qualities demanded by the market; and establish a broodstock conditioning system for these oysters.

2. Transfer and integrate accepted knowledge to key BCMI staff from established hatcheries to improve technical capability at the facility, improving quality spat supply services for Western Australia (WA) industry.

3. Produce spat from the selected Akoya host and saibo donor oysters in the hatchery and growout in spatially diverse sites at the Abrolhos Islands to assess the viability of developing the industry on a commercial scale. Pre-seeding conditioning trials will also be undertaken.

4. To investigate the impact of environmental factors on key pearl characteristics including growth rate, mortality, shell size, lustre and nacre for Akoya (*P. imbricata* = *P. fucata* = *P. martensii*) by monitoring spatially diverse growout sites at the Abrolhos Islands.

OUTCOMES ACHIEVED TO DATE

NON-TECHNICAL SUMMARY:

The technical feasibility and commercial potential for Akoya culture in Western Australia was demonstrated by this project. It not only helped facilitate the establishment of reliable pearl oyster hatchery production for non-maxima species in WA but provided an intensive analysis of factors affecting the successful production of pearls, not all of which are environmental. Significant factors included husbandry practices, technician routines and site variations. Benefits generated by the project have already been adopted by the non-maxima pearl industry through improved hatchery spat production and better husbandry activities and directly applied to the blacklip industry. Farm management practices for Akoya can now utilise the extensive knowledge gained in pre-operative conditioning of oysters and relationships between oyster size, nucleus and subsequent pearls. Further work should investigate other environmental aspects that clearly must be involved in stimulating the variations that were seen between oysters and pearls produced at the 5 different research sites at the Abrolhos Islands.

Prior to the commencement of the current project, all Akoya oysters used in pearl culture work at the Abrolhos Islands were wild-caught spat or naturally occurring animals collected from the wild. This project has developed an appropriate broodstock selection protocol, capable of identifying and utilising desirable oyster characteristics for the long term benefit of the industry. This involved a mathematical assessment, based upon morphological oyster measures, which is difficult to apply at this early stage. Nevertheless, this is an important baseline aspect that is likely to yield benefits in the longer term, once production from subsequent generations is assessed. For the current project, intensive analyses of the pearl harvest may well provide further insight into this aspect.

Techniques for assessing and monitoring broodstock condition and the necessary holding and conditioning practices/protocols on farm and at the hatchery were identified. The regular availability of broodstock in reproductive condition, at the Abrolhos Islands, has generally negated the need for a routine broodstock conditioning program either on farm or in a hatchery.

Established hatchery knowledge has been transferred to staff on this project with subsequent improvements in hatchery capability and success.

Furthermore, establishment of effective hatchery expertise for a reliable supply of hatchery spat in WA has meant that hatchery operating periods are now forecast to be short in time span, and efficient with production. Current technical ability in WA is such that a high quality, reliable spat supply is available for the WA industry.

During this project spat from selected (host) broodstock were produced in a hatchery and grown out at 5 spatially diverse sites at the Abrolhos Islands. An extensive array of data was collected during regular monitoring trips and this information has been utilised in assessing whether the industry can be developed on a commercial scale. The

comparative success of this culture work suggests that from a commercial aspect, at least several sites should be acceptable.

Important trials were conducted on pre-operative conditioning of oysters and the seeding of conditioned oysters by Japanese technicians. These trials have provided detailed guidelines for future conditioning techniques and desirable oyster parameters for the use of specific nuclei sizes and therefore subsequent pearl sizes which should also provide insights for the black pearl industry. Additionally, sites more suited to such pre-operative (pre-seeding) conditioning work, as opposed to oyster growout or pearl culture, were identified. This information will collectively allow pearl farmers to select preferred locations for the various stages of Akoya culture and to maximize the potential success rate of pearl culture operations and hence the subsequent return on investment.

The impact of the environmental factors monitored herein on key pearl characteristics, has been examined. Environmental characteristics of good culture sites (within the scope of this project) have been identified and their impact on pearl production assessed. Statistical analysis of data has shown that the main environmental factor influencing the quality of pearl produced was water temperature as the other parameters showed no discernible differences. This project has successfully shown that Akoya pearls can be produced within a 3.5-year culture cycle and potentially, within a shorter time frame. The commercial potential of Akoya pearl oysters at the Abrolhos Islands has been demonstrated and the potential for production of high quality pearls has also been shown. The relationships between culture sites, environmental variables and pearl quality may be significant and need to be incorporated in farm management to maximize the potential of the region, for all pearl culture.

Industry should now utilise outcomes from the current project to develop strategies for improving the commercial Akoya opportunities further. Identifying the best pearls and oysters currently will assist industry in selectively breeding future oyster generations, which should improve pearl values and possibly the culture time frame. Utilising site characteristics to benefit various stages of pearl culture should reduce culture costs and further enhance pearl values. The ability to conduct ongoing environmental monitoring also exists and investigating such aspects as dissolved sediments at each site may reveal additional factors behind site variations.

Summary

This research project focused on the need to investigate the techniques required for a commercial Akoya pearl industry in WA. Previous work on Akoya oysters in NSW had shown their potential to produce quality pearls in Australian waters. A reduction in Japanese Akoya pearl production also stimulated market interest in Australian Akoya pearls, including the potential to produce Akoya pearls of larger sizes. The established South Sea Pearl industry in tropical Australian waters had also demonstrated long term viability utilising the silver-lip pearl oyster, *Pinctada maxima* to produce even larger and more valuable pearls for a different market segment.

A fledgling pearling industry established at the Abrolhos Islands, off Geraldton, in WA was initially focused on producing black pearls from the naturally occurring blacklip pearl oyster. This industry began to expand somewhat using an ad hoc supply of

hatchery produced spat (juveniles). The NSW Akoya work suggested that the short culture cycle and limited husbandry costs made Akoya culture commercially attractive in Australia. By utilising existing infrastructure, two small, established pearling companies, Latitude Fisheries and Abrolhos Pearls, conducted sufficient initial culture trials with native Akoya oysters at the Abrolhos Islands, to suggest that the production of Akoya pearls may be viable.

The research project entitled, "Develop the non-maxima pearl industry at the Abrolhos Islands (*Pinctada imbricata*)" commenced in July 2007 with the development of selection protocols for desirable oyster breeding stock or broodstock as the first objective. Establishment of a system for assessing gonad condition in oysters, followed.

Subsequently, methods for monitoring and improving gonad condition up to spawning were set up and documented such that the ability to source suitable broodstock in spawning condition was developed. Utilising selected host and donor oysters from the Abrolhos Islands as broodstock, a batch of Akoya spat was produced by the Blue Lagoon Pearl hatchery in December 2007. These spat were transferred by plane to the Abrolhos Islands in January 2008 and disseminated to 5 different culture sites at the Islands. They were ongrown for a period of about 20 months during which time monthly monitoring of growth and survival of the oysters was conducted. At the same time, a range of environmental factors were monitored, both by collecting monthly water samples and by automatic data loggers located at several depths at all the sites.

Suitable sized oysters were conditioned in conventional WA culture baskets at high density to make them weaker and less likely to reject the nucleus at seeding time. Further Pre-Operative Conditioning Trials were conducted to clarify the effect of a range of variables relevant to WA farmers.

Experienced Japanese Akoya technicians seeded the oysters which were then located again at the 5 culture sites. After 20 months of culture and monitoring, pearls were harvested from the oysters in May 2011. They were graded and assessed by the Japanese technicians and valued at the Farm Gate in WA.

Monitoring data showed that several of the sites had higher oyster growth rates than others while one of the slower growth sites was the best for pre-operative conditioning of stock. Subsequently, several of the sites with faster growing oysters also revealed more rapid pearl growth and hence larger pearls, even bigger than 9mm in size. The summer of 2011 was remarkably warm with temperatures exceeding typical levels by 3 - 4 degrees. These trends were observed consistently across sites and depths. Temperature was the only environmental factor found to be significantly different between sites and some relationship between cooler temperatures and better pearls was found to exist. The recovery rate of pearls from seeded oysters was 35.2% overall.

This project also demonstrated that the success rate for seeded oysters producing pearls can be increased by up to 15% through appropriate site choice, timing of seeding operations and better seeding operations. This translates directly to the same percentage increase in pearl revenue.

KEYWORDS: Akoya pearl oysters, environmental effects, conditioning.

ACKNOWLEDGEMENTS

This has been an industry instigated and driven project, lead from the beginning by pearl farmers Pia Boschetti and Murray Davidson. It has been supported by the local industry association - AMWING (Albina, Margaritifera and Winged Pearl Producers Association, WA).

Scientific expertise was provided by Dr Wayne O'Connor Department of Primary Industry NSW, and Dr Brian Jones Department of Fisheries, WA who, as members of the Steering Committee, provided extensive and valuable guidance and advice for the duration of the project. Their assistance was also invaluable in facilitating technology exchange with industry and project extension work. Their input was a great asset to the project and both were exceptionally generous with their time and knowledge and were a pleasure to work with.

Principal Investigator Derek Cropp has been outstanding in keeping this project on track. His organisational skills and professional approach have greatly assisted industry in applying some structure and rigorous methodology to questions surrounding production issues which have previously been dealt with in an informal ad hoc manner. We particularly wish to thank Derek for stepping up from Co-Investigator to Principal Investigator mid way through this project and we acknowledge his tireless efforts. Finally Derek has always been very willing to work closely with industry in the field, sometimes under difficult conditions, and his dedication is greatly appreciated.

This project was assisted by staff from the Batavia Coast Maritime Institute (BCMI), part of Durack Institute of Technology, Geraldton, WA. In particular, thanks are extended to Steve Webster and Bert Beevers for their enthusiasm in supporting the project from inception.

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Erica Starling guided this project from its inception and ensured all aspects were completed as required. Roseanne Oliveri expertly collated, compiled and formatted all documentation to produce a professional Final Report.

FRDC is thanked for their support throughout the project and for their valuable assistance in guiding industry towards achieving worthwhile industry outcomes.

1. BACKGROUND

Culture of Akoya pearl oysters and pearls is relatively new to Australian waters although pearl culture with other species (*Pinctada maxima* - South Sea Pearls) has been underway since 1959. The first recorded trials involving Akoya pearl oysters commenced in NSW with spat monitoring, gonad assessments and hatchery production in the late 1990's (O'Connor et al., 2003). The NSW Akoya pearl oysters were referred to as *Pinctada imbricata* Røding but these oysters have been more recently referred to as *P. fucata* (Gould*)* in WA and are also known as *P. martensii* in many overseas regions (Shirai, 1994). A review of the nomenclature used for Akoya pearl oysters is detailed in Pit (2004) and as his work supports the findings of this project, *Pinctada fucata* will be used in reference to the WA species. Nevertheless, they are the same species and to avoid confusion they will be collectively referred to as Akoya pearl oysters herein.

Akoya pearl oysters are widespread throughout Asia and have been used for pearl culture since the 1920s. They are similarly, quite widespread in Australia ranging from Shark Bay in the West (and the Abrolhos Islands) round northern Australia and down the east coast to Victoria (Hynd, 1955; O'Connor et al., 2003). Akoya pearl oysters are one of the smallest commercial pearl producing oysters but can produce white pearls of 3-10mm in diameter of a superior quality to freshwater pearls, but of a lesser value than the larger Tahitian (*Pinctada margaritifera*) or South Sea Pearls (Kripa, et al., 2007).

Production difficulties associated with pollution and disease have occurred over recent years in major Akoya pearl producing countries such as Japan and China. Volume and quality declines have been serious and have generally resulted in smaller pearls with thinner nacre coatings being landed on the international market. This has provided a large window of opportunity for Australia to not only place Akoya pearls into the marketplace (Pit, 2004) but to lift the standard by marketing relatively large Akoya pearls with a comparatively thick nacre. India also appears able to compete in this sector and research by Kripa et al., (2007) has noted the industry preference for larger Akoya pearls, preferably above 6mm, the thin nacre coatings of current Akoya pearls in the marketplace and the important relationships occurring between environmental variables and pearl quality, aspects critical to the current study.

The Abrolhos Islands pearling industry operates on aquaculture leases granted by the WA Department of Fisheries. 15 years ago the first blacklip oyster shell was found and the industry has slowly grown since, producing black pearls from blacklip oysters (*P. margaritifera*) and pearls and shell products from the lee shell (*P. albina*) and the wing shell (*Pteria penguin*).

Culture of Akoya pearl oysters and pearls is also a relatively recent activity for WA pearl farmers. There have been limited trials with the Akoya oyster (*P. imbricata* = *P. fucata* = *P. martensii*) seeding wild shell, which is naturally occurring in the area. These small-scale commercial trials have been underway at the Abrolhos Islands for a number of years and have principally been conducted by Latitude Fisheries and Abrolhos Pearls. Ad hoc collection of naturally settling wild spat has been conducted since about 2001. Initial oyster culture and pearl production has been extremely encouraging in WA and the increasing worldwide demand for Akoya pearls, due to a decline in Japanese

production, has stimulated Australian interest. The initial work conducted on Akoya pearl oysters in NSW has illustrated the benefits of a structured R & D program in developing the industry. Subsequently, the WA sector commenced a jointly funded Industry-FRDC program on Akoya pearl oysters in 2007.

Key objectives were to determine desirable broodstock characteristics and establish hatchery production techniques within WA. Once spat were available, they were to be grown out at several spatially diverse sites with concurrent monitoring of the environment to determine the impact of environmental factors on key pearl characteristics.

Spat were produced at a Shark Bay hatchery in December 2007 and transferred to 5 selected farm sites at the Abrolhos Islands in mid-January 2008. Spat were grown out at each of the 5 sites with regular monitoring of oysters and environmental data conducted. At about 20 months of age (August 2009), oysters of suitable size were placed into conditioning baskets for approximately 6 weeks prior to being seeded by Japanese technicians (September, 2009). Further assessments and monitoring was conducted right through to the harvest of pearls in May 2011, when the oysters were approximately 41 - 42 months old. The pearls were then assessed, graded, and valued by experienced personnel.

The change in seawater temperature over time (Caputi et al., 2009) and the predicted influence on Akoya pearl culture at the Abrolhos Islands is also considered.

Accurate feedback on pearl market demands and the knowledge and ability to maximize culture efficiency in farming operations are critical aspects in a developing pearl industry. Effective usage of multiple culture sites, with varying physical characteristics becomes possible once the environmental variations inherent in each site are understood. Although the environmental monitoring conducted within this study was relatively basic, it was comprehensive enough to cover the entire grow-out period for the Akoya pearl oysters and identified the effects that some of the key variables have on oyster culture and pearl production. Many of the outcomes of this work have already been incorporated into current farm management practices (including for black pearls) of pearling companies operating at the Abrolhos Islands and have also been fed back to the NSW industry.

The pearl farmers form a cohesive industry group and engage in dialogue with the WA Department of Fisheries, industry groups AMWING Pearl Producers Association Inc (AMWING), Aquaculture Council of WA (ACWA) and the WA Fishing Industry Council (WAFIC). AMWING members have recently formed the Abrolhos Pearlers Association (APA) to work together to develop the industry though improved production techniques and marketing.

Commercial production from the Abrolhos industry is at low levels (34,500 black pearls in 2006) with increased production forecast for the coming years (potentially 220,000 black pearls by 2011). A visit to Japan in July 2006 by a delegation of 5 people (on behalf of Abrolhos pearl producers), led by Austrade, met with pearl producers, importers and traders in Tokyo and Kobe. The purpose was to explore the market in Japan and see production from other areas. The trip highlighted the fact that we need to improve our

quality of black pearls in order to compete in Japan and other markets. A positive outcome of the market visit by the group was the strong interest shown by Japanese customers in our trial Akoya pearls from seeded wild shell. Japan is the world's main Akoya market and in previous years, the largest producer. Their production is declining due to disease and other environmental and cost factors. China is rapidly becoming a large producer of low cost Akoya. However China is producing smaller sizes and focuses on quantity, not quality. There is a commercial opportunity in producing larger pearls in the Abrolhos area.

A significant part of this project has involved the refinement of hatchery services in Geraldton, based at the newly built Batavia Coast Maritime Institute (BCMI). This facility is the primary source of training for students of aquaculture and marine operations in the Mid West area. At the start of this project, the facility had a new hatchery and was keen to support the pearl growers in the area. Industry has a very constructive relationship with the BCMI and both parties wished to expand pearl aquaculture in the area. Refined hatchery services will enhance and ensure continuity of supply of quality spat at affordable levels, a factor which has limited the industry development in the past. Unfortunately, operational issues at BCMI meant that broodstock conditioning trials in hatchery facilities could not be completed. Furthermore, hatchery spat production for the project was not possible and a commercial hatchery facility in Shark Bay was contracted to produce Akoya spat for the project, utilising selected Abrolhos Islands broodstock.

The project also aimed to reduce production costs by assessing a range of sites to identify those that result in the lowest levels of mortality and the highest growth rates and yields. The Abrolhos Islands area is unique and pearl farmers have found a highly variable environment amongst and within various sites. It is critical that field research be conducted to determine the impact of local environmental variability on pearl production and how to manage the farms to optimise the positive impacts and minimise the negative impacts.

2. NEED

Research is needed to investigate the development of an Akoya industry in WA.

Limited trials of seeded wild Akoya shell have shown promising results and market feedback lead industry to believe there is a very real opportunity for slightly larger Akoya pearls of better quality. NSW has built a successful Akoya industry and established black pearl farmers in WA would like to assess the commercial viability of this model in the Abrolhos area. The economics of farming Akoya appear attractive in terms of total cycle and husbandry costs when compared with black pearls and offer the ability to leverage existing infrastructure into the new species, creating economies of scale.

Part of the project involves developing broodstock selection protocols to choose desirable broodstock and create a broodstock conditioning system both on farm and in the hatchery. Research into the use of hatchery bred stock and development of broodstock selection protocols and broodstock holding and conditioning systems will be very beneficial to this developing industry.

The BCMI has a full time hatchery technician that will assist in developing these protocols. This technician has limited experience of other hatcheries and the project proposes to build the knowledge base of the technician through hands on supervised experience at two East Coast hatcheries. This will improve the technical capacity of the hatchery facility to increase quality spat production. Due to the BCMI operational issues, several WA industry members participated in technology exchange with Port Stephens hatchery and the NSW industry.

There is a need to assess the impact of the environmental conditions at various farm sites on animal health, growth rates and pearl quality. Production costs must be managed carefully for the species to be commercially viable therefore site selection at various stages of the life cycle is important.

3. OBJECTIVES

1. Develop broodstock selection protocols for Abrolhos pearl oysters both on farm and in the hatchery to identify desirable broodstock (*P. imbricata* = *P. fucata* = *P. martensii*) with the qualities demanded by the market; and establish a broodstock conditioning system for these oysters.
   A protocol for the selection of broodstock has been developed for Abrolhos pearl oysters (Akoya). As a result of broodstock conditioning trials and the monitoring of gonad condition in cultured oysters, a protocol for conditioning oysters for use as broodstock has been established.

2. Transfer and integrate accepted knowledge to key BCMI staff from established hatcheries to improve technical capability at the facility, improving quality spat supply services for WA industry. Due to operational changes required in the early stages of the project, hatchery work was conducted by an Industry hatchery in WA, rather than at BCMI. Hence, it was deemed more appropriate for industry members to improve their technical capabilities by gaining knowledge and experience from established hatchery facilities in NSW. As a direct and in-direct result of this project,

WA industry members now have experience in the successful production of Akoya pearl oyster spat, and, in separate activities, have successfully produced blacklip pearl oyster spat.

3. Produce spat from the selected Akoya host and saibo donor oysters in the hatchery and growout in spatially diverse sites at the Abrolhos Islands to assess the viability of developing the industry on a commercial scale. Pre-seeding conditioning trials will also be undertaken.
   Due to the lack of spat production at BCMI, a necessary change in hatchery facility (to Blue Lagoon Pearls Pty Ltd at Shark Bay) at the start of the project meant that only one production run could be conducted and hence only selected host broodstock were utilised for the production of spat for the project. The spat were transferred to the Abrolhos Islands and grown out at 5 spatially diverse sites. Results indicate that it would be commercially viable to grow Akoya pearl oysters at a number of these sites. As part of the culture process, conditioning (pre-operative) trials were conducted to identify the optimum factors for successful conditioning of mature oysters, prior to the seeding operation. Again, site proved to be a key factor, given the apparent variation in environmental aspects inherent at each site.

4. To investigate the impact of environmental factors on key pearl characteristics including growth rate, mortality, shell size, lustre and nacre for Akoya (*P. imbricata* = *P. fucata* = *P. martensii*) by monitoring spatially diverse growout sites at the Abrolhos Islands.
   Initial environmental monitoring results suggested that significant within site variations might have been occurring; hence the decision was made to increase the number of sample sites and the number of automatic data loggers. Subsequently, monitoring of environmental factors has continued throughout the project with the benefit of additional data points. Sites which experienced the lowest range in daily water temperatures and fewer deviations from the mean temperature displayed lower post-seeding mortality. Sites with a more stable and cooler temperature profile also generated higher pearl production rates from seeded oysters and better nacre growth. For the environmental parameters measured, the research sites were almost indistinguishable from one another, although some temperature variations between the sites were seen. Significant differences in pearl production were found between the sites and it was not explained by temperature alone or any of the other environmental parameters measured. Clearly, some other environmental factor was influencing oyster growth, survival and pearl production at the sites. This aspect requires further investigation and may well involve a variable such as suspended solids or fouling organisms.

4. RESEARCH AND DEVELOPMENT

4.1 Details of the Five Research Sites

4.1.1 Introduction

The Houtman Abrolhos Islands (Abrolhos Islands) are a chain of 122 islands located about 60 kilometres west of Geraldton, Western Australia. The islands are located in the Indian Ocean and positioned in the path of the southward-flowing Leeuwin Current (Collins et al., 1991). The current funnels warm, low nutrient, tropical water from the Pacific Ocean down past Indonesia and along Western Australian's continental shelf allowing a range of tropical marine species to be found at unusually high latitudes. The Abrolhos Islands consist of three island groups, the Wallabi Group, Easter Group and Pelsaert Group. The most northerly group is the Wallabi Group. Lying to the southeast of the Wallabi Group and separated by a 9 kilometre wide, open ocean channel, is the Easter Group. Further to the southeast, across 12 kilometres of open ocean and the Zeewijk Channel, lies the Pelsaert Group, where the most southerly true coral reef in the Indian Ocean lies (Teichert, 1947; Wilson and Marsh, 1979; Hatcher, 1991). Prevailing currents and wave action are from the southwest or southeast direction with winds in excess of 11 knots for 76% of the time and exceeding 17 knots for 44% of the time, during the summer month. (Wells, 1997). Within the three main island groups, semi-enclosed lagoons exist, with patches of coral and some macroalgae present. Coral develops the greatest inside the lagoons and water depths ranging down to 30 metres are also common in these areas (Wells, 1997).

Farming of pearls first commenced at the Abrolhos Islands in 1996 when the first Aquaculture pearling licences were issued to farm the blacklip pearl oysters (*Pinctada margaritifera*). The industry has grown and currently there are 8 aquaculture pearling licences issued by the Western Australian Fisheries Department that now include the farming of Akoya oysters. Currently there are 4 licences located at the Southern Group and 2 at each of the Easter and Wallabi Groups. At the commencement of the project, industry representatives identified 5 different farm leases at the Abrolhos Islands where trials could be conducted. The farm sites represented the range of different habitats found throughout the current pearl farm leases, with 3 farm sites in the Southern Group and 2 in the Easter Group, identified as Sites 1 to 5. At three of the farms a second research site was established (1 & 1a, 3 & 3a, 4 & 4a) due to inherent variation of physical aspects within the relevant pearl lease (farm). This meant that additional site and environmental data was collected within the one pearl lease.

The location and research focus for each site is outlined below:

- ***Site 1 and 1a - Located at Easter Group***
  Spat Growth Trial, Seeding / Pearl Growth Trial, Initial Broodstock Conditioning, Broodstock Condition Trial, Pre-Operative Conditioning Trial.
- ***Site 2 - Located at Easter Group***
  Spat Growth Trial, Seeding / Pearl Growth Trial.
- ***Site 3 & 3a - Located at Pelsaert Group***
  Spat Growth Trial, Seeding / Pearl Growth Trial, Broodstock Condition Trial, Pre-Operative Conditioning Trial.
- ***Site 4 & 4a - Located at Pelsaert Group***
  Spat Growth Trial, Seeding/ Pearl Growth Trial, Broodstock Condition Trial, Pre-Operative Conditioning Trial.
- ***Site 5 - Located at Pelsaert Group***
  Spat Growth Trial, Seeding / Pearl Growth Trial.

Prior to research commencing at the Abrolhos Islands, selected Akoya oysters were transferred from the Islands to the Blue Lagoon Pearl Hatchery in Shark Bay, Western Australia, where they were spawned. The Blue Lagoon Pearl hatchery is located 350 km north of the Abrolhos Islands in a large shallow, protected bay, 1 km northwest of Monkey Mia.

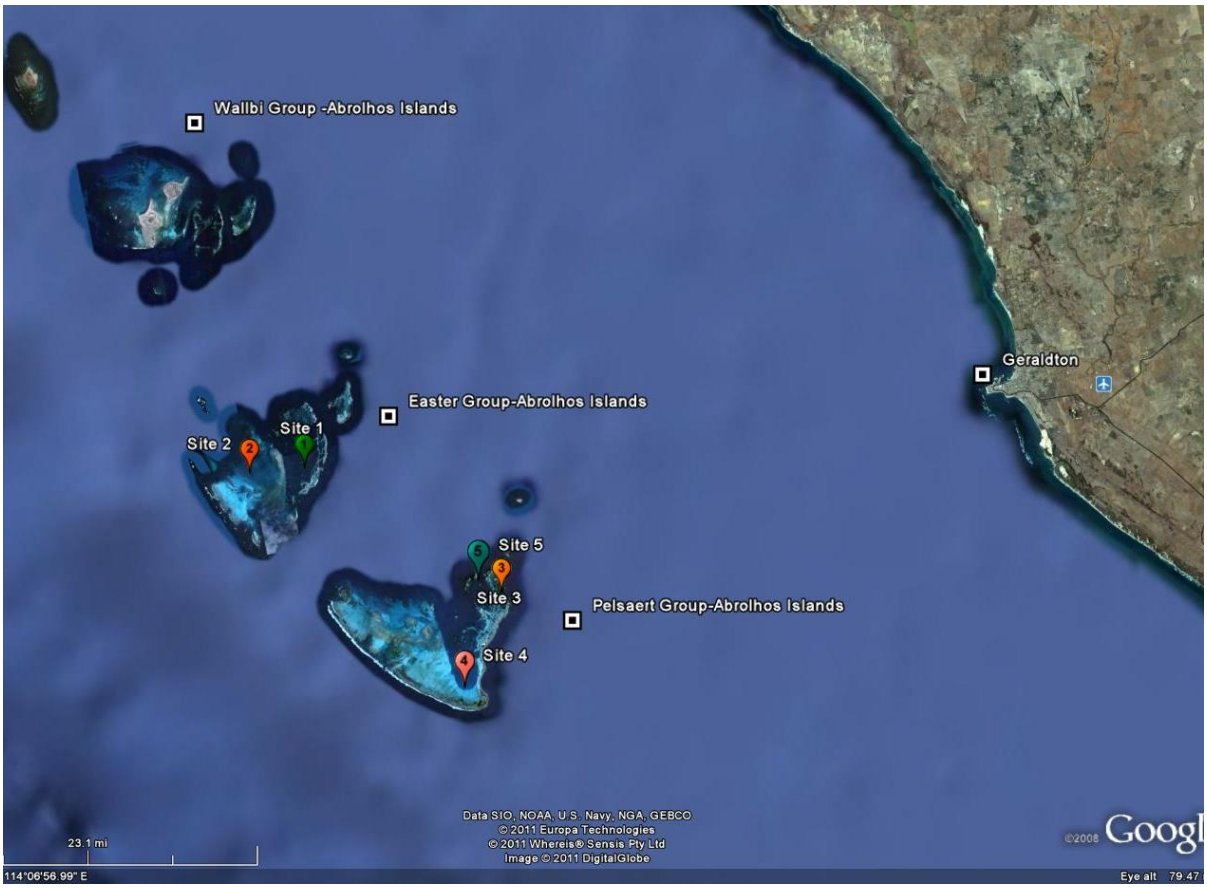


Figure 1 - The Houtman Abrolhos Islands.
(Source: Google Earth 2011).

Figure 2 - Abrolhos Islands and Shark Bay (Monkey Mia).
(Source: Google Earth 2011).

4.1.2 Research Sites

Site 1		
Farm	Abrolhos Pearls Pty Ltd Easter Group, Rat Island	
Description	Site 1 is located at the Easter Group, approximately 1.4 nm (nautical miles) south east of Rat Island. The farm site is deep (40 metres) with a sandy bottom and a number of coral bommies (outcrops) located randomly throughout the farm. This farm site is located in the main lagoon area at the Easter Group and is protected by shallow reefs and islands.	
Farm Position	A - 28 42.526' S 113 49.287' E B - 28 42.676' S 113 49.587' E C - 28 43.026' S 113 49.337' E D - 28 43.166' S 113 49.607' E E - 28 42.076' S 113 48.987' E F - 28 44.076' S 113 48.137' E	
Trials Conducted at Site	Spat Growth Trial Initial Broodstock Conditioning	
Site Position	S 028° 43.716' E 113° 48.715'	
Site Depth	Site 1 - s (surface)	1.7 metres
	Site 1 - m (mid)	4.0 metres
	Site 1 - d (deep)	7.5 metres
Site Seabed Depth	4 metres	
Farm Site Depth	Ranging from 0.2 to 40 metres.	
Farm Seabed	Ranging from coral lumps to deep sandy bottom.	
Nearest Land to Site	Morley Island lies 1.1 nm miles south. Rat Island is located 1.33 nm north west.	

Site 1a		
Farm	Abrolhos Pearls Pty Ltd Easter Group, Rat Island	
	Site 1a is located at the Easter Group, approximately 1.5 nm south east of Rat Island. The farm site is deep (40 metres) with a sandy bottom and a number of coral bommies (outcrops) located randomly throughout the farm. This farm site is located in the main lagoon area at the Easter Group and is protected by shallow reefs and islands.	
Farm Position	A - 28 42.526' S 113 49.287' E B - 28 42.676' S 113 49.587' E C - 28 43.026' S 113 49.337' E D - 28 43.166' S 113 49.607' E E - 28 42.076' S 113 48.987' E F - 28 44.076' S 113 48.137' E	
Trials Conducted at site	Broodstock Condition Trial Seeding / Pearl Growth Trial	
Site Position	S 028° 43.716' E 113° 48.715'	
Site Depth	Site 1a - s	1.7 metres
	Site 1a - m	4.0 metres
	Site 1a - d	7.5 metres
Site Seabed Depth	40 metres	
Farm Site Depth	Ranging from 0.2 to 40 metres.	
Farm Seabed	Ranging from coral lumps to deep sandy bottom.	
Nearest Land to Site	Morley Island lies 0.83 nm miles south. Rat Island is located 1.31 nm north west.	

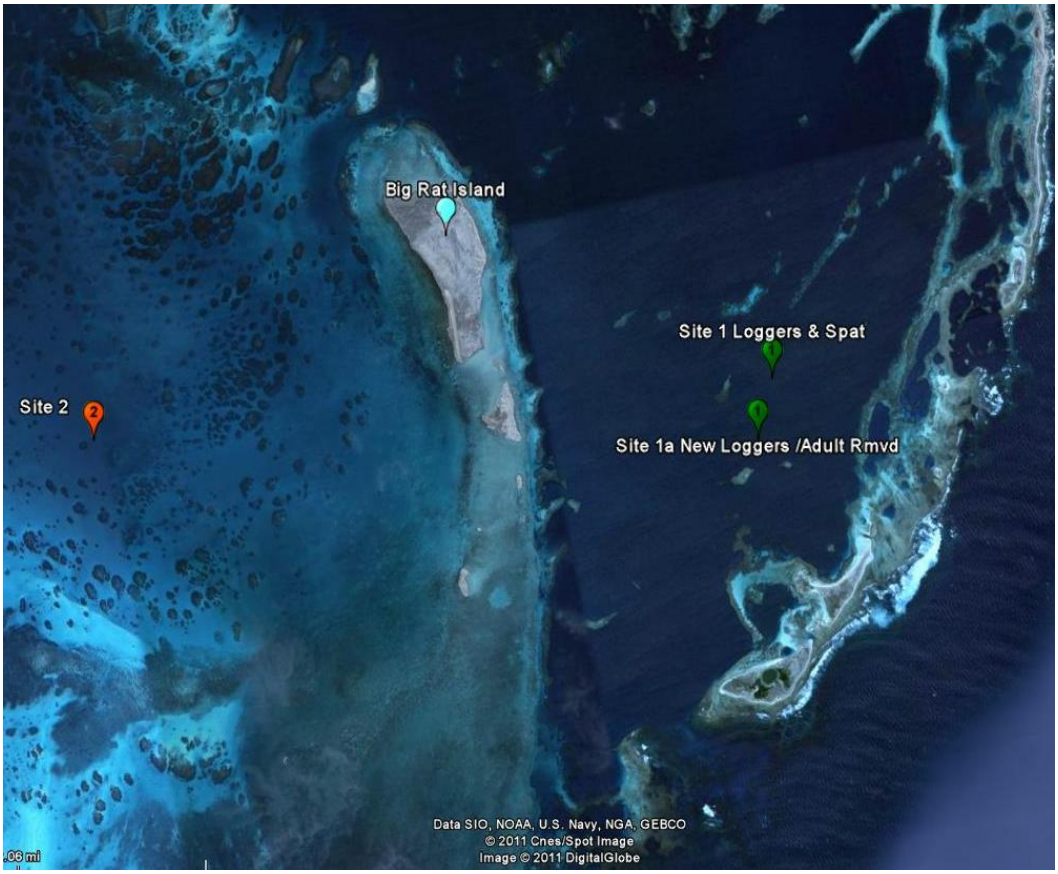


Figure 3 - Site 1**,** Easter Group, Rat Island, Abrolhos Pearls Pty Ltd. (Source: Google Earth 2011).

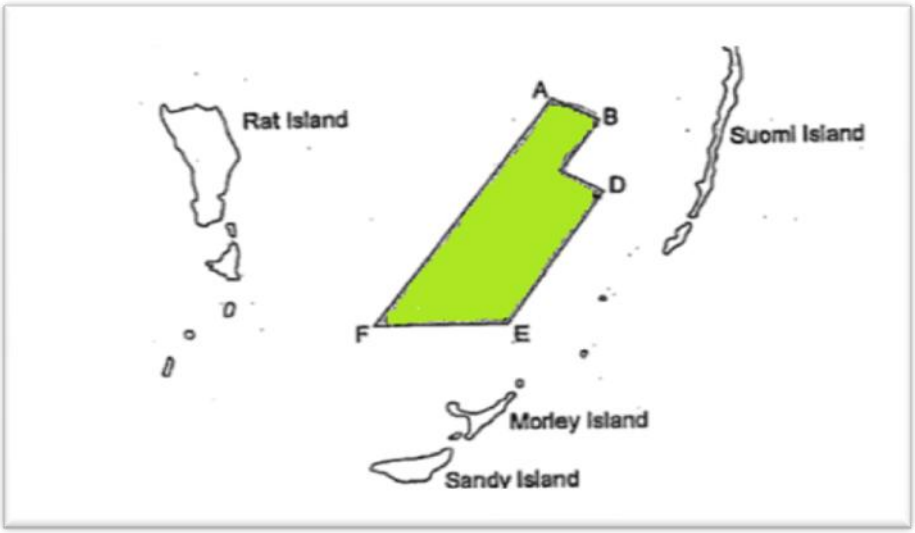


Figure 4 - Site 1 and 1a farm site boundaries. (Source: Abrolhos Pearls Pty Ltd Aquaculture Licence Number 1574).

Site 2		
Farm	Radar Holdings Pty Ltd Easter Group, Rat Island	
Farm Description	Site 2 is at the Easter Group, located approximately 1.77 nm to the west side of Rat Island. The farm bottom is mainly sandy and the depth is consistently around 13 metres. This site is located to the west of the Easter Group lagoon area and to the west of the site lies a large area consisting of shallow sandy bottom before the outer reef that shelters the site from the open ocean.	
Farm Position	A - 28 43.341' S 113 44.639' E B - 28 43.076' S 113 44.639' E C - 28 42.648' S 113 45.296' E D - 28 43.563' S 113 45.094' E	
Trials Conducted at Site	Spat Growth Trial Seeding / Pearl Growth Trial	
Site Position	S 028° 43.364' E 113° 45.010'	
Site Depth	Site 2 - s	1.7 metres
	Site 2 - m	4.0 metres
	Site 2 - d	7.5 metres
Site Seabed Depth	12 - 13 metres	
Farm Site Depth	12 - 13 metres	
Farm Seabed	Sandy bottom with little depth variation.	
Nearest Land to Site	Rat Island is located 1.77 nm west.	

Figure 5 - Site 2 Easter Group, Rat Island, Radar Holdings Pty Ltd.
(Source: Google Earth 2008).

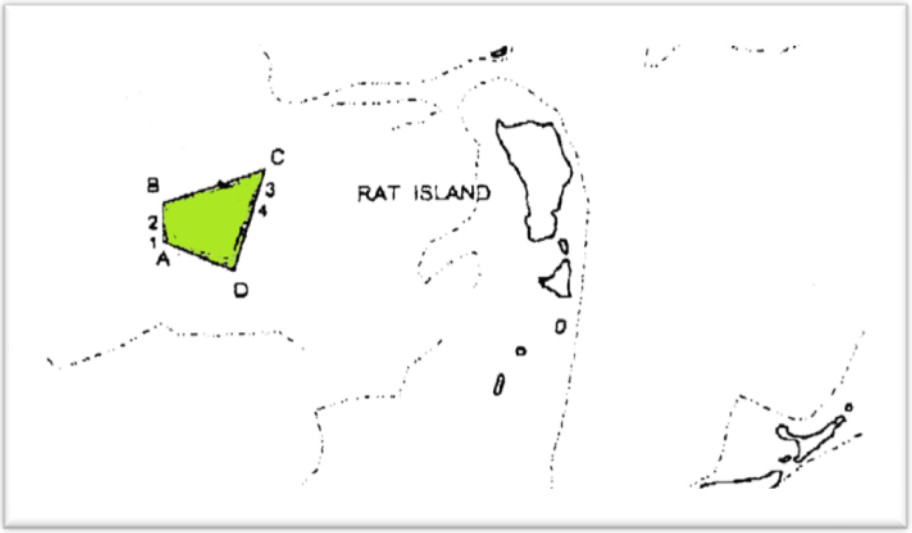


Figure 6 - Site 2 farm site boundaries.
(Source: Radar Holdings Pty Ltd Aquaculture Licence Number 1592).

Site 3		
Farm	Abrolhos Pearls Pty Ltd Pelsaert Group, Pelsaert Island	
Farm Description	Site 3 is located at the Pelsaert Group. The farm is located approximately 0.8 nm on the north west side of Pelsaert Island. The farm site has many coral bommies located throughout. This farm site is located in the main lagoon area at the Pelsaert Group and is protected by shallow reefs and islands.	
Farm Position	K - 28 52.630' S 113 59.790' E L - 28 52.630' S 114 00.140' E M - 28 53.006' S 114 00.195' E N - 28 53.640' S 114 00.430' E O - 28 53.730' S 113 59.800' E P - 28 53.350' S 113 59.640' E Q - 28 52.980' S 113 59.800' E	
Trials Conducted at Site	Broodstock Condition Trial Seeding / Pearl Growth Trial Conditioning Trial	
Site Position	S 028° 53.110' E 114° 00.051'	
Site Depth	Site 3 - s	1.7 metres
	Site 3 - m	4.0 metres
	Site 3 - d	7.5 metres
Site Seabed Depth	6 - 10 metres	
Farm Site Depth	Ranging from 0.2 to 35 metres.	
Farm Seabed	Sand and coral bommies located under the site.	
Nearest Land to Site	Coronation Atoll lies 1.1 nm north west. North tip of Pelsaert Islands is located 0.94 nm south east. Robertson Islands is located 0.3 nm east.	

Site 3a		
Farm	Abrolhos Pearls Pty Ltd Pelsaert Group, Pelsaert Island (North)	
Farm Description	Site 3a is located at the Pelsaert Group. The farm is located approximately 1 nm on the north west side of Pelsaert Island. The farm site has many coral bommies located throughout. This farm site is located in the main lagoon area at the Pelsaert Group and is protected by shallow reefs and islands.	
Farm Position	K - 28 52.630' S 113 59.790' E L - 28 52.630' S 114 00.140' E M - 28 53.006' S 114 00.195' E N - 28 53.640' S 114 00.430' E O - 28 53.730' S 113 59.800' E P - 28 53.350' S 113 59.640' E Q - 28 52.980' S 113 59.800' E	
Trials Conducted at Site	Broodstock Condition Trial Seeding / Pearl Growth Trial Pre-Operative Conditioning Trial	
Site Position	S 028° 53.350' E 114° 59.698'	
Site Depth	Site 3a - s	1.7 metres
	Site 3a - m	4.0 metres
	Site 3a - d	7.5 metres
Site Seabed Depth	6 - 10 metres	
Farm Site Depth	Ranging from 0.2 to 35 metres.	
Farm Seabed	Sand and coral bommies located under the site.	
Nearest Land to Site	Coronation Atoll lies 1.1nm north west. North tip of Pelsaert Island is located 0.98 nm south east. Robertson Islands is located 0.6 nm east.	

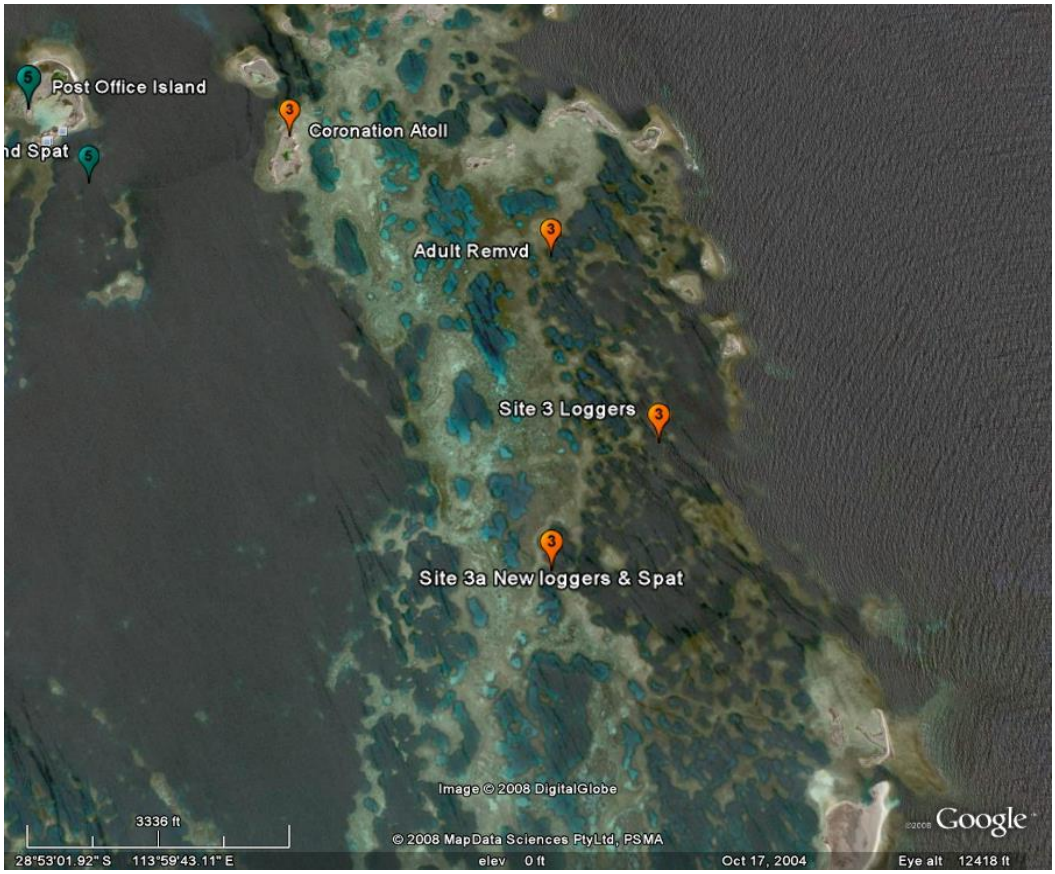


Figure 7 - Site 3 & 3a, Pelsaert Group, Pelsaert Island, Abrolhos Pearls Pty Ltd. (Source: Google Earth 2008).

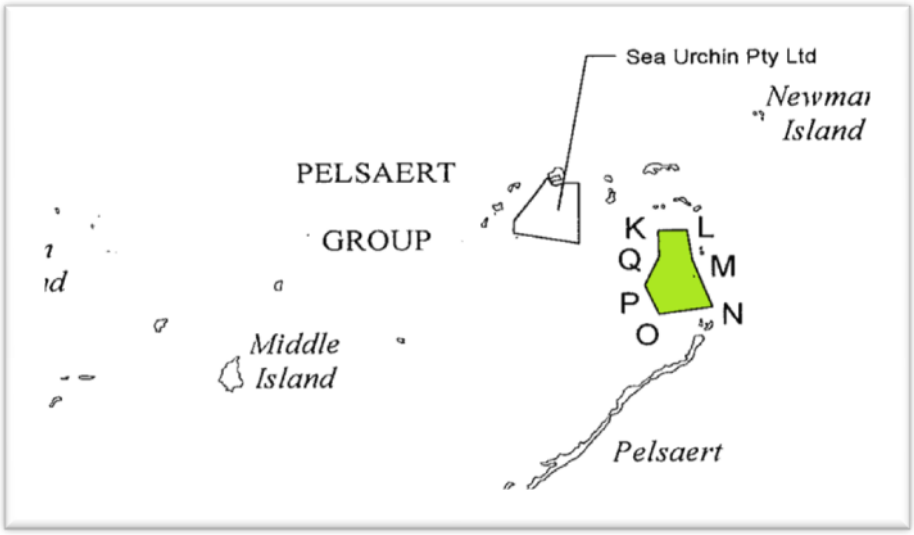


Figure 8 - Site 3 and 3a farm site boundaries.
(Source: Abrolhos Pearls Pty Ltd Aquaculture Licence Number 1347).

Site 4		
Farm	Pelsaert (WA) Pty Ltd (Latitude Fisheries) Pelsaert Group, Pelsaert Island (south)	
Farm Description	Site 4 is the most southern site, located 0.5 nm west of the southern tip of Pelsaert Island. The sea bed is mostly sand and the depth ranges from 2 – 25 metres. This site is located to the south of the Pelsaert Group lagoon area and to the south and west of the site lies a large area consisting of shallow sandy bottom before the outer reef that shelters the site from the open ocean.	
Farm Position	A - 28 57.576'S 113 55.116' E B - 28 57.546'S 113 55.936' E C - 28 58.426'S 113 57.136' E D - 28 58.426'S 113 55.146' E	
Trials Conducted at Site	Spat Growth Trial Broodstock Condition Trial Pre-Operative Conditioning Trial	
Site Position	S 028° 58.582' E 113° 56.712'	
Site Depth	Site 4 - s	1.7 metres
	Site 4 - m	4.0 metres
	Site 4 - d	7.5 metres
Site Seabed Depth	25 metres	
Farm Site Depth	2 - 25 metres	
Farm Seabed	Sand	
Nearest Land to Site	Pelsaert Islands lies 0.86 nm west.	

Site 4a		
Farm	Pelsaert (WA) Pty Ltd (Latitude Fisheries) Pelsaert Group, Pelsaert Island (south)	
Farm Description	Site 4a is at the most southern farm, located 0.5 nm west of the southern tip of Pelsaert island. The sea bed is mostly sand and the depth ranges from 2 – 25 metres. This site is located to the south of the Pelsaert Group lagoon area and to the south and west of the site lies a large area consisting of shallow sandy bottom before the outer reef that shelters the site from the open ocean.	
Farm Position	A - 28 57.576'S 113 55.116' E B - 28 57.546'S 113 55.936' E C - 28 58.426'S 113 57.136' E D - 28 58.426'S 113 55.146' E	
Trials Conducted at Site	Spat Growth Trial Broodstock Condition Trial Conditioning Trial	
Site Position	S 028° 57.611' E 113° 55.961'	
Site Depth	Site 4a - s	1.7 metres
	Site 4a - m	4.0 metres
	Site 4a - d	7.5 metres
Site Seabed Depth	25 metres	
Farm Site Depth	2 - 25 metres	
Farm Seabed	Sand	
Nearest Land to Site	Pelsaert Islands lies 1.5 nm west.	

Figure 9 - Site 4 & 4a, Pelsaert Group, Pelsaert Island, Latitude Fisheries. (Source: Google Earth 2008).

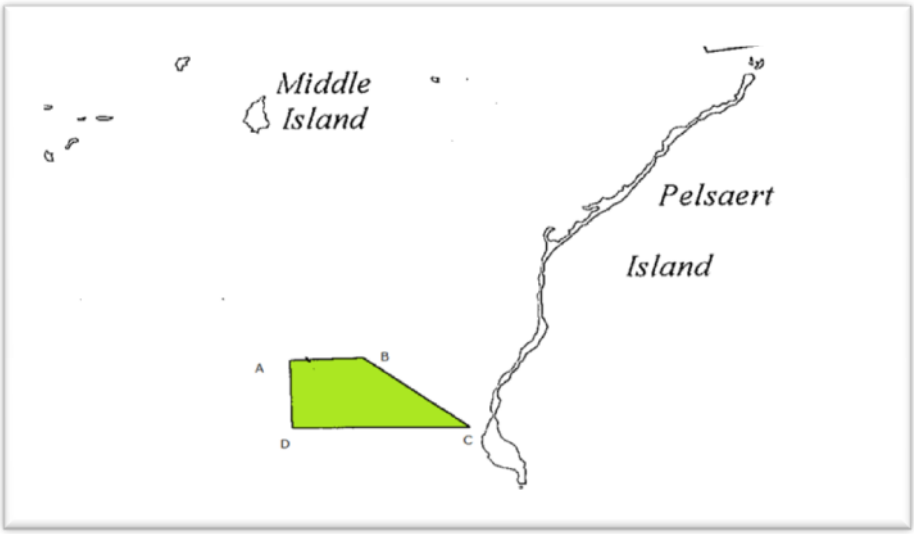


Figure 10 - Site 4 and 4a farm site boundaries. (Source: Pelsaert (WA) Pty Ltd Aquaculture Licence Number 1540).

Site 5		
Farm	Sea Urchin Pty Ltd Pelsaert Group, Post Office Island	
Farm Description	Site 5 is located on the west side of the Sea Urchin farm lease. The water is deep close to the site but coral bommies are located throughout the farm lease to the east of Site 5. This farm site is located in the main lagoon area at the Pelsaert Group and is protected by shallow reefs and islands although there is a large channel area to the east of the site.	
Farm Position	1 - 113° 58.8696' E 28° 51.9996' S 2 - 113° 58.8696' E 28° 52.8000' S 3 - 113° 58.0998' E 28° 52.6596' S 4 - 113° 58.0998' E 28° 52.5000' S 5 - 113° 58.4496' E 28° 52.0098' S 6 - 113° 58.5000' E 28° 51.9300' S 7 - 113° 58.5564' E 28° 51.9998' S 8 - 113° 58.6932' E 28° 51.9996' S	
Trials Conducted at Site	Spat Growth Trial Seeding / Pearl Growth Trial	
Site Position	S 028° 52.137' E 113° 58.708'	
Site Depth	Site 5 - s	1.7 metres
	Site 5 - m	4.0 metres
	Site 5 - d	7.5 metres
Site Seabed Depth	40 metres	
Farm Site Depth	Ranging from .2 to 40 metres.	
Farm Seabed	Ranging from coral lumps to deep sandy bottoms.	
Nearest Land to Site	Coronation Atoll lies 0.4 nm miles west. Post Office Islands is located 0.1 nm north.	

Figure 11 - Site 5 Pelsaert Group Pelsaert Island, Sea Urchin Pty Ltd.
(Source: Google Earth 2008).

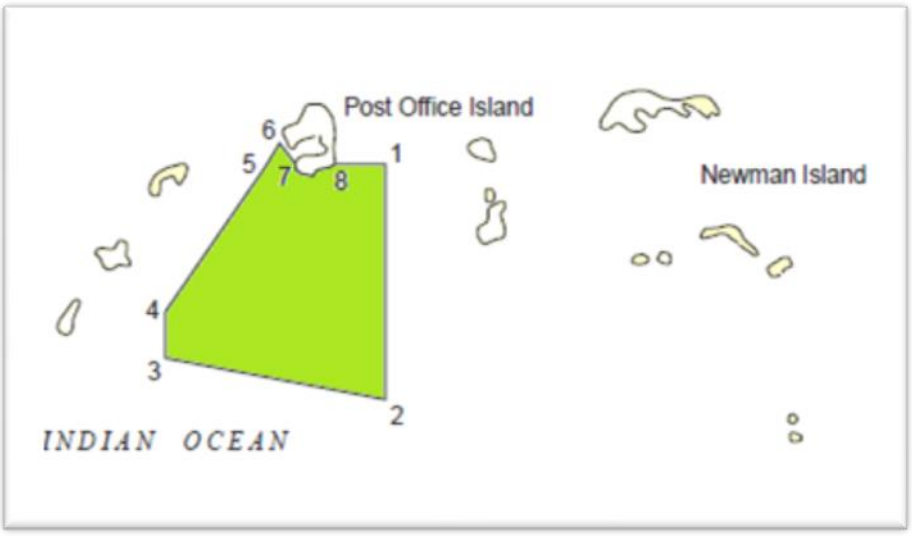


Figure 12 - Site 5 and 5a farm site boundaries.
(Source: Sea Urchin Pty Ltd Aquaculture Licence Number 1577).

4.2 Pearl Oysters – Broodstock Selection and Gonad Development

4.2.1 Introduction

Akoya pearl oysters, referred to herein as *Pinctada fucata*, but variously known as *P. imbricata*, or *P. martensii* in the southern hemisphere, are the target species for culture in this project. Akoya pearl oysters appear to be naturally occurring at the Abrolhos Islands (WA) and became the focus of various pearl culture trials around 1999. Their reputation as a fast growing species, able to be seeded/nucleated at a small size, and produce a relatively large pearl, makes them an attractive species for pearl culture. Their ability to produce small to medium sized white pearls also provides significant commercial potential for the species, something which has been proven in major pearl producing countries such as Japan and China. Consequently, a developing Akoya pearl industry requires the reliable availability or supply of sufficient quantities of juveniles each year to become sustainable and commercially viable. While natural spat settlement from wild oysters has occurred repeatedly in recent years, it has been both irregular in timing and highly variable in number. Therefore, the only way to ensure a high quality supply of juveniles/spat is maintained annually is to artificially breed these oysters in a hatchery. Knowledge of the reproductive cycle is therefore essential and was extensively examined in this project.

Although there is a paucity of information on Akoya pearl oysters in Australian waters, various overseas studies have shown that the reproductive behaviour varies markedly according to location (O'Connor & Lawler, 2004). Akoya pearl oysters are generally found as single sex animals, but they have the ability to change sex (hermaphrodite); this usually occurs as they age. There is a tendency for smaller animals to be male and the percentage of females to increase (to over 60%) as they grow/age, similar to a number of other pearl oyster species. Individual animals mature sexually within their first year and at relatively small sizes (<35mm shell height) although neither age nor shell height ensure maturity (O'Connor et al., 2003). Akoya pearl oysters are known to mature sexually during their first year but the actual timing varies quite significantly and is affected by a range of factors not the least of which is environmental. Given that this project has monitored environmental data on a monthly basis for the duration of the project (discussed elsewhere) we are able to examine the inter-relationships between environmental variables and the growth, survival, and development of the pearl oysters, including gonad development.

The gonad of the Akoya is wrapped around the digestive gland, as with other pearl oyster species, and is easily observable. The reproductive condition of each oyster can be visually assessed but unlike other pearl oyster species, colour is an unreliable indicator of the sex of the oyster (Tranter, 1958). However, the female gonad colour is most frequently yellow but the male can appear to be orange as frequently as the common cream colour. Closer examination reveals that the orange colour is actually an external epithelium and not the internal gametes (Tranter, 1958). While histological sections can clarify sexes and sexual development stages, a simple and practical system of accurately assessing gonad condition was required for use in the developing WA industry. This system is an integral part of establishing reliable and cost-effective spat production in WA as the option of collecting wild-caught spat is, as mentioned, a limiting factor for the Abrolhos Islands.

As part of the hatchery process, mature adult oysters were required as broodstock. It is important, for the long term success of the local industry, that broodstock exhibiting desirable characteristics are used for reproductive purposes. The difficulty initially, was to clarify market demands for pearls and attempt to translate this into appropriate desirable characteristics of broodstock oysters, without knowledge of genetic makeup of the oysters or relationships between genotypes and phenotypes in the oysters. Two types of potential broodstock may be selected based on different criteria for pearl production. These two types reflect two sets of phenotypic traits required for pearl production, the host oyster and the donor (saibo tissue) oyster.

The culture of pearls in Akoya oysters (as in other species) requires the insertion of a pearl nucleus and a piece of mantle tissue ('saibo') from a donor oyster into a recipient or host oyster. This operation/implantation is often referred to as 'seeding' and has been acknowledged as the most important step in pearl culture (Inoue et al., 2010). The donor (saibo) tissue is one of the important factors in determining pearl quality (Acosta-Salmón et al., 2004). Acosta-Salmón and Southgate (2005) also showed that saibo tissue can be removed from donor oysters without killing them, and the oysters can then be used as broodstock in future; a potentially significant benefit in improving pearl quality overall. Wada and Komaru (1996) demonstrated the influence of using saibo tissue from inbred Akoya pearl oysters with white shells rather than saibo from normal brown shelled oysters. In recent xenograft work (graft from different oyster species), McGinty et al., (2010) found that pearl colour and surface complexion were strongly influenced by the donor oyster species. While the issue of utilising donor tissue from a different pearl oyster species complicates the issue, the work also showed that there was a negative correlation between surface complexion and nacre deposition.

4.2.2 Materials and Methods

4.2.2.1 Broodstock Selection

4.2.2.1.1 Host

The first and most numerous oysters are those that are to be nucleated to produce pearls (host oysters). Initially these oysters were selected primarily upon shell shape and while there are equations to quantify suitability, the focus for this project was on the actual shell dimensions. Primarily the equations assess the convexity of the shells; shells with broader shell cavities increase the likelihood of insertion of larger nuclei, which have the potential to produce large and more valuable pearls.

The following is a key to the measurement abbreviations that also relate to Figure 1, and an asterisk (*) is shown next to those that are most important (O'Connor et al., 2003). Also see Figures 1 - 4.

*APM	max width of shell
*DVM	max length (height) of shell
WN	max width of nacre
HN	max length (height) of nacre
AML	distance of the edge of the adductor muscle scar from the posterior lateral margin of the nacre
AMD	distance of the edge of the adductor muscle scar from the ventral margin of the nacre
AM	length of adductor muscle
AMM	length of posterior part of adductor muscle from edge of constriction
WM	width of the adductor muscle
HL	hinge length
AH	length of posterior hinge line
BN	length of byssal notch
* WD	inflation, width of both valves
HD	heel depth
G	width of hinge line gape

Note: See Figure 1 for diagrammatic representation of measurements.

The basic equation used for selecting Akoya hosts is:

WD/ (WD + APM + DVM) - the higher the number the better
i.e. Host Selection Criteria Value (CV) = WD/ (WD+APM+DVM)

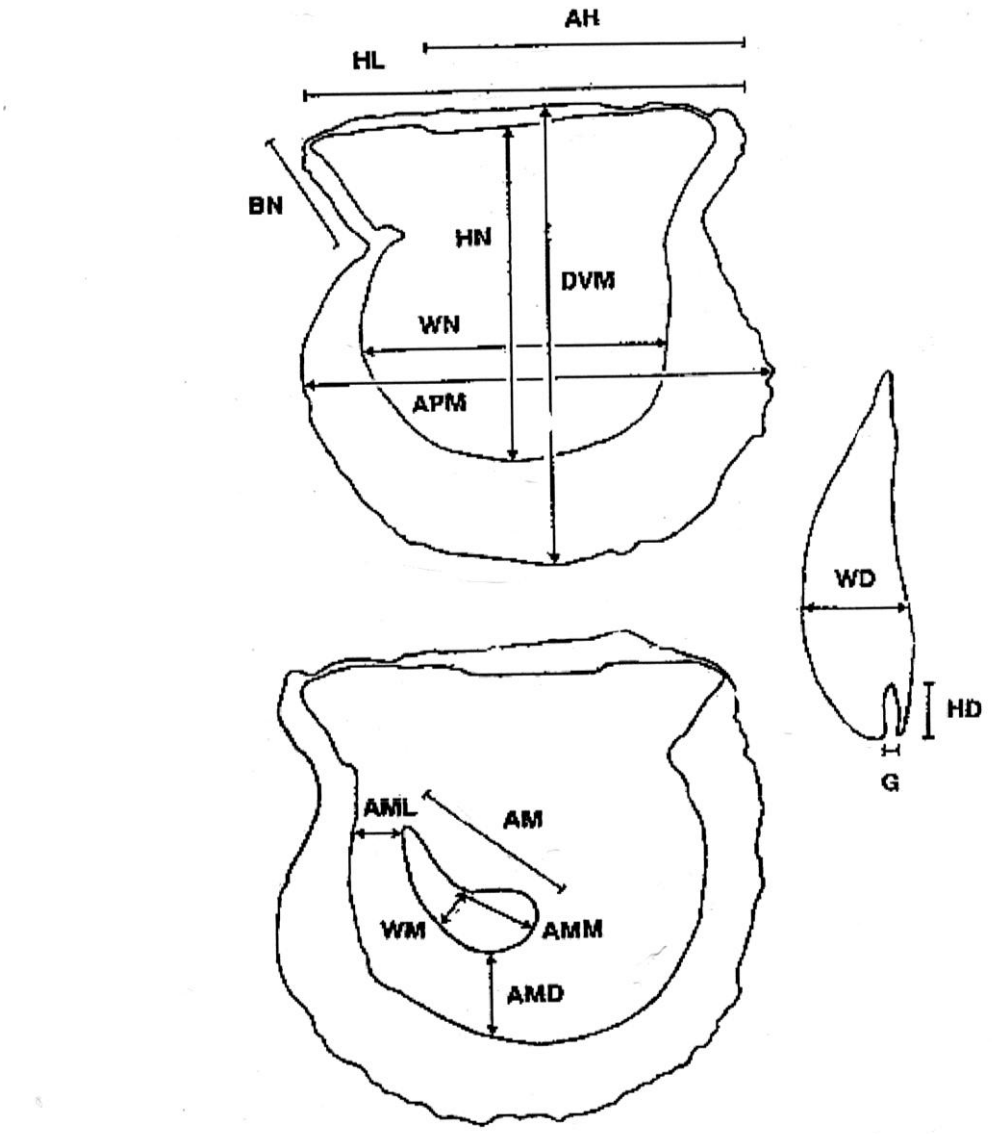


Figure 1 - Measurements taken on shell (see text for details).

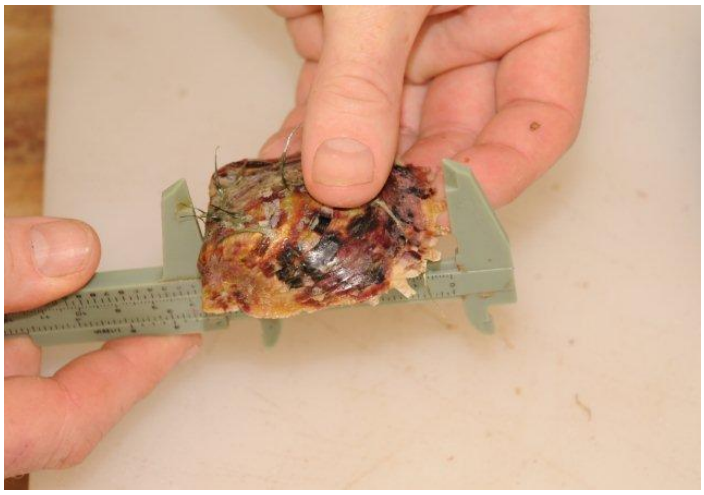


Figure 2 - Measuring height (length - DVM) of shell.

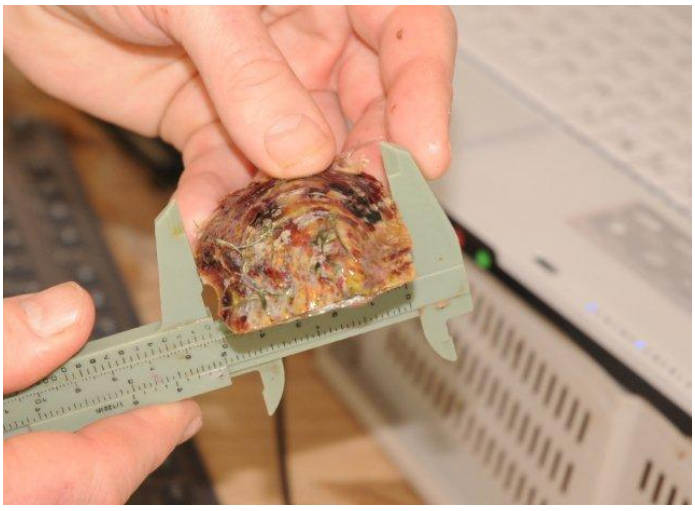


Figure 3 - Measuring width (APM) of shell.

Figure 4 - Measuring width of both valves (inflation/thickness - WD) of shell.

Additionally, it has also been common practice in NSW to use only oysters of around 50-60g wet weight or more for broodstock. This is not essential, but is done primarily to ensure a reasonable number of females are present and because there is a concomitant increase in fecundity with size. The latter gives greater flexibility during spawning to ensure control of parental crosses. Females release eggs gradually. Although females are isolated to separate containers when spawning begins, it is common for fertilization to occur due to sperm already present in the water on and within the shell cavity of females moved from the spawning table. Therefore, the first batch of eggs released in isolation is commonly discarded, because there is a greater risk of an unknown male being involved. With small females producing fewer eggs, discarding the first release of eggs can leave you with too few eggs for larval rearing. Hence, a range of oyster sizes is utilised.

4.2.2.1.2 Saibo

The second group of oysters are those that provide the mantle tissue or 'saibo' for the seeding operation (Donor oysters). For this group it is the colour and quality of the nacre produced by the oyster that is being targeted. To assess this, an area of the darker, prismatic outer layer of the live shell can be removed using sandpaper to expose the pearly nacre layer (O'Connor et al. 2003, & Assael, 1991). Provided the shell is not damaged the oyster should not suffer unduly from this action.

4.2.2.2 Gonad Condition Monitoring - Macroscopic

A concurrent system of assessing gonad condition was set up and commenced at the start of the broodstock conditioning and holding trials for both the hatchery and farm. This was based on visual assessments whereby a rating from 1-5 was used with 1 being no development and 5 being fully mature and ready to spawn. As such a photo rating scheme was developed using photos of Akoya oysters (with one shell removed) from the Abrolhos Islands. This photo series of Akoya oysters displaying the 5 stages described above, for the current project is shown in Appendix 3.

Additionally, this project developed further assessment schemes to rate Glycogen (mucoprotein) covering and byssal threads in potential broodstock. Hence the schemes that were developed are:

(a)	Gonad Condition:	1 – undeveloped to 5 - mature.
(b)	Glycogen Covering (of the gonad):	1 - clear/nil to 5 - fully.
(c)	Shell Nacre Colour.	
(d)	Byssal Threads:	1 - none to 5 - numerous/large.

Standard photo protocols were followed to ensure that each of the 5 numbered photos in each scheme displayed an identical view of the oyster so that the relevant characteristic can be easily and directly compared visually.

In order to monitor gonad condition, and the seasonal and environmental influences upon it, groups of selected wild oysters were established at commercial densities in pearl nets at Sites 1, 3 and 4 at the start of the project (July 2007). Random samples of

30 oysters held at Sites 1, 3 and 4 were collected on an approximately monthly basis over a 12 month period from November 2007 through to November 2008. Morphological data on each oyster was collected sufficient to allow a host selection Criteria Value (CV) to be derived (as outlined earlier). This data included whole weight, meat (soft tissue) weight, height (DVM), width (APM) and thickness (WD) as shown on Figure 1 and displayed in Figures 2 – 4. Additionally, oysters were then visually assessed for Gonad condition and Glycogen condition using the relevant Photo Series prepared during this project. Randomly selected soft tissue samples from these oysters were then sent to the Animal Health Laboratories in Perth for histological assessment.

The criteria used for macroscopic scoring of gametogenic stages were developed by O'Connor et al., (2003) and based upon criteria described by Tranter, (1958) for *Pinctada albina*. The criteria for ranking here are based upon the portion of the body exposed when the mantle and gill are folded back, that is the area anterior and ventral to the urogenital papilla, (O'Connor et al., 2003, p15).

Table 1. Criteria for macroscopic scoring of gametogenic stages.

Stage	Description	Score
Inactive	Gametes are absent. The gonadal area is translucent and the digestive diverticulae are visible.	1
Developing 1	Gonads are filling, development is patchy and appears to be emanating from the posterior forward, males and females are indistinguishable.	2
Developing 2	Gonads less patchy in appearance as follicles spread and begin to fill. A pattern of development toward the anterior is less apparent; however the anterior edges of the body remain translucent. Gonad and body thickening.	3
Developing 3	Gonad and body turgid and consistent in colour, the development of follicles is no longer apparent with the exception of an occasional translucent strip at the base of the foot. Sex can be generally differentiated on the basis of colour. The digestive diverticulae are no longer visible.	4
Ripe	Gonad highly turgid and consistent in colour, follicles not apparent.	5

4.2.2.3 Gonad Condition Monitoring - Juvenile Gonad Development

As a result of the growth monitoring work, it was possible to determine that the hatchery produced oysters, in the current project, began to mature at a range of sizes, but generally from about 8 months of age onwards. However, overall oyster growth and development was slower than anticipated and the trial monitoring reproductive condition of juveniles did not commence until April 2009 when oysters were some 16 months old. At that stage juvenile oysters, at the sites where higher growth rates were apparent, had reached a minimum seedable size. The trial was conducted to determine if any differences in reproductive stages existed between the wild and the hatchery produced oysters. Due to many of the hatchery oysters still being too small to visually inspect the gonads, the 10 largest oysters from each site were selected from within the 30 sample shell, collected during each monitoring trip to monitor growth rates. The 10

oysters were then measured (Width-APM, Height-DVM, Thickness-WD, all in mm) and total weight in grams (g) recorded. After the juvenile oysters were weighed and measured they were opened and a visual assessment based on the 1 to 5 ranking systems (Photo Series) was conducted for gonad, byssus and glycogen. The oysters were then photographed for future reference. Colour of the shell was difficult to determine due to the difference in natural light availability at the different times of monitoring trips and therefore inconsistencies were evident. Data on shell colour was therefore not collected for this trial. The monitoring was conducted for 12 months from April 2009 to April 2010.

Prior to this, two preliminary Photo Series were prepared to allow assessment of gonad, byssus, glycogen and colour stages in the oyster, based on a 1 to 5 ranking system (see Appendix 3). Photos for this series were obtained (after each oyster was weighed and measured) by opening and photographing a range of larger pearl oysters, with varying stages of maturity, held on the various farms.

4.2.2.4 Gonad Condition Monitoring - Microscopic

Histological Methods (Jones, 2009)
Oysters were shucked and the whole meat was fixed in 10% formalin seawater before transfer to the Animal Health Laboratories in Perth. Oysters were dissected, tissue dehydrated and embedded in paraffin wax using standard histological techniques. Sections of 5 µm thickness were cut, stained with haematoxylin-eosin and examined using a light microscope.

Oyster gonads were scored histologically using the following scale, derived from Saucedo et al., (2001) and Normand et al., (2008).

Table 2. Histological assessment of Akoya gonads.

0	No trace of sex. Follicles non-existent or elongated and consist of undifferentiated germinal epithelium.
1	Early growth. Follicles small and isolated with numerous spermatogonia or oogonia.
2	Late growth. Follicles actively developed with primary gametocytes and some free spermatozoa and oocytes.
3	Mature. Near ripe, densely packed with maturing gametes, some mature gametes present.
4	Spawned or resorbing. Follicles distended, gametes remain.
5	Spent. Follicles empty and being resorbed.

4.2.3 Results

4.2.3.1 Broodstock Selection

Using the selection protocol established previously, in excess of 1,400 oysters were selected from existing stocks and held in culture cages at Sites 1, 2 and 3. These oysters were subsequently utilised in broodstock conditioning trials and hatchery larval culture trials and production. Only selected host oysters were used in the actual production of larvae/spat for the current project as just one commercial spawning and hatchery batch was reared. Selection of donor (saibo) oysters was made at various seeding times utilising the protocols established earlier. In the current project, potential saibo oysters were selected by technicians based upon the visual appearance of their nacre only; no sanding or cutting of the shell was conducted.

As part of the selection criteria, photo assessment scales were developed that allowed, gonad stage, glycogen stage and nacre colour to be determined (see Appendix 3).

4.2.3.2 Gonad Condition Monitoring – Macroscopic

For results on gonad condition monitoring see Table 3 over.

4.2.3.3 Gonad Condition Monitoring – Juvenile Gonad Development

For results on juvenile gonad development see Table 4 over.
Statistical analysis of the data revealed differences in relative shell dimensions and measurements but interestingly, there was no significant difference in the average Host Selection Criteria Value (CV) between the three sites. However, gonad condition and glycogen condition did vary significantly between the sites with Site 1 recording the highest average conditions while Site 4 recorded the lowest.

Table 3. Monitoring of Oyster Shell and Gonad Condition in Wild Oysters at the Abrolhos Islands, November 2007 – November 2008.

	Trip/Date	4-Nov-07	4-Dec-07	10-Feb-08	20-Mar-08	26-Apr-08	25-May-08	29-Jun-08	3-Aug-08	25-Aug-08	28-Sep-08	1-Nov-08
	Site	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Whole weight (g)	1	75	54	52	69	86	36	62	34	41	21	46
	3	51	64	28	36	61	65	71	81	46	60	58
	4	80	65	21	47	32	76	57	92	66	47	51
Meat weight (g)	1	25	16	15	23	25	11	20	12	15	5	17
	3	12	22	6	10	21	24	24	31	14	17	18
	4	26	13	7	13	8	26	16	30	22	11	14
Height (DVM) (mm)	1	81	74	76	82	85	61	71	57	64	52	63
	3	70	79	61	66	79	77	80	81	71	80	71
	4	87	71	58	70	62	79	73	81	77	68	67
Width (APM) (mm)	1	79	69	74	78	86	60	73	58	68	53	67
	3	69	75	59	63	82	78	81	84	71	81	74
	4	84	68	55	67	62	83	74	83	79	69	69
Thick (WD) (mm)	1	31	27	28	34	31	23	27	22	26	17	27
	3	24	31	21	23	32	29	32	30	25	33	29
	4	32	25	19	27	22	33	27	33	33	26	24
Akoya host selection criteria value	1	0.16	0.16	0.16	0.17	0.15	0.16	0.16	0.16	0.16	0.14	0.17
	3	0.15	0.17	0.15	0.15	0.16	0.16	0.17	0.16	0.15	0.17	0.16
	4	0.16	0.15	0.15	0.17	0.15	0.17	0.16	0.17	0.17	0.16	0.15
Gonad	1	3.3	2.5	2.1	2.2	2.2	3.8	3.7	4.4	4.3	3.3	4.6
	3	1.4	2.2	1.9	1.7	2.5	3.1	3.4	3.1	4.7	3.1	2.8
	4	1.6	1.2	1.8	1.9	2.2	3.2	2.4	2.1	2.3	2.5	2.4
Glycogen	1	2.8	2.2	2.2	2.3	1.5	2.5	1.9	1.6	2.1	1.3	2.7
	3	1.0	2.1	1.7	1.6	2.1	2.8	1.6	1.4	1.7	1.0	1.3
	4	2.1	1.2	2.0	1.4	1.6	2.1	1.1	1.0	1.0	1.0	1.0

Table 4. Monitoring of Hatchery Produced Juvenile Akoya Oysters at the Abrolhos Islands, April 2009-April 2010.

		Spawned	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07	6-Dec-07
		Trip Date	25-Apr-09	17-May-09	2-Jul-09	31-Jul-09	6-Sep-09	26-Sep-09	7-Nov-09	28-Nov-09	29-Dec-09	6-Feb-10	28-Feb-10	9-Apr-10
		Age Days	506	528	574	603	640	660	702	723	754	793	815	855
		Age Months	16.6	17.4	18.9	19.8	21.0	21.7	23.1	23.8	24.8	26.1	26.8	28.1
	Site	Trip #	17	18	19	20	21	22	23	24	25	26	27	28
Height (DVM)	1		57.50	59.30	59.10	63.30	64.10	63.20	56.50	63.70	63.60	66.40	66.60	67.00
(mm)	2		48.90	51.90	51.10	52.20	49.30	55.60	49.30	51.80	53.20	54.70	58.60	60.80
	3		46.70	51.20	48.90	52.70	50.70	48.30	49.70	50.90	52.70	54.00	57.50	56.90
	4		50.00	52.70	52.50	52.70	53.10	53.40	53.80	54.50	57.30	57.90	59.20	61.50
	5		61.00	61.10	62.80	62.20	64.30	66.70	62.90	64.10	64.80	68.00	67.80	71.70
Width (APM)	1		61.00	61.40	62.70	67.90	67.00	69.90	64.20	69.60	67.70	69.60	71.00	73.18
(mm)	2		50.10	53.00	52.90	55.10	53.50	57.80	51.30	53.90	55.70	56.70	60.80	62.60
	3		48.20	51.00	49.70	55.00	51.30	51.10	52.30	53.70	54.50	56.70	60.70	60.30
	4		52.30	54.90	56.20	56.50	54.70	55.50	56.40	58.40	59.70	60.80	61.30	64.00
	5		63.50	65.20	66.20	66.90	68.00	70.40	65.70	69.20	67.30	71.90	71.70	76.70
Thick (WD)	1		24.60	23.70	23.70	26.90	24.90	25.30	25.60	28.60	27.10	28.30	28.00	26.27
(mm)	2		18.10	18.40	19.10	19.20	17.90	19.20	18.20	19.90	19.50	20.80	20.70	21.80
	3		15.80	15.90	15.50	19.40	17.00	16.40	16.70	18.50	19.50	19.90	20.70	20.60
	4		19.40	18.20	18.70	20.10	18.20	18.50	18.70	19.90	22.70	22.10	22.30	21.40
	5		24.80	24.90	25.70	26.70	27.10	25.60	25.50	25.20	27.10	28.40	27.90	29.90
Total Weight	1		27.00	38.10	36.50	45.13	49.00	39.60	43.50	45.25	45.30	47.30	57.10	57.40
	2		14.00	24.00	21.00	20.00	25.60	25.10	24.60	29.25	28.10	26.00	27.55	32.30
	3		13.50	19.70	15.90	21.20	21.80	17.80	22.70	25.20	24.20	27.30	32.95	39.40
	4		17.00	26.30	22.60	20.90	20.50	26.10	26.40	28.80	28.90	30.40	35.45	40.00
	5		35.00	47.10	40.70	36.60	50.80	42.30	45.30	49.35	43.30	49.10	50.15	60.00
Byssus	1		2.30	2.10	2.40	2.90	3.00	3.20	3.30	3.40	4.20	2.70	3.60	4.80
	2		1.60	2.00	2.40	2.20	2.30	2.80	2.60	3.10	3.60	3.80	3.05	2.40
	3		2.80	1.90	2.50	3.10	2.30	2.50	3.40	3.75	4.10	3.80	3.55	3.60
	4		2.10	3.00	2.20	3.40	2.90	2.70	2.00	2.85	3.50	1.90	2.70	3.40
	5		1.90	2.50	3.10	2.90	3.60	3.00	3.70	3.90	3.80	2.20	2.65	4.00
Glycogen	1		3.00	3.60	3.30	4.10	3.60	3.30	3.50	3.30	3.10	4.10	4.00	3.90
	2		1.30	1.30	1.40	1.00	1.00	1.10	1.10	2.25	1.20	1.70	2.40	1.70
	3		1.20	1.60	1.20	1.20	1.10	1.10	1.00	1.50	1.70	1.80	2.40	2.90
	4		2.20	2.30	2.20	1.80	1.30	1.30	1.20	1.75	1.90	2.80	3.20	3.70
	5		3.90	4.00	4.30	4.30	4.20	3.30	3.90	4.10	4.20	4.10	4.30	5.00
Gonad	1		3.30	3.60	3.80	4.50	3.80	4.40	4.40	3.95	3.40	2.90	3.55	4.20
	2		1.90	3.00	2.00	2.80	3.00	1.80	2.40	2.20	2.40	2.00	2.05	2.40
	3		1.10	2.00	3.00	3.00	2.80	3.30	3.30	3.10	2.90	2.80	2.95	2.90
	4		3.10	3.20	3.60	2.30	2.00	2.20	2.70	3.00	3.30	2.80	2.85	2.90
	5		4.00	4.10	3.60	4.00	4.10	4.20	3.60	3.30	3.20	3.60	3.80	3.50

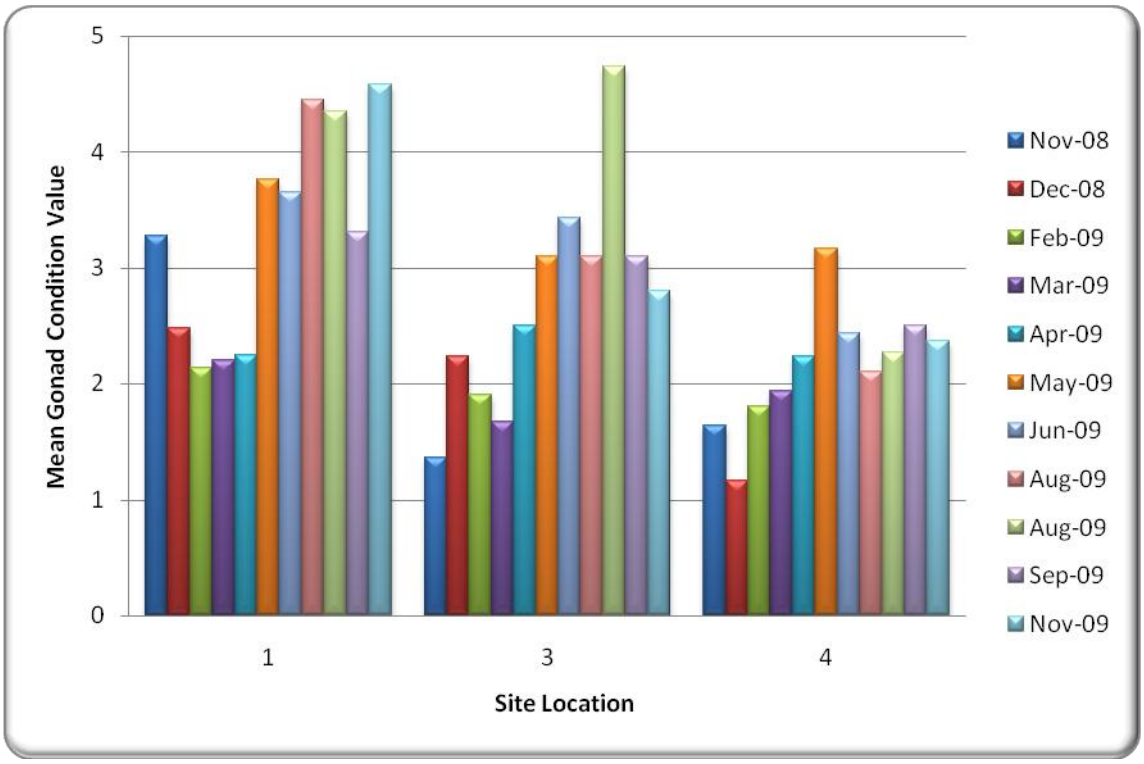


Figure 5 - Mean Macroscopic Gonad Condition at Sites 1, 3 & 4; Nov 08 – Nov 09.

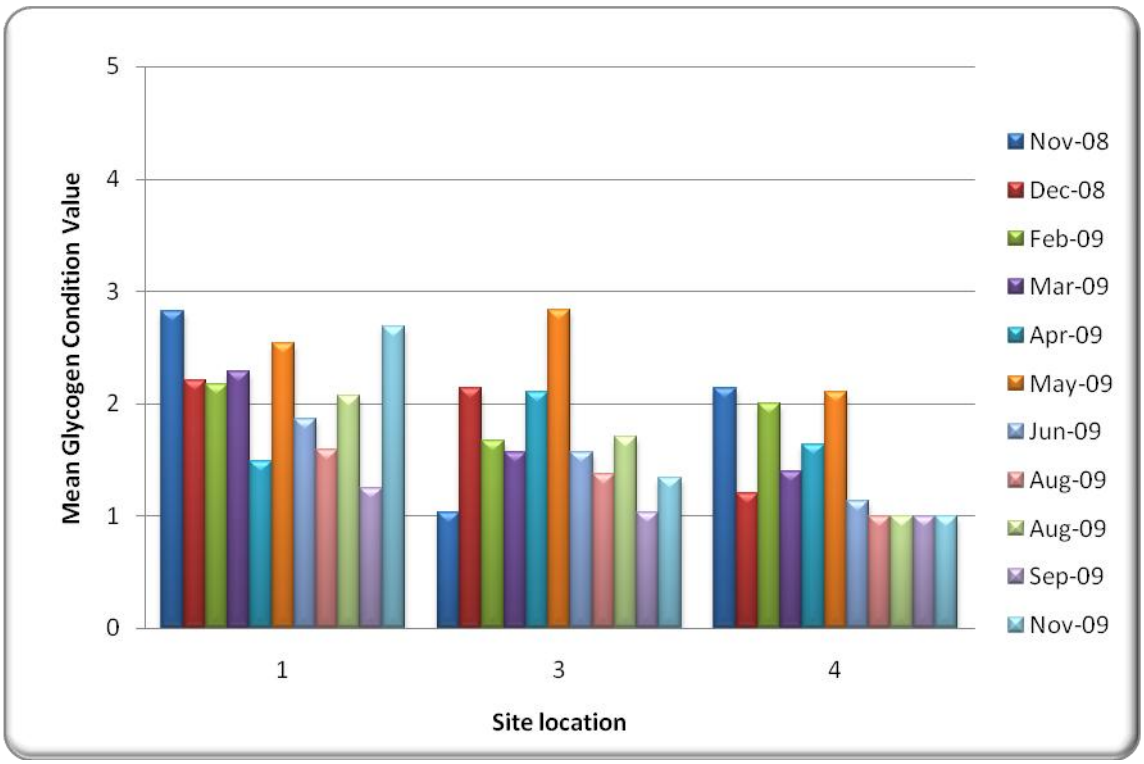


Figure 6 - Mean Macroscopic Glycogen Condition at Sites 1, 3 & 4; Nov 08 – Nov 09.

Figures 5 and 6 suggest a seasonal variation in both condition assessments (using macroscopic Photo Series) may exist as well as apparent variation in overall condition between sites. Site 1 is likely to support oysters in spawning condition for at least several months per year, whereas at the other sites, such animals will be more difficult to source and in fewer months, if at all at Site 4. Statistical analyses were conducted to examine the various aspects.

4.2.3.4 Gonad Condition Monitoring – Microscopic

The histological assessment of gonad samples taken during the period November 2007 to November 2008 showed possible seasonal and site variations although statistical analyses were required to evaluate this.

Table 5 - Condition and Sex of oyster gonads from culture Sites 1, 3 & 4 by histological assessment.

Date	Monitoring Trip No.	Site 1 Gonad Score	Site 1 Sexes	Site 3 Gonad Score	Site 3 Sexes	Site 4 Gonad Score	Site 4 Sexes
4/11/07	1	4.0	2M,2F,1MF	4.0	3M,1F,1MF	4.0	1MF,0F,4MF
4/12/07	2	4.0	2M,3MF	4.0	2M,1F,2MF	4.0	2M,2F,1MF
10/2/08	3	3.54	2M,4MF	4.0	1M,1F,1MF,1I	4.0	2M,1F,2I
27/04/08	5	4.0	1M,1F,4MF	4.0	3M,1F,1MF	4.0	5M
24/05/08	6	4.0	1M,2F,1MF	4.0	3M,1F,1MF	5.0	1M
29/06/08	7	2.2	1M,4MF	2.0	2M,3MF	2.0	1M,1F,3MF
02/08/08	8	3.0	2M,1F,2MF	3.25	1M,4MF	3.2	2M,3MF
25/08/08	9	3.27	4M,1MF	3.0	2M,1F,2MF	3.18	1M,4MF
28/09/08	10	3.25	4M,1MF	3.5	3M,1F,1MF	3.38	4M,1MF
01/11/08	11	3.0	6M,2F,1I	3.38	4M,3F,1MF	2.71	7M

Note: No samples were able to be collected on Trip 4.
M: Male, F: Female, MF: both Male & Female tissue present

Table 6 - Health Assessment of histological Akoya samples
C/- Animal Health Laboratories, Perth, WA, B. Jones. 2009

Case Number	Collection Date	Comments
AS-07-4034	December 2007	One oyster infected with papova-like viral inclusions.
AS-08-1028	February 2008	Two of the oysters had a focal infection of a gram positive grocotts negative branched organism consistent with Nocardia.
AS-08-1627	April and May 2008	Oysters from the three sites do not show any deleterious infectious or parasitic agents. They are probably remarkable for the low numbers of commensal parasites, with only small numbers of rickettsiales bacteria present in several animals.
AS-08-3011	June, August, September 2008	No significant findings.
AS-08-3692	November 2008	No significant findings.

Stages of development of Akoya oysters from Histology (Jones, 2009)

Stage 0
Resting. No trace of sex. Follicles non-existent or elongated and consist of undifferentiated germinal epithelium.

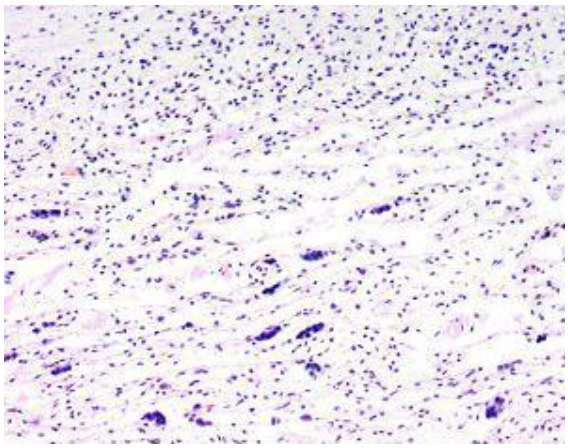
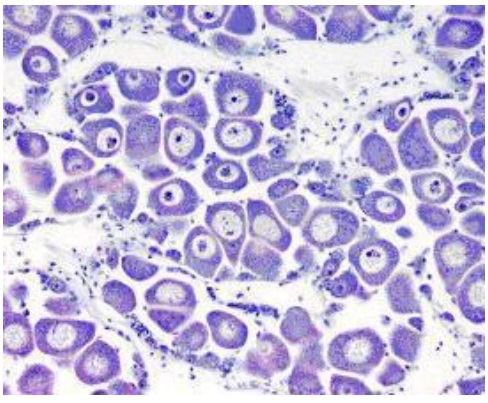


Figure 7 - Resting phase, germinal epithelium forming x 200.

Stage 1
Early. Follicles small and isolated with numerous spermatogonia or oogonia.

Not seen.

Stage 2
Late growth. Follicles actively developed with primary gametocytes and some free spermatozoa and oocytes.



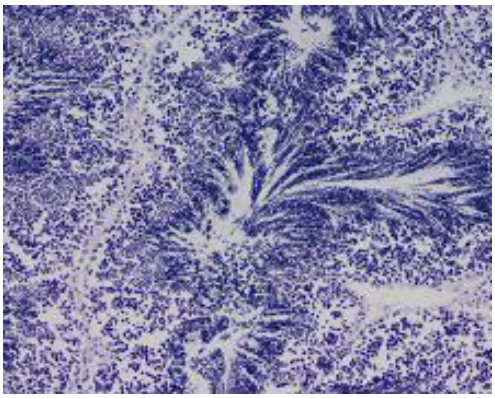


Figure 8 - Female (left) and male (right) x 200.

Stage 3
Mature. Near ripe, densely packed with maturing gametes, some mature gametes present.

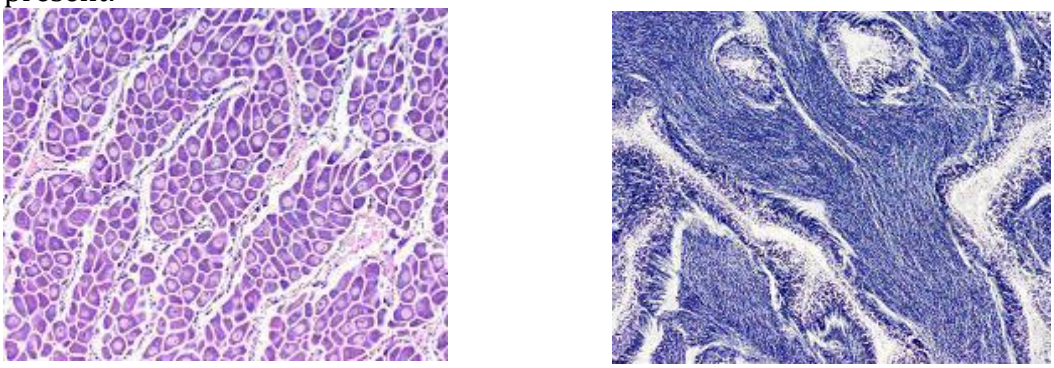


Figure 9 - Female (left) and male (right) x100.

Stage 4
Spawned and resorbing. Follicles distended, gametes remain.

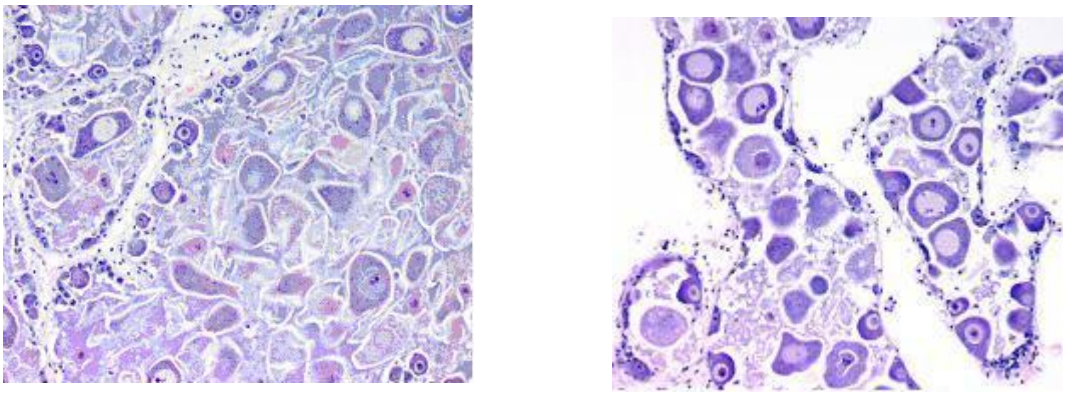


Figure 10 - Resorbing eggs x 200.

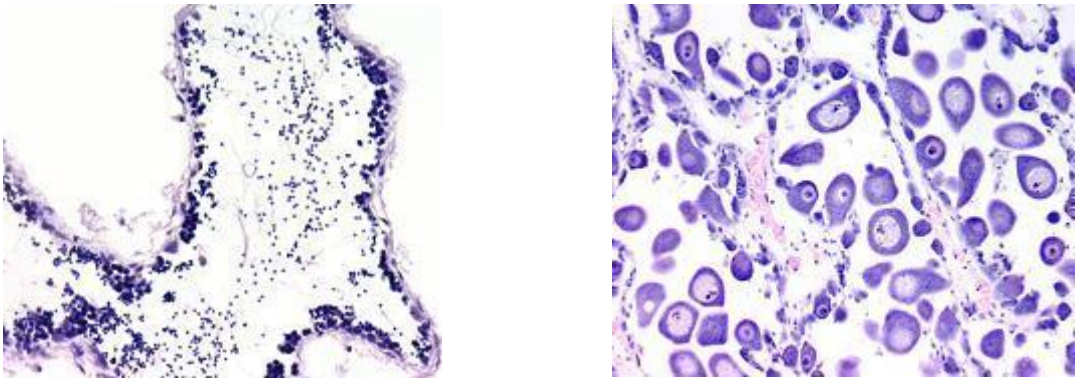


Figure 11 - Male (left) female (right) spawned x 200.

Stage 5
Spent. Follicles empty and being resorbed.

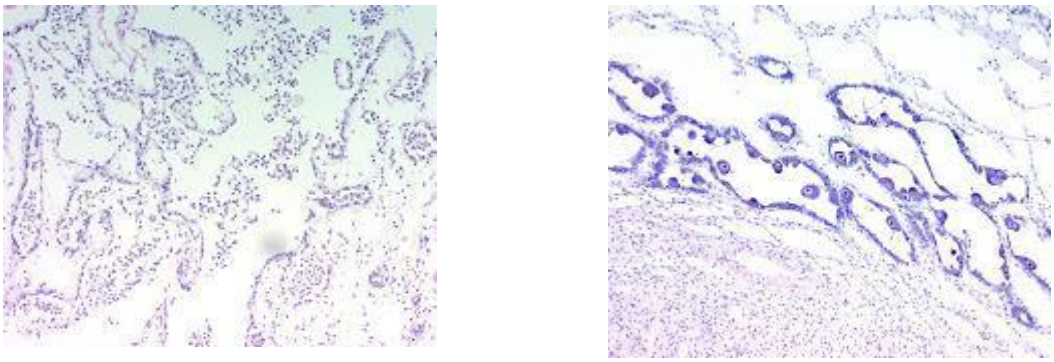


Figure 12 - Empty gonad follicles x 100.

Health Assessment of Akoya Oysters (Jones, 2009).
The general lack of parasites and commensal organisms was surprising, given the numbers of such organisms seen in *Pinctada maxima* from the northwest shelf. However, a few pathogens of interest were seen, all rare. These included Papova-like virus inclusions (Figure 13); a Nocardia-like organism in the digestive gland (Figure 14), and a rickettsia-like organism in the gonad (Figure 15).

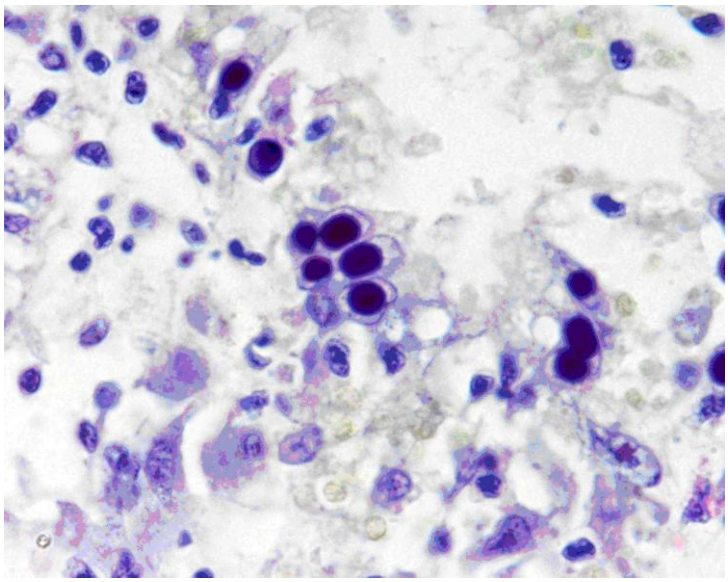


Figure 13 - Papova-like viral inclusions.

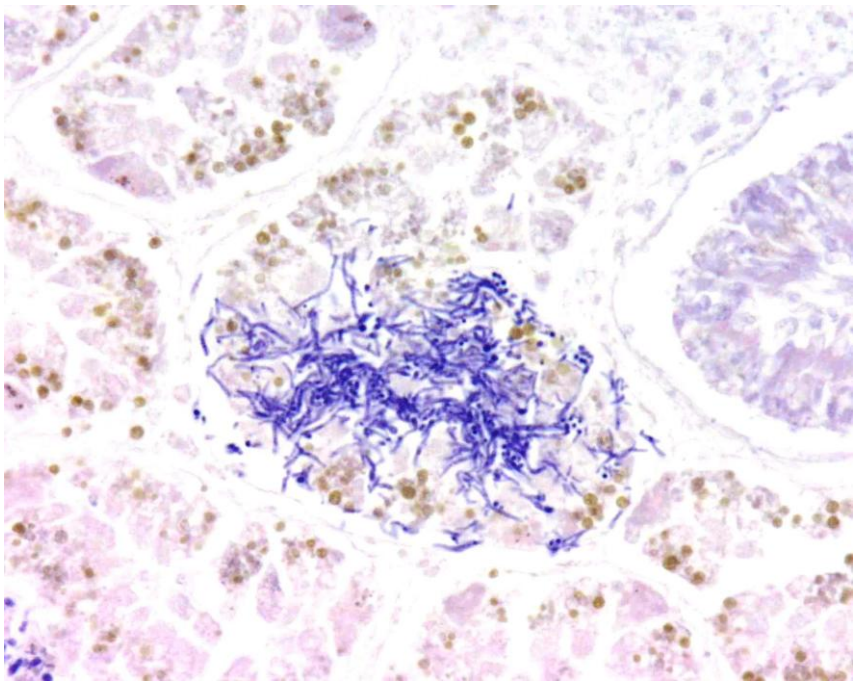


Figure 14 - Nocardia-like colony in digestive gland tubule.

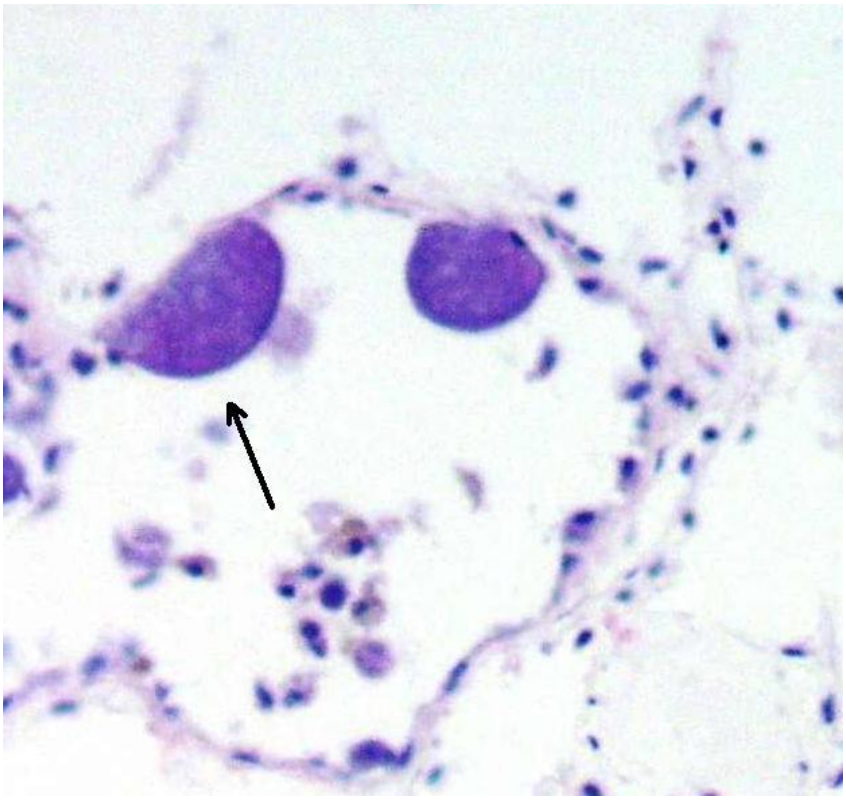


Figure 15 - Rickettsia-like organisms associated with gonad follicle epithelium (large ovoid purple bodies).

Statistical Analyses

Macroscopic and microscopic gonad assessments were conducted on wild oysters at Sites 1, 3 and 4 simultaneously on several trips. This provides a comparison between the two methods. Unfortunately individual oysters were not assessed by both methods and it is therefore necessary to compare average ratings for the two methods on a given site for a given trip. Figure 16 shows a lack of correlation between the two methods. Category 5 on the microscopic assessment identifies gonads that are spent; this cannot be assessed accurately using macroscopic assessments. Consequently the analysis was revisited, omitting microscopic assessments with gonad scores of 5. This did not improve the correlation between the gonad indices.

The time series of macroscopic and microscopic gonad assessments on the wild oysters is shown in Figure 17. The macroscopic gonad index and glycogen tracked closely until May-June when they diverged, after this they continued to exhibit similar patterns with a constant difference between the indices.

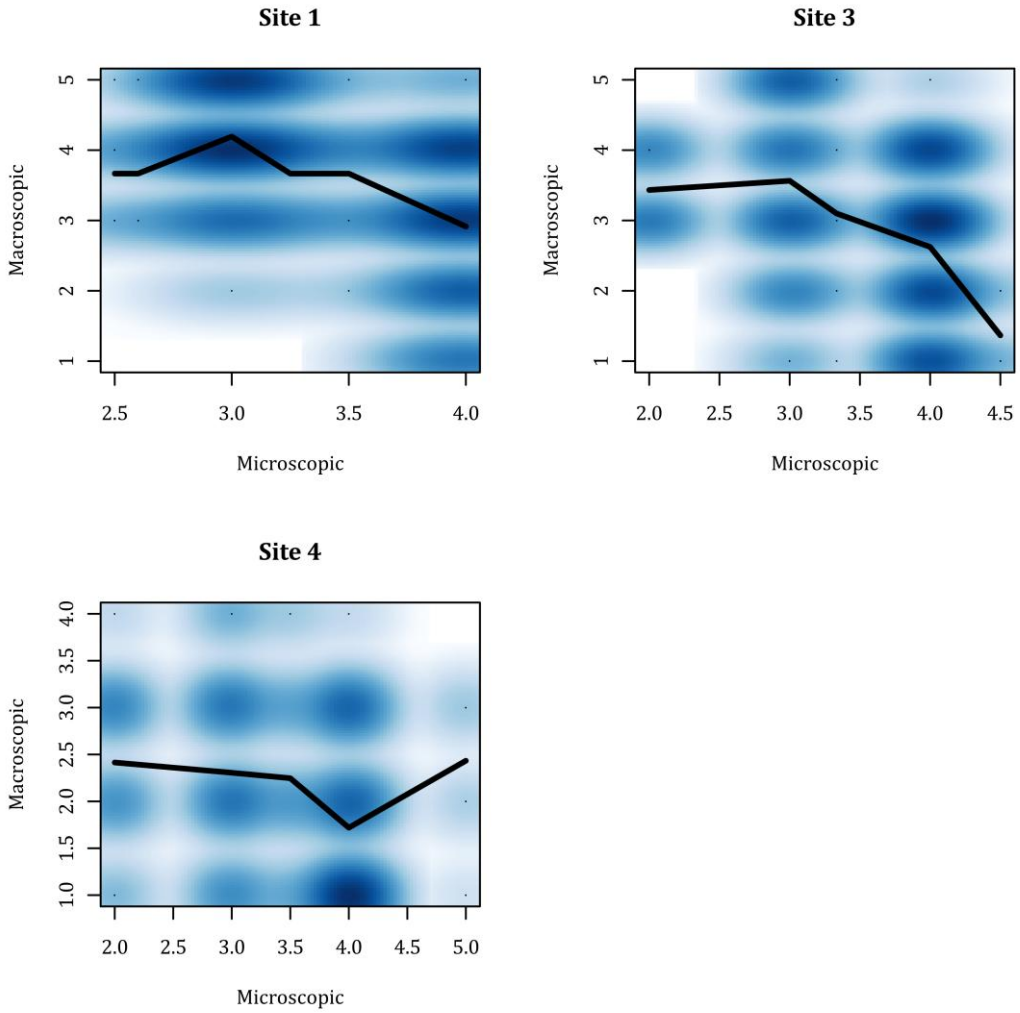


Figure 16 - The correlation between the macroscopic and microscopic gonad indices.

Darker blue indicates more observations for that combination of indices. Black indicates the mean relationship between the two indices. A positive correlation would present as a dark blue running from bottom left to top right and a black line running the same direction, this was not observed here.

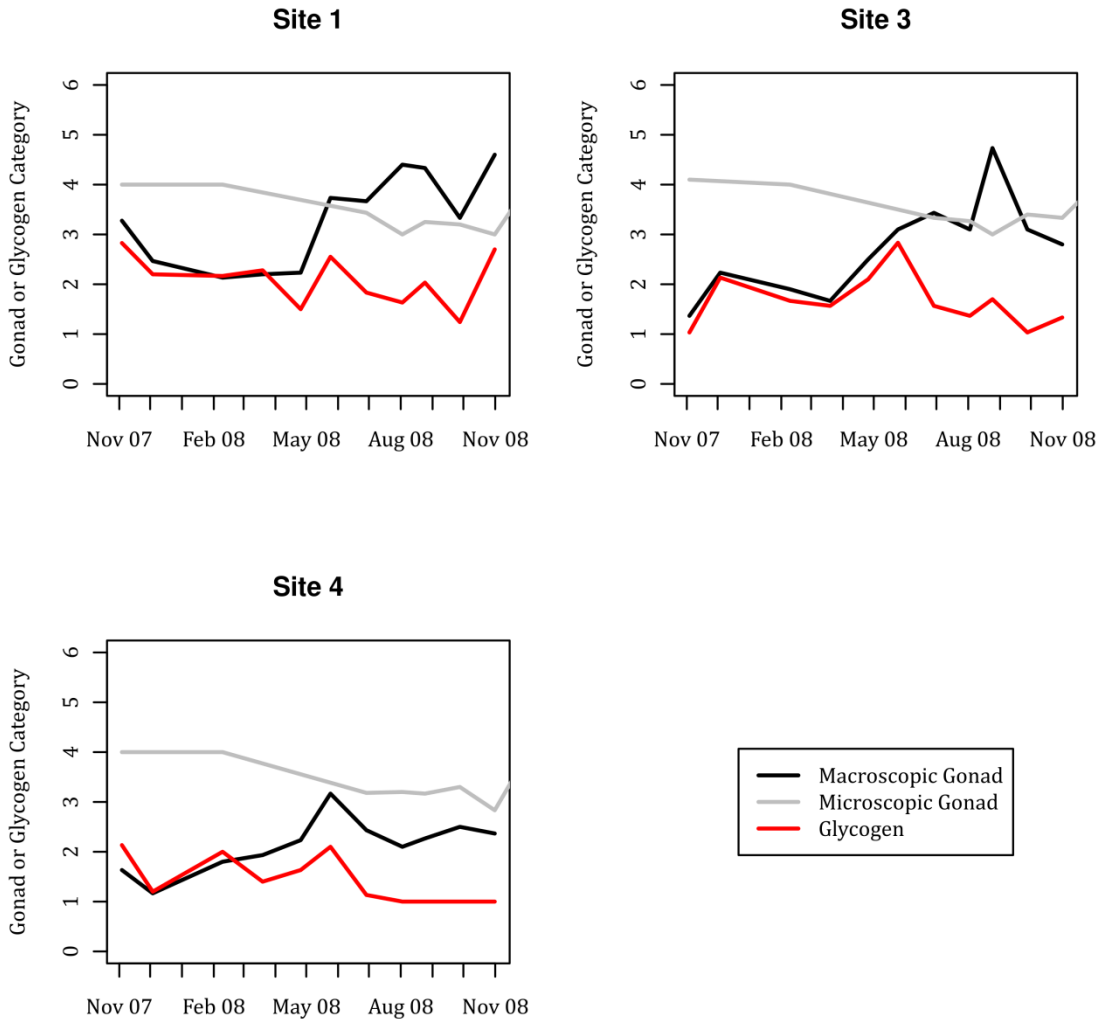


Figure 17 - A time series of glycogen and macroscopic and microscopic gonad indices for the wild oyster study.

Linear modelling revealed that Gonad, Glycogen, Weight and Byssus are all positively correlated ($p<0.01$). The correlation of Gonad and Glycogen indices could be due to a bias in the visual assessment. Weight and size were not measured for the oysters scored microscopically. This measurement would have allowed this bias to be assessed and would be a worthwhile exercise if the visual assessment is to be used in future.

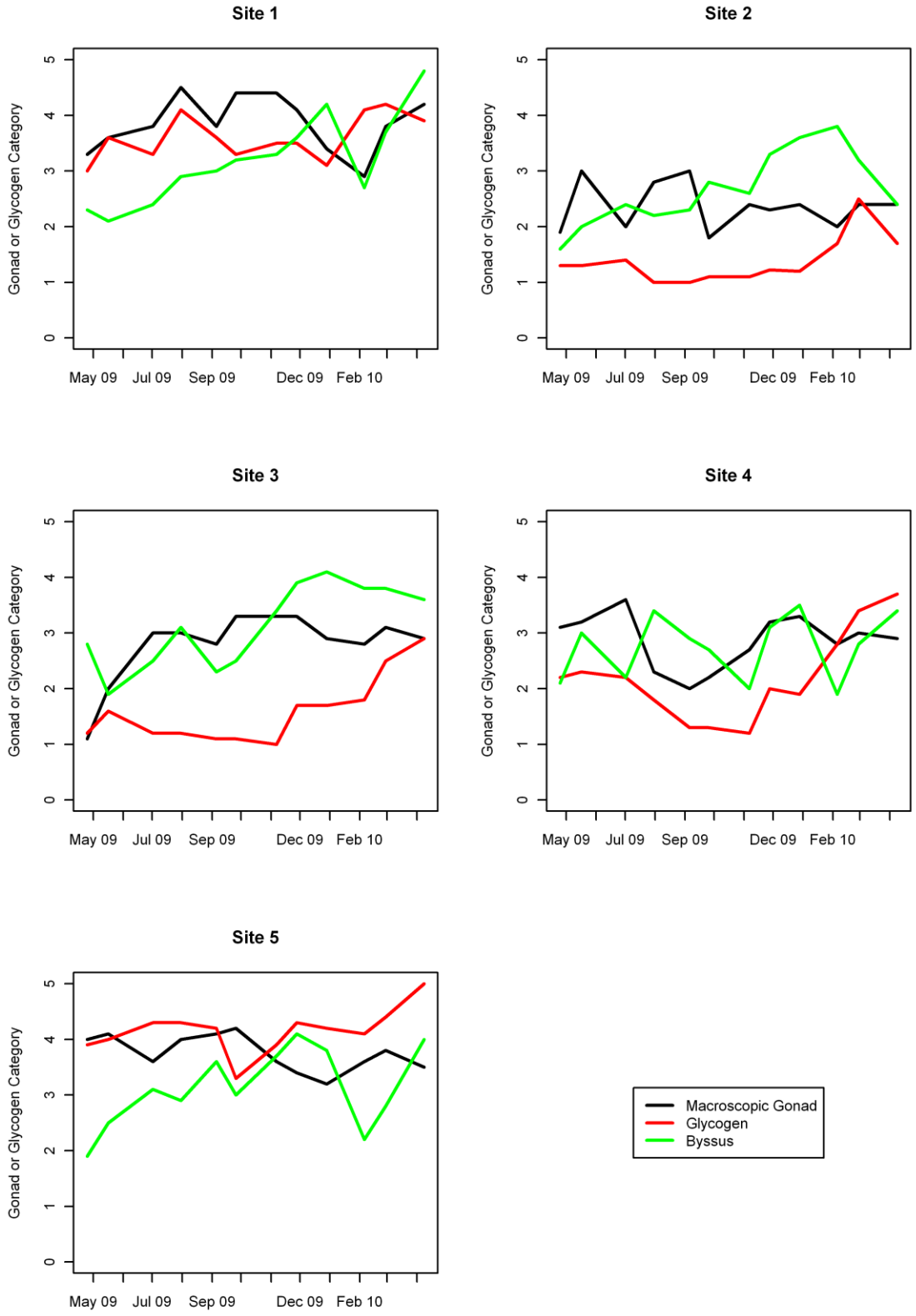


Figure 18 - A time series of glycogen, byssus and gonad indices for the hatchery produced oysters.

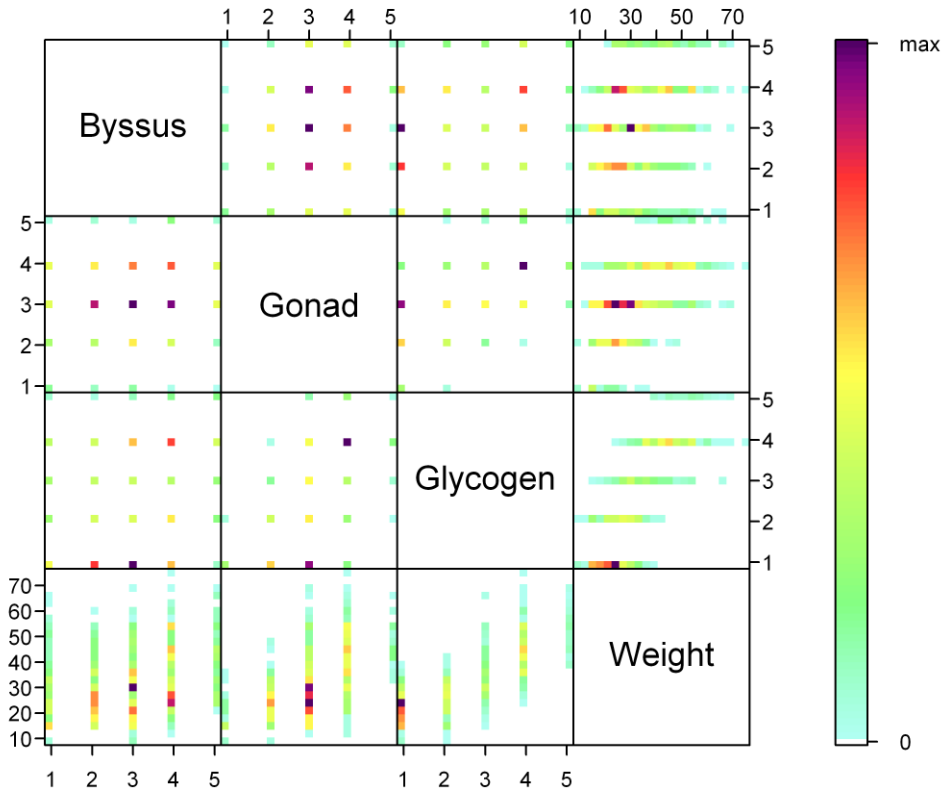


Figure 19 - A scatter plot matrix with the colour indicating the density of points.

Strong positive correlation would be evident as a series of high density points from the bottom left to the top right in one of the panels. The strongest relationship is between Glycogen and Weight.

4.2.4 Discussion

The macroscopic and microscopic gonad indices were not correlated. The relationship could be determined with much greater confidence by assessing the same animals with both methods. Given the lack of correlation the two indices may actually be assessing different gonad properties. Since this study did not collect extensive data on spawning events it is not possible to determine which index may be more appropriate. Nevertheless, the microscopic index is a better method of assessing actual gonad condition over time.

Macroscopic gonad and glycogen indices for the wild oysters diverged in all sites between May and June 2008. This may well be indicative of animals approaching spawning condition and using glycogen reserves. Induced spawnings of Akoya oysters have been successfully conducted in recent years by the Abrolhos Islands industry during May-June.

Hatchery produced oysters at Sites 1 and 5 had the highest gonad indices with a significant proportion of the oysters in condition to spawn during the entire study period. Site 1 also had the highest wild oyster gonad index (Site 5 was not assessed). Sites 2 and 4 had evidence of spawning in September and July respectively. Sites 1 and 5

suggested gradual spawning between October and February with Site 1 delayed by approximately one month.

Sites 1 and 5 are therefore the best sites to source oysters in spawning condition for hatchery requirements. At either site, routine gonad monitoring would be required to assist in targeting oysters in good reproductive condition. Monitoring of glycogen condition appears unlikely to have much additional benefit in identifying good potential broodstock at these sites.

This project has developed a macroscopic or visual method of assessing gonad and glycogen condition that can be used by industry to assist in sourcing broodstock for hatchery breeding or when assessing gonads during the pre-operative conditioning stages.

The use of a calculated Host Selection Criteria Value was completed, but the results at this stage, display minimal variability and appear to be inconclusive. However, over time, further work on this aspect may well reveal effects related to such physical aspects and therefore establish relationships which do not yet appear to be present. The Selection Value is reconsidered after the pearl harvest has been discussed later in this report. From the histological assessments, the development of the reproductive tissues was unremarkable, following closely the pattern seen in other pteriid oysters (Rose et al., 1990). Bisexual oysters were not seen (Jones, 2009). These microscopic assessments showed periods of good gonad condition without distinct peaks, followed by periods of poorer gonad condition. General similarities of seasonal gonad development between the sites appeared to be evident.

4.2.4.1 Health Aspects (Jones, 2009)

The papova-like virus inclusion is apparently identical to those seen in *P. maxima* and is not associated with any pathology (Norton et al., 1993; Humphrey & Norton 2005). The Nocardia-like organism in individual digestive gland tubules was also not associated with an inflammatory response and its effect on the oyster is uncertain. The rickettsiales-like organism is similar to those seen in *Pinctada maxima* gonads (Humphrey & Norton 2005).

4.3 Pearl Oysters – Broodstock Conditioning

4.3.1 Introduction

The ability to condition Akoya oysters in a hatchery environment has been investigated in Australia (NSW) by O'Connor et al., (2003) and has provided valuable guidance for the WA project. Temperature has been found to be of particular importance although it is still only one of a series of exogenous factors involved (O'Connor & Lawler, 2004). The time taken to condition broodstock has been determined experimentally for Japanese stocks of *P. imbricata* (Akoya) and is estimated to be between 700-800 degree days (Wada, 1991). This is calculated by summing the number of degrees over 13°C per day based upon the equation:

(Water Temp. °C - 13°C) x No. days = Total No. degree days.

For example, oysters held at 23°C for 10 days would accumulate 100 degree days:
e.g. (23°C - 13°C) x 10 days = degree days.

As part of the hatchery process, mature adult oysters were required as broodstock. It is important, for the long term success of the local industry, that broodstock exhibiting desirable characteristics are used for reproductive purposes. The difficulty initially, was to clarify market demands for pearls and attempt to translate this into appropriate desirable characteristics of broodstock oysters, without knowledge of genetic makeup of the oysters or relationships between genotypes and phenotypes in the oysters. This trial was aimed at identifying desirable Akoya broodstock based on the selection protocol developed as the first part of the current project. The establishment of a suitable broodstock conditioning system was then conducted.

4.3.2 Materials and Methods

4.3.2.1 Broodstock Conditioning System (BCS)

Broodstock originated as wild shell, collected as juveniles at the Abrolhos Islands and held in culture cages up until their selection and transport to the BCMI Hatchery. While stress factors during selection and transport were minimised, no additional conditioning techniques were used for the broodstock oysters prior to the transfer to BCMI. Ideally, obtaining mature oysters from culture cages in a ready-to-spawn condition is the preferred option, but if this is not possible then conditioning (induced development of the gonads) is necessary.

The satisfactory completion of broodstock conditioning can be indicated by a successful spawning and larval production, in addition to visual (or histological) gonad assessment. Hatchery spawning of broodstock was aimed at utilising a minimum of 30 males and 30 females to maintain genetic diversity, and commenced with host oysters due to their priority status. To end up with this number actually spawning, an allowance of 112 male and 112 female oysters (host) was made to start a broodstock conditioning trial (Group 1).

The hatchery-based conditioning system consisted of 6 x 1,400 L conical bottom tanks set up in the insulated and temperature controlled hatchery room at BCMI (1-2 tanks used for water changes). Oysters were held in commercial orange string panels at 56 oysters per panel (8 rows x 7 oysters).

- Holding water was filtered to at least 20µm and was preheated, when necessary, in 4,500 L tanks within the BCMI facility before it was pumped into the BCS. This maintained stable water temperatures even during water changes. A 50% water exchange was conducted on the first day and a full water change was conducted every second day when the panels were lifted into an adjacent clean tank, filled with pre-heated water.
- Oysters in the first group brought into the BCS were spread between 4 tanks initially. Once other oysters were brought in, the first group was placed into just 2 tanks. Monitoring of actual feed rates in each tank and subsequent condition of oysters may provide useful information in relation to broodstock holding density.
- Water temperature commenced at ambient (around 18-20°C) to start (when broodstock were first brought in) and was gradually brought up to 24°C over the first 7 days and then kept constant for the broodstock conditioning period right up to spawning trials.
- Algal diet was based upon the species *Chaetoceros muelleri* (50%), Tahitian *Isochrysis*-Tiso (30%), *Pavlova lutheri* (20%) and *Tetraselmis chui* (10%) with percentages able to vary slightly based upon algal availability each day. Basically the same diet composition was used from start to finish in the BCS with *Chaetoceros muelleri* used every day if possible. However, if difficulties arose then the diet was reduced to 2 species when absolutely necessary. The satiation feeding rate varies between 2-4 x 10^9 cells/oyster/day with oysters of 55-75mm in shell height (O'Connor et al., 2003). The trial started at 2 x 10^9 cells/oyster/day and was adjusted accordingly subject to actual consumption by oysters.
- Monitoring of oyster gonad condition commenced when the oysters entered the hatchery. An initial Gonad Photo Series with oysters displaying all the various major stages of development, as discussed in Section 4.2 earlier, ranked 1 (undeveloped) to 5 (mature), was utilised (Appendix 3). A sub-sample of the oysters was inspected and the oysters were then distributed at random to the panels in the BCS. Assuming that on arrival the oysters were not in 'ripe' ready to spawn condition, for the first couple of weeks, a panel could be pulled out of the water (from each tank) and 10 gaping oysters gently inspected visually and assessed (and recorded) at weekly intervals. This process would allow assessment of whether the oysters were all conditioning at a similar rate. As condition improved, and the risk of spawning increased, sampling was reduced to only one panel (per group) and 10 oysters per week, with a different tank sampled in each successive week (should inadvertent spawning occur, this would not risk the loss of all broodstock). Careful attention was paid to the rate* at which the oysters were improving in reproductive condition and if the oysters reached a mean visual rating of >3.5, they would not be inspected until a spawning was conducted.
- Once used for spawning trials, oysters would go back to the farm-based broodstock holding longlines (BHS) in their tagged panels.

*Note: improvement in condition is not necessarily linear, i.e. the time taken to progress from stage 1 to stage 2 is not necessarily the same as the time taken to move from stage 4 to stage 5. In addition, these scales are useful but not absolute indicators of egg quality or ability to spawn. Oysters do not need to reach stage 5 to spawn.

4.3.3 Results

Prior to the selection of broodstock, based on specific criteria (outlined earlier), a large pool of potentially suitable Akoya oysters was made available by Abrolhos Pearls and Latitude Fisheries. Selected broodstock were chosen from this pool and transported to the BCMI hatchery facilities at Geraldton on 4 September 2007. The oysters were stocked in orange string panels at 56 oysters per panel (8 rows of 7 oysters), placed so that byssal threads attach to an adjacent shell and with coloured and numbered tags on every panel. Hence the Group 1 Oysters comprised a total of 224 oysters. Note: sexes were still not identifiable in live oysters until spawning occurred.

As staff did not want to stress the oysters unduly, only 10 random animals were visually inspected for gonad condition at that stage, resulting in an average score of 3.7, higher than expected. Oysters were fed and maintained as per the protocol discussed above.

Unfortunately on 6 September 2007, a spontaneous mass spawning of oysters occurred in the holding tanks after a water exchange. A total of 89.7×10^6 fertilised eggs were obtained and placed into 2 prefilled larvae tanks. After 24 hours a total of 42.1×10^6 D larvae resulted. These larvae were cultured through to day 16 with gradually reducing numbers due to mortalities. At that stage the batch of larvae (AK0107) was discarded due to its poor condition and lack of sufficient numbers (a few thousand). Various water quality issues (related to heavy metals) were identified and after a series of analyses were conducted, various strategies were adopted by BCMI and the project staff, to try and improve the water quality sufficiently, to allow normal larval development. A second group of Akoya broodstock was brought into the hatchery on 3 October and used for spawning attempts. Subsequent spawning trials confirmed that problem issues with water quality could not be resolved adequately and the project at BCMI was discontinued. Additionally, the broodstock conditioning work was compromised and although the protocol detailed above was utilised, and gonad development was apparently promoted, the subsequent larval outcomes could not be considered as an accurate reflection of the treatment.

In late November 2007, project staff contracted the Blue Lagoon Pearl hatchery, located in Shark Bay, to commercially produce a batch of Akoya spat for the project. For the planned hatchery spat production run at Blue Lagoon Pearls, 100 Akoya broodstock were selected (using the established protocol) from adults held on various farms at the Abrolhos Islands and flown to the hatchery on 4 December 2007. This part of the project is discussed in Section 4.4. As good quality broodstock were readily identified, further broodstock conditioning work was not pursued. Additionally, gonad monitoring work (Section 4.2) would provide more information on the condition of Akoya adults at the 5 research sites.

4.3.4 Discussion

This aspect of the project was focused on the conditioning of selected broodstock for use in hatchery spawnings and subsequent production of larvae and spat. Unfortunately, aspects that could not be controlled by project staff impacted heavily on this work and it had to be discontinued, even though it may well have achieved its objective. Techniques aimed at promoting gonad development in Akoya oysters were utilised and early results were encouraging. Similar techniques have subsequently been used by the WA industry to hold broodstock in hatchery facilities, prior to induced spawnings. Although not reported herein, this work has been successful and resulted in commercial quantities of Akoya spat (juveniles) being produced in WA.

4.4 Pearl Oysters - Hatchery Production and Growout

4.4.1 Introduction

Any pearling industry requires a consistent and reliable supply of high quality animals to be host and donor individuals for the culture of the pearl. Various countries, including Australia, rely on some collection of animals (oysters) from the wild, to be placed into culture and used as host and donor animals. While this has occurred in the past for Akoya pearl culture at the Abrolhos Islands, production has been highly variable in quantity and quality. Selection of broodstock (as discussed earlier) and hatchery culture of juveniles provides the opportunity for significant improvements to be made in the physical characteristics (phenotype) of oysters. No genetic (genotype) assessments were conducted on Akoya oysters in the current project, only the visual selection of individuals by project members utilising the selection protocols developed for the project.

The BCMI (Batavia Coast Maritime Institute) at Geraldton (WA) was scheduled to produce Akoya spat for this project (at the completion of broodstock conditioning trials); however operational difficulties caused complete mortalities in the larval stage (and difficulties in conditioning broodstock). Subsequently, another facility had to be contracted to conduct the work. The facility used to produce Akoya spat (juveniles) for this project was the Blue Lagoon Pearl hatchery, principally located near Monkey Mia, in Shark Bay (WA).

4.4.2 Materials and Methods

The facilities at Blue Lagoon Pearl hatchery included a large, aluminium, floating barge with onboard generator, pumps, air conditioners, larvae tanks, spawning table and laboratory, located on moorings some 1 km offshore. Water for the hatchery was drawn by suction pump through an intake located adjacent to the barge. Micro-algae was cultured at an airconditioned on-shore building with autoclave located near Denham (Shark Bay) and transported daily to the barge as required. Culture temperature for the algae was 20°C with carboy algae fed after 6-9 days of culture. The water supply for algal culture was filtered to 0.2µm after it was drawn from Denham Lagoon. The salinity was reduced (by adding clean freshwater) and it was then autoclaved. For the planned hatchery spat production run at Blue Lagoon Pearls in Shark Bay, 100 Akoya broodstock were selected from adults held on various farms at the Abrolhos Islands and flown to the hatchery on 4 December 2007.

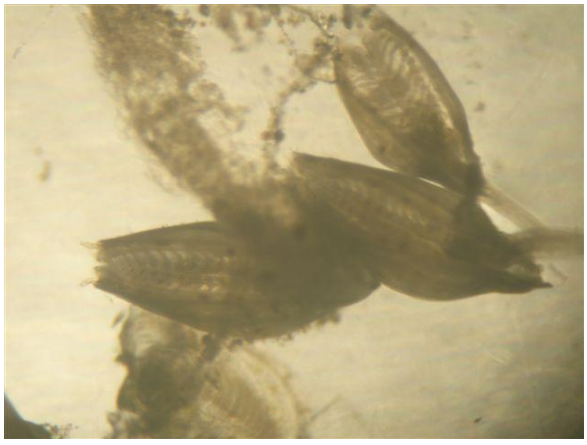
4.4.3 Results

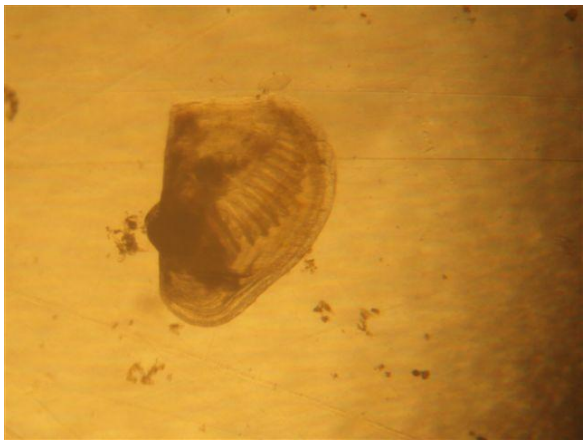
4.4.3.1 Spawning

Upon arrival at the hatchery, the selected (host) broodstock were cleaned and inspected briefly so that injured or stressed animals could be removed. As a result of this inspection only 80 oysters were subsequently placed on the spawning table for inclusion in the spawning attempts. Spawning attempts commenced at about 1300 hours on 4 December 2007 with flow through water connected to the spawning table. Some 40 hours later, on 6 December, between 0700 and 0800 hours, a significant spawning event occurred. As a result, some 160 million fertilized eggs were obtained (Day 0) and these were poured through a 105µm screen to remove spawning debris and then poured into larvae tanks for culture.

4.4.3.2 Larval Culture

On Day 1 the water in the tank was dropped and all material <37µm was discarded, with some 20 million (20 x 10^6) D larvae retained. Overall larval survival and growth is shown in Figure 3. Apart from the significant losses from Day 0 to Day 1, survival was satisfactory up to the pedi-veliger stage. Larvae were fed a mixture of *Pavlova lutheri* and *T. Isochrysis* for Days 1 - 6, then *Chaetoceros calcitrans* was added to the feed mixture on Days 7 - 22. From Day 23 - 42 *Chaetoceros muelleri* was also added to the diet.





Figures 1 and 2 - Hatchery produced Akoya oysters - settling.

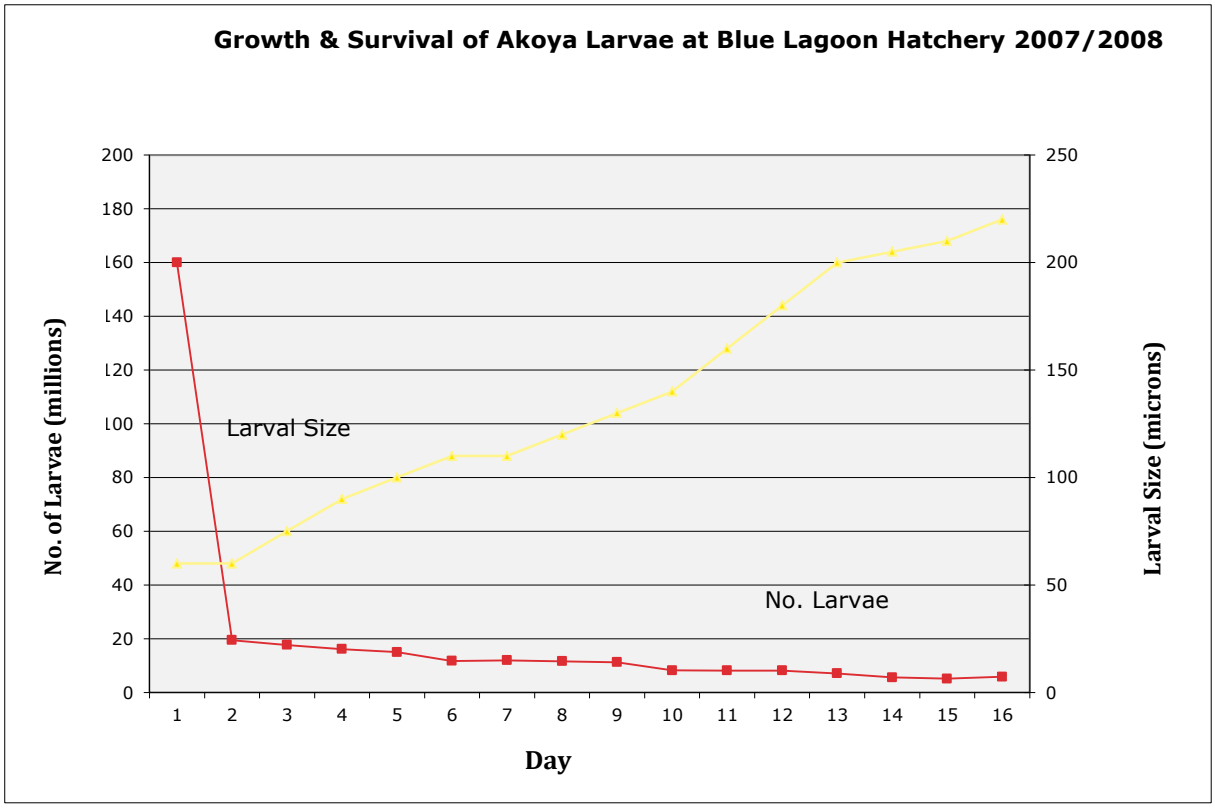


Figure 3 - Growth and Survival of Akoya larvae at Blue Lagoon Pearl Hatchery 2007/2008.

4.4.3.3 Settlement

Larvae grew well and settled after 15 days at 28°C and 36 ppt salinity. Approximately 4 million ready to set larvae were placed into Tank 1 (2,000 L) with 25 x 10m sections of power loop rope cable tied to separate milk crates while another 2 million larvae were placed into Tank 2 (2,000 L) with 15 x 10m sections of loose power loop rope. Tank 1 was fed 80-100 L of cultured algae per day in 3 separate feeds. Tank 2 was fed similar amounts of algae until Day 23 when algal supplies became insufficient to maintain the feed rate. After this, Tank 2 was only provided food via flow-through water passing through the tank for 8 hours per day. Spat was an average of about 700µm (shell height-DVM) on 5 January 2008 (Day 30).

4.4.3.4 Transfer to Farm Sites

Spat averaged 1.42mm on 17 January 2008 (Day 42); the day spat were transferred from Shark Bay to Rat Island at the Abrolhos Islands by plane. An estimated 500,000 spat were transferred on this day. On the day of transfer, air temperature was around 23.5°C. Water temperature at the hatchery in Shark Bay was 21°C when spat were removed and packed into damp hessian at 0600 hours.

In sealed polystyrene boxes, spat were transported by boat and car to the Denham Airport where a small plane flew them at a maximum altitude of 6,500 feet to Rat Island at the Abrolhos Islands (Easter Group). The polystyrene boxes were immediately unloaded and transferred to the vessel '*Cochon'* which ferried them to the Abrolhos Pearls barge moored at Site 1.

Random sections of rope were sampled to ascertain spat numbers and size range. Rope was then cut into 1m lengths and attached to panels and then into bags and cages, which were subsequently hung in the water. Various replicates of the various culture systems were established such that sufficient spat were organised for each of the 5 research sites. Spat were then deployed to Sites 1 and 2 before the remaining spat were transferred to the vessel '*Business Class*' for transport to the Southern Group of Islands. Spat were subsequently deployed at Sites 3, 4 and 5 the same afternoon. The total time spat were effectively out of water was 7.5 to 11.5 hours.

Figures 4 and 5 - Hatchery produced Akoya oysters being sorted from initial culture cages.

4.4.4 Spat Culture and Growout of Oysters

4.4.1.1 Culture System

As mentioned above, once spat arrived at the Abrolhos Islands, they were attached to panels and placed into a series of culture bags and cages. The culture bags were 92 x 54 cm; the panels 88 x 54 cm and the culture cages dimensions 92 L x 58 W x 20 H cm, with a steel mesh frame and black mesh lining. These cages were the conventional pre-operative conditioning cages used by the WA industry, and discussed in the following section.

Figure 6 - Initial fine mesh pearl nets.

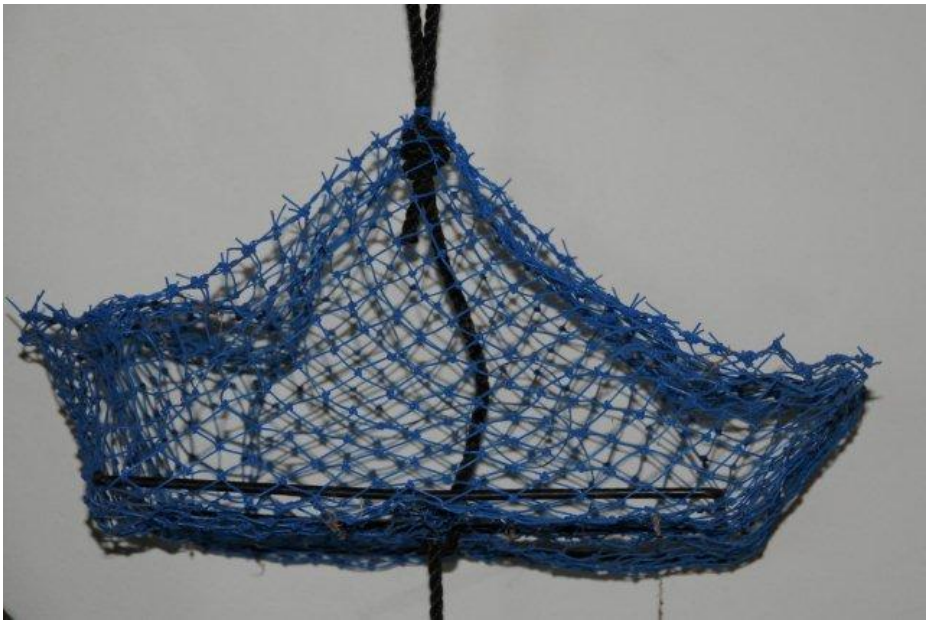


Figure 7 - Intermediate large mesh pearl nets.

The amount of spat established for the culture trial was 13,932 per site or a total of 69,660 spat as shown in Table 1 over. Once the 'Research' spat was organised, the remaining spat was placed into various culture cages and transferred to Sites 1, 3 and 4 at the same time, providing a ready source of additional oysters should they be required.

Table 1. No. of Akoya spat placed at each site in cages and bags (17.01.08).

SITE	No. Spat in Cages	No. Spat in Bags	No. Spat at Each Site
1	3 x 3,096 = 9,288	3 x 1,548 = 4,644	13,932
2	3 x 3,096 = 9,288	3 x 1,548 = 4,644	13,932
3	3 x 3,096 = 9,288	3 x 1,548 = 4,644	13,932
4	3 x 3,096 = 9,288	3 x 1,548 = 4,644	13,932
5	3 x 3,096 = 9,288	3 x 1,548 = 4,644	13,932
TOTAL	**46,440**	**23,220**	**69,660**

As the spat grew they were placed in larger mesh culture cages known as pearl nets, commencing in April 2008. Fine mesh (5mm) pearl nets were initially used with 350 spat per net placed in each net at each site with 6 nets used per site. Stocking densities were gradually reduced (down to 50/net) as the oysters grew and oysters were subsequently spread into more identical pearl nets. From March 2009, oyster juveniles were placed into larger mesh (20mm) pearl nets at 40 oysters per net until the completion of the growout stage in May 2010.

4.4.1.2 Growth

A sample of oysters was collected for growth monitoring every month in line with environmental monitoring trips. Whole wet weight and shell dimensions were collected and recorded along with a photographic record of each group of shells from each site. This data was collated to form growth and survival information for oysters grown at each of the sites over a period of 2.4 years from 17 January 2008 until 8 May 2010; when oysters reached an age of 884 days from spawning and fertilization. At that stage (Trip 29, on 8 May 2010), size monitoring was discontinued.

At the beginning of the trial the oysters were allocated to each site from the same pool, therefore the sites began with similar size distributions. The first measurement of the oysters was conducted in April (Trip 5), an ANOVA conducted on this data revealed significant differences between the sites ($p<0.001$ for APM and DVM). This is illustrated in Figure 8. Sites 3 and 4 form the smallest size group; Sites 1 and 2 form another group with larger oysters and Site 5 has the largest.

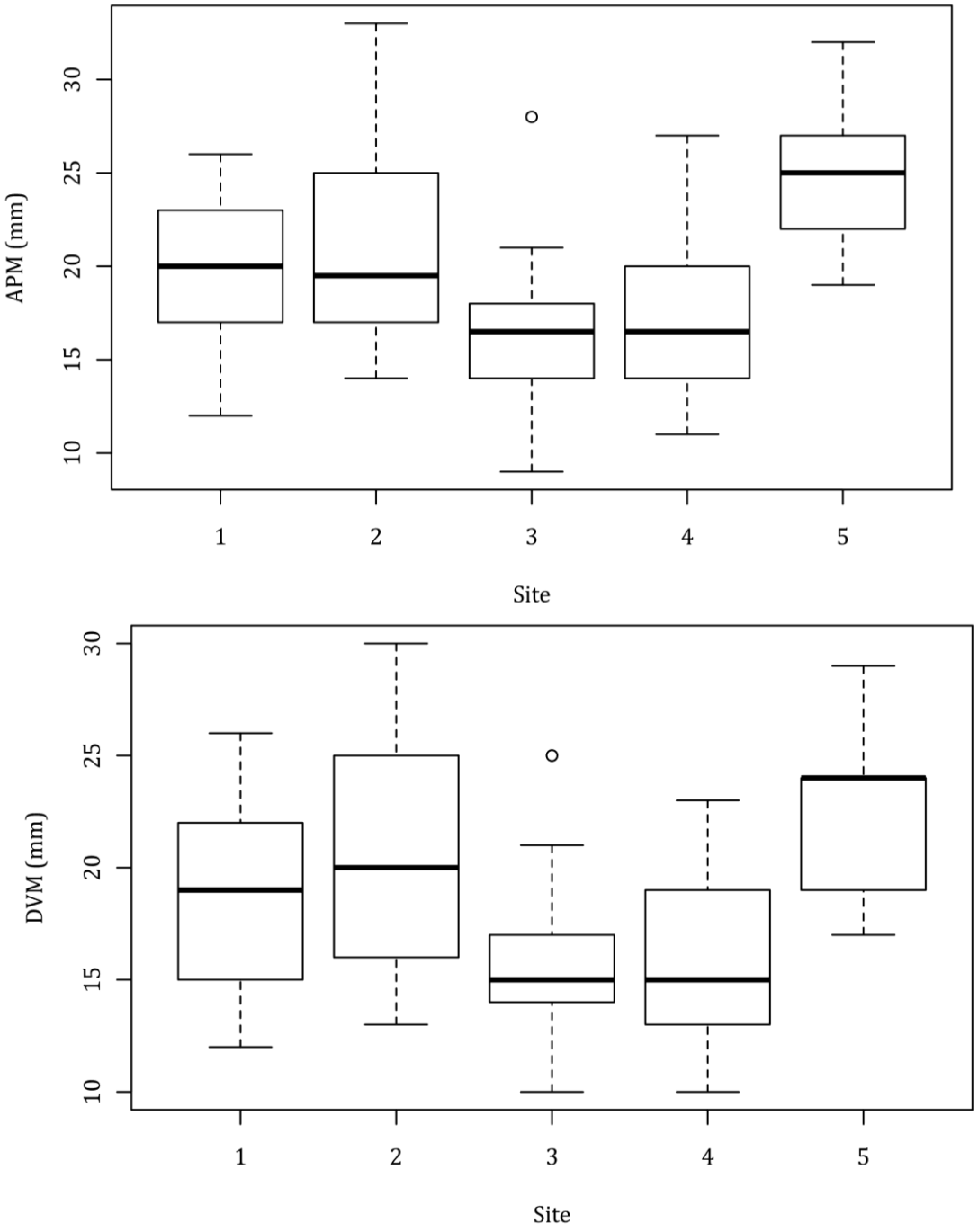


Figure 8 - Box plots of APM (top panel) and DVM (bottom panel) as sampled on Trip 5.

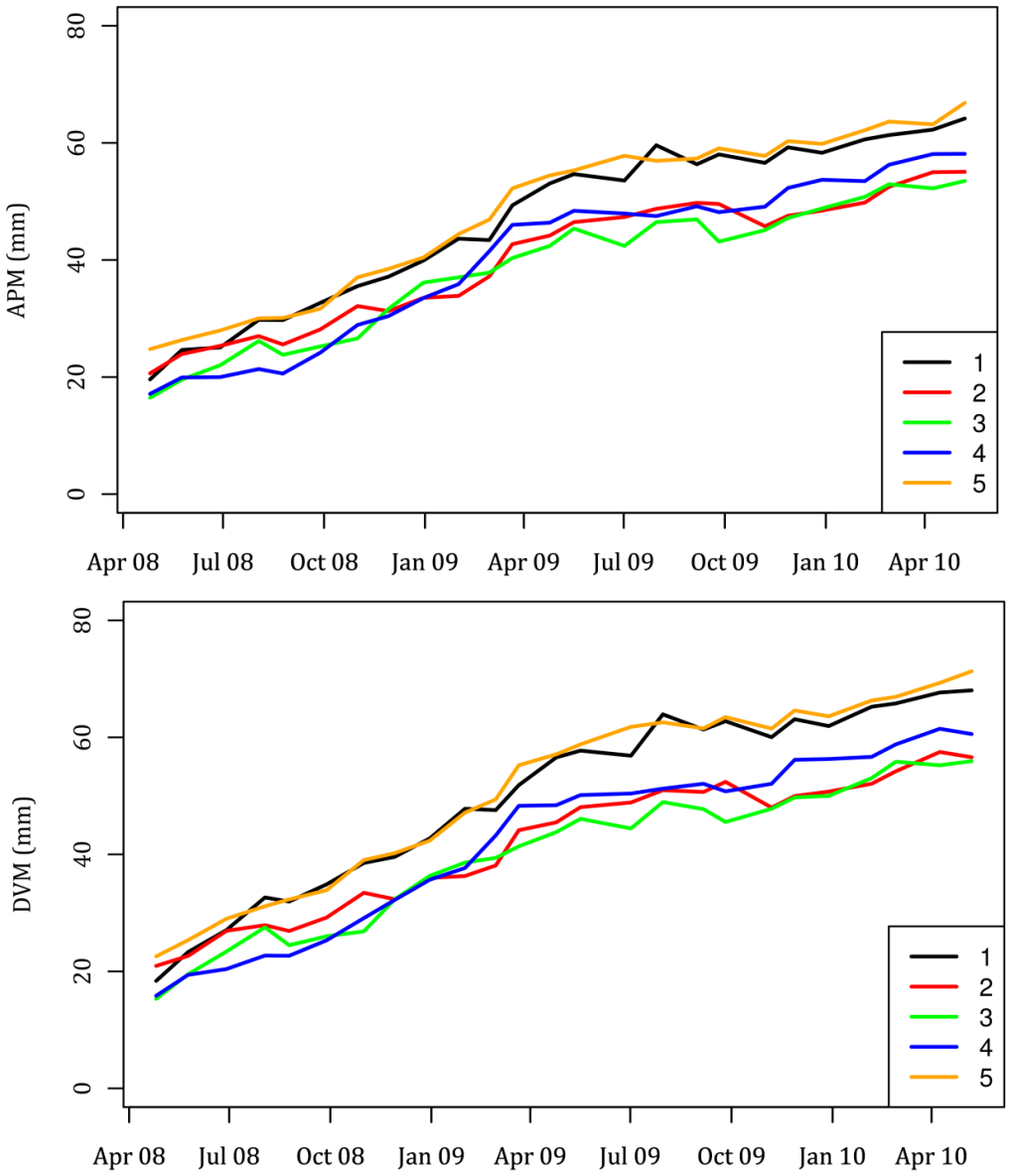


Figure 9 - Growth throughout the study period as measured in APM (top panel) and DVM (bottom panel).

Figure 9 shows growth through time. The points on this figure were obtained by calculating the median APM or DVM at each site for each sampling trip. There is a clear difference between sites, which is consistent over both measurements. From Figure 9 it is apparent that some of the differences in growth rates observed on the first monitoring trip changed over time.

To formalise this a GLM* was developed. First the data was de-trended by subtracting the overall mean from each sampling trip. The de-trended size of a oyster at site i, sampled at time t (days since the start of this study) is denoted by Di(t), this was then fitted with the following model:

$$D_i(t)=a_i+b_i t.$$

*GLM – Generalised Linear Model (GLM; see Dobson and Barnett 2008 for an introduction).

The first coefficient, ai, indicates whether the oysters at site i were consistently larger or smaller than the overall mean. The second coefficient, bi, indicates whether oysters at site i grew faster or slower than oysters elsewhere (after the first monitoring trip). Figure 10 shows the fitted model. A seasonal trend is clearly evident. A model incorporating several variations of temperature and degree days was attempted, with little success. The relationship between growth and temperature must be explored further to provide a useful model.

Coefficient estimates for the model are shown in Table 2. This indicates that Sites 1, 4 and 5 had a higher growth rate after the first monitoring trip. The same model was also applied without de-trending the data, this provides coefficients that are easier to interpret (see Table 2). This model indicates that initial growth varied by 8mm and growth rates after the first monitoring trip by 0.013mm/day.

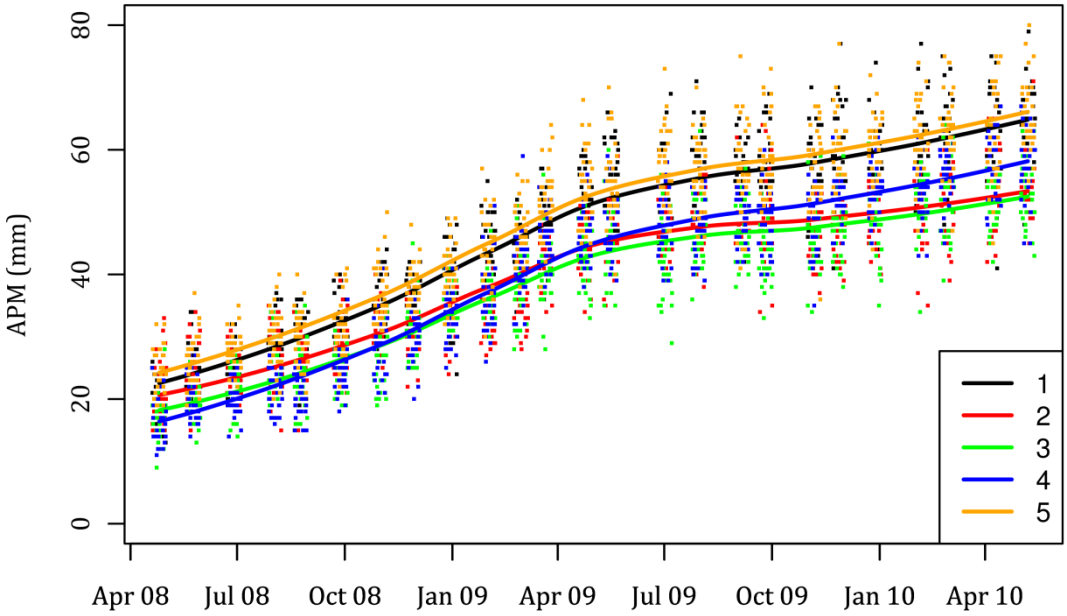


Figure 10 - The lines show the model fit to APM. The points show actual measurements, these are slightly randomly spread out (jittered) to reduce overlap.

Table 2 - Model coefficients.

	De-trended Model		Standard Model	
	a_i (size deviation)	b_i (growth deviation)	Initial Size	Growth rate (mm/day)
Site 1	2.1	0.0048	25	0.059
Site 2	0.2	-0.0080	23	0.046
Site 3	-2.3	-0.0059	20	0.049
Site 4	-4.1	0.0042	19	0.059
Site 5	3.7	0.0043	27	0.059

Model coefficients for the de-trended APM model are shown on the left. These are interpreted as differences from average size and growth rate. Model coefficients for non-de-trended APM data have a straightforward interpretation as the initial size (in April 2008) and the subsequent growth rate (last column).

4.4.1.3 Survival

The number of dead animals present at each sample was also documented for the same monitoring period as growth (below). Due to the necessary nature of the sampling, overall mortalities (no. dead) were calculated for each site for the duration of the oyster growout period, not on a monthly basis.

Table 3 - No. of Akoya spat in culture at each site.

Spat at Each Site	No. Spat	Site	Growth			No. Dead (Morts)	Type & No Units	Total No. Spat at Each Site
Period	Per Unit		Height (DVM)	Width (APM)	Thick (WD)			
Jan 08- Apr 08 Spawning – Trip 5	3,096 1,548	S 1	1.42				Cages x 3 Bags x 3	9,288 + 4,644 = 13,932
		S 2	1.42					
		S 3	1.42					
		S 4	1.42					
		S 5	1.42					
Apr 08- July 08 Trip 5- Trip 8	350	S 1	19.60	18.37	5.67		Pearl Nets x 6 5mm Mesh	6 x 350 = 2,100
		S 2	20.63	20.93	4.87			
		S 3	16.47	15.30	3.57			
		S 4	17.13	15.83	3.67			
		S 5	24.77	22.57	7.67			
Aug -08- Nov 08 Trip 8 & 12	110 Approx	S 1	29.77	32.63	12.47	30	Pearl Nets x 18 5mm Mesh	17x 110 =1980
		S 2	27.00	27.90	8.97	24		18x 110 =1980
		S 3	26.17	27.53	7.80	31		16x 110 =1760
		S 4	21.37	22.70	6.47	26		17x 110 =1870
		S 5	30.03	31.13	11.20	17		17x 110 =1870

Spat at Each Site	No. Spat	Site	Growth			No. Dead (Morts)	Type & No Units	Total No. Spat at Each Site
Period	Per Unit		Height (DVM)	Width (APM)	Thick (WD)			
Nov 08-Feb 09 Trip 12 & 15	50 Approx	S 1	35.53	38.53	15.50	16	Pearl Nets x 30 5mm Mesh	28 nets x 50= 1400
		S 2	32.13	33.43	11.47	42		36 nets x 50= 1800
		S 3	26.60	26.83	8.37	26		30 nets x 50= 1500
		S 4	28.90	29.10	9.87	27		34 nets x 50= 1700
		S 5	37.03	39.03	15.77	41		30 nets x 50= 1500
Mar 09-Apr 10	40	S 1	43.40	47.57	19.27	7	Pearl Nets x 30 20mm Mesh	31 nets x 40= 1240
		S 2	37.20	38.10	14.03	33		38 nets x 40= 1520
		S 3	37.83	39.40	14.07	15		41 nets x 40= 1640
		S 4	41.50	43.23	14.87	17		40 nets x 40= 1600
		S 5	46.90	49.40	20.40	12		34 nets x 40= 1320
Apr 10	30	S 1	62.27	67.67	26.13	37	Pearl Nets x 20 20mm Mesh	Total Shell 835
		S 2	55.00	57.50	20.57	57		Total Shell 772
		S 3	52.23	55.23	18.93	97		Total Shell 790
		S 4	58.10	61.47	21.47	35		Total Shell 930
		S 5	63.17	69.31	27.59	35		Total Shell 840
8 May 10	20	S 1	64.17	68.03	26.07	Shell not graded, shell growth trial completed		
		S 2	55.07	56.60	20.13			
		S 3	53.50	55.93	19.30			
		S 4	58.13	60.57	21.57			
		S 5	66.83	71.30	27.83			

Table 4 - Akoya oyster size at 8 May 2010 - 884 days old.

Site	Height (DVM)	Width (APM)	Thick (WD)
1	64.17	68.03	26.07
2	55.07	56.60	20.13
3	53.50	55.93	19.30
4	58.13	60.57	21.57
5	66.83	71.30	27.83

Table 5 - Total mortality at each site as at April 2010.

Site	Initial number of oysters April 08	Number of oysters removed for growth samples	Number of oysters remaining after sampling	Number of oysters remaining after sampling	Survival %
1	13,932	750	1350	835	62
2	13,932	750	1350	772	57
3	13,932	750	1350	790	59
4	13,932	750	1350	930	69
5	13,932	750	1350	840	62

4.4.5 Discussion

During the initial high-growth period oyster growth varied by 8mm between sites. Site 5 had the highest growth followed by Sites 1 and 2 and then 3 and 4. Subsequent to this initial period the growth rates varied differently between the sites. Growth rates at Sites 1, 4 and 5 were approximately 0.01mm/day higher (APM growth) – a 20% difference.

Without replication it is unclear whether the initial differences were due to chance conditions or whether the oysters require different conditions during the initial high growth phase relative to the remaining growout period. For example, high levels of fine silt, which may be present in some sites, is known to detrimentally affect growth rates in young oysters. Additionally, it is possible that low spat densities on some 1m sections of rope from the hatchery allowed spat to grow much faster initially than spat present at higher densities.

Sites 1 and 5 provided the highest initial and long term growth. In May 2009 oysters at these sites had reached a size that was only reached at Sites 2 and 3 towards the end of the experiment in May 2010. Site 4 had the lowest initial growth and similar long term growth as Sites 1 and 5. If the initial low growth was due to chance conditions that are atypical, then it could be a site of similar quality to Sites 1 and 5. Sites 1, 4 and 5 also had the highest survival rates. In summary:

- Sites 1 and 5 provided the highest growth and high survival rates.
- Site 4 provided high long term growth rates and the highest survival rates. Relocating oysters here after an initial period at Sites 1 or 5 may be beneficial. It may also be possible that the initial low growth period was due to chance in which case relocation would be unnecessary.
- Sites 2 and 3 had the lowest initial and long term growth rates and lowest survival.
- The difference between the sites amounted to almost a year of growth time.

4.5.1 Pearl Oysters – Pre-Operative Conditioning

4.5.1 Introduction

Conditioning of Akoya oysters prior to seeding (operations) is aimed at reducing the overall physical condition of each oyster. This process serves to increase the success rate of the seeding process and reduce rejection rates (of the nucleus). As a result of conditioning, the oyster is easier to open and peg (weaker adductor muscle), the byssus is absent or reduced in size, the exterior of the gonad is easier to cut and technicians may be able to use larger nuclei. The conditioning process, which incorporates high-density stocking rates, actually reduces the water flow and hence feed, to individual oysters, in small culture cages/baskets. It causes the oyster to use up glycogen reserves and resorb gonad material, but, it is important for these processes to occur without causing the death of the animal, excessive stress or disease outbreaks. Hence the attributes of the conditioning site and the holding method are important. Additionally, it is highly likely that good sites for oyster culture may not be good pre-operative conditioning sites.

The overall aim was to determine optimal conditioning protocols for WA Akoya pearl oysters prior to seeding. These conditioning trials assessed the importance of density, depth, duration, cage type, site location and byssal removal on the soft tissue condition of the oysters. It should be noted that of necessity, conditioning of oysters for the main seeding trial (discussed elsewhere), which was conducted in September 2009, was a standardised process uniformly carried out for the entire group of oysters involved. That trial was focused on examining the factors affecting seeding success, not the previous conditioning process. In the current trial, pre-operative conditioning is the focus and seeding is assumed to be the standardised process that follows; and is the measure of the success of the conditioning technique used.

4.5.2 Materials & Methods

This experiment utilised oysters cultured at Site 1 (Rat Island) to provide spat for conditioning prior to seeding. Spat were at least 50mm in shell height (DVM). Approximately 16,000 oysters stocked in pearl nets and panels were collected and transferred damp, by boat, to sorting/seeding facilities near Site 3 (Coronation Island) on 21 March 2010. The pearl nets and panels were emptied, oysters cleaned and grouped into the various amounts required to set up each of the conditioning trials; an action that may have stimulated oysters to spawn if they had fully developed gonads. Ad-hoc spawning of oysters as a result of the handling is seen as beneficial to the conditioning process, in that gonadal material is significantly reduced.

Two types of culture equipment were utilised; a standard black, conditioning basket (B) as used in the NSW Akoya industry, and the larger black cages used in the WA industry (P). The standard unit was a rigid black plastic basket containing 60 oysters occupying approximately 50% of the available volume. The standard NSW basket (B) was 26 x 15 x 34.5 cm (Length-L x Width-W x Height-H) at the base and 31 L x 15 W x 38.5 H cm at the top rim. The available surface area (SA) was therefore 0.36 m^2 and the average volume of the basket was about 15.7 litres. In comparison the large basket (P) had dimensions

of 92 L x 58 W x 20 H cm with an available SA of 1.67 m^2 and a total volume of 106.7 L which was divided into 2 units of approximately 53.3 litres each, for the conditioning trial. Hence each unit of the large basket (P) had a volume some 3.4 x more than the standard basket (B) and 4.6 x the SA when divided horizontally (top-bottom). In comparison, each unit of the large basket (P) divided vertically (left-right) only had a surface area 2.3 x more than the standard basket (B) while the volume was still 3.4 x more.

Figure 1 - Standard basket (B).

Figure 2 - Standard basket (B) with lid.

Figure 3 - Large basket (P) showing vertical (L-R) separation internally.

Figure 4 - Large basket (P), with horizontal separation (T-B) internally.

Within site variations were assessed using standard baskets located at surface and deep-water positions holding a similar number of oysters. Additionally, a density effect was assessed using standard baskets located at 2.0m deep. Cages of similar sized oysters were filled with high density (90 oysters), standard density (60 oysters) and low density (45 oysters).

Equipment was set up to test between site variations using the standard basket (B) located on long-lines at approximately 2.0m deep adjacent to the Hobo Data Loggers. The sites used to identify whether any between site variations existed, were Site 3 and Site 4.

At the time of sorting out juvenile oysters for the Pre-Operative Conditioning Trial, and just prior to the commencement of the Trial, the byssal thread of every oyster was cut. Subsequently, byssal threads on the oysters were thereafter cut on an approximately weekly basis except for two trial groups where they were cut either fortnightly or only the once at the start of the trial.

Oysters were stocked into standard conditioning baskets at the allocated amounts and then tied up in their groups of 3 baskets (except for the two large cages used by industry at the Abrolhos Islands) and held on a floating barge until they were deployed to their culture site for the trial. Two different versions of the large cage were used for testing. One was divided horizontally (top-bottom; T-B) using an existing culture panel into two groups of 200 oysters while the second one was divided vertically (left-right; L-R) using black mesh, into two groups of 200 oysters. The structure of the various trials at Site 3 was replicated for Site 4 and the conditioning protocol subsequently followed for each group is shown in Table 3 following.

All oysters to be conditioned at Site 3 were transferred by boat to the culture longline on 22 March 2010, referred to as Day 0 for the trial at that site. Oysters to be conditioned at Site 4 were transferred by boat, to the designated culture longline on March 25, Day 0 for that site. All handling activities for the oysters were related to the number of days from the start of conditioning at each site.

A regular routine of byssus cutting/breaking was then conducted on a weekly basis, subject to weather. At the designated assessment time (5, 6 or 8 weeks, about 30 April, 5/6 May and 21 May respectively), oysters were transferred to the seeding room at Coronation Island and inspected by an experienced Japanese technician. A sample of 35 oysters was randomly selected from the entire group of oysters subjected to each different treatment. Oysters were then individually pegged and inspected by the technician at an operating cubicle. They were allocated a gonad score and byssal thread score based on the Photo Series scale developed for Akoya assessment (see Figures 5 and 6 and Appendix 3). The technician then made an overall condition judgment based on whether the oyster was suitable to be seeded or not. If seeded, the nuclei size was recorded along with any other comments. Each oyster was given a sequential number from 1 - 35 and then the live wet weight and shell dimensions were recorded. If the oyster was not in suitable condition to be seeded (reject), then a reason for this decision was recorded. These reasons were deemed as too much gonad material, too many byssal threads, deformed animal/gonad or damaged shell. If one lot of 35 oysters was found to be not suitable for seeding then the remaining oysters (from the group of oysters held in 3 baskets) were placed into just two baskets and the group were returned for further conditioning at Site 3. For the groups of oysters that were assessed as being in suitable condition, all the remaining oysters in the group were seeded and placed in panels as well. All oysters assessed were then placed in tagged panels in the same sequential order as the numbering system (including non-seeded oysters) and each panel returned to Site 3. Tag details also recorded what treatment the oysters had

experienced as part of the conditioning process. This was conducted to allow assessment of seeding success at a later date, which would also confirm the accuracy of conditioning assessments. A total count of mortalities from each different trial was recorded at the completion of conditioning (at seeding time) for each site to collate overall mortalities.

A visual assessment of mortalities was conducted for each tagged group on 1 October 2010, at the second cleaning of the shell, approximately 5 months after the seeding of conditioned oysters was completed. The location of each dead shell in the panel and the panel number was recorded for all mortalities. Dead shells were left in the panel until the final pearl harvest.

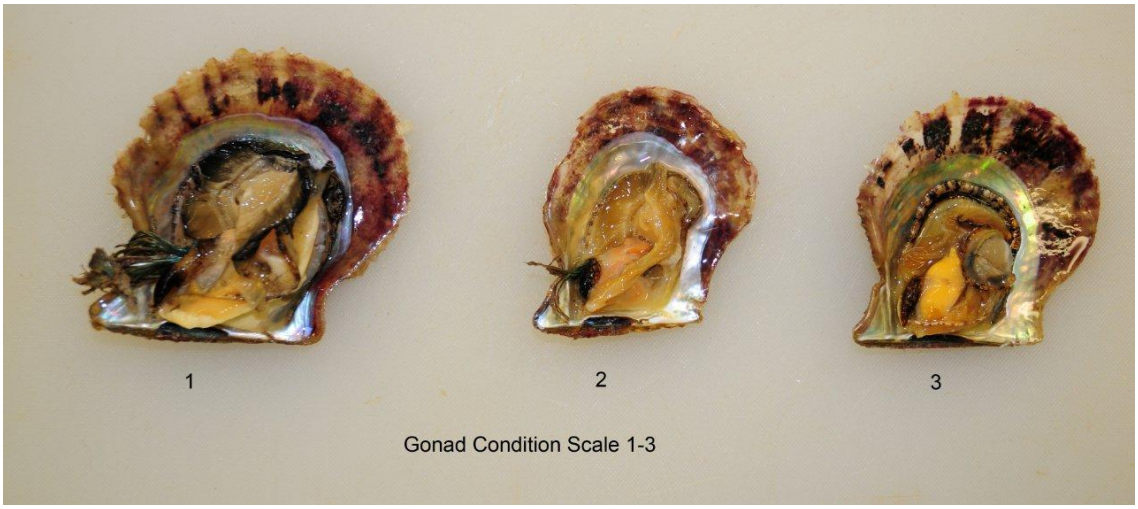


Figure 5 - Gonad Condition Photo Series 1-3.

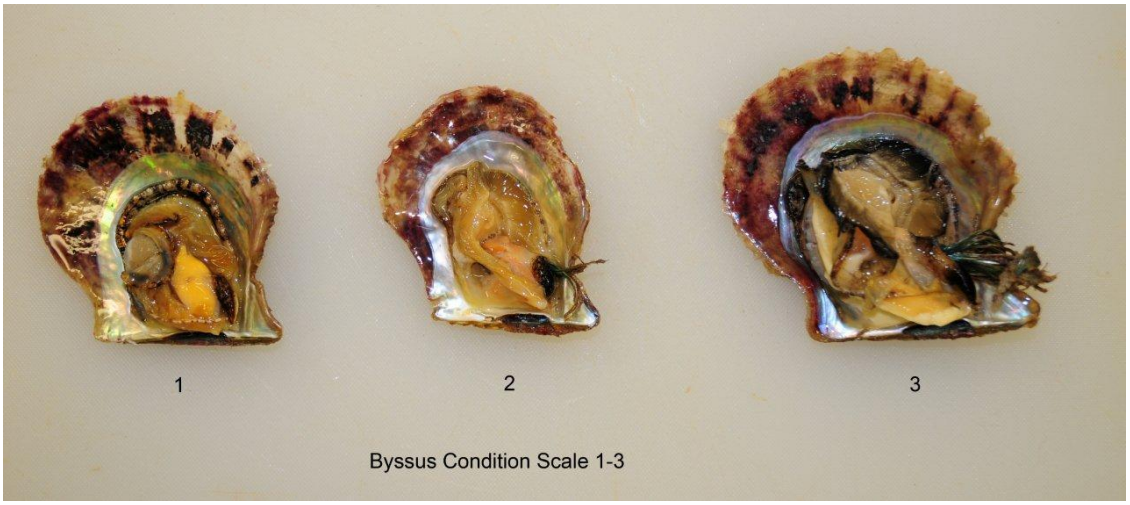


Figure 6 - Byssus Condition Photo Series 1-3.

Table 1. Pre-Operative Conditioning Trial Protocol - Site 3 & 4.

Group	Panel	Type of Basket	No. of Shell	Depth (m)	Cond. Period (weeks)	Byssus Cut	Treatment Test
1	1	Small	60	2	6	weekly	Standard
	2	Small	60	2	6	weekly	Standard
	3	Small	60	2	6	weekly	Standard
2	4	Large	400	2	6	weekly	Cage Type,T-B
3	5	Large	400	2	6	weekly	Cage Type,L-R
4	6	Small	45	2	6	weekly	Low Density
	7	Small	45	2	6	weekly	Low Density
	8	Small	45	2	6	weekly	Low Density
5	9	Small	90	2	6	weekly	High Density
	10	Small	90	2	6	weekly	High Density
	11	Small	90	2	6	weekly	High Density
6	12	Small	60	6	6	weekly	Depth
	13	Small	60	6	6	weekly	Depth
	14	Small	60	6	6	weekly	Depth
7	15	Small	60	2	5	weekly	Short Period
	16	Small	60	2	5	weekly	Short Period
	17	Small	60	2	5	weekly	Short Period
8	18	Small	60	2	8	weekly	Long Period
	19	Small	60	2	8	weekly	Long Period
	20	Small	60	2	8	weekly	Long Period
9	21	Small	60	2	6	start	Byssus once only
	22	Small	60	2	6	start	Byssus once only
	23	Small	60	2	6	start	Byssus once only
10	24	Small	60	2	6	2 weekly	Byssus fortnightly
	25	Small	60	2	6	2 weekly	Byssus fortnightly
	26	Small	60	2	6	2 weekly	Byssus fortnightly

Table 2. Pre-Operative Conditioning Trial Treatment Schedule - Site 3.

Group	Type of Basket	No. of Shell	Depth (m)	Cond. Period (weeks)	Byssus Cut	Treatment Test	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
1	Small	180	2	6	w	Standard	D9	D19	D25	D31	D39	D42-ASS		
2	Lg T-B	400	2	6	w	Cage Type	D9	D19	D25	D31	D39	D44-ASS		
3	Lg L-R	400	2	6	w	Cage Type	D9	D19	D25	D31	D39	D44-ASS		
4	Small	135	2	6	w	Low Density	D9	D19	D25	D31	D39	D42-ASS		
5	Small	270	2	6	w	High Density	D9	D19	D25	D31	D39	D42-ASS		
6	Small	180	6	6	w	Depth	D9	D19	D25	D31	D39	D42-ASS		
7	Small	180	2	5	w	Short Period	D9	D19	D25	D31	D39-ASS			
8	Small	180	2	8	w	Long Period	D9	D19	D25	D31	D39	D41	D56	D60-AS
9	Small	180	2	6	w	Byssus once	D9	D19	D25	D31	D39	D42-AS		
10	Small	180	2	6	w	Byssus f	D9	D19	D25	D31	D39	D42-ASS		

w = weekly f = fortnightly

- Each Group consisted of 3 baskets tied one under the other except for the large basket which was only a single one.
- AS: means Assessment of Condition then Seeding of some of the 35 oysters.
- ASS: means Assessment of Condition and Seeding of the majority of the group as they were conditioned suitably.
- D9: means Day 9 from the start of conditioning period.

Table 3. Pre-Operative Conditioning Trial Treatment Schedule - Site 4.

Group	Type of Basket	No. of Shell	Depth (m)	Cond. Period (Weeks)	Byssus Cut	Treatment Test	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8
1	Small	180	2	6	weekly	Standard	D7	D17	D23	D27	D37	D42-AS		
2	Large	400	2	6	weekly	Cage Type	D7	D17	D23	D27	D37	D42-AS		
3	Large	400	2	6	weekly	Cage Type	D7	D17	D23	D27	D37	D42-AS		
4	Small	135	2	6	weekly	Low Density	D7	D17	D23	D27	D37	D42-AS		
5	Small	270	2	6	weekly	High Density	D7	D17	D23	D27	D37	D42-ASS		
6	Small	180	6	6	weekly	Depth	D7	D17	D23	D27	D37	D42-AS		
7	Small	180	2	5	weekly	Short Period	D7	D17	D23	D27	D36-AS			
8	Small	180	2	8	weekly	Long Period	D7	D17	D23	D27	D37	D42	D53	D57-AS
9	Small	180	2	6	start	Byssus once	D7	D17	D23	D27	D37	D42-AS		
10	Small	180	2	6	2 wks	Byssus fortnightly	D7	D17	D23	D27	D37	D42-AS		

- Each Group consisted of 3 baskets tied one under the other except for the large basket which was only a single one.
- AS: means Assessment of Condition then Seeding of some of the 35 oysters.
- ASS: means Assessment of Condition and seeding of the majority of the group as they were conditioned suitably.
- D7: means Day 7 from the start of conditioning period.

4.5.3 Results

Oysters were assessed for seeding in a group of 35 that they had been conditioned in. Data for each of the oysters assessed was collected and subsequently averages for each conditioning treatment and seeding results were calculated. These are shown in the summary tables following.

Mortalities were removed and recorded during the cutting of the byssus and at the end of the conditioning a total for each treatment was tallied and recorded.

Table 4. Conditioning Trial Assessment Averages at Site 3.

Group	Cond Period wks	Wet Wt g	Shell APM mm	Shell DVM mm	Shell Thick mm	Gonad Score 1-3	Bys Thr 1-3	% Seeded	Nucl Size bu	Reject Cause			
										Spawn	Byss	Def	No. Sh.D
1. St.	6	44.34	64.03	67.89	24.86	1.89	1.00	80.00	2.19	4	0	2	1
2. LgT-B	6	40.59	59.43	64.80	23.89	2.43	1.11	54.29	2.11	13	2	1	0
3. LgL-R	6	37.47	58.54	62.80	23.24	2.40	1.00	60.00	2.02	13	0	1	0
4. Low	6	43.15	63.71	67.37	24.31	2.11	1.11	74.29	2.19	7	2	0	0
5. High	6	40.99	62.03	65.54	24.06	1.60	1.00	88.57	2.11	4	0	0	0
6. Deep	6	46.30	61.60	67.77	24.91	1.63	1.00	88.57	2.24	3	0	1	0
7. Short	5	44.05	63.37	67.49	23.34	2.23	1.03	68.57	2.10	9	0	0	2
8 . Long	8	41.23	59.37	64.40	24.29	1.63	1.00	82.86	2.02	6	0	0	0
9. By-s	6	46.09	64.89	68.83	24.43	2.46	1.11	48.57	2.16	15	2	1	0
10.By-fn	6	34.56	56.23	60.29	23.34	2.17	1.00	71.43	2.00	14	0	1	0

- St.: Standard basket with 60 oysters, held at 2m depth
- LgT-B.: Large cage; 400 oysters, held at 2m depth, divided horizontally into 2 x 200 oysters
- LgL-R.: Large cage; 400 oysters, held at 2m depth, divided vertically into 2 x 200 oysters
- Low: Standard basket with 45 oysters - low density, held at 2m depth
- High: Standard basket with 90 oysters - high density, held at 2m depth
- Deep: Standard basket with 60 oysters with 4m dropper, held at 6m depth
- Short: Standard basket with 60 oysters, held at 2m for only 5 weeks
- Long: Standard basket with 60 oysters, held at 2m for 8 weeks
- By-s.: Standard basket, byssus cut at start of trial only
- By-fn.: Standard basket, byssus cut every fortnight

Reject Cause: Spawn - too much sperm or egg Byss - too much byssus
Def - deformed gonad No. Sh. D - damaged shell

Table 5. Mortalities during the pre-operative conditioning period.
The number of mortalities is shown along with the percentage. Significant differences from the standard trial (6 weeks) are indicated in bold.

	Number per Trial	Site 3 Mortalities		Site 4 Mortalities	
		Number	%	Number	%
Deep	60	8	13	5	8
High Density	90	13	14	12	13
Low Density	45	6	13	4	9
No Cut	60	5	8	4	7
2 Week Cut	60	6	10	4	7
Right - Left	400	**18**	**5**	**15**	**4**
Top - Bottom	400	**25**	**6**	**18**	**5**
5 Weeks	60	8	13	6	10
6 Weeks	60	8	13	6	10
8 Weeks	60	**12**	**20**	5	8
Total	1295	109	8	79	6

Table 5 shows the mortalities experienced during the pre-operative conditioning period. Mortalities varied between 4 and 20%. It is likely that the 20% mortality rate is not representative of the true rate for that trial (8 week conditioning) as the 8 week conditioning at the other size and a separate undocumented site did not increase.

The large cages significantly reduced mortality. Other effects are too small to provide conclusive answers. A guide to the variability of this data is provided by the fact that there are unexpected effects of the order of 3-4% (e.g. both no cut and fortnightly cuts reducing mortality). This prevents conclusions being made about the effect of the remaining trials on mortality.

Table 6. Number of mortalities post-seeding from the Pre-Operative Conditioning Trial as at 1 October 2010.

	No. of Mortalities Post-Seeding	
Panel	**Site 3**	**Site 4**
1. Standard/6 weeks	5	4
2. Large Basket/Top-Bottom	5	4
3. Large Basket/Left-Right	1	3
4. Low Density	6	2
5. High Density	4	3
6. Deep	5	6
7. Short Period/5 weeks	3	3
8. Long Period/8 weeks	2	3
9. Byssus-S/No cut	1	4
10. Byssus-fn cut	3	6
Total	**35**	**38**

The effect of the different conditioning techniques produced differing mortalities between the sites tested, but not for all treatments.

The number of mortalities post-seeding are low and the variation between experiment groups/techniques is low (the total range is between 1 and 6). In addition there are unexpected trends (e.g. in both sites, experiments with both shorter and longer conditioning periods had fewer mortalities than the reference experiment) which can only be attributed to the natural variability in mortality in the absence of any experimental techniques. The only conclusion to be drawn from this data is that no individual techniques caused significant increases in mortality or unusually low mortalities.

Table 7. Conditioning Trial Assessment Averages at Site 4.

Group	Cond Period wks	Wet Wt g	Shell APM mm	Shell DVM mm	Shell Thick mm	Gonad Score 1-3	Bys Thr 1-3	% Seeded	Nucl Size Bu	**RejectCause** Spawn	Byss	Def	No. Sh.D
1. St.	6	40.18	59.86	64.43	23.46	2.69	1.57	31.43	2.05	14	10	0	0
2. Lg T-B	6	41.72	61.46	67.51	23.11	2.43	1.06	57.14	2.12	14	1	0	0
3. Lg L-R	6	42.26	59.83	64.71	24.49	2.86	2.31	14.29	2.08	8	22	0	0
4. Low	6	39.11	56.86	62.80	23.37	2.86	1.51	14.29	2.02	23	7	0	0
5. High	6	40.25	59.91	64.31	23.83	2.23	1.06	71.43	2.12	9	1	0	0
6. Deep	6	39.40	60.11	64.57	23.34	2.74	1.29	25.71	2.17	19	5	2	0
7. Short	5	36.85	59.00	63.74	23.46	2.60	1.06	40.00	2.02	20	0	0	1
8. Long	8	40.56	58.09	78.57	24.14	2.34	1.23	51.43	2.12	12	4	1	0
9. By-s	6	38.46	58.71	61.66	23.94	2.77	1.34	20.00	2.10	21	6	1	0
10.By-fn	6	36.23	57.74	62.49	23.17	2.66	1.06	34.29	2.02	22	1	0	0

4.5.4 Analysis of Results

The model used to analyse seedability data is a probit linear regression. This is a linear regression that takes into account the binary response nature of this data (an oyster is either seedable or not). The linear regression gives a probit index for each experiment which relates to the probability of an oyster being seedable as illustrated in Figure 8. For example, an oyster from an experiment with a probit index of 0 has a 50% chance of being seedable, whereas an index of -1.1 indicates a 10% chance of being seedable.

The model requires a single experiment to be (arbitrarily) chosen as a reference. In this case Site 3 with a density of 60 in a small basket at 2m, weekly cutting, and a 6 week conditioning period was selected (the standard). All other experiments vary from this by a single factor and possibly the site.

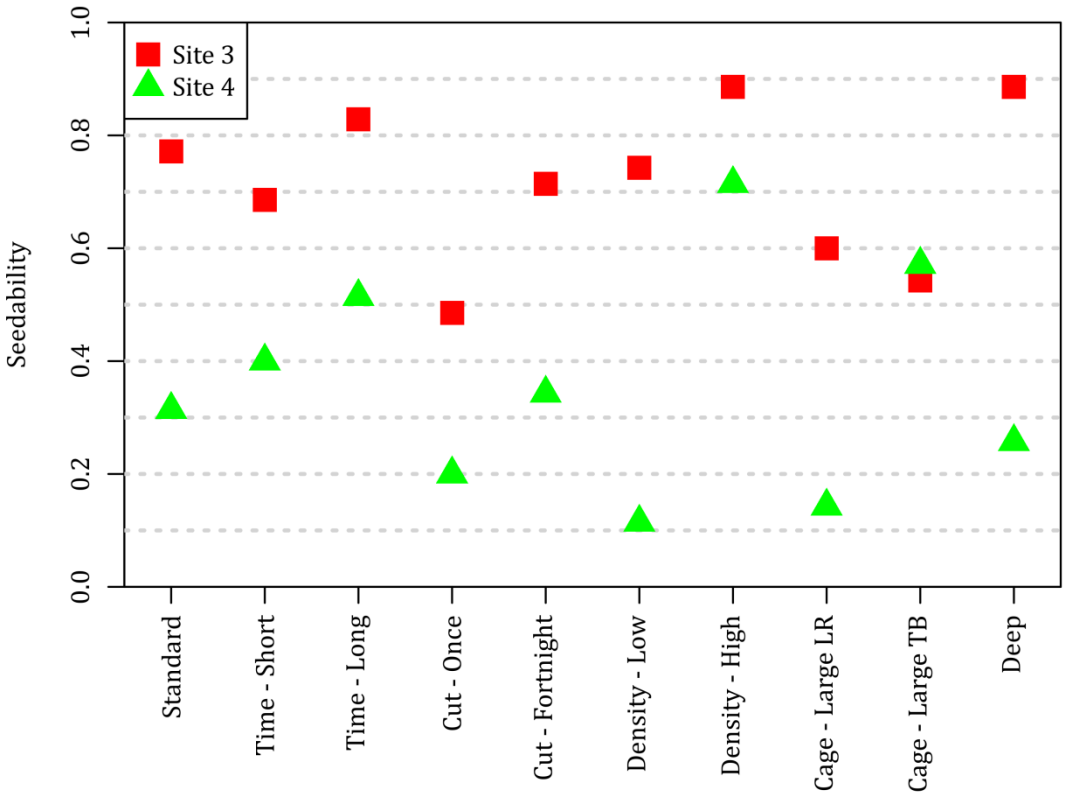


Figure 7 - Seedability for each experiment.

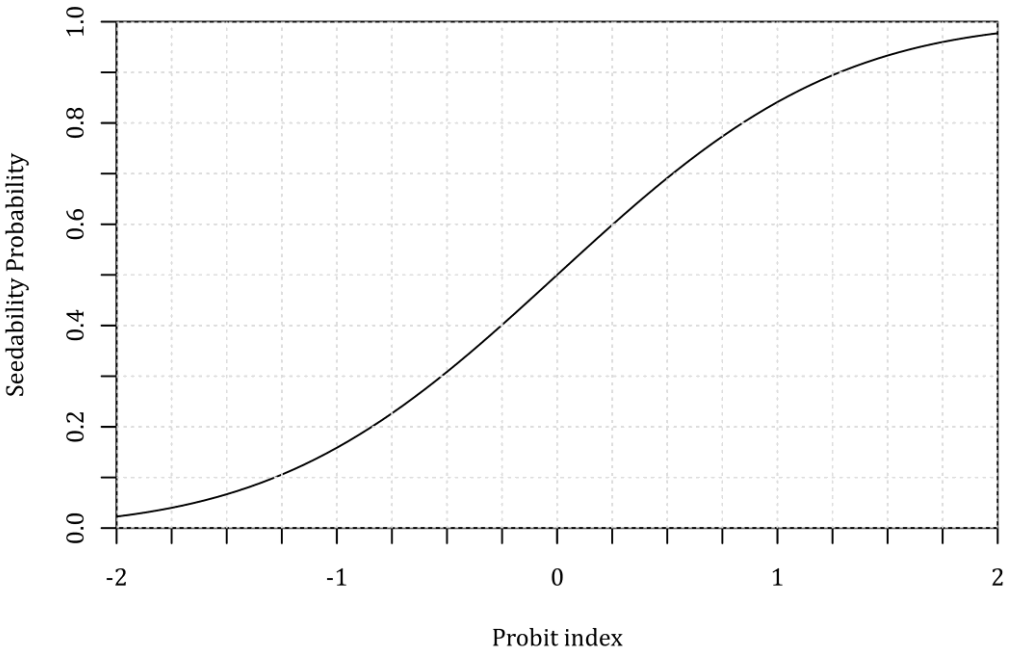


Figure 8 - The relationship between the probit index and the probability of an oyster with that index value being seedable. (Note this is a cumulative normal probability distribution with mean 0 and standard deviation of 1).

Table 8. Model Coefficients for Seedability. Significant coefficients indicated in bold.

	Coefficient	Significance
Reference	0.70	
Site: 4	-1.02	**0.000**
Cutting: Fortnightly	-0.11	0.530
Cutting: Once	-0.64	**0.000**
Conditioning: 5 weeks	-0.14	0.062
Conditioning: 8 weeks	0.27	0.062
Density: 45	-0.38	**0.000**
Density: 90	0.76	**0.000**
Site: 4 Basket: Large L-R	-0.45	0.059
Site: 4 Basket: Large T-B	-0.75	**0.007**
Site: 3 Basket: Large L-R	-0.59	**0.012**
Site: 3 Basket: Large T-B	0.50	**0.033**
Site: 3 Depth: 6m	0.51	0.088
Site: 4 Depth: 6m	-0.33	0.177

Table 8 shows the model parameters for a fit to an exhaustive model that included all experimental factors. The interaction between a factor and the site was considered for those factors that showed substantial variation across sites. This table indicates that the previously discussed reference experiment (standard) has a probit index of 0.7 which (referring to Figure 8) corresponds to a ~75% chance of seedability (which matches that seen in Figure 7 – the red dot on the far left). The remaining rows show how much the probit index is adjusted relative to the reference experiment for each variable. For example, the probit index for a 6m basket located at Site 4 is derived by taking the reference index (0.7) adding the site 4 index (-1.02) and adding the Site 4, 6m index (-0.33) giving a total of -0.65; a seeding probability of ~25%.

4.5.4.1 Sites

Seedability at Site 4 was substantially lower (-1.02 probit units; $p<0.001$) than at Site 3. This was the strongest and most significant effect.

4.5.4.2 Density

Across the two sites, increasing density had the strongest and most significant effect ($p<0.001$).

4.5.4.3 Conditioning

The conditioning period was weakly significant ($p=0.062$) with longer conditioning periods increasing seedability. The short conditioning experiment at Site 4 violated this trend (increasing seedability), however the overall test indicates that this is probably due to chance alone.

4.5.4.4 Cutting

There was no detectable difference between fortnightly and weekly cuts (p=0.53), however performing just a single cut at the start of the trial, decreased seedability substantially (p<0.001). These results were consistent across both sites.

4.5.4.5 Baskets

The large left-right basket (P, L-R) consistently decreased seedability across the sites (p=0.06 at Site 4 and p=0.01 at Site 3). The large top-bottom basket (P, T-B) decreased seedability at Site 3 and increased it similarly at Site 4 (respectively, p=0.01,p=0.03).

4.5.4.6 Depth

At Site 4, depth did not affect seedability (p=0.18) and at Site 3 it had a weak positive effect (p = 0.09). Combined, this indicates depth was not significant (p=0.88; not shown in table). However the different response to a top (surface)-bottom split at the sites may suggest that depth has a different effect at the sites and pooling depth is inappropriate.

Table 9. Summary of Significant Effects on Seedability.

	Density	Conditioning	Cutting Once	Basket L-R	Basket T-B	Depth
Site 4 (-)	+	+	-	-	+	-
Site 3 (+)	+	+	-	-	-	+?

(+ indicates positive effects, - indicates negative effects.)

4.5.4.7 Growth

Growth differed significantly between the sites (p<0.001). Site 3 had larger oysters with a mean difference of 2.4g, 2.2mm APM, 1.8mm DVM and 0.44mm WD.

4.5.4.8 Nucleus Size

The nucleus data shows the size of nuclei that was implanted into seedable oysters by the technicians at the completion of the Pre-Operative Conditioning Trial. The smallest nuclei used were 2.0bu (6.0mm) and the largest 2.5bu (7.6mm)[bu is a Japanese unit-equivalent in mm is shown]. The number of oysters seeded with the nuclei of various sizes is shown in Figure 9. The data is heavily skewed with over 70% of oysters seeded with 2.0 or 2.1bu nuclei. Figure 10 shows that the size of nuclei implanted is strongly correlated with the weight of the oyster.

Similar relationships hold for APM, DVM and WD, results for these are shown in more detail later.

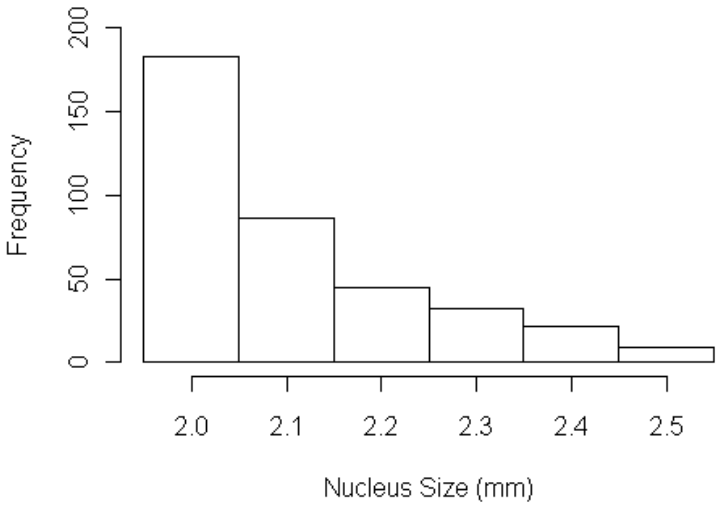


Figure 9 - The number of oysters with nuclei of each size that were seeded.

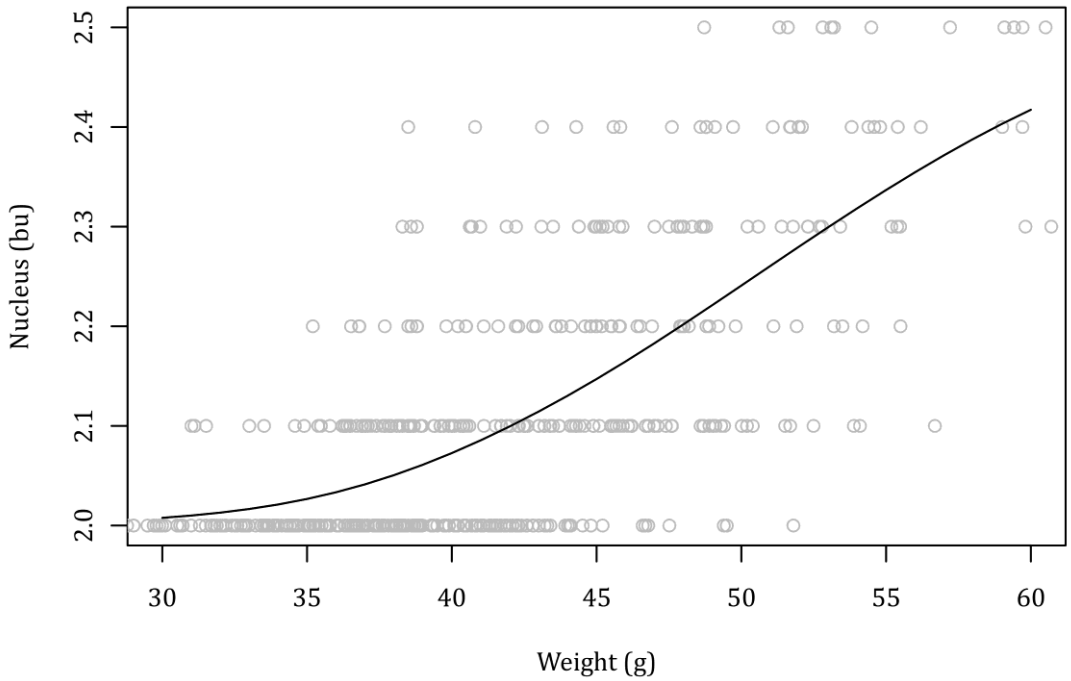


Figure 10 - Nucleus size as a function of oyster weight (each circle corresponds to a single oyster). The solid line indicates a model fit which will be discussed in more detail in the following chapter.

4.5.5 Discussion

The number (percentage) of oysters that could be seeded when they were assessed was the indicator for a successful conditioning technique. Overall, Site 3 produced a much higher seeding percentage of oysters and was therefore a better site to condition oysters pre-operatively; this may mean that the site is actually not as good for oyster growth as Site 4, although this aspect is considered elsewhere.

Overall, site selection had the single most significant effect on results. Increased density increases seedability, but the study has not determined a critical limit beyond which this may decrease (there was no significant increase in mortality). Single cutting is unlikely to be worth the cost saving, but fortnightly cutting produces the same results as weekly cutting.

Generally the small conditioning basket (B) produced better results, but the large conditioning basket (P) divided horizontally (top-bottom) may have a site dependent positive effect. Longer conditioning periods increased seedability, however the study did not determine a critical limit. The observed decrease in seedability due to the large cages exceeds the gain from the reduced mortality they provide.

It should be noted that experiments looking at interactions between these conditioning effects were not conducted. Therefore it is possible that applying a suite of measures (e.g. Site 3 high density, and long conditioning period) may result in increased mortality instead of further increasing seedability as the model developed here would suggest. Also, there was no temporal repetition of the trial, hence site refers to the environmental conditions experienced at the sites during this study, not the sites or typical conditions experienced therein.

Seeding of oysters at the completion of the trial showed that heavier oysters (generally larger) were seeded with larger nuclei, which is to be expected, given that technicians try to maximise the individual value of each pearl.

This experiment has shown that the success of pre-operative conditioning of oysters is likely to vary between sites especially where those sites may be subjected to differing environmental conditions. Evaluation of the variation in environmental conditions existing at each site may assist in explaining why the oysters conditioned at different rates. These aspects are considered elsewhere in the report.

4.6 Pearl Oysters – Seeding and Pearl Harvest

4.6.1 Introduction

The majority of Australia's pearl production (South Sea Pearls - SSP) comes from *Pinctada maxima* which produces large pearls between 10 and 20mm in diameter. The market competition with Indonesia, which also produces SSP, has previously been considered as not threatening Australia's production due to Indonesia's poorer quality and yellowish coloured pearls and their usual size ranges. In the past it has been considered that Indonesia filled a convenient gap in world pearl production, i.e. 9-12mm. In general the pearl market was dominated by Japanese Akoya pearls up to 9mm in size, Indonesian pearls at 9-12mm and Australian pearls from 12mm and larger (Assael, 1993). More recently, this market has undergone some minor changes according to Nick Paspaley, (Australia's largest producer); with Chinese Akoya pearls up to 6mm in size; Japanese Akoya pearls from 6-8mm; Indonesian SSP of 8-11mm and Australian SSP of 11mm upwards (Dietrich, 1995a). Mr Paspaley also commented that Australian pearlers do not want to, nor could they afford to produce an 8-10mm pearl (from *P. maxima*) even though there was a perceived 'vacuum' in the market for such pearls.

Gem quality pearls are initially sorted into 3 groups based upon whether they are unmarked, have one major blemish or more than one major blemish. These pearls are then further graded in relation to quality with the main considerations being:

1. Thickness of the Nacre
2. Lustre
3. Colour
4. Shape
5. Surface Perfection
6. Size

(Farn, 1986.)

In relation to South Sea Pearls (SSP) from *Pinctada maxima*, in particular, Australian SSP, the feature of nacre thickness is not assessed as all are expected to be much thicker than accepted minimums of 0.6 – 1.0mm. Thinner layers of nacre are generally associated with deficiencies in the other features displayed by pearls. Overall, pearls tend to be assessed by growers and then graded using a sequence of the quality criteria in the following order.

1. Lustre. This is the result of reflected and refracted light from the inner layers of nacre as well as from the surface; it cannot be measured quantitatively. Also known as 'orient'. The most beautiful lustre displays a rectangle-reflection of a light source and has the sharpest contour (Doumenge et al., 1991).

2. Size. The size of a pearl is the smallest diameter measured in millimetres. However the length of a necklace in the U. S. or the U. K. is measured in inches.

3. Shape. The most desirable and valuable high quality pearls are spherical and in general the rounder the pearl, the more valuable. However, other specific non-round shapes such as teardrop, pear, oval, button and then baroque are in demand but are worth less than round pearls. Near round but not perfectly round pearls, in a string, can be identified by rolling the pearls in a semi-circle whilst on a flat surface.

4. Surface Perfection. Any imperfections can also be identified whilst conducting rolling tests as above. Categories of imperfection are generally classified: Clean; Small spots: Spotted; Very Spotted.

5. Colour. The most popular colours are white and pink rose although there is a wide range of colours and shades including cream, grey, green, blue and pink. The richness and evenness of the colour are most important. Almost 100 % of Akoya pearls are bleached or dyed (Dietrich, 1995 b).

6. Thickness of the Nacre. Largely determined by the length of time the nucleus is in the host animal. Usually, the thicker the nacre, the higher the value, and the better the lustre. The Japanese Akoya pearl has a nacre of about 0.2mm thick whereas the South Sea Pearl has nacre about 3.5mm thick (Dietrich, 1995 b).

A range of aspects combine to determine when to harvest the pearl from its host oyster and they can vary year to year (O'Connor et al., 2003). The recommended period for harvest of Japanese Akoya pearls is 16 to 24 months (Shirai, 1970 in Kripa et al., 2007). Key aspects are the duration/time span of pearl culture since the seeding operation, the environmental conditions existing at the culture site throughout that period and the water temperature leading up to the harvest. In order to clarify these aspects, pearl harvesting was conducted over a period of months towards the end of the pearl culture period when water temperatures were relatively low, with samples being taken from each of the five sites. Other determining factors such as conditioning and the initial seeding operation have been considered earlier, but the overall impact of all factors affecting pearl production that have been assessed during this project, are considered later in the report. Of relevance to this trial is the supposition that, in general, nacre growth increases with higher water temperatures, but lustre and colour improve with cooler temperatures.

The production of nacre or deposition of layers of nacre is known to vary with environmental factors, particularly temperature. This trial was aimed at firstly collecting information on the range of nacre produced by Akoya pearl oysters at the Abrolhos Islands from the separate aspects of nacre quality, colour and lustre although naturally, inter-relationships exist between these three. The variability in nacre production and quality with season and over time was examined and a Photo Series of the various colours was developed to encompass the range encountered. The assessment of nacre and lustre are more difficult to qualify and grade or rank, hence a visual assessment was conducted by experienced pearl graders at the completion of the trial. Given that this project has monitored environmental data on a monthly basis for the duration of the project we are able to examine the inter-relationships between environmental variables and the colour and quality of nacre produced. This is analysed elsewhere although results from the current trial assessing nacre quality, colour and lustre over time are documented below.

Seeding operations involve a number of key components, namely conditioned host oysters, donor or saibo oysters, and the nuclei to be implanted. To achieve the optimum results (i.e. a large percentage of high value pearls) from the group of oysters to be seeded it is important to ensure that oyster selection and pre-operative conditioning has been properly conducted, that operating hygiene is of a high standard and that nuclei are both of suitable quality and suited to the size of the host oyster. By utilising clean operating facilities with experienced technicians, purchasing nuclei from reputable sources and ensuring post-operative culture is appropriate, pearl farmers provide the most opportunity to produce good results. This trial was aimed at clarifying the importance of some of the key aspects affecting the seeding operation and its subsequent success rate. Additionally, information on the relationships between various shell parameters and nuclei size was sought.

4.6.2 Materials and Methods

Cultured Akoya oysters from Site 1 were cleaned, sorted and transferred to Site 3 in August 2009 ready for pre-operative conditioning. As part of the conditioning process, the byssus in each oyster was cut with knives on the first occasion then broken by hand approximately every 5 days thereafter for a period of 4-5 weeks. The conditioning technique was the same for all oysters involved in this seeding trial.

Oysters were placed into standard WA conditioning baskets (P) at a density of 400 oysters per basket. These baskets/cages had dimensions of 92 L x 58 W x 20 H cm with an available Surface Area (SA) of 1.67 m^2 and a total volume of 106.7 L which was divided horizontally (Top-Bottom) into 2 units of approximately 53.3 Litres each (200 oysters each).

Figure 1 - Large basket (P) with horizontal separation (T-B) internally.

Once oyster condition was deemed to be suitable, seeding operations commenced on 20 September 2009 and continued through to 25 September 2009 utilising the services of two skilled Japanese technicians (M and K) seeding oysters for one site per day (none seeded on 23 rd September). While seeding operations were also conducted at the completion of the Pre-Operative Conditioning Trial (discussed elsewhere), those operations were aimed at assessing the effectiveness (or otherwise) of the various conditioning treatments conducted. The seeding operations were deemed to be conducted uniformly at that stage. In the current trial, oysters seeded by each technician were tagged accordingly and therefore were assessed separately, but the hygiene and operating facilities were deemed to be uniform for each technician.

For each site, 420 individual oysters were seeded by the first technician (M) and 315 oysters per site were seeded by the second technician (K), on a daily basis over 5 days. From this seeding, detailed data was collected on 175 individual oysters seeded by the first technician (M) and 140 oysters per site, seeded by the second technician (K), on a daily basis over 5 days.

At seeding time, live wet weight and shell dimensions (Width-APM, Height-DVM and Shell Thickness-WD) were recorded as well as the size of the nuclei implanted in each oyster. The oysters were placed in sequential order into standard tagged panels for culture through to the pearl harvest. The seeding details (and tags) also included which technician completed the operation so that subsequent pearl harvest data could be related back to the seeding data. Saibo (donor tissue) was cut from the cultured oysters, selected on each day by the individual technicians based upon established criteria.

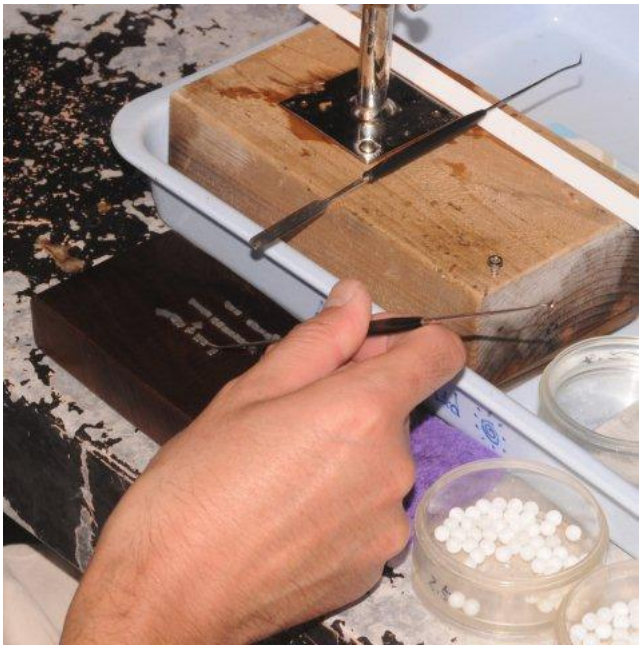


Figure 2 - Technician preparing saibo (donor) tissue for seeding operations.

Oysters rejected by the technicians for an operation were retained and similar data was collected from each oyster including why it had been rejected. That is, whether there was too much gonad material (spawn) present, too much muscle condition, a deformed gonad or a damaged shell. Additionally, two other panels (Panel A and Panel B) containing conditioned oysters, were placed at either end of the trial oysters but were not seeded. Oysters in these panels were used to clarify the percentage of mortalities at each location, seeded and unseeded.

The replicate groups of seeded oysters in tagged panels were placed at each of the 5 culture sites within days of the operations being conducted. Panels were then maintained and cleaned on a regular basis as per normal farming practices at the Abrolhos Islands. A visual assessment of mortalities in each of the tagged panels was conducted in conjunction with a Japanese technician, at each site on 1 April 2010, 187-192 days after seeding. The location of each dead shell in the panel and the number of the panel was recorded, as was the total mortality per panel. Dead oyster shells were left in the panels and were removed during the final pearl harvest when total mortalities were also assessed.

In early May 2010 (7.5 months after seeding), 5 panels of seeded oysters were transferred from Site 3 to Site 1 to determine whether the transfer mid-way through pearl culture produced different results to those transferred immediately after seeding.

As a result of the growth monitoring/sampling work, it was possible to collect shells representative of the entire range of nacre produced at the Abrolhos Islands. Preliminary Photo Series were prepared to document the range of colours in the oyster shell, based on the most commonly occurring colours. Photos for this series were also obtained by opening and photographing a range of larger pearl oysters, held on the various farms. These photos were developed for use during the final assessment of the pearl harvest and are shown in Appendix 3.

The assessment and grading of harvested pearls was conducted by two skilled Japanese technicians during the major harvest on 29 and 30 May 2011, some 616 days after seeding. This grading placed every pearl into A, B, C or D grade pearls with D grade being non-saleable/non-marketable. C Grade pearls were subsequently divided into C and C- groups to simplify the valuation process for the farmers. Data on rejected nuclei (no pearl), nuclei only, Keshi pearls, mortalities and missing shells were recorded.

The Grades are shown in Table 1.

Table 1. Akoya Pearl Grading Scheme for Project

Grade	Lustre	Shape	Blemishes	Nacre
A:	Excellent	Round	None	
B:	Good	Round	Tiny	
C: C	Dull	Roundish	>2 blemishes, white colour	
C: C-	Poor	Includes baroques	On surface, bluish colour	Thin
D:	Very Poor	All	All over, calcium stain, organic material	Very thin

Pearls harvested from each site were subsequently valued, in the various grades, by experienced pearl jewellers from the Geraldton (WA) area. An estimated Farm Gate price was given to each grade of pearls and then a subsequent value calculated using the pearls of each grade harvested from each site. This generated a Total Farm Gate value for the pearls that allowed direct comparisons of the success rate at each site to be quantified. The Farm Gate Value is based upon raw clean pearls which have not been processed or value added.

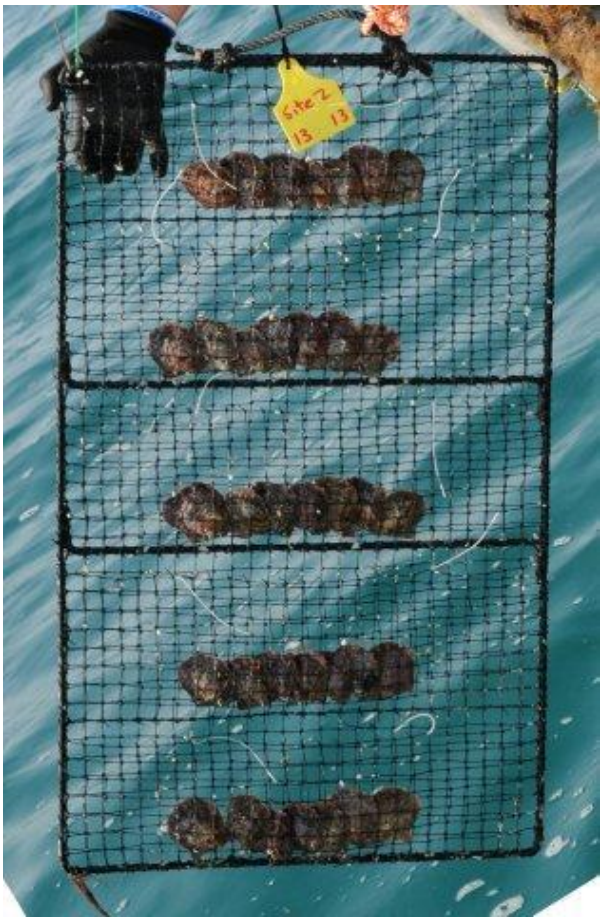


Figure 3 - Tagged and seeded oysters established at each of the 5 sites.

Sample harvesting of pearls commenced in June 2010, some 9 months after the seeding operations were conducted. An indicative sample of seven pearl oysters was collected from Panel 5 at each site, to monitor the progress of the pearls. Oyster size and weight dimensions were collected for each host oyster; the oyster was cut open and the pearl removed. Individual shells were cleaned, numbered and retained with a corresponding number for each pearl produced. These shells (valves) were stored until the major pearl harvest was completed in May 2011. Further samples of 7 oysters were collected from Panel 5 in July, August and September 2010; 10, 11 and 12 months after seeding.

A key sampling was conducted in October 2010 (about 13 months post-seeding) to determine the viability of conducting the major harvest later in 2010. Samples consisted of all oysters from Panel 12 and 15 oysters from Panel 13, i.e. 50 oysters per site. These oysters were seeded at the same time as all the monitored oysters and held on the same longline, but did not have morphological data collected. The use of these oysters therefore, did not compromise oyster numbers for the final harvest nor affected the veracity of the data collected. When each sample was collected, the pearl and its corresponding host oyster shell were retained in numbered sample bags.

At the final harvest all seeded shell in each tagged panel were opened; the pearl removed (if present) and one valve cleaned and retained. Each pearl was then cleaned before its size, quality and grade were assessed. Each pearl was accurately measured with a stainless steel digital micrometer. Once all pearls had been harvested, measured and graded and their corresponding shells collected, a valuation of the pearls was conducted by a panel of experienced jewellers. Additionally, the nacre thickness was calculated utilising the original nuclei size in relation to the final pearl size. Each pearl was graded based upon nacre quality, colour and lustre, then placed accordingly into A, B, C or D with D being non-saleable. Subsequent to this relative Farm Gate values were allocated for each of the pearls.

Table 2. Oysters Seeded by Technicians.

Panel	Technician	Data	Time Handled
Panel A	Control Shell	Sample Data	Morning
1	M	Collected	Morning
2	K	Collected	Morning
3	M	Collected	Morning
4	K	Collected	Morning
5	M	Harvest Data Only	Morning
6	M	No Data	Random
7	M	Harvest Data Only	Morning
8	K	No Data	Random
9	M	Collected	Afternoon
10	K	Collected	Afternoon
11	K	Collected	Afternoon
12	M	No Data	Random
13	M	No Data	Random
14	K	No Data	Random
15	K	No Data	Random
16	M	No Data	Random
17	M	No Data	Random
18	K	No Data	Random
19	K	No Data	Random
20	M	No Data	Random
21	M	No data	Random
Panel B	Control Shell	Sample Data	Random

4.6.3 Results - Seeding

Table 3. Summary of Akoya oysters seeded at each site.

Site/ Parameter	Total Weight (g)	Shell Width APM: (mm)	Shell Height DVM: (mm)	Shell Thickness WD: (mm)	Nuclei Size (bu)	Mortalities as at 1/4/10
Site 1	28.20	55.72	60.15	22.47	1.94	50
Site 2	27.82	55.91	59.89	22.37	1.95	114
Site 3	27.53	55.83	59.86	22.35	1.97	107
Site 4	27.41	55.70	59.73	21.63	1.98	77
Site 5	27.03	55.38	59.71	21.55	2.02	80
Mean	27.60	49.08	59.87	22.07	1.97	85.60

4.6.3.1 Seeding Nucleus Size

The oysters were seeded with nuclei ranging from 1.8bu to 2.1bu (bu is a Japanese unit - equivalent in mm is shown). Sizes were in 0.1bu increments, consequently there are four distinct size categories: 1.8bu - 5.4mm (240 oysters), 1.9bu - 5.7mm (175 oysters), 2.0bu - 6.0mm (1070 oysters) and 2.1bu - 6.3mm (88 oysters). Due to the small number of size categories, size cannot be treated as a continuous variable. To examine the relationship between nucleus size and oyster characteristics an ordinal logistic regression as implemented in the R routine "lrm" from the "Design" package (Harrell, 2011) was used.

The parameter estimates for a comprehensive model are shown in Table 4. The first three indicate the cutoff points between Nucleus categories. Their significance indicates that our independent variables can differentiate between them and that no two nuclei sizes are alike. These parameters are not explained further here.

The remaining coefficients show the relationship between that independent variable and nucleus size, a positive value indicates a larger nucleus size. The first variables relate to the physical characteristics of the oyster – weight, width, height and thickness. These are all strongly significant and are considered in more detail later. The remaining coefficients examine the sources of variation across the sites and technicians.

4.6.3.2 Variation Across Sites and Technician

4.6.3.2.1 Technician

Technician M seeded oysters with bigger nuclei ($p<0.001$). A separate ANOVA (not shown here) indicates that the technicians were supplied with oysters with the same physical characteristics; this suggests that the technician effect is due to the technician's method, not due to a difference in oysters supplied to them. A model with just weight and technician indicated that technician M provided the same effect as a 0.5g increase in oyster weight.

4.6.3.2.2 Site

The site was significant (overall, $p<0.001$) with later sites resulting in larger nuclei. A difference was not expected as the oysters for all sites were supplied from the same source. One explanation may be the technician's method improving/changing during this intensive seeding period (sites were seeded sequentially). A model with just weight and site indicated that site provided the same effect as a 2.5g increase in oyster weight.

4.6.3.3 Shell

The shell number (starting from the first oysters seeded being No. 1) was also significant ($p<-0.001$) with later shells in a given seeding trial receiving bigger nuclei. This is an unexpected effect as the shells should have been randomly selected. A possible explanation is that the technicians became more confident during each trial. A separate linear model also indicated a significant correlation between shell number and the size of the shell ($p<0.01$).

Table 4. Parameters from the full ordinal logistic regression model.

	Coefficient	Significance
Nucleus>=1.9	-19.19	0.000
Nucleus>=2	-20.06	0.000
Nucleus>=2.1	-25.27	0.000
Weight	0.16	0.000
Length	0.11	0.000
Height	0.12	0.000
Thickness	0.14	0.003
Technician M	0.24	0.034
Site=2	0.26	0.156
Site=3	1.17	0.000
Site=4	0.96	0.000
Site=5	0.88	0.000
Shell	0.01	0.000

4.6.3.4 Predictive Model - Nucleus Size

Weight, width and height are highly correlated (pair wise Pearson correlation of 0.72-0.73). Ordinal logistic regressions with several of these parameters provide a marginally improved fit over a regression with a single parameter (e.g. weight). Thickness has a lower correlation (0.4-0.45), however its predictive abilities are limited – despite being a significant parameter, inclusion in the regression does not increase the fit substantially.

For practical application, this implies that measurement of a single parameter – weight, width or height – will give the best prediction of nucleus size. Figure 4 shows the median nucleus size for oysters with different weights. For an oyster weighing 30g the median is approximately 1.99bu indicating that half the oysters would be seeded with a larger nucleus and half the oysters with a smaller nucleus.

Figure 5 considers the composition of nucleus size for oysters of a given weight. It indicates the proportion of oysters predicted by the model to be seeded with each nucleus size as a function of weight. For example, a 30g oyster has approximately a 5% chance of being seeded with a 1.8bu (5.4mm) nucleus, a 5% chance of a 1.9bu (5.7mm) nucleus, an 85% chance of a 2.0bu (6mm) nucleus and a 5% chance of a 2.1bu (6.3mm) nucleus.

Similar relationships hold for width (APM) and height (DVM) but for brevity they are not displayed here. They are shown over in Figure 6 which combines current data with that from the seeding of oysters in the Pre-Operative Conditioning Trial.

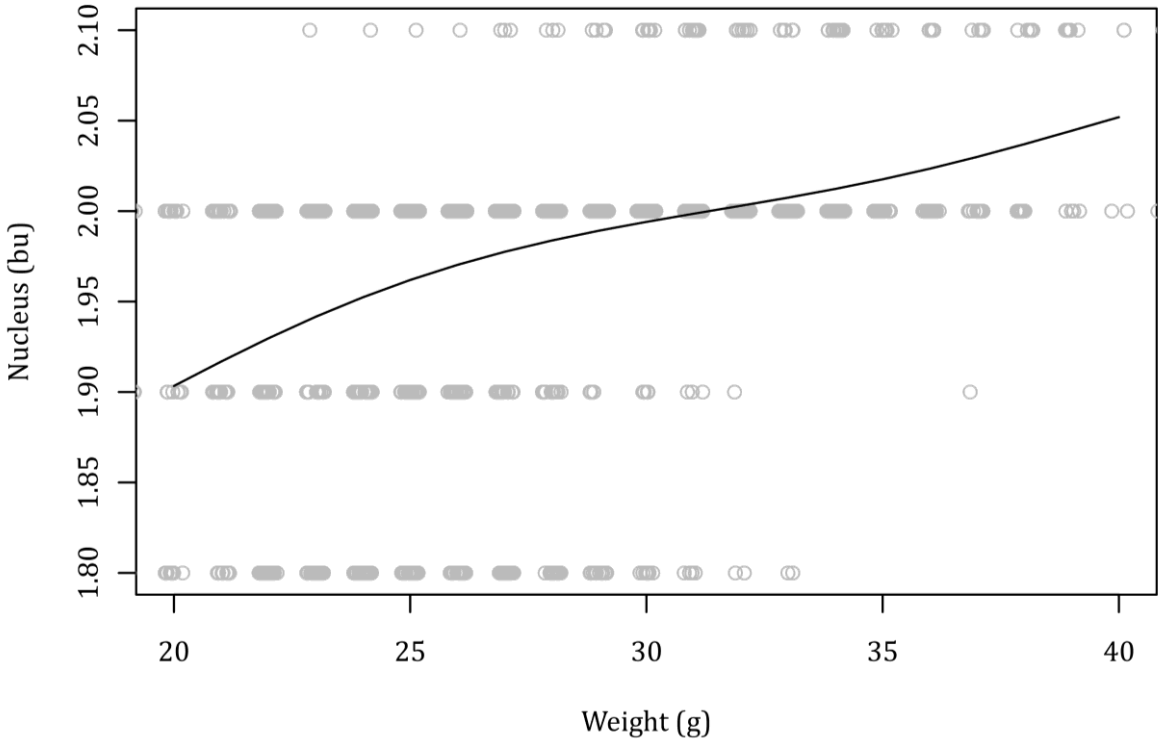


Figure 4 - Nucleus size as a function of oyster weight (each circle corresponds to a single oyster). The solid line indicates a model fit of the mean nucleus size.

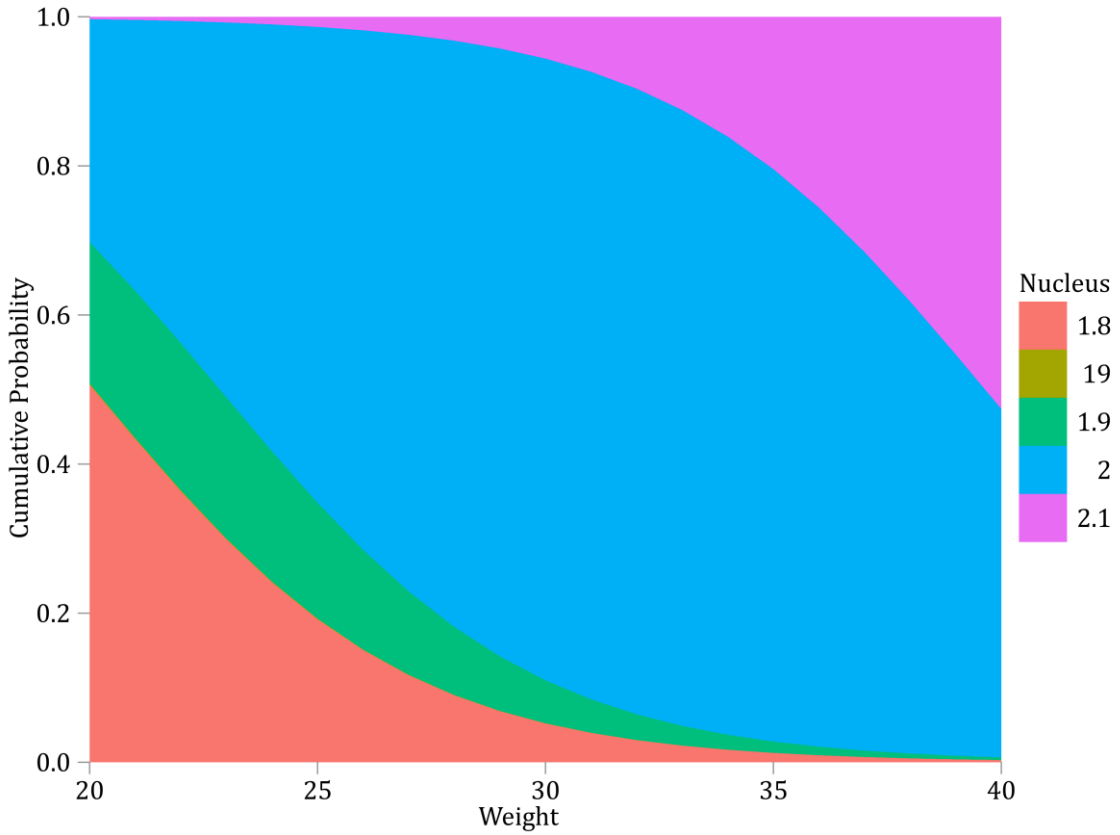
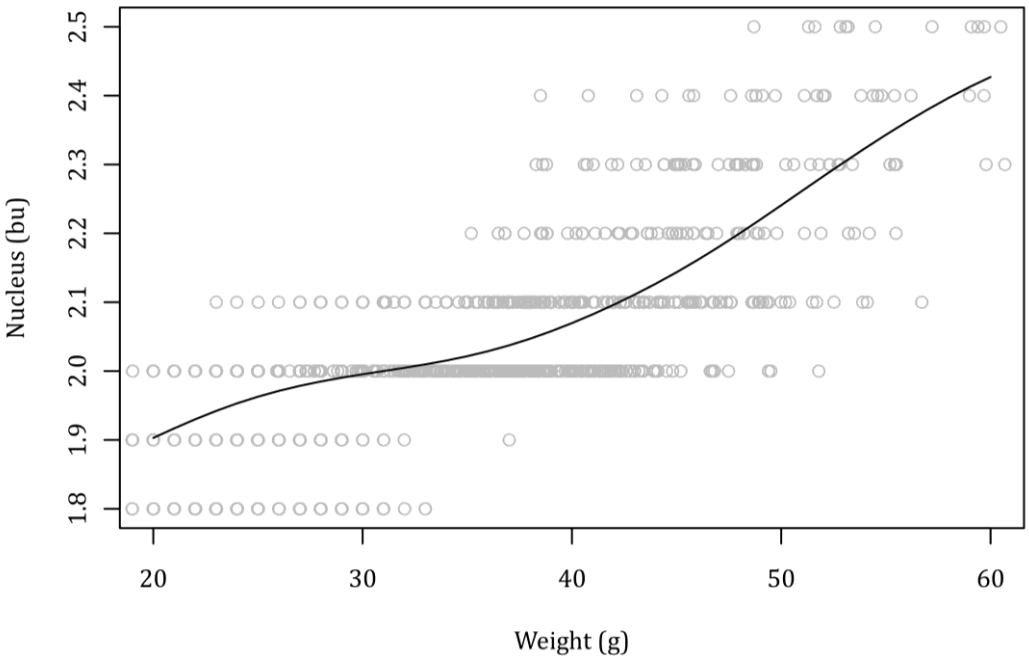


Figure 5 - The predicted proportion of oysters at a given weight seeded with each Nucleus. For a given weight, the size of the coloured slice above that weight indicates the likelihood of that oyster being seeded with the corresponding nucleus size.

4.6.3.5 Combined Seeding

Due to the size of the oysters in this experiment, it was only possible to seed the oysters with four nucleus sizes: 1.8, 1.9, 2.0 and 2.1bu. In the Pre-Operative Conditioning Trial larger oysters were seeded, these could be seeded with nucleus sizes ranging from 2.0 to 2.5 bu. Combining the two datasets it is possible to analyse the oyster size / nucleus size relationship from 1.8 to 2.5bu (Figure 6). Figure 7 depicts the relationship of Nucleus size with Weight, APM and DVM using the previously discussed model.

PANEL A:



PANEL B:

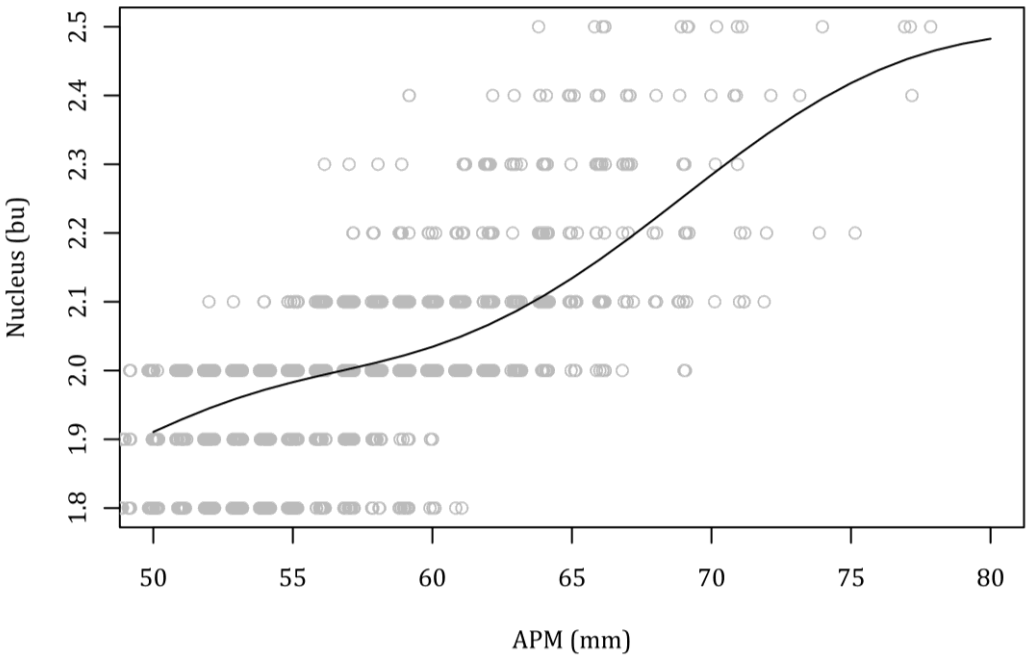


Figure 6 - Continued over

PANEL C:

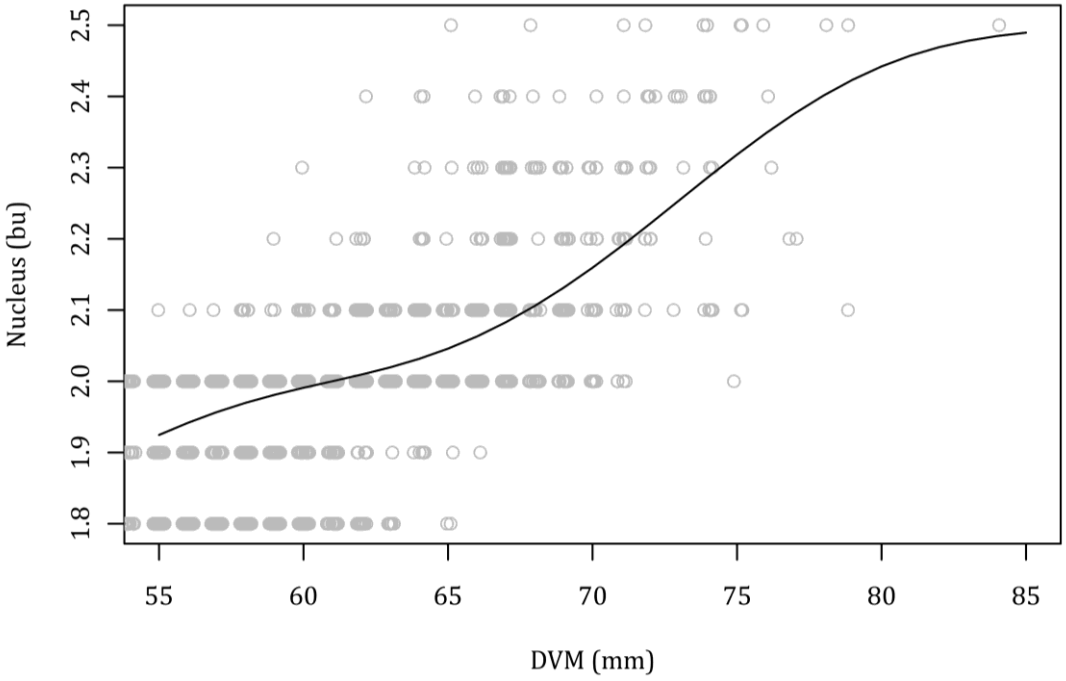


Figure 6 - Nucleus size as a function of oyster weight (Panel A), APM (Panel B) and DVM (Panel C). Each circle corresponds to a single observed oyster. The solid line indicates a model fit of the mean nucleus size.

PANEL A:

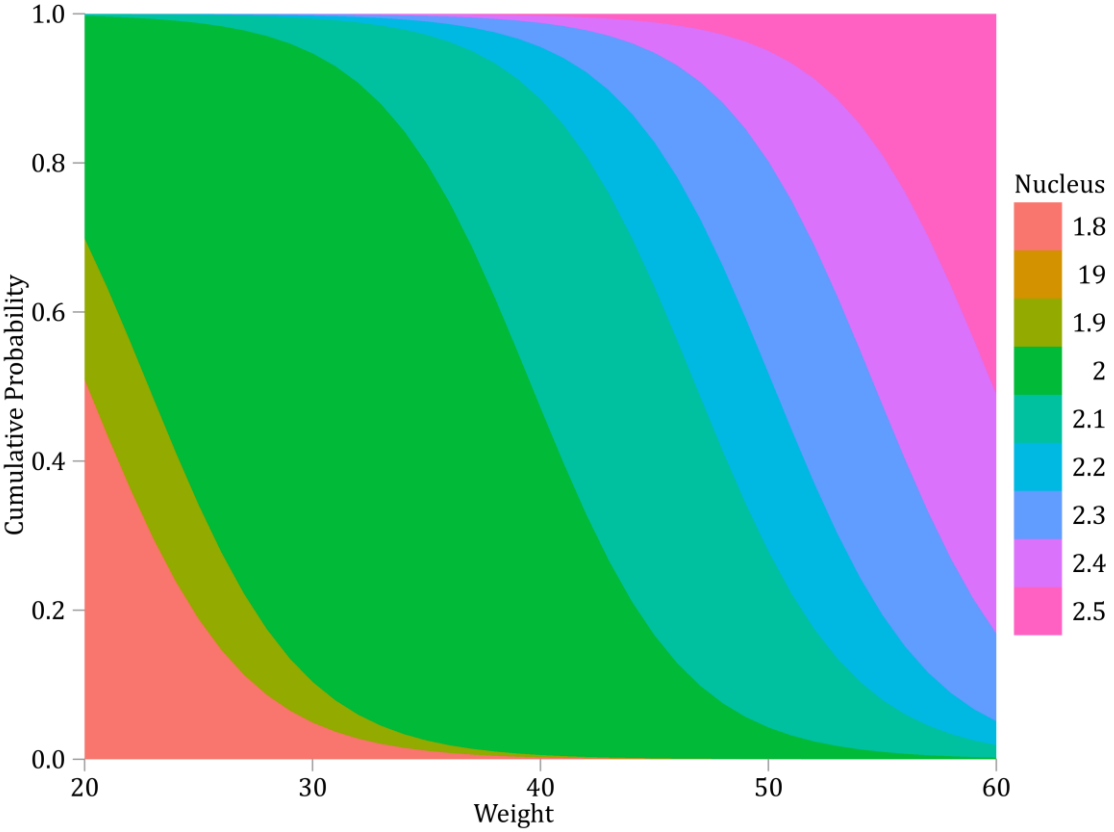
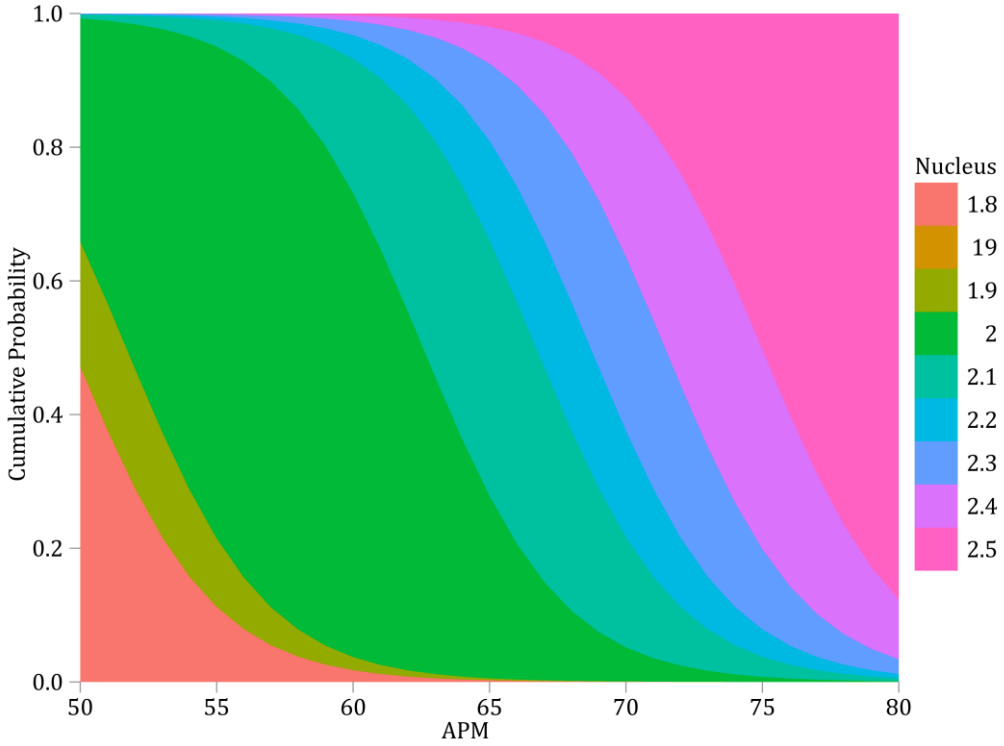


Figure 7 - Continued over page

PANEL B:



PANEL C:

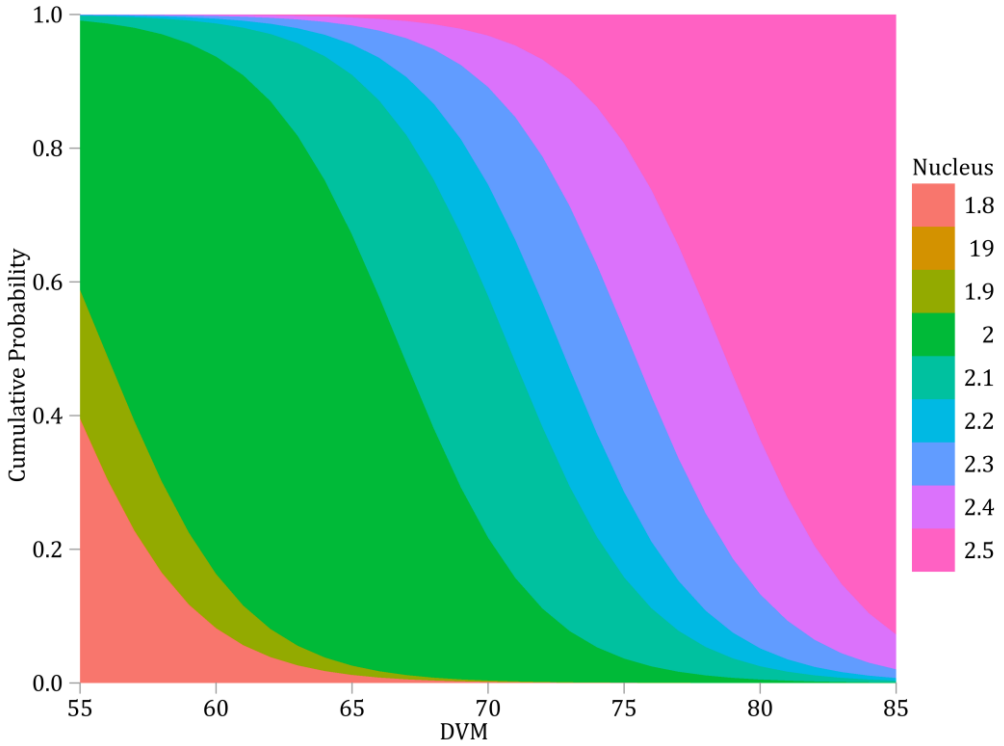


Figure 7 - The predicted proportion of oysters at a given weight (Panel A) or APM (Panel B) or DVM (Panel C) seeded with each size nucleus. For a given x-value, the size of the coloured slice above indicates the likelihood of an oyster of that size being seeded with the corresponding nucleus size. (e.g. For a 65mm DVM oyster, the biggest slice is green (from Panel C) indicating a 2bu nucleus is most likely).

4.6.3.6 Results - Harvest Analysis

This section considers the factors influencing the size and quality of the harvested pearls. The potential sources of variation include characteristics of:

i) the individual seeded shells;
ii) the seeding operation; and
iii) the sites.

Due to the large number of factors that can be considered and the complexity of the experimental process, there are certain limitations on the effects that can be explored with this dataset. This section initially considers the limitations inherent in the data, in detail.

However, before the final pearl harvest is examined, the earlier pearl sampling is noted. Ad hoc sampling of oysters from Panel 5 (see Table 2) involved 7 oysters per month (May, July, August, September) before a larger assessment was made in October 2010, 13 months after seeding operations.

As expected, the size of the pearl produced was larger with a longer culture period. However, the colour of the pearl was found to be similar throughout five months of culture when sampling was conducted. A difference was noted between the lustre exhibited by pearls harvested from month 8 to month 13 after seeding although small sample sizes mean no statistical significance can be demonstrated. Additionally, nacre growth was insufficient in many cases for a saleable pearl hence no valid conclusions could be made.

Table 5. Sample Harvest in October 2010 - 13 months after seeding.

Site	No. of Shell	No. Dead	No. Rejects	Non-Saleable Pearl	Blue/ Baroque Pearl	Need More Time	Currently Saleable	Potentially Saleable	Potentially Saleable
1	50	9	10	6	1	15	9	24	48
2	50	8	15	10	4	10	3	13	26
3	50	13	6	4	5	9	13	22	44
4	50	18	10	8	1	5	8	13	26
5	50	8	15	9	3	8	7	15	30

Results of pearls sampled 13 months after seeding showed quite clearly that more culture time was required at the existing sites to produce saleable quality pearls. Additionally, the actual amount of time required to yield a high proportion of saleable pearls from each site is quite different and further reflects the known site differences. The final pearl harvest was targeted for the cooler months of May/June 2011 as a result of the October sample harvest and because of unusually high water temperatures over the summer months.

4.6.3.6.1 Data Description

ANOVAs were conducted to compare differences in seeded oyster size and weight between sites, panels, seeding technician and seeding time. (The routine for seeding oysters at each site was shown earlier in Table 2.) Ideally none of these factors should be significant, as the same population of oysters was used for all seeding operations. However due to the practicalities of seeding (e.g. the infeasibility of mixing all oysters prior to seeding and selecting one at a time at random) some differences between sites may be expected.

Table 6 shows the significance of each explanatory variable for each oyster size parameter. This shows that thicknesses varied significantly between sites, with Sites 4 and 5 having 0.8mm thinner oysters (see Table 3). The other significant effect was a variation between panels for oyster width.

Table 6: Significance of ANOVAs examining the effect of explanatory variables (columns) on oyster size characteristics (rows). As many tests are being conducted without a priori expectations, an adjusted p-value should be used to reduce false positives. The adjusted Bonferroni and Sidak adjustments reduce a p value of 0.05 to 0.003 and 0.0031 respectively. Significant results are indicated in bold.

	Technician	Time	Site	Panel
Weight	0.11	0.86	0.02	0.01
Height (DVM)	0.3	0.03	0.3	0.15
Width (APM)	0.52	0.5	0.26	**0.003**
Thickness	0.23	0.43	**0**	0.51

Seeding nuclei were chosen by experts to provide the best pearl outcome. For the oyster sizes utilised herein, nuclei varied from 1.8 to 2.1bu (5.4 to 6.3mm), with 68% of nuclei sized at 2.0bu (or 6mm). The limited variation in nucleus size resulted in a high correlation between the final pearl size and nacre thickness as seen in Figure 8. In other words, larger pearls will have a thicker nacre as all pearls began at a similar size. The correlation between nacre thickness and pearl size prevents a robust assessment of the individual effect of these factors on the pearl grade.

In summary:

- Shell thickness at Sites 1, 2, 3 differed significantly to Sites 4 and 5. Consequently consistent differences between these two sets of sites may be due to either site differences or shell thickness differences.
- The initial nucleus sizes had a limited range (as is normal practice) resulting in only four distinct nacre thicknesses for each pearl size. This makes it impossible to distinguish between the effects of pearl size and nacre thickness on pearl grade. Furthermore conclusions regarding growth and seeding operations may not hold true outside of the initial range of oyster and nucleus sizes.
- Due to the limited variation in initial nucleus size and the fact that they were chosen to provide optimal results, there will be limited power to test the effect of nucleus size for a given shell characteristic on the final pearl outcome.

- Analysis of pearls resulting from seeding at the end of the Pre-Operative Conditioning Trial (discussed earlier) is likely to provide a greater insight into relationships between nucleus size, oyster size and the resulting pearl. That harvest is not expected to occur until at least September 2011 and is not part of the current project.

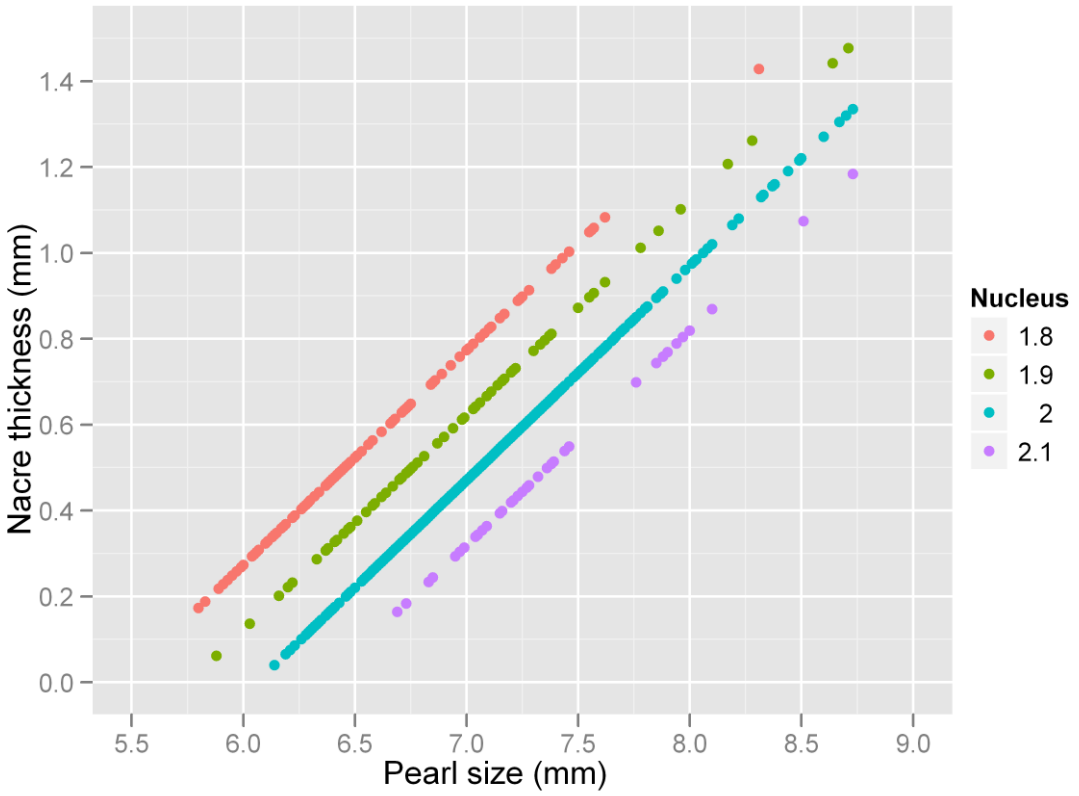


Figure 8 - Nacre thickness versus pearl size. As a result of the limited seeding nuclei sizes used, there are only four possible nacre thicknesses for a given pearl size (one for each original nucleus size). These are indicated by the different colours.

4.6.3.6.2 Pearl Production

In this section the aspects influencing pearl production from a seeded oyster are considered. The stages are divided into:

i) early survival (post-seeding);
ii) overall survival;
iii) nucleus retention; and
iv) pearl production from a retained nucleus.

4.6.3.6.2.1 Post-Seeding Oyster Survival

Early oyster survival (post-seeding) is considered using mortality data recorded after 7 months. During this initial period, seeding related mortality is expected to be most significant. This analysis is therefore expected to be more sensitive to the effects of the seeding operation than an analysis of overall mortality which is more influenced by the site.

At each site there were two panels of unseeded oysters and two panels of seeded but unmeasured oysters (oysters not measured at seeding time). Using these panels it is possible to investigate the effect of both measuring and seeding oysters. A regression model with a gamma link function was used to relate mortality rate to site, shell measurement, whether an oyster was seeded and if so, the time of the seeding operation (morning/afternoon) and the seeding technician. The outcome of this analysis is shown in Table 7. The early (initial) mortality rate was significantly increased by seeding (an increase from 5% to 10%) and varied significantly between the 5 sites (ranging from 5% to 14%). The other factors did not cause the mortality rate to vary more than expected by chance. The survival of individual oysters was not tracked in this sample; hence inferences about the effects of oyster size on early survival cannot be made.

4.6.3.6.2.2 Overall Survival

Table 7 shows the mortality rate (both missing and dead oysters) at harvest. Probit regression models were used to establish significant differences between the levels of a given factor of interest. Seeded oysters continued to show higher mortality than unseeded oysters (25% compared with 17%). There was a mass mortality event at Site 3 due to a whale carcass drifting through the site, compromising the survival data. If Site 3 is excluded, the total mortality rates for seeded / unseeded oysters reduce to 22% / 16%. Site variations in mortality were strongly significant, even excluding Site 3. The mortality rate (for seeded, measured oysters) across all sites except 3 varied between 19% and 26%.

Interestingly there was a strongly significant effect for the time of day that the seeding operation took place. Morning seeding resulted in a mortality rate of 22% versus 30% in the afternoon. Relative to unseeded oysters, morning seeding increased the mortality rate by 5% and afternoon seeding by 13%. This effect is confounded with the technician effect; technician K did a greater proportion of the afternoon seeding than technician M, hence a similar pattern is observed for the technician effect. The statistical approach determined that this effect was due to the time of seeding ($p=0.01$) and not the technician ($p=0.28$).

The effect of measuring oysters on overall survival or pearl production could not be determined as only information regarding the oysters produced on these panels was recorded (thereby it is impossible to differentiate between oysters that rejected the nucleus and those that died). In this trial there was no post-seeding x-raying of oysters to determine nucleus retention/rejection rates as there is in the South Sea Pearling industry; the costs of x-raying Akoya oysters cannot be justified.

4.6.3.6.2.3 Nucleus Retention

Retention of the nucleus by surviving seeded oysters was investigated using the same method as for overall survival. Groups with low overall mortality consistently had high pearl retention rates. Site and seeding time effects were again significant. However the technician effect was the most significant effect for nucleus retention with an 11% difference between the technicians.

4.6.3.6.2.4 Pearl Production

This part of the analysis considers the production of a pearl (Grades A, B, C or D) from all surviving oysters, hence it is an extension of the nucleus retention analysis. The results are very similar to the nucleus retention analysis, however the effect of the technician was further amplified with a 17% difference between the technicians.

4.6.3.6.2.5 Pearl Success

The success rate of producing a pearl (Grade A, B, C or D) from a seeded oyster is also shown. This is simply the overall survival percentage for a given group multiplied by the pearl production rate.

4.6.3.6.2.6 Oyster Characteristics

The effects of the oyster – weight, width (APM), thickness, height (DVM) – and the size of the seeded nucleus were also considered. However the relationship between these factors and mortality rates, nucleus retention and pearl production was insignificant in all cases ($p>0.05$).

In summary:

- Seeding increased oyster mortality by 8% (to 25% total).
- Measuring oysters resulted in no early mortality increase.
- Afternoon seeding mortality was 8% higher than morning seeding mortality.
- The technician had very strong effect on nucleus retention and pearl production (11% and 17% respectively).
- The highest pearl success rate (66%) was obtained by technician M at Site 5.
- The size of the seeded nucleus did not affect any aspect of oyster survival and pearl formation.

Table 7. This table shows early (post-seeding) and overall mortality rates, nucleus retention and pearl production for surviving oysters and lastly, the probability of a seeded oyster producing a pearl (grades A-D). The p value indicates whether there was a significant effect for each explanatory variable; significant outcomes are highlighted.

		Early Mortality		**Total Mortality**		**Retention**		**Pearl Production**		**Pearl Success**
		Rate	p	Rate	p	Rate	p	Rate	p	
Seeded	TRUE	**10%**		**25%**		74%		66%		49%
	FALSE	**5%**	**0.0052**	**17%**	**0.008**	NA				
Measured	TRUE	10%								
	FALSE	12%	0.63							
Technician	K	11%		27%		**65%**		**56%**		41%
	M	9%	0.35	23%	0.28	**76%**	**<0.001**	**73%**	**<0.001**	56%
Time	Morning	9%		**22%**		**75%**		**70%**		54%
	Afternoon	11%	0.39	**30%**	**0.01**	**64%**	**0.02**	**57%**	**0.04**	40%
Site	1	**5%**		**26%**		**79%**		**73%**		54%
	2	**13%**		**23%**		**69%**		**62%**		48%
	3	**14%**		**34%**		**64%**		**61%**		40%
	4	**11%**		**22%**		**73%**		**66%**		52%
	5	**7%**	**<.001**	**19%**	**0.01**	**71%**	**0.01**	**66%**	**0.01**	53%

4.6.3.6.3 Pearl Quality

4.6.3.6.3.1 Pearl Size

The primary factor influencing pearl size is the nucleus size. A linear regression showed that this effect is highly significant ($p<0.0001$) with a slope of 2.5±0.24SE. This means that for each 0.1bu (or 0.3mm equivalent herein) increase in nucleus, the expected pearl size increases by 0.25mm (see Figure 9). (Note: Figures utilise measurements all in mm so they are directly comparable). Oysters were seeded with the largest nucleus that the technicians could physically insert without causing tissue damage to the oyster. There is therefore a possibility that the nucleus size is a proxy for a physical trait of the oyster that resulted in the large pearl size, instead of being the mechanism itself. To address this aspect a GLM was conducted including the initial oyster size (weight, width (APM), height (DVM) and thickness); as explanatory variables (in addition to pearl size) this was insignificant ($p>0.25$ for all variables). The possibility remains that some unmeasured aspect of the oyster (visually assessed by the technician) is the major influence on pearl growth, but this seems unlikely.

A further GLM was conducted to investigate the effects of site and oyster growth on the pearl size. In addition to the nucleus size, this model showed that weight increase and the site are both highly significant ($p<0.001$). The weight increase has a coefficient of 0.014±0.001SE, indicating that for each 10g increase in oyster weight, the pearl will be 0.14mm larger in diameter. Figure 10 shows the pearl size distribution at the five sites; Sites 1 and 5 produced the largest pearls. The remaining parameters were highly insignificant ($p>0.3$).

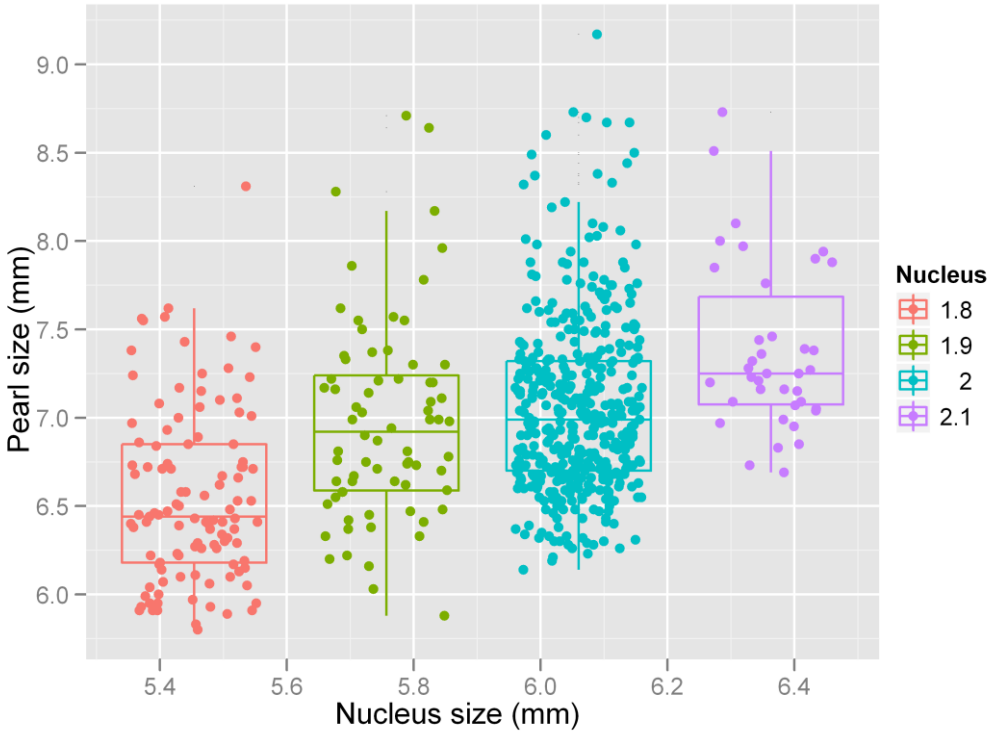


Figure 9 - Pearl sizes as a function of the four original nucleus sizes. For each nucleus size a box and whisker plot is shown and all pearls are indicated by the dots (which are jittered to reduce overlap).

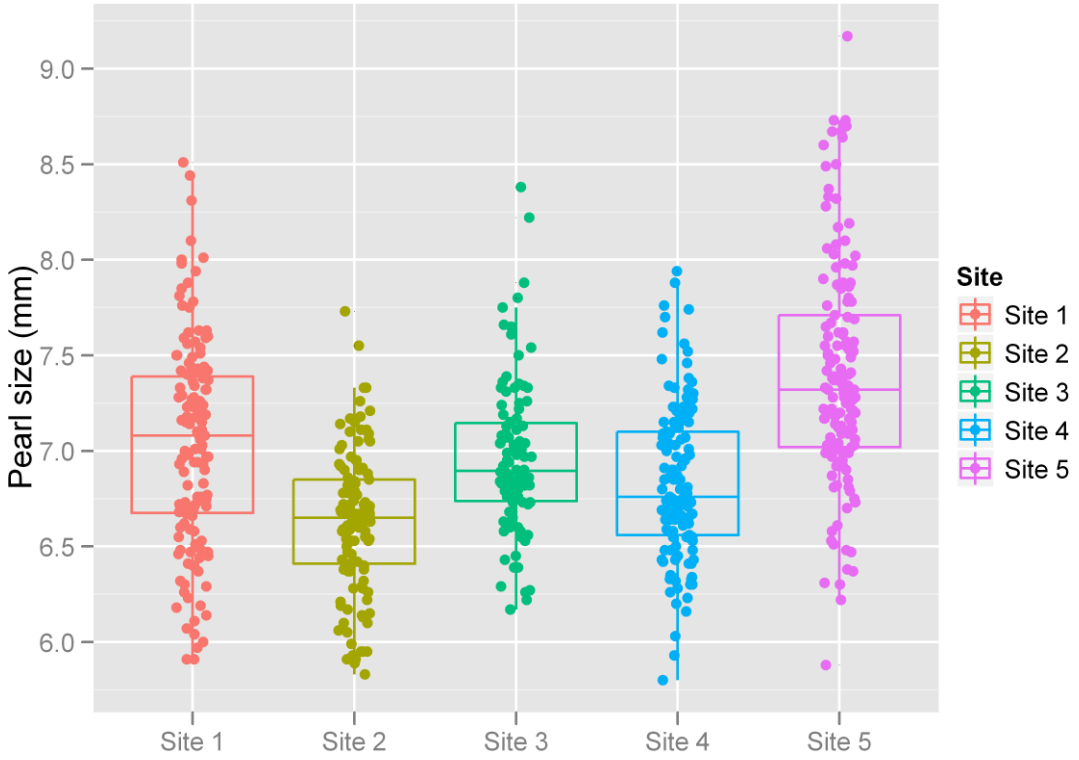


Figure 10 - Pearl size at the five sites. For each site a box and whisker plot is shown and all pearls are indicated by the dots (which are jittered to reduce overlap).

4.6.3.6.3.2 Nacre Growth

The growth of nacre at the 5 sites was compared by calculating the size of pearl against the initial nuclei size in mm. This provided an overall increase in diameter (Table 8) averaging 1.09mm, which translates to an actual nacre growth of 0.546mm around the nucleus in 20 months or approximately 616 days. Thus the average/mean nacre growth per day is 0.886 microns. Mean nuclei size (Table 3) was 1.97bu or approximately 5.91mm.

Table 8. Calculate Growth in Nacre at each Site.

Site	Pearl Size	Increase in Diameter from Nucleus (mm)	Actual Nacre Growth (mm)	Nacre Growth/day (microns)
1	6.81	1.27	0.64	1.039
2	6.31	0.79	0.40	0.649
3	6.75	1.02	0.51	0.828
4	6.64	0.88	0.44	0.714
5	7.09	1.49	0.74	1.201
Mean	6.72mm	1.09mm	0.546mm	0.886

4.6.3.6.3.3 Pearl Grade

Logit regression models were conducted to investigate factors influencing pearl grade. Factors considered included oyster size, oyster growth, seeding parameters, nacre thickness and site. None of these factors – including nacre thickness – were significant ($p>0.1$ for all factors). This suggests that pearl grade is robust to all these factors; to influence it something beyond the scope of this study must be investigated. Table 9 provides a summary of harvested pearls.

Table 9. Summary of harvested pearls. The top panel shows the number of pearls of each grade at each site and the bottom panel, the percentages.

Grade	Site 1	Site 2	Site 3	Site 4	Site 5	All Sites
A	3	5	1	1	3	13
B	26	17	22	10	25	100
C	167	136	66	89	149	607
D	44	40	15	81	29	209

Grade	Site 1	Site 2	Site 3	Site 4	Site 5	All Sites
A	1%	3%	1%	1%	1%	1%
B	11%	9%	21%	6%	12%	11%
C	70%	69%	63%	49%	72%	65%
D	18%	20%	14%	45%	14%	22%

In summary:

- Nucleus size had the largest influence on pearl size. A small weight and site effect was also noted.
- Pearl grade was unaffected by any measured parameters including nacre thickness. The study did not identify any factors, in this section, affecting pearl grade. Potential environmental effects on pearl quality are considered later in this report.

4.6.3.6.4 Economics

The Farm Gate price for the harvested pearls is shown Table 10 and Figure 11; pearls graded "D" had no value. The number harvested is also shown in Table 10, combining this with the price data yields total revenue of $7,986. (The total value can be found by multiplying the number harvested in each cell by the corresponding price then adding the result.) A total of 2585 oysters were seeded, the revenue per oyster is therefore $3.10. A total of 910 pearls were harvested (including "D" grade pearls), thus revenue of $8.78 per harvested pearl was obtained. The recovery rate of pearls from seeded oysters was therefore 35.2%.

Table 10. The Farm Gate price for pearls of different sizes (in mm) and grades are given in the first columns. The remaining columns give the grade-size breakdown of the harvest from this study.

	Price			Number Harvested			
Pearl Size	A	B	C	A	B	C	D
6.0-6.4	15	10	5	1	12	73	47
6.5-6.9	20	13	10	5	41	233	77
7.0-7.4	25	18	11	4	26	211	51
7.5-7.9	35	22	13	3	11	59	20
8.0-8.4	40	24	12		7	11	5
8.5-8.9	45	30	18		2	5	3
9.0-9.4	55	33	19		1	1	1
9.5-9.9	70	36	23				
10.0-10.4	100	39	24				

The grade composition of the pearls in this study was unaffected by all treatments, however the size and number of produced pearls can be influenced. The success rate for seeded oysters producing pearls can be increased by up to 15% through appropriate site choice, timing of seeding operations and better seeding operations. This translates directly to the same percentage increase in pearl revenue.

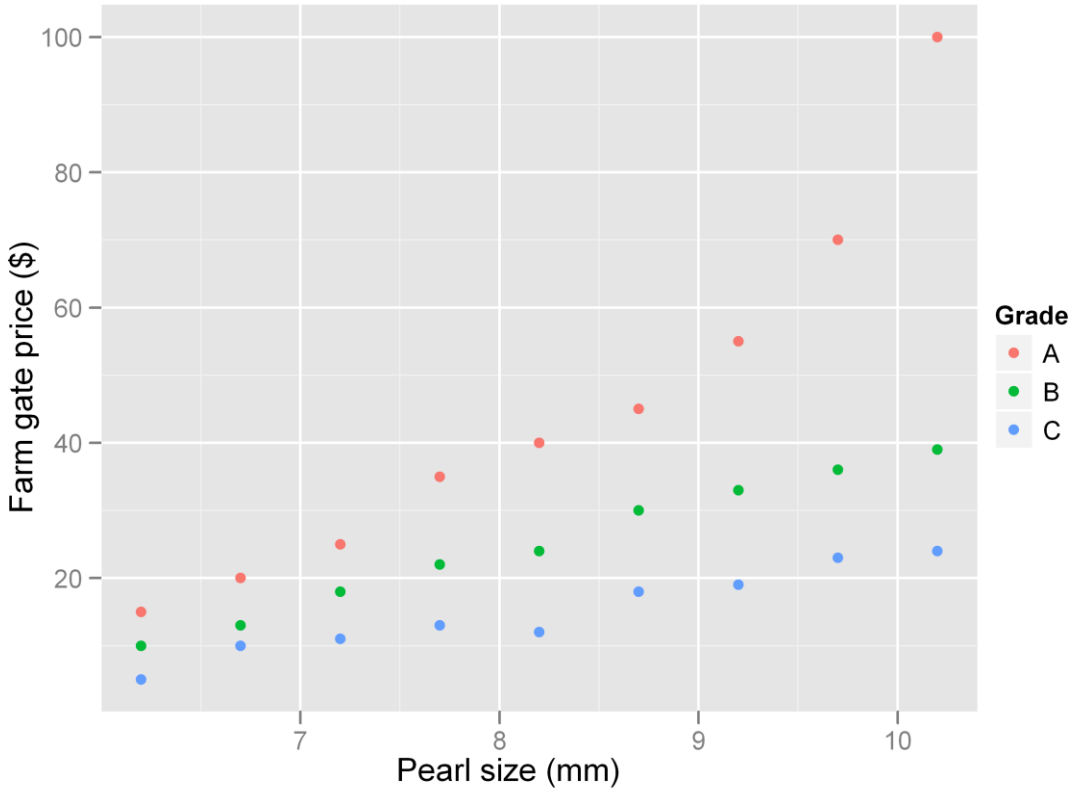


Figure 11 - The Farm Gate price as a function of pearl size, coloured by pearl grade.

Pearl size can be readily increased by seeding oysters with larger nuclei, but this requires larger oysters. The previously established relationship between oyster size and seeding nucleus, combined with the relationship between pearl size and nucleus size allows us to determine the size distribution of pearls for a given oyster size. Figure 12 shows the results from this analysis. For example, the oysters used had a mean weight of 27.5g, at this size, 68% were seeded with a 2.0bu (6mm) nucleus and only 5% accepted a 2.1bu (6.3mm) nucleus. Increasing mean oyster weight from 27.5g in this study to 30g would reduce the number of pearls below 7mm and increase the number above 7mm. To reiterate, if farmers wish to increase the size of nuclei used, and the size of resulting pearls, then they need to use larger oysters at seeding.

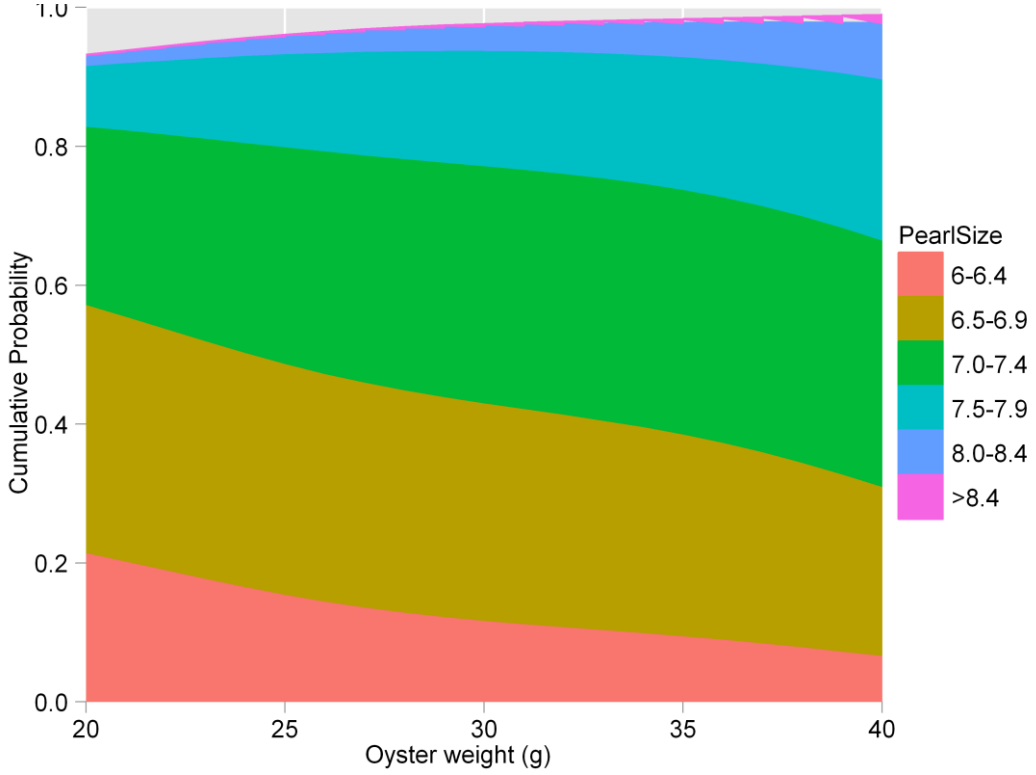


Figure 12 - The composition of pearl size as a function of seeded oyster weight.

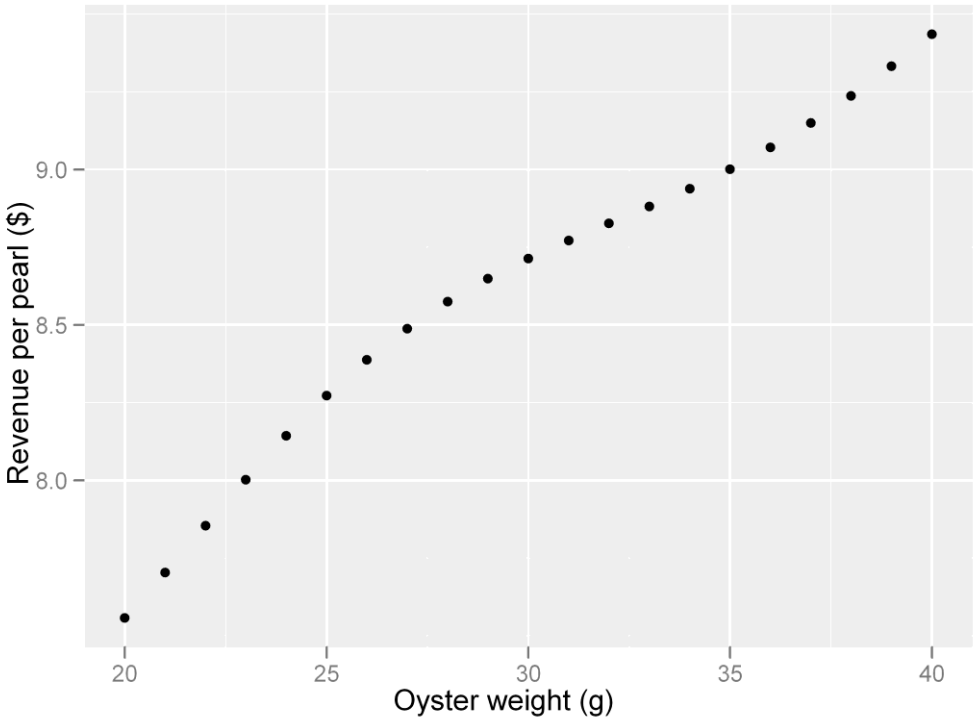


Figure 13 - Revenue per pearl as a function of oyster weight.

Combining the price data in Table 10 and the size composition in Figure 12, it is possible to determine the expected revenue per pearl produced by an oyster of a given size. This result is shown in Figure 13. This figure suggests that the marginal benefit of using larger oysters is at its low point near the oyster weight seen in the current study (27.5g). This marginal increase in revenue is the slope shown in Figure 13, which is at a minimum of about $0.06 / gram at 30 g. This analysis would need to be combined with costs of growing oysters to a larger initial size (the marginal cost of the additional growth) to determine the point at which profit is maximised. However, the cost of growing oysters up to seeding size was not examined in the current study.

In summary, revenue per seeded oyster can be increased by:

- Raising pearl production using the best site and seeding practises.
- Seeding larger oysters that can take larger nuclei – the cost effectiveness of this needs to be balanced with the cost of growing oysters to a larger initial size. Note that size-dependent mortality effects were not examined in this study.

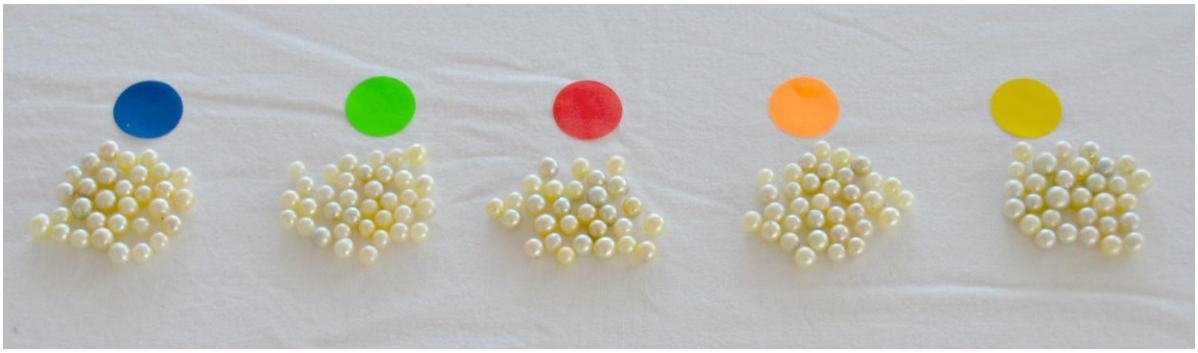


Figure 14 - A random sample of the C grade pearls harvested from each of the research sites.
Blue – Site 1; Green – Site 2; Red – Site 3; Orange – Site 4; Yellow – Site 5.

4.6.3.6.5 Discussion

This trial helped to clarify the importance of various oyster parameters and sites utilised in the seeding of Akoya oysters and culture of the pearl at the Abrolhos Islands. More specifically, at determining the optimum relationship between oyster size and nuclei size by assessing the size and quality of the pearl produced. Additionally, the preferred month for harvesting pearls was investigated with the sequential sampling of pearls from 8 -13 months after the seeding operation. The harvest-sampling period was initially targeted at the months with cooler water temperatures when lustre was expected to be at its best. However, the slower than anticipated nacre growth meant that the pearl harvest had to be delayed until May 2011, 20 months after seeding, so that marketable/saleable pearls could be produced.

In summary:

- Measurement of weight, APM or DVM is sufficient to provide a good prediction of nucleus size.
- The target weight can be readily determined from Figure 5. For example if 90% of oysters are to be seeded with >=2bu nuclei, then a 30g target weight is appropriate.
- The data suggests that technicians seeding oysters over a period of several days will increase the nucleus size within that time.
- Shell thickness at Sites 1, 2, 3 differed significantly to Sites 4 and 5. Consequently consistent differences between these two sets of sites may be due to either site differences or shell thickness differences.
- Seeding increased oyster mortality by 8% (to 25% total).
- Measuring oysters resulted in no early mortality increase.
- Afternoon seeding mortality was 8% higher than morning seeding mortality.
- The technician had very strong effect on nucleus retention and pearl production (11% and 17% respectively).
- The highest pearl success rate (66%) was obtained by technician M at Site 5.
- The size of the seeded nucleus did not affect any aspect of oyster survival and pearl formation.
- Nucleus size had the largest influence on pearl size, a small weight and site effect was also noted.
- Nacre growth varied from 0.40mm to 0.74mm between the sites
- Pearl grade was unaffected by any measured parameters including nacre thickness.
- Revenue per seeded oyster can be increased by raising pearl production using the best site and seeding practices.
- The cost effectiveness of seeding larger oysters with larger nuclei needs to be balanced with the cost of growing oysters to a larger initial size.

It is interesting to note that as a result of trials conducted in NSW, O'Connor et al., (2003) found that Akoya oysters could be seeded successfully when they had achieved a shell weight of approximately 30g or around 60mm in shell height. The current trial indicated similar results, with seeded oysters averaging 27.60g in weight and 59.87mm in shell height.

Mortality of oysters after seeding (grafting) has been documented at 10% in *Pinctada margaritifera* with a further 20% rejecting the nucleus (Ellis and Haws, 1999). Higher numbers were suggested as indicating poor technical skill (for the operation) or the onset of disease. Kripa et al., (2007) found that survival in Indian Akoya oysters seeded with 6mm (2bu) was 85.6% initially, (1 month after implantation) and 46.4% survival after 317 days of culture. Interestingly, no such relationship was shown in the current study and it is possible that technician skill at visually assessing the size of nucleus to insert and the technician experience in the seeding procedure were influential factors in the Indian work.

Kripa et al., (2007) also found that the nucleus retention rate was only 24% for oysters seeded with a 6mm nucleus while retention rates for all size nucleus in the current trial were higher. Furthermore, the average pearl produced from a 6mm nucleus after 317 days was 7.24 $\pm$ 0.382mm, with a total nacre coating of 1.23 $\pm$ 0.38mm (increase in

diameter). However, if the actual nacre growth around the nucleus is considered, this is 0.615mm or 1.94µm nacre/day. If this is extrapolated to the current study where 5.4 – 6.3mm (1.8-2.1 bu) nucleus were implanted and the culture period was 616 days, (from 20 September 2009 - 29 May 2011) the average nacre coating should have been 1.195mm and the average pearl size about 7.105mm. This approximates the pearl size displayed at Site 5 but is higher than the mean pearl size of the 5 sites in the current study. The nacre coating overall is highly important as "*the minimum required for Akoya pearls is generally 450 – 500µm*." (O'Connor et al., 2003) or 0.4 - 0.5mm thick (Kripa et al., 2007). "*A pearl with 0.40mm of nacre should last through a lifetime of normal wear*" (Ward, 1995). In the current study, only 2 sites produced pearls with coatings less than this; with the mean nacre coating for the 5 sites being 0.546mm. Mean deposition rate for nacre in the current study ranged from 0.649 to 1.201µm/day at the 5 sites. This is higher than documented Japanese deposition rates but below documented values for India (Kripa et al., 2007). With a recommended harvest period for harvest of Japanese Akoya pearls being 16 to 24 months (Shirai, 1970 in Kripa et al., 2007), the 20-month culture period is comparable to results obtained in similar trials in India (Kripa et al., 2007).

In the current project, revenue of $8.78 per harvested pearl was obtained with a recovery rate of 35.2% pearls from seeded oysters. While the recovery rate of pearls appears low, it compares favourably with the recent Akoya work in India (Kripa et al., 2007). Importantly, it allows for mortalities during production and results in pearls with a good nacre coating. Thick nacre coatings have given Australian South Sea Pearls an outstanding international reputation for quality; and contributes to raising the profile and value of such pearls, certainly an aspect that Australian Akoya pearls can also focus on. Revenue per harvested pearl was not high in the current study, however, costs of producing Akoya oysters are not high and given the short time span from hatchery to harvest (3.5 years in this case), the return on expenditure is acceptable. Nevertheless, with experience gained from this project, it is quite conceivable that the time span from hatchery to harvest can be significantly reduced and the percentages for each stage of culture can be improved, allowing for more, high quality and larger pearls being harvested.

4.7 Environmental Monitoring Within and Between Sites

4.7.1 Introduction

Environmental conditions are a key factor in the growth and quality of the pearl and pearl oyster. Prior to the current FRDC Project, little was known about the environmental conditions relating to pearl oyster culture at the Abrolhos Islands. Variations between farms sites within the island groups were known to exist although no relevant data was available to quantify these. Previous data recorded by Fisheries Western Australia showed temperature extremes at Rat Island of 24.0°C in March and 17.7°C in August of 1993 (Pearce 1997). Pearce (1997) also recorded salinities of 35.74ppt in January and 35.37ppt in July, a range of just 0.4ppt during 1993.

Early consultation within industry identified the need to gather water quality data at a range of sites, depths and over a period of time to understand the different environmental conditions present at the individual farms. Temperature, pH, Salinity, Dissolved oxygen (DO), Conductivity and Chlorophyll A were recognised as influential environmental parameters in the development of an oyster pearl. These parameters are also relatively easy to measure in remote locations.

4.7.2 Materials and Methods

Monitoring trips were conducted every 4 - 6 weeks so that sampling could occur during every month of the year. Two main methods of sampling water quality were used to gather water quality data. Hobo loggers measuring temperatures and light sensitivity were placed on each site. The Hobo loggers are a programmable pendent that allows water quality parameters to be measured continually at set intervals. During the monitoring trips Hobo loggers were downloaded, hand held water quality meters were used and water was collected at each depth and stored for further analysis. The hand held multi meter was used to provide instant water quality parameters at each site. The water samples were collected using a 12 volt pump and stored in sample jars for subsequent chlorophyll A analysis.

Also collected during the monitoring trips were the sample oysters used in various trials (gonad development, growth etc.) being conducted as part of the project. Cleaning of the oysters was conducted at sea (using mechanical pressure cleaners) when required during the monitoring trips. Trips were conducted around favourable weather conditions and used the current pearl farm vessels and staff during the trips.

4.7.2.1 Hobo Loggers

Hobo loggers recording temperature and light sensitivity were attached to empty panels and deployed at all five sites. Initially two per site were attached to the main longline where the oysters (shell) for the project, at each site, were to be grown out at. Droppers were measured to ensure the loggers were at of 1.7 and 4.0m. Shortly after a third logger was deployed at a depth of 7m at each of the sites. The loggers were programmed to sample water quality at five minute intervals. In April 2008 another three new sites were identified. These were sites within three of the existing five farms site and allowed

data to be collected and compared within a farm. The new sites were at Farm Sites 1, 3 and 4 and were named Site 1A, 3A and 4A. Also in April 2008, the Hobo loggers were reprogrammed to measure data at 30 minute intervals allowing longer time spans to be covered by the data stored on the loggers. Loggers that were lost or malfunctioned during the project were replaced with new loggers at the most convenient time.

Figure 1 - Hobo logger and portable shuttle/interface to laptop computer.

Figure 2 - Hobo logger attached to panel.

Figure 3 - Hobo shuttle with logger being downloaded.

4.7.3 Water Quality

During each monitoring trip a hand held multi meter water quality device was used to measure dissolved oxygen, pH, salinity, conductivity and temperature. At each site the probe was lowered from the vessel to depths of 1.7m, 4.0m and 7.0m and the data collected. The set logger depths were also the same depths used to collect data with the Horiba W-20 and YSI 556 meters. Similarly, water was collected from the same depths using a 12 volt pump and weighted hose, with the water collected in plastic sample jars before being stored for analysis. The samples were analysed within 72 hours of collection for Chlorophyll A using an Aquafluor hand held fluorometer.

Figure 4 - Horiba (www.horiba.com) and **Figure 5 -** YSI (www.ysi.com) Multi Meters.

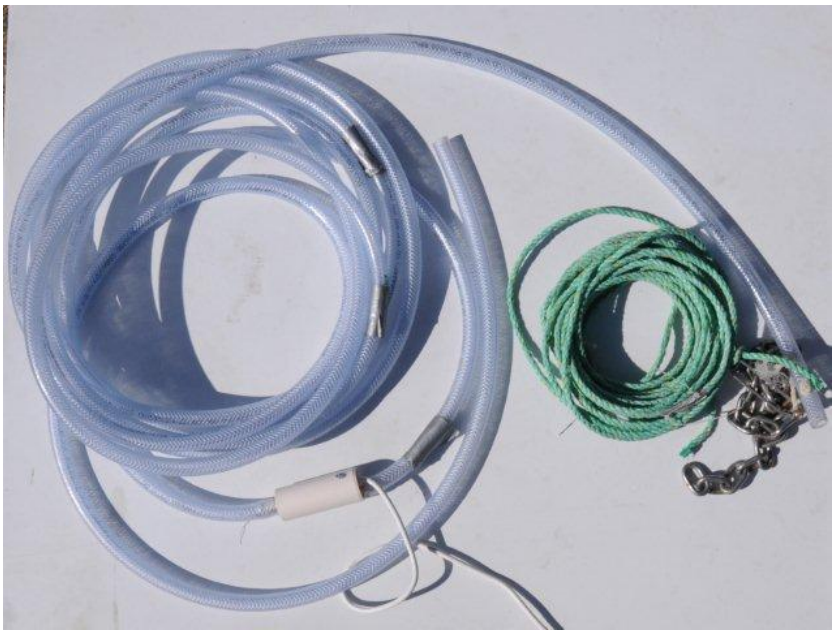


Figure 6 – 12 volt pump and weighted hose.

4.7.4 Results

4.7.4.1 Temperature

Long term water temperature data shows a temperature range seasonally of approximately 4-5°C at each site (between 20 and 25°C), with Sites 2 and 4 generally displaying regular wide fluctuations in mean water temperature, probably related to the shallow nature of those sites. Statistical analyses were conducted to evaluate differences between and within sites.

4.7.4.2 Between Site Variation

Figure 7 shows the mean daily temperature during the growth period at the three depths across the eight monitoring sites. To smooth these curves a twenty-day rolling mean was calculated. The recorded temperatures across all sites were generally within 0.25 degrees. With some exceptions, the largest differences were less than one degree. The exceptions to this were likely to be attributable to instrument error. For example the deep data logger at Site 1 recorded record lows for the time of year in January 2010, however this differed by more than 1.5 degrees from the logger at 1a which showed typical temperatures.

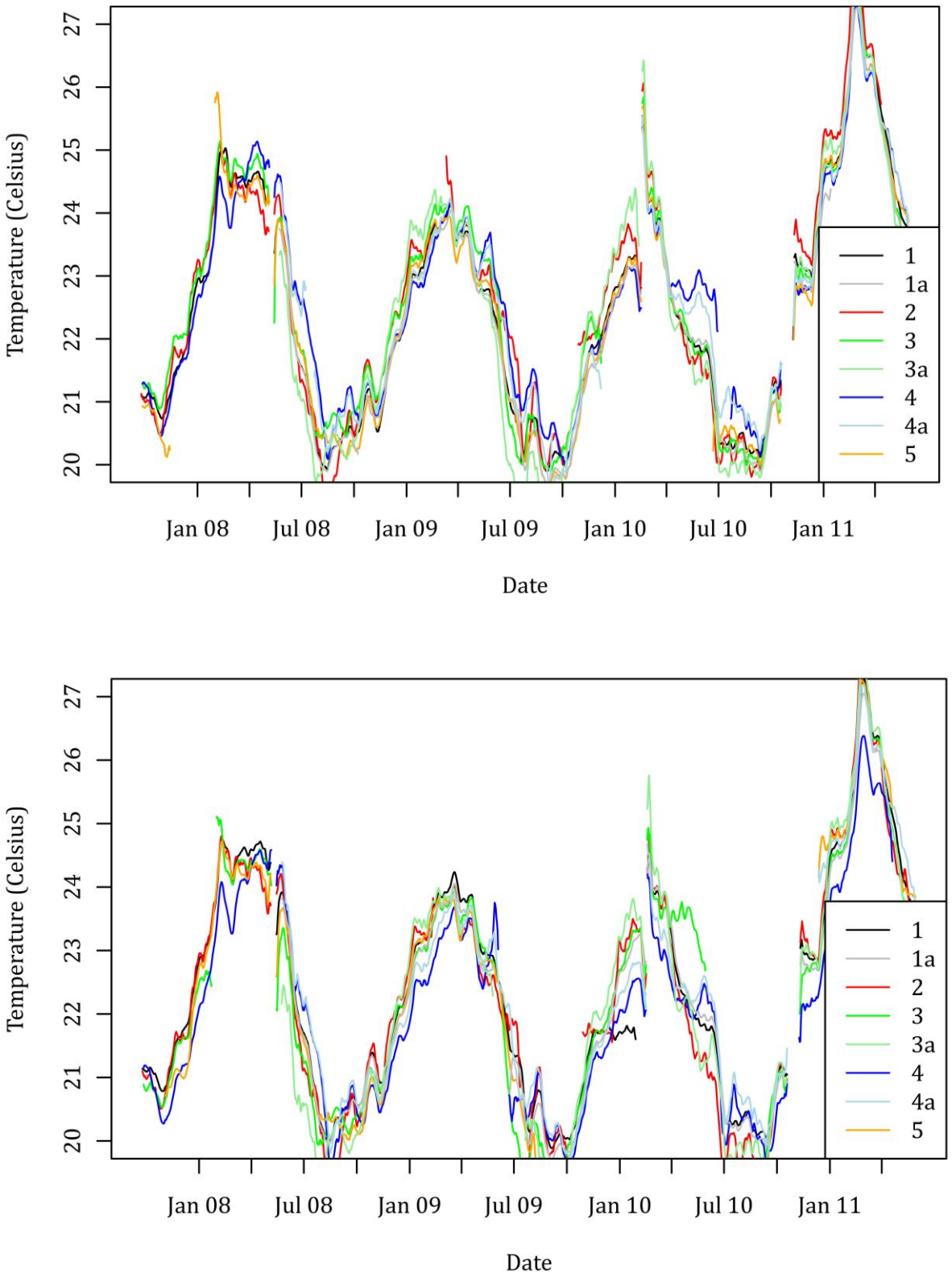


Figure 7 - The twenty-day running mean temperature at all sites.
The top panel shows the logger at 1.7m, the bottom panel at 7m. The legend indicates the site number.

To quantify the differences between sites, the pair wise mean difference between the daily temperatures was calculated, this result is shown in Table 1 for each depth and for the overall difference. The biggest overall difference was 0.35 degrees between Sites 3a and 4 (4 was warmer). This difference is largely attributable to the spike in June 2010 for 4m at Site 4. The validity of this spike is questionable as it was not captured by the shallow or deep loggers at that site or the loggers at Site 4a.

Table 1. The mean difference in temperature between sites.

Site	1a	1	2	3a	3	4a	4	5
1a	0.00	-0.06	-0.09	0.09	-0.06	-0.15	-0.07	0.01
1	0.06	0.00	0.00	0.10	0.00	-0.10	-0.01	0.08
2	0.09	0.00	0.00	0.18	0.02	-0.14	-0.03	0.09
3a	-0.09	-0.10	-0.18	0.00	-0.16	-0.22	-0.25	-0.08
3	0.06	0.00	-0.02	0.16	0.00	-0.09	-0.01	0.04
4a	0.15	0.10	0.14	0.22	0.09	0.00	0.01	0.16
4	0.07	0.01	0.03	0.25	0.01	-0.01	0.00	0.04
5	-0.01	-0.08	-0.09	0.08	-0.04	-0.16	-0.04	0.00

The numbers indicate how much warmer the site in the row is compared to the site in the column. e.g. Site 4 is 0.06 °C warmer than Site 1.

Figure 8 shows the cumulative number of degree days (base 13 °C) for all sites from the 1.7m depth logger. These curves have been truncated to the first day when logging began recording at all sites (in June 2008), missing temperature data was by necessity linearly interpolated. Visually from this figure the difference between sites appears minimal.

Table 2 shows the total number of degree days at each site and depth along with the deviation from the mean for that depth. Sensor accuracy is typically < 0.2 °C, which over 3 years corresponds to 219 degree days (3*365*0.2). Deviations in the total number of degree days exceeding this may be attributable to genuine site differences; these are indicated in bold in Table 2.

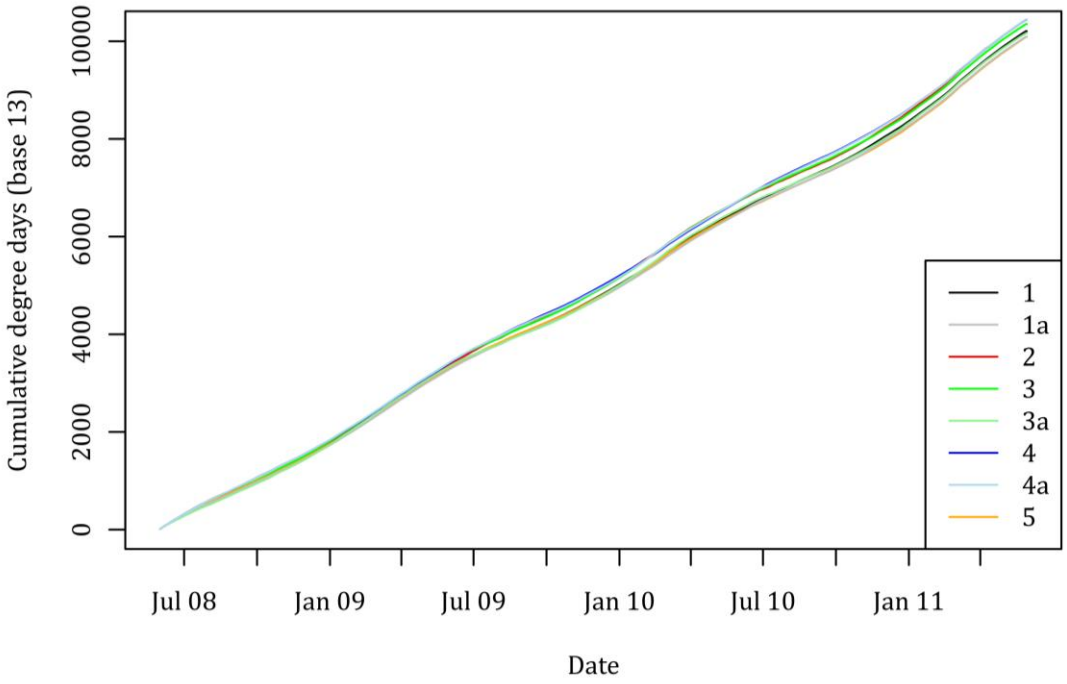


Figure 8 - The cumulative degree days at 1.7m during the period where all data loggers were deployed. The reference temperature used was 13°C.

Table 2: The number of degree days at each site (columns 2, 3 and 4) and the deviation from the average number of degree days at that depth (columns 5, 6 and 7). Deviations above a threshold of 219 degree days are indicated in bold.

	Degree days			Deviation		
Site	1.7m	4m	7m	1.7m	4m	7m
1	10206	10407	10136	-3	199	160
2	9884	10203	10015	**-325**	-5	39
3	10351	10133	10115	142	-75	138
4	10441	10451	9607	**232**	**243**	**-370**
5	10088	10064	10244	-121	-144	**268**
1a	10092	10070	9522	-117	-139	**-454**
3a	10164	9970	9966	-45	**-238**	-10
4a	10446	10366	10205	**237**	158	**228**

4.7.4.3 Depth Variation

Table 3 shows the mean temperature difference between sensors at different depths in the different Sites. Sites 1a and 4 showed a minimal increase in temperature from 1.7m to 4m which is attributable to random variation. The remaining sites showed decreasing temperature with depth. The largest vertical stratification was recorded at Site 4 with a 0.50°C degree difference between 1.7m and 7m.

Table 3: The difference in temperature by depth at each site (in °C). The first row shows the temperature at 1.7m minus the temperature at 4m. The second row shows the temperature at 1.7m minus 7m.

	1a	1	2	3a	3	4a	4	5
1.7m – 4m	-0.09	0.04	0.14	0.17	0.21	0.12	-0.05	0.09
1.7m – 7m	0.00	0.03	0.27	0.21	0.30	0.22	0.50	0.10

4.7.4.4 Temporal Variation

The mean temperature during the peak summer period in 2009 was 0.3-0.6 degrees cooler than 2008 across all depths. In 2010 there is an unfortunate gap in the temperature data. The peak period appears similar to 2009 at the beginning of the gap. When logging resumed, temperatures were at a level similar to 2008 after an initial peak of record highs. The three winter temperature troughs were at consistent levels across sites and depths.

The summer of 2011 was remarkably warm with temperatures exceeding typical levels by 3 - 4 degrees. These trends were observed consistently across sites and depths. Figure 9 shows the temperature in deep and shallow water at all sites during this period. Site 2 was up to 1.2 degrees warmer than other sites during this period across all depths. Site 3a was up to 1 degree warmer than other sites at 7m and 4m depth. Site 4 was significantly cooler at 1.2 meters depth but typical at other depths.

This suggests that Sites 2 and 3a, may be less resilient to abnormally warm events due to the thermal stress placed on the oysters.

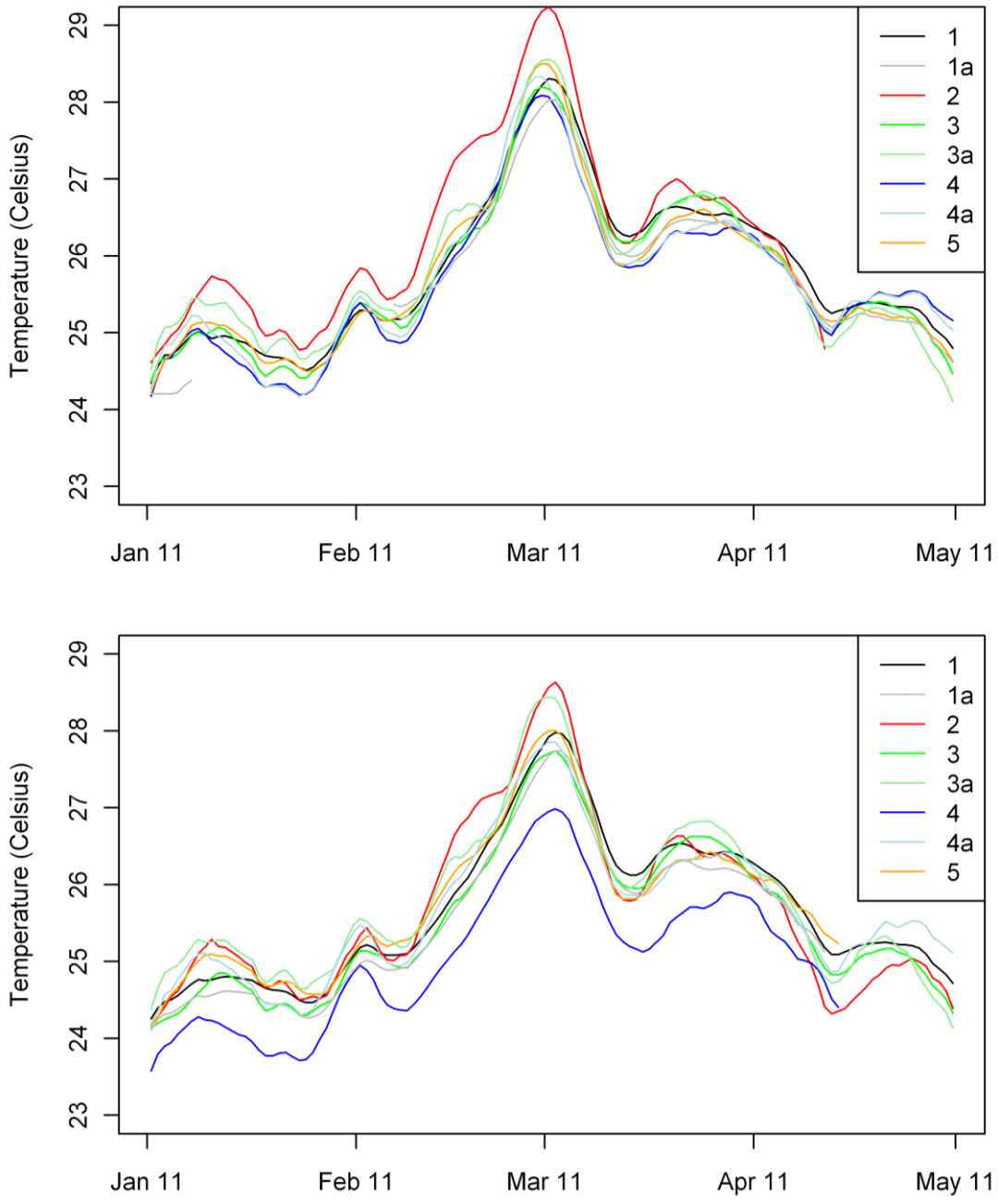


Figure 9 - The mean temperature at 1.7m depth (top) and 7m depth (bottom) during the warm summer event.

4.7.4.5 Light Intensity

Figure 10 shows the luminous flux at the 1.7m sensor across all sites. This data is extremely noisy, making even the annual trend in luminous flux difficult to ascertain. Apparent patterns and anomalies are inconsistent across depth and relative trends are inconsistent over time. This suggests a fundamental problem with this data.

Luminous flux is notoriously difficult to measure underwater due to fouling of the optical sensors and inconsistent sensor orientation.

4.7.4.6 Chlorophyll A

There were initial problems with the Chlorophyll A (ChlA) sensor such that results are only reliable after April 2009 for ChlA. There was an overall pattern in ChlA. Thereafter this was removed by subtracting the mean value recorded on each monitoring trip from those samples. An ANOVA was conducted on the de-trended data, this indicated that depth was not significant ($p=0.99$) but the site was strongly significant ($p<0.01$). A pair wise comparison revealed that Sites 1, 1A, 4, 4A and 5 had elevated readings relative to Sites 2, 3 and 3a. This effect was between 0.5 and 1.5 units of Chlorophyll A.

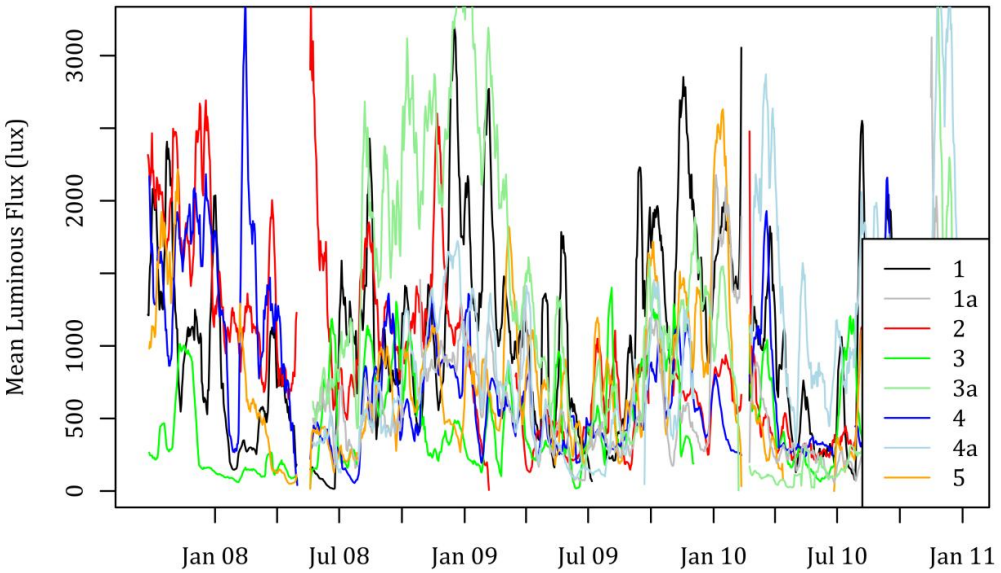


Figure 10 - The twenty-day running mean of luminous flux at 1.7m sensors across all sites.

4.7.4.7 Remaining Parameters

There were initial problems with the salinity sensor such that results are only reliable after May 2009 for salinity.

Salinity ranged from 34.07 to 34.98ppt. This is within the optimum range for Akoya oysters. Experiments conducted to determine effects of salinity on oysters generally vary salinity by several ppt to observe any effect. Consequently the observed salinity variation is unlikely to have had an effect on this project.

The remaining data did not exhibit a strong temporal pattern and was consequently not de-trended. An ANOVA was conducted for each environmental variable with the site and depth as factors. The results are shown in Table 4. Dissolved Oxygen (DO) was the only parameter to give a positive effect, however closer examination did not reveal clear differences between groups.

Interestingly the manual sampling approach did not detect a temperature difference at the sites.

Table 4. Mean Environmental Values and Significance.

	Significance	Median Value								
	Site	Depth	1	1A	2	3	3A	4	4A	5
Chlorophyll A	**<0.01**	0.99	5.65	5.63	4.51	4.55	5.05	6.06	5.52	6.16
pH	0.1	0.07	8.19	8.16	8.13	8.18	8.12	8.16	8.19	8.15
Salinity	0.06	0.99	34.60	34.61	34.64	34.65	34.68	34.60	34.57	34.63
Conductivity	1	0.7	162.50	163.20	159.20	165.70	158.70	163.20	162.90	159.10
DO_mg	**0.004**	0.7	7.23	7.15	7.31	6.90	6.90	7.12	7.16	7.02

The first two columns indicate the significance of site and depth for the measured parameters. The two significant effects are indicated in bold. The remaining columns show the mean values for each parameter.

4.7.5 Discussion

4.7.5.1 Water Temperature

The 2008 year was marginally warmer in summer than 2009. There is some uncertainty about 2010 due to missing data; however it appears similar to 2008. Temperature differences between sites, years and depths were on a similar scale. Some decreases (0.25 - 0.31°C) in water temperature from 1.7m to 7m were seen at Sites 2, 3, 3a, 4, 4a with the largest decrease of 0.50°C, seen at Site 4. This suggests that water mixing at all sites is good, with some stratification occurring at Site 4.

The summer of 2011, particularly, from late December 2010 – January 2011 was 3 - 4 degrees warmer than previous years. Sites 2 and 3a were the hottest with temperatures up to 1.2 degrees warmer than at other sites. This suggests that these sites are less resilient to such hot events, an issue that may be of increasing importance in future years. Their proximity to sandy shallow areas may well explain why such warm temperatures were experienced in comparison to the other sites.

4.7.5.2 Light Intensity

The light intensity data did not provide any meaningful results due to excessive noise. Stability of sensors in the water column and varying amounts of fouling appear to be the cause. Further refinement work is clearly needed to establish light sensors correctly in the water column and then intensive cleaning (more regular than monthly) would be required to ensure more reliable data was recorded. Such aspects were not possible within the constraints of the current project and its monthly monitoring program.

4.7.5.3 Biogeochemical

Possible problems were detected in numerous salinity and Chlorophyll A readings and these were omitted. Chlorophyll was significantly lower at Sites 2, 3 and 3a than elsewhere. There is a possible difference in dissolved oxygen but the exact nature was not identified. Closer examination of data did not reveal clear differences between groups although Sites 3 and 3a displayed somewhat lower readings. The remaining factors did not vary significantly between sites.

4.8 Effect of Environmental Factors on Pearl Oysters and Pearls

4.8.1 Introduction

A wide range of influential factors interact to determine the final quality and hence value of a pearl. The 5 key criteria used to assess the value of a pearl are lustre, size, shape, surface and colour (Ward, 1995). The issue of nacre thickness, which used to be ignored as high quality pearls always had more than an adequate coating of nacre over the nuclei, has more recently needed to be assessed. Nacre thickness is largely determined by the length of time the nucleus is in the host animal. Usually, the thicker the nacre, the higher the value, and the better the lustre. The Japanese Akoya pearl has a nacre of about 0.2mm thick whereas the South Sea Pearl has nacre about 3.5mm thick (Dietrich, 1995 c). The nacre thickness in Australian Akoya pearls does not appear to have been documented as yet, but it is known to vary quite substantially. Hence, the current assessment of pearl quality and value will incorporate assessment of nacre thickness.

While earlier discussion has examined the effect of various culture, handling, technician and site differences, the current section will examine the effects that environmental parameters (as discussed in the preceding section) have upon the pearl oyster and the pearl. The environmental conditions existing at the culture site throughout the pearl culture period, from the implanting or seeding operation right through to the harvest have been monitored and analysed. Other determining factors such as conditioning and the initial seeding operation have been considered earlier, but the overall impact of all environmental factors affecting pearl production that have been assessed during this project, are considered herein.

The Japanese researcher Machii (1958) documented the significant influence of factors such as the size of the host, the nucleus and the piece of mantle tissue (saibo) but qualified the whole process by stating that the influence of environmental factors such as water temperature, were much more significant (than the size of nuclei or mantle piece) to the process of pearl-sac formation.

An examination of the rate of pearl formation by Wada (1969) in Ago Bay (Mie Prefecture), Japan showed that it was apparently governed by water temperature. His three main findings were that: the growth rate of pearls was at a maximum in September although the water temperature peaked in August; at water temperatures below about 12°C the weight of pearls does not increase but eventually decreases; and the growth rate of pearls is usually more rapid in the period after breeding than in the period during breeding.

In general then, deposition of layers of nacre, or nacre growth, increases with higher water temperatures, but based upon the experience of the Australian pearling industry (and others) lustre and colour improve with cooler temperatures. For example, Gervis and Sims (1992) noted that slower nacre deposition and therefore thin nacre layers results in better pearl quality. Further they reported that lower temperatures resulted in better pearl lustre and harvests were usually timed to this.

The size of the pearl is largely related to growth duration and environmental factors while shape and surface are additionally influenced by technician skill during operations and husbandry practices. The current assessments will provide further insight into how the environmental parameters at the Abrolhos Islands affect these pearl characteristics.

4.8.2 Materials and Methods

The current section utilises data and information documented earlier for gonad monitoring, spat growth and survival, pre-operative conditioning, seeding and pearl production in combination with the previous section on environmental monitoring. As a result of the monitoring and sampling work, the entire period of nacre production for pearls in Akoya oysters at the Abrolhos Islands has been covered by the various datasets (up to the pearl harvest in May 2011). This section aims to identify relationships between various environmental parameters and subsequent oyster and pearl effects and characteristics by comparing data collected from all areas of the entire project.

4.8.3 Results

4.8.3.1 Gonad Environmental Correlation

Figure 1 shows the temperature during the hatchery oyster gonad trial. There is high correlation between the sites with differences primarily in the timing and intensity of short term temperature fluctuations. Figure 2 shows the gonad and glycogen indices during this period, these are highly uncorrelated. Sites 2 and 4 appear to have experienced a spawning event in September and July respectively. Both of these events occurred after a 3°C drop in temperature over the previous two months. A temperature drop of this magnitude was also evident at Site 3; however the gonad index was low prior to this event suggesting that spawning would not have been possible.

Sites 1 and 5 lack temperature data during the May-August period of cooling waters, hence it is difficult to ascertain if oysters at these sites were exposed to similar sharp temperature declines. Monitoring at Site 1a suggests that the oysters at Site 1 may have been exposed, however Figure 2 shows no impact on the gonad index. Site 1 and 5 had the highest condition oysters (this is consistent with findings in the growth chapter); it is therefore possible that these oysters responded differently.

The data suggests that:

- Oysters with a low gonad index (<2.5) will not spawn in response to the observed temperature drops.
- Oysters with a gonad index 2.5 - 3.5 may be brought to spawn by a temperature decrease of greater than 3°C in less than two months.
- Oysters with a high gonad index (>3.5) may not spawn in response to environmental signals, and may instead spawn gradually with increasing water temperatures from November to January.

Due to the low sample size, lack of temperature contrast between sites and uncertainty regarding the visual gonad scoring, these findings should be taken as purely suggestive.

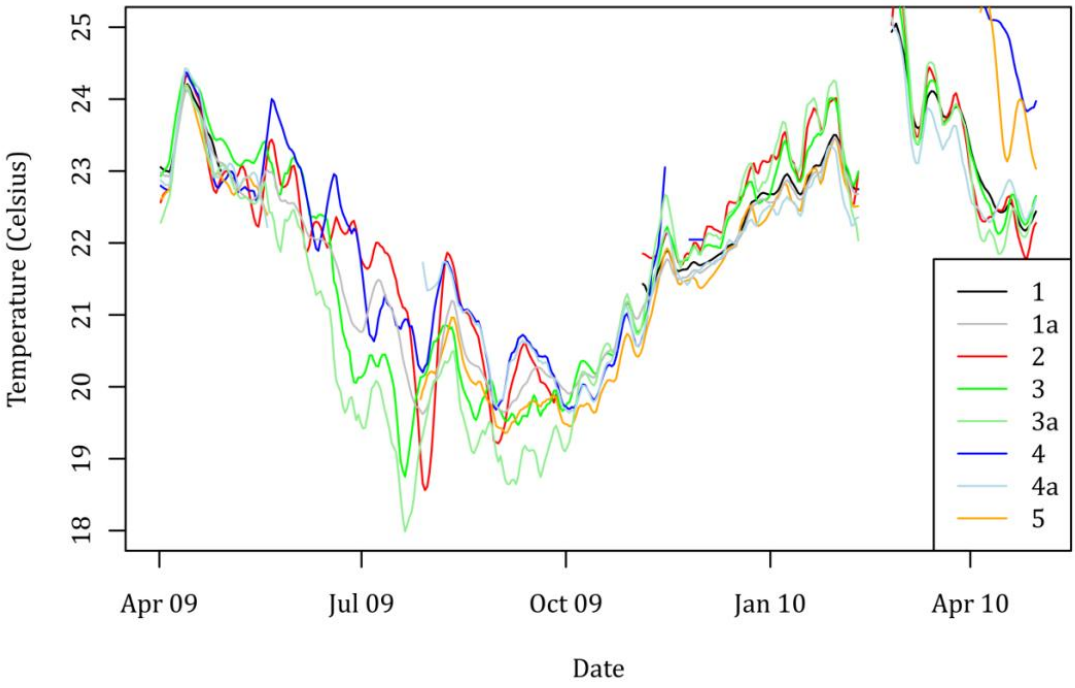


Figure 1 - 7 day temperature averages during the hatchery oyster gonad trial at 4m depth.

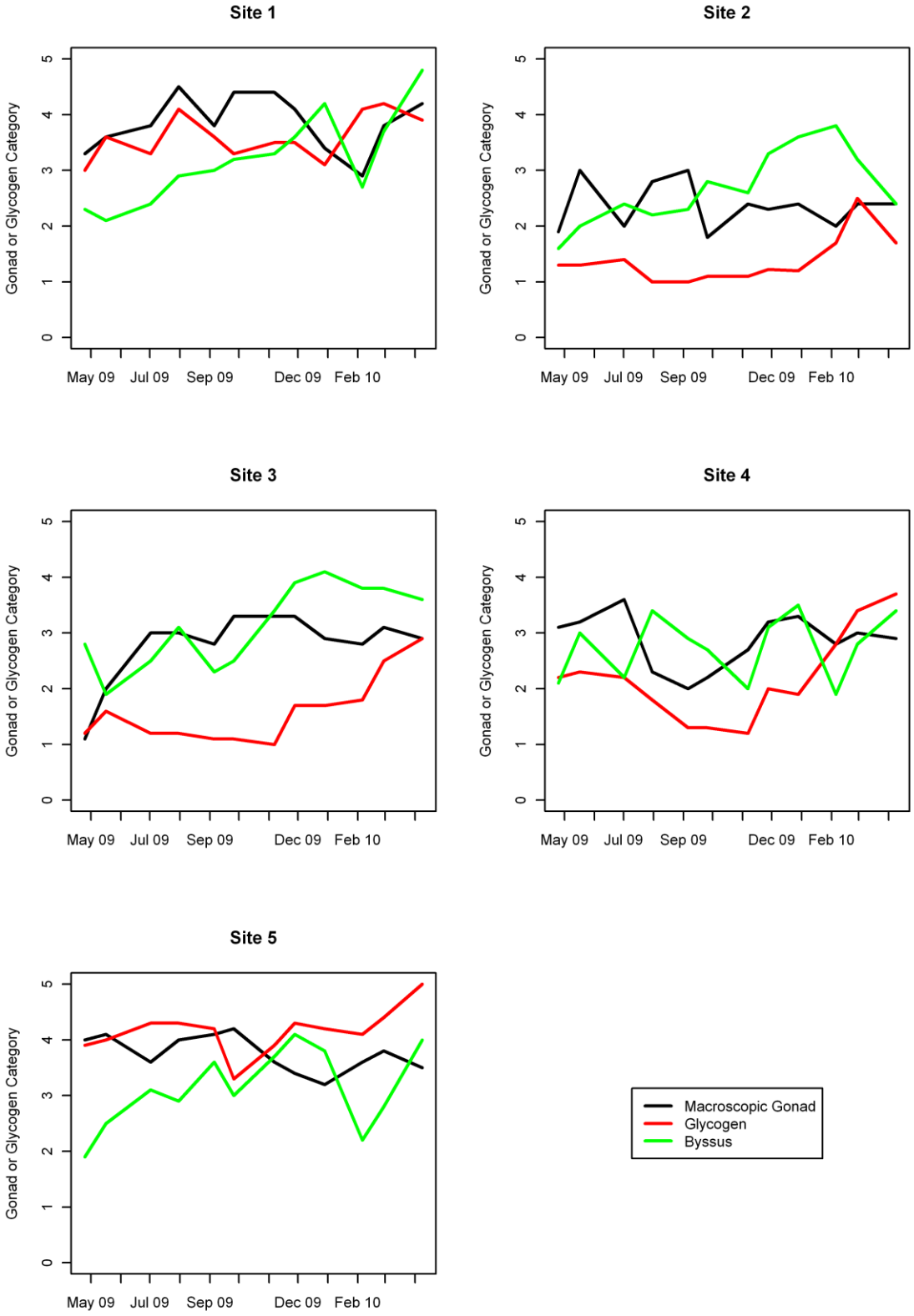


Figure 2 - A time series of glycogen, byssus and gonad indices for the hatchery produced oysters.

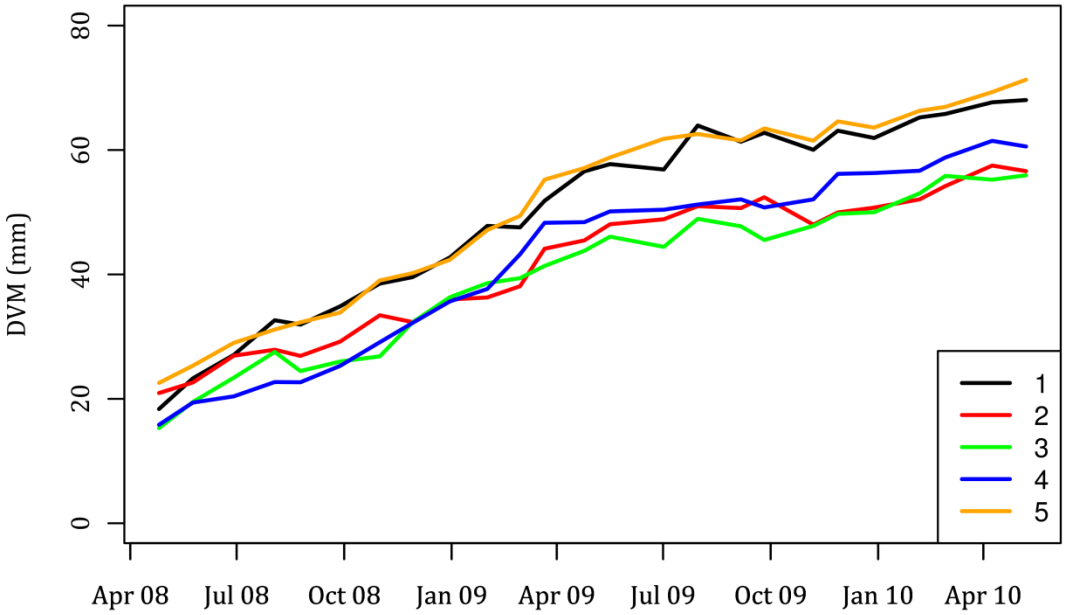


Figure 3 - Growth throughout the study period as measured in DVM.

Figure 3 shows growth through time. The points on this figure were obtained by calculating the median DVM at each site for each sampling trip. There is a clear difference between sites, which is consistent over both DVM and APM measurements. From Figure 3 it is also apparent that some the differences in growth rates observed on the first monitoring trip reversed.

In summary:

- Sites 1 and 5 provided the highest growth and high survival rates.
- Site 4 provided high long term growth rates and the highest survival rates. Relocating oysters here after an initial period at Sites 1 or 5 may be beneficial. It may also be possible that the initial low growth period was due to chance in which case relocation would be unnecessary.
- Sites 2 and 3 had the lowest initial and long term growth rates and lowest survival.
- The difference between the sites amounted to almost a year of growth time.

4.8.3.2 Pre-operative Conditioning

Pre-operative conditioning was conducted at Sites 3 and 4 from 25 March to 21 May 2010. The major difference observed was that Site 3 provided better conditioning results which suggests poorer growing conditions at Site 3.

Figure 4 shows the temperature measured by the shallow (1.7m) and deep (7m) loggers. The temperature measured at medium depth at Site 4 during this period is erroneous (the sensor consistently measured 3 degrees higher than all other loggers deployed during this period). The elevated temperature in 7m at Site 3 from early April onwards also seems likely to be erroneous given the normal temperature measured in 1.7m (and at the medium temperature; results not shown).

The temperature at 1.7m (at which most trials were conducted) was similar during the first few weeks of the trial after which Site 3 cooled down whilst Site 4 remained at a similar temperature for the rest of the trial. The final difference was approximately one degree celsius.

Two environmental samples were conducted during the trial period. Initially dissolved oxygen was 0.5mg higher at Site 4 but during the second sample trip no difference was detected. No difference in Chlorophyll A was detected.

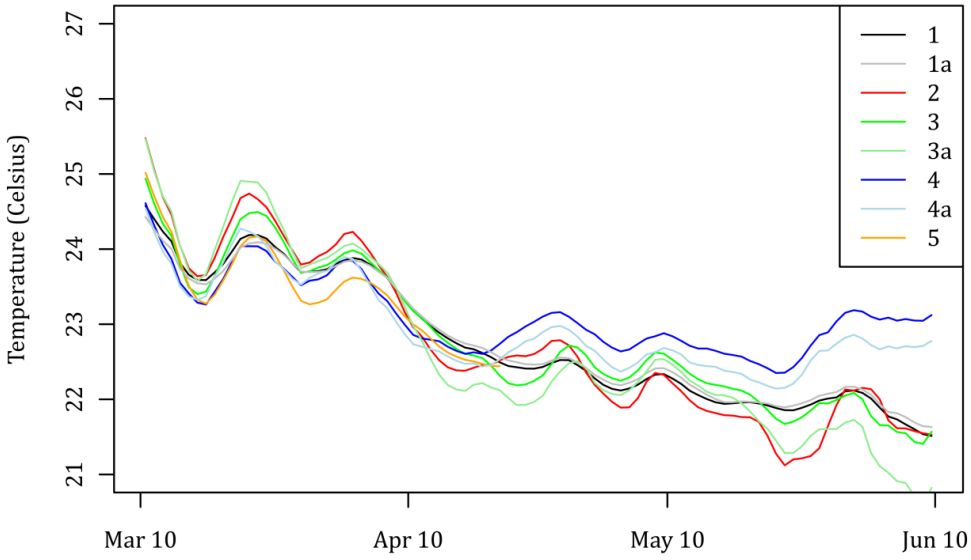
The data suggests that:

- Site 4 had higher temperatures (at the relevant depth); and
- higher dissolved oxygen levels.

This is indicative of better growing conditions and hence consistent with the trial findings of lower seedability at Site 4.

Due to the infrequent sampling of DO and ChlA and problems with the temperature loggers these results are only suggestive. The difference observed between these sites appears to be atypical (see Environmental Section). Consequently it is suggested that temperature be monitored during the pre-operative period to determine whether the conditioning period should be extended. To determine an appropriate system for this it would be necessary to collect data using the same conditioning method over a range of different temperature regimes.

PANEL A:



PANEL B:

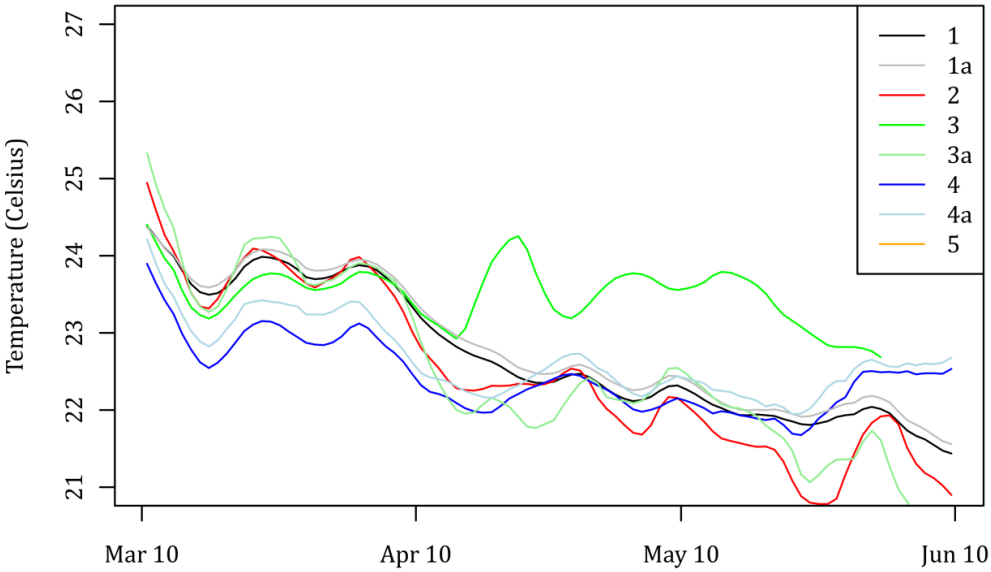


Figure 4 - Temperature measured at 1.7m (panel A) and 7m (panel B) during the pre-operative conditioning period. Note: Data from Site 5 was not available for the entirety of this period.

A pair wise comparison revealed that Sites 1, 1A, 4, 4A and 5 had elevated readings of Chlorophyll A, relative to Sites 2, 3 and 3a.

4.8.3.3 Environmental Effects of Pearl Production

4.8.3.3.1 Pearl Production Variation Between Sites

Table 1 summarises site characteristics affecting pearls. Early mortalities were low at Sites 1 and 5 and high at Sites 2, 3 and 4. Early mortalities can be attributed to seeding and normal mortality rates. The "Seeding" column shows the mortality rate attributed directly to the early seeding effect as obtained by subtracting site specific normal mortality rates from the early mortality rate.

Total mortalities were lowest at Sites 2, 4 and 5 with Site 3 having substantially higher mortality. By subtracting the seeding mortality the normal mortality rate is obtained – this would be of primary interest when considering the effect of longer growth periods on mortality.

The conversion rate (pearl production rate/success rate) of seeded oysters into pearls was highest at Sites 1 and 5 and lowest at Site 3 (due to the mass mortality event). The size of the pearls was largest at Site 5, followed by Sites 1 and 3. Pearl production combined with pearl size resulted in the largest revenue per seeded oyster at Sites 1 and 5.

Table 1. Pearl production characteristic summary of the 5 sites. The best sites for each category are highlighted in bold.

	Early Mortalities		Total Mortalities		Pearls		Revenue per
Site	Total	Seeding	Total	Without Seeding	Production	Size (mm)	Seeded Oyster
1	**5%**	**0%**	26%	26%	**54%**	7.03	**$4.37**
2	13%	8%	**23%**	**15%**	48%	6.69	$3.28
3	14%	7%	34%	27%	40%	6.98	$3.04
4	11%	7%	**22%**	**15%**	52%	6.82	$2.47
5	**7%**	**3%**	**19%**	**16%**	**53%**	**7.32**	**$4.77**

Sites 1 and 5 were coolest during this period (to April 1, 2011), suggesting that cooler temperatures may reduce early mortalities. There is no clear temperature explanation for overall mortality, in particular Site 1 which had the highest mortality, (excluding whale mortality) had temperatures throughout the period that were average compared to the other sites.

The difference between sites is substantial, with almost twice the revenue (per seeded oyster) at the best performing site (Site 5) than at the worst performing site in this particular project (Site 4). Nevertheless, this is a comparative result for Akoya oysters and does not necessarily show that the poorer performing sites are not economically viable. Neither does it indicate how the sites may perform when producing pearls from other species such as the blacklip (*P. margaritifera*). Understanding the environmental factors influencing this difference would enable appropriate sites to be selected for future operations and provide insight for managing oysters in response to environmental conditions.

4.8.3.3.2 Site Variability in Pearl Size

The earlier analysis of seeding in Section 4.6 found that pearl size was significantly related to nucleus size, site and oyster growth. A GLM was conducted of pearl size against site and nucleus size. The coefficients are shown in Table 2 (Site model). This indicates that, for example a pearl at Site 5 is on average 0.27mm larger than a pearl at Site 1 (0.71-0.44=0.27) and for each 0.1bu (0.30mm) increase in nucleus size the pearl becomes 0.24mm larger (2.40 x 0.1=0.24). Nucleus sizes varied by 0.3bu (0.9mm) indicating that nucleus size explains 0.72mm of total variation in pearl size (2.4 x 0.3=0.72). This is a similar magnitude to the difference between sites (0.71mm between Sites 2 and 5) indicating that the nucleus size range used in this study is as important as the chosen site.

The seeding analysis in Section 4.6 also showed that oyster growth had a significant effect on pearl size. A GLM that includes width increase in addition to site and nucleus size was conducted. The coefficients are shown in Table 2 (Site and growth model). With this model, the part of the site effect due to site differences in growth is now captured by width increase. Note that width increase captures pearl variability due to both variations in growth between, and within sites. This GLM indicated that site choice alone affected pearl size by 0.55mm (the difference between Sites 2 and 5), whereas growth affected pearl size by 0.76mm (the difference between an oyster with no increase in width and an oyster with a 30mm width increase). The difference due to nucleus size was similar to the previous model at 0.75mm, indicating that all three effects in the range considered or observed, are of equal importance for pearl size. Crucially, oyster growth is as important as some other, unknown difference between the sites.

Table 2. Coefficients for the GLMs discussed in the text. The mean value and standard error for each coefficient estimate are provided.

	Site Model		Site and Growth Model	
Effect	Mean	SD	Mean	SD
Intercept	2.5	0.4	1.7	0.4
1	0.44	0.05	0.33	0.05
2	0.00	0.06	0.00	0.05
3	0.26	0.06	0.27	0.06
4	0.13	0.06	0.08	0.05
5	0.71	0.05	0.55	0.05
Nucleus	2.40	0.22	2.50	0.2
Width Increase	-	-	0.025	0.0026

4.8.3.3.3 Environmental Data

The temperature data during the pearl growth period is shown in Figure 5. This period was characterised by a cold year followed by an extremely warm year. Unfortunately growth data was not collected during this period – only the initial and final sizes were measured – hence the effect of these two different seasons cannot be examined in any detail.

The other measured biogeochemical parameters were recorded manually on each trip (approximately once per month), hence this data series is sporadic and unable to detect small variations between sites. In the previous chapter it was established that no statistically significant difference between the sites could be detected from this data. Hence, in the following analysis we concentrate solely on temperature.

4.8.3.3.4 Temperature Trends

Overall, the differences between temperatures were limited and the range between the warmest and coolest site was generally less than 1 degree with a few notable exceptions – some of which are suspected to be erroneous.

During the early mortality period the temperatures at Sites 1 and 5 were the lowest of all sites. This suggests that low temperatures may help reduce post-operative mortalities as these were also lowest at Sites 1 and 5.

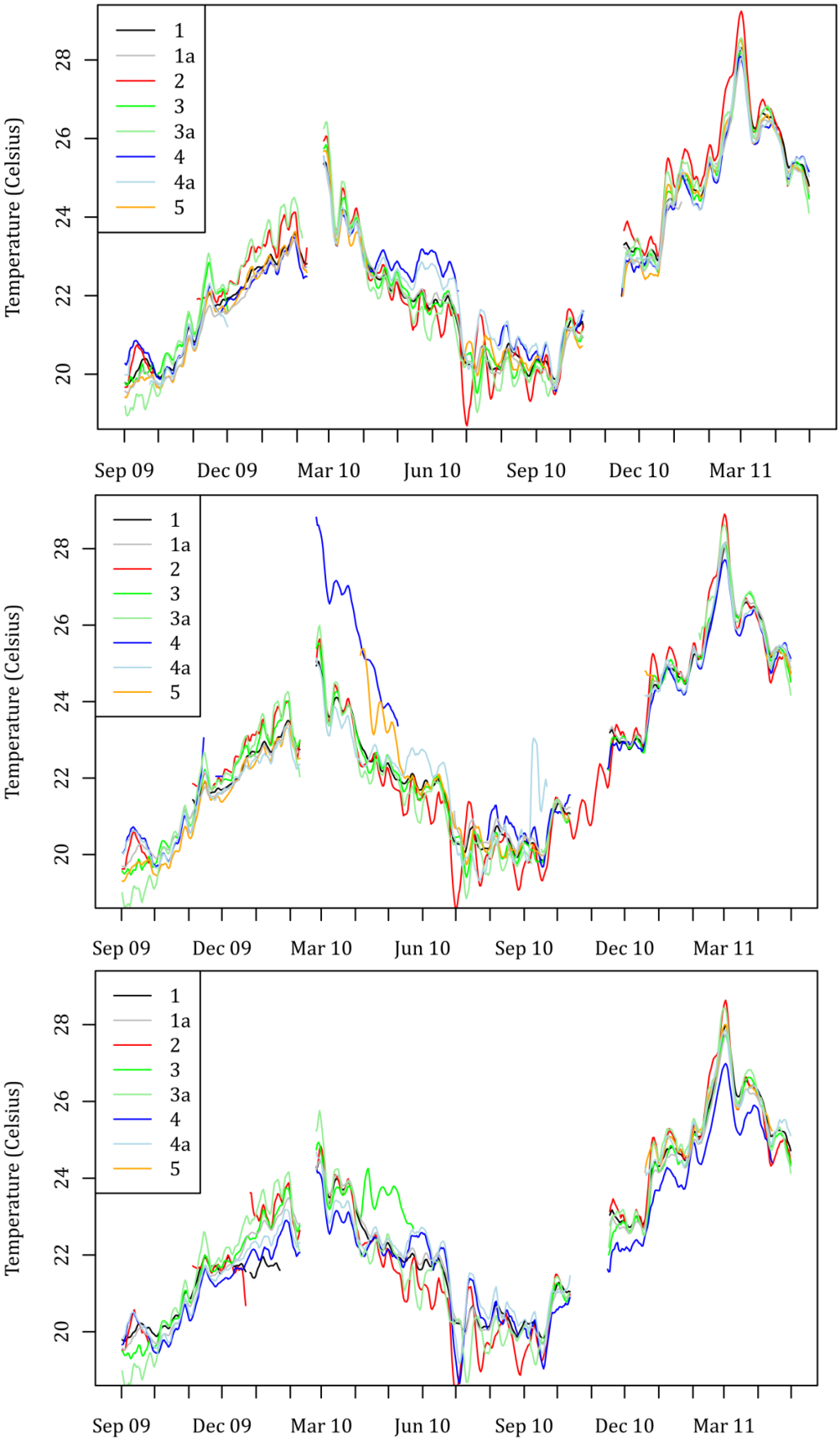


Figure 5 - Temperatures at 2m (top panel), 4m (middle panel) and 7m (bottom panel) from seeding to harvest.

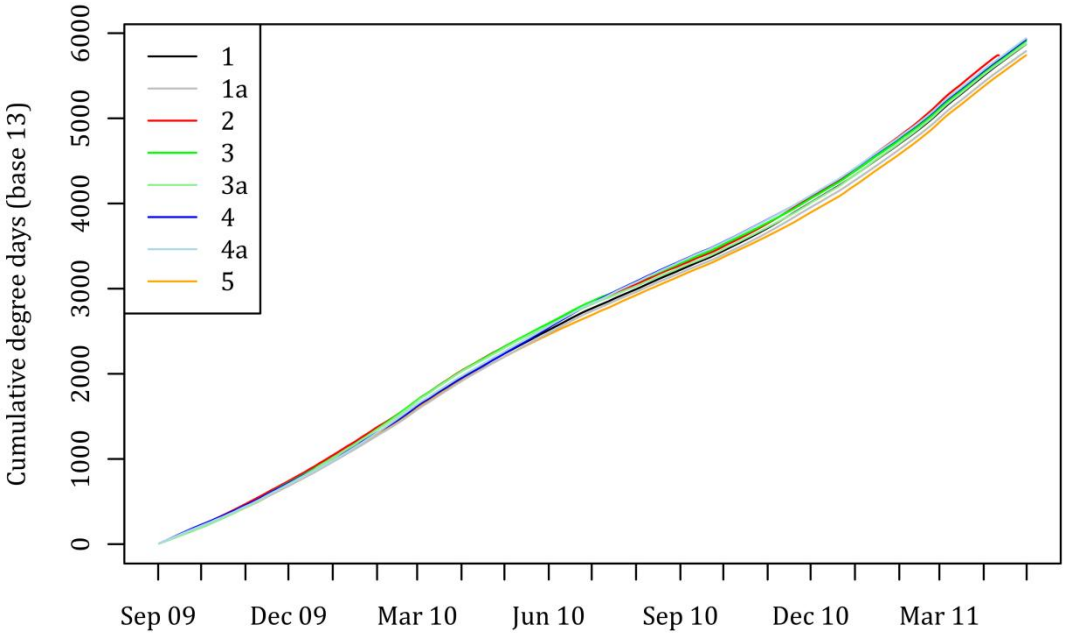


Figure 6 - Cumulative degree days (base 13) at 2m during the pearl growth period.

Table 3. Columns 2, 3 and 4: Mean difference from daily mean temperature of all sites. A positive value indicates a site was consistently warmer than all other sites, a negative value indicates it was consistently cooler. For clarity negative values are in bold. Columns 5, 6 and 7: The mean daily temperature range (highest temperature minus lowest temperature).

Column #	2	3	4	5	6	7
	Mean Difference			Mean Daily Range		
Site	2m	4m	7m	2m	4m	7m
1a	**-0.15**	0.06	0.02	0.69	0.55	0.40
1	**-0.01**	**-0.08**	0.05	0.80	0.52	0.49
2	0.12	**-0.06**	**-0.05**	0.89	0.75	0.62
3a	**-0.01**	**-0.13**	0.05	1.27	1.03	0.84
3	0.00	**-0.04**	**0.14**	0.80	0.61	0.58
4a	0.11	**-0.04**	0.07	1.27	1.45	0.86
4	0.05	0.40	**-0.28**	1.21	1.03	0.76
5	**-0.10**	0.01	0.14	0.80	0.69	0.60

4.8.3.3.5 Temperature Deviations

Table 3 shows the average difference in the temperature at each site from the mean temperature at all sites. These differences are negligible, however Sites 1, 1a and 5 (which had the lowest mortality and largest pearls) appear to be cooler in shallow water and warmer in deeper water. However due to the small temperature differentials and the accuracy of the instruments, this should be taken as purely suggestive.

Table 3 also shows the mean difference between the daily high and daily low temperature. Across all depths, Sites 1, 1a and 5 routinely had the lowest temperature range/variability.

4.8.3.3.6 Accumulated Temperature Differences

Figure 6 shows the cumulative degree days at the sites. As previously discussed, this gives an indication of the potential growth at each site. From this figure it is apparent that all sites tracked similarly, in fact, the largest difference of 130 degree days was between Site 1 and Site 1a.

4.8.3.3.7 Conclusions

It was shown that nucleus size, oyster growth and site variations not captured by oyster growth were all of equal importance in determining final pearl size. By grouping oysters of a similar size and seeded with similar size nuclei, farmers could estimate pearl size without sampling (which costs $2.50 to $5.00 lost revenue per sampled oyster) and selective harvesting would also be a possibility.

Due to the similarity of the sites it was difficult to determine the environmental factors driving the differences in pearl growth and oyster survival. The biogeochemical data was not collected with sufficient temporal resolution to determine site differences; consequently the only explanatory parameter was temperature.

Sites 1, 1a and 5 are slightly cooler in shallow water and warmer in deeper water than the remaining sites (although Site 3 also followed this trend). More importantly, Sites 1 and 5 also had the lowest range in daily temperatures and showed fewer deviations from the mean temperature across all sites. This was the main difference found that was consistent with the pattern of lower mortality and higher growth at these sites.

In summary:

- Nucleus size, oyster growth and site variations had equal impact on pearl size.
- Nucleus size and oyster growth combined could provide a good estimate of pearl size.
- Sites were nearly indistinguishable for the monitored environmental parameters.
- Low daily temperature ranges and resistance to small local scale temperature fluctuations appears to be linked to higher pearl production and pearl growth.

4.8.4 Discussion

The examination of pearl production at a range of culture sites and the relationships with environmental variations shown at those sites has revealed several interesting findings. Water temperature appears to affect post-seeding mortality with a more stable and cooler temperature profile also being more conducive to higher pearl production rates (from seeded oysters) and faster nacre growth. This is interesting in that nacre growth is generally more rapid at higher temperatures and slower at lower temperatures when lustre improves. Hence the industry focus on harvesting pearls during annual periods of low water temperatures. In the current project, recent water temperatures have been extremely high and this may well have been too high for oysters to function normally

without being temperature stressed. Therefore it would then make sense for cooler sites during the period to actually perform better as the lower temperatures were still within comfortable tolerance limits for the Akoya oysters.

The range of environmental parameters monitored during this project does not appear to have been extensive enough to identify clearly the factors causing substantial differences in the pearl production capability at each site. While our data clearly shows differing economic returns for effort in the current pearling trials, the variability in environmental parameters that were measured between sites has been minimal. This suggests that other factors, which tend to vary between sites, such as suspended or dissolved solids and fouling, may be affecting oyster survival and growth as well as nacre growth. Future development work should incorporate research that considers such aspects more thoroughly than could be attempted in this project. For example, the South Sea Pearl industry conducts enormous amounts of oyster cleaning, costing millions of dollars annually, to ensure that fouling organisms do not inhibit pearl growth.

5. BENEFITS AND ADOPTION

Prior to this project, there was very limited data available on water temperatures at the Abrolhos Islands gathered simultaneously from different locations. The monitoring of environmental parameters by this project has provided environmental data that has been utilised by pearl farmers to modify existing husbandry techniques. Furthermore, it has allowed farmers to carry out structured experiments aimed at optimising culture activities at different sites.

These culture activities or operations include:

- Annual seeding time.
- Timing for hatchery production runs.
- Broodstock monitoring which potentially allows a hatchery run at any time of year.
- When to harvest pearls, i.e. pearl growing time (time after seeding).
- Identifying the best times to handle shell during the year.
- Pre-operative conditioning of shell.

Choosing a time to conduct a hatchery run is critical. Shell size at seeding can be increased, or age at seeding reduced, by placing oysters on the farm at a time that will allow fast growth, so growth occurs over two summers and one winter instead of two winters and one summer. Getting spat out of the hatchery at the right time increases the opportunity of shortening growout time and getting them to a seedable size as soon as possible. Hence, a short operational time for the hatchery at the optimum time of year, means reduced costs for shell growout and a quicker time span to the market for investors. In the current project, oysters were seeded mainly at 20 - 21 months of age and then after a further 20 months of culture, pearls were harvested. However, some of the faster growing oysters, not reared as part of the research trial, were seeded by farmers at around 17 months of age with good results.

Basic broodstock conditioning and monitoring work has provided an insight that allows farmers to increase development of gonads within the oyster thereby increasing the potential success of hatchery work and extending the possible time span for hatchery production runs. Data on gonad development and assessment has been analysed to provide improved knowledge of optimum spawning times, a key factor in determining hatchery culture success.

Water temperature has an effect on the whole cycle of shell growth and pearl production. Traditional summer months in Australia – December to February – do not necessarily reflect the warmest water temperatures throughout the year. The current project has meant that farmers can now analyse water temperature data and decide the best times to carry out each activity. In light of the recent unusually warm water experienced at the Abrolhos Islands, and its effect on pearl production at the various sites, farmers are more aware of how to manage such situations and to benefit from them. With good nacre and lustre quality being especially dependent on water temperature, this project has allowed farmers to analyse the water temperature profiles and harvest pearls at the time when nacre should be at its best. Unfortunately, environmental monitoring did not identify any direct causes of variation between the sites but nevertheless, has provided substantial information on the indirect impact of a

range of factors at the culture sites. This has raised the level of knowledge and made farmers much more aware of the need to carefully control pre-seeding and post-seeding oyster handling during each day, given the daily variations illustrated by the data herein. Akoya oysters are sensitive to fluctuating water temperatures, especially when being handled. By identifying unsuitable conditions and being able to predict water temperatures, the risk of any oyster mortalities can be minimised.

Identification of a more efficient oyster conditioning program will now allow determination of the best times of year to commence pre-operative conditioning. By monitoring gonad condition, scheduling of seeding technicians for the best possible time, is achievable.

The project also provided an opportunity to visit an existing Akoya hatchery in NSW. Information was exchanged and will be used to further develop hatchery techniques in WA. Having access to experts from Japan to undertake seeding and harvesting has also been invaluable. Forging relationships, both national and international is beneficial in increasing the network of shared information.

Information gathered from project monitoring has shown that all sites are different in some way and need individual consideration, thereby potentially providing different and varying benefits. Some shell (oysters) grow well at one site, whereas they may condition better at another. With this knowledge, farmers can now decide when and if they should move shell subject to the environmental conditions, and ensure they are getting the best out of each site, while decreasing production time and producing better quality pearls. It also provides an opportunity for farmers to work cooperatively to achieve better results during each culture stage and maximise the characteristics of individual sites.

All research has focused on determining whether Akoya oysters are suitable for commercial pearl production at the Abrolhos Islands in WA. The potential may be a new industry offering economic benefits to operators through leveraging its existing investment in black pearl infrastructure, offering economies of scale and improved cash flow, turnover and profitability due to lower cycle time from spat to pearl in Akoya oysters. The current project has shown that the success rate for seeded oysters producing pearls can be increased by up to 15% through appropriate site choice, timing of seeding operations and better seeding operations. This translates directly to the same percentage increase in oyster revenue.

6. FURTHER DEVELOPMENT

The collective information gained can be adapted to techniques for the development of current aquaculture activities and the data produced will assist in establishing new aquaculture opportunities for other species at the Abrolhos Islands.

The extensive and detailed environmental research undertaken at several established aquaculture sites has already been useful in further developing the existing blacklip pearl oyster industry at the Abrolhos Islands. Monitoring of sites also included some examination of the physical attributes of each site, sea floor types i.e. sand, coral; and site depths. While temperature variability between and within sites was quite minimal, various trials indicate that sites generate different results for the various stages of oyster and pearl culture, which is presumably related to other aspects. Hence, future environmental work at the different sites should focus on identifying the causal factors at each site that may be responsible for different results. Strategies for quantifying such aspects then would need addressing. As seabed sediments, depths and currents vary somewhat between sites, one possible area of investigation should include dissolved and suspended sediments. Suspended silts loads are known to impact on oyster growth, especially juveniles. Similarly, proximity to hard seabed (including coral reef) and the communities they support, is also a factor in determining the level of biological fouling that occurs. Fouling is known to detrimentally affect pearl production and clearly more attention to this aspect is required.

With the existence of a reliable hatchery facility, the development of selective breeding programs can now begin - based on utilising the best oysters from those already produced for this project. Data has been collected from oysters of known age – a huge benefit in initiating successful oyster production.

There are many benefits in breeding two lines of shell as differing aspects can be targeted, including aspects relevant to the two main lines of oysters utilised in pearl culture:

1. Host oysters (shell to produce pearl) which may have faster nacre laying quality; and
2. Saibo oysters (shell with good nacre used for mantle tissue).

One of the keys to successful Akoya pearl production is shortening the time from hatchery to producing a pearl. If this can be done in two years, using approaches such as a selective breeding program, then this will be of enormous benefit to pearl farmers.

Ongoing environmental awareness and monitoring will assist all pearl farmers and aquaculturists at the Abrolhos Islands in their understanding of environmental factors affecting Akoya oysters (and other species) in both positive and negative ways. This project has demonstrated a range of significant factors which have affected pearl production at the various sites. While some are directly driven by the environment present at each site, some aspects such as warm water, cool water and high primary productivity can be utilised at different stages of the culture cycle to optimise production potential. Other factors, indirectly linked to the existing environment (elevated holding temperatures and reduced DO levels in tanks), need to be managed and allowed for in handling of oyster stock, to ensure that current variability in conditioning and seeding practices is improved substantially in future.

7. PLANNED OUTCOMES

All outcomes will benefit the commercial sector, in developing non-maxima pearling at the Abrolhos Islands.

The research has focussed on determining whether Akoya is suited to commercial production at the Abrolhos Islands in Western Australia. The outcome may be a new industry offering economic benefits to operators through leveraging its existing investment in fishing ventures or black pearl infrastructure, offering economies of scale and improved cash flow, turnover and profitability due to lower cycle time from spat to pearl in Akoya oysters. Outcomes from the Akoya Project may easily be translated into benefits for the blacklip pearl industry at the Abrolhos Islands and may well provide further insight into the importance of certain parameters, including environmental ones, to the South Sea Pearl industry in Australia.

Development of appropriate broodstock selection, holding and conditioning practices on farm and at the hatchery includes the establishment of:

1. Selection criteria for oysters expected to satisfy projected market demands for Akoya

OUTCOME: The physical attributes, growth rates and internal nacre colour of shell have been recorded for our local species located at the Abrolhos Islands. This information helps us identify fast growing shell, good quality shell, acceptable gonad shape and good nacre colour of shell for mantle tissue which affects pearl colour greatly. Industry is currently targeting pearls averaging 9mm in size. Important relationships between oyster size and subsequent pearl size have been demonstrated with clear site differences evident. Such relationships are of considerable benefit in farm management and financial planning.

2. Identifiable potential broodstock

OUTCOME: The physical attributes recorded have allowed us to easily identify the potential broodstock with desirable characteristics from a wide range of shell, by referring to data that has been collected.

3. A system for the on farm holding, monitoring and conditioning of broodstock

OUTCOME: Broodstock holding, monitoring and conditioning systems have been designed and tested with results including spat production for the current project and in recent times, industry production of selected Akoya spat for direct industry use.

4. A holding system and operational technique for hatchery conditioning of pearl oysters in WA

OUTCOME: A hatchery based system was developed to feed and maintain oysters to ensure gonad quality is at its optimum level before spawning. Also by having shell in a controlled water temperature system, the timing of a spawning event can be controlled to some extent. While operations for conditioning were quite limited, the technique implemented has shown to be effective, especially where oysters have gonads that are not in an undeveloped state.

5. A practical scheme of gonad condition assessment and operational protocol for hatchery-based and farm-based broodstock

OUTCOME: This project has shown that gonad condition has great variability over the year, particularly between different sites. Correlation between visual and microscopic assessments of gonad condition were shown to be of minimal use in this project, however, further work on direct analysis of individual oyster samples by both methods is expected to clarify relationships between the two and allow a practical system to be implemented. A Photo Series scale was developed that easily allows gonad condition to be visually assessed by industry members.

6. Optimum pre-spawning and pre-seeding conditioning protocols for WA Akoya oysters

OUTCOME: Our trials have demonstrated techniques that have achieved successful outcomes both with hatchery spawnings (pre-spawning/broodstock conditioning) and with high seeding rates (pre-seeding/pre-operative conditioning). There is now a preferred system of conditioning shell for seeding which will be more efficient at ensuring the oyster is in optimum condition for seeding technicians. The conditioning is very important as it directly affects the operation, nacre quality and hence value of the pearl.

Development of formalised protocols and systems will be transferable from Akoya to black pearl culture and will assist industry development through improved spat supply capacity.

Exposure to proven pearl hatchery culture systems will increase hatchery staff knowledge of species variations and ability to adapt established culture systems to suit local species.

Completion and documentation of focused environmental monitoring and animal health, growth rates and pearl quality will lead to an assessment of the relationship between various environmental parameters and oyster and pearl production to maximise efficiency in site growout and husbandry practices.

8. CONCLUSIONS

The basis for any shellfish industry is a reliable supply of juveniles or spat and a developing Akoya industry in Western Australia is no different. Naturally occurring spat and older oysters have been used as a source of oyster stock in past years. The current project has investigated a range of aspects associated with the reliable production of suitable hatchery spat for the industry. As part of the hatchery process, mature adult oysters are required as broodstock. It is important, for the long term success of the local industry, that broodstock exhibiting desirable characteristics are used for reproductive purposes. Part of this project was aimed at identifying desirable Akoya broodstock based on the selection protocol developed as the first part of the current project. The use of a calculated Host Selection Criteria or value was completed but the results displayed minimal variability and appeared to be inconclusive at the time.

A macroscopic or visual method (Photo Series) of assessing gonad condition can be used effectively by industry to assist in sourcing broodstock for hatchery breeding or when assessing gonads during the pre-operative conditioning stages.

Even though broodstock trials could not be completed, they were sufficient to demonstrate that such work is time consuming, and costly but likely to provide at least some potential broodstock from which spat production is possible, but not guaranteed. Alternatively, utilising potential broodstock held on various farm sites, by monitoring water temperature and gonad condition is likely to be sufficiently reliable to be the most cost-effective approach provided the best broodstock sites are used; in this case, Sites 1 and 5.

Industry hatchery capability has been proven. Sufficient quantities of spat for the project were produced by one major spawning event at a commercial hatchery, demonstrating the benefit of using oysters with well developed gonads. Induced spawnings of Akoya oysters have been successfully conducted in recent years by the Abrolhos Islands industry during May - June.

Hatchery produced oysters at Sites 1 and 5 had the highest gonad indices with a significant proportion of the oysters in condition to spawn during the entire study period. Site 1 also had the highest wild oyster gonad index (Site 5 was not assessed). Sites 2 and 4 had evidence of spawning in September and July respectively. Sites 1 and 5 are the best sites to source oysters in spawning condition for hatchery requirements. Monitoring of glycogen condition appears to be of limited benefit in identifying good potential broodstock at these sites.

For oyster growout, Sites 1 and 5 provided the highest growth and high survival rates. Site 4 provided high long term growth rates and the highest survival rates. Sites 2 and 3 had the lowest initial and long term growth rates and lowest survival. The difference between the sites amounted to almost a year of growth time.

Furthermore, the best 3 growing Sites (Sites 1, 4 and 5) also had the best survival rates over the monitoring period.

The number (percentage) of oysters that could be seeded when they were assessed was the indicator for a successful pre-operative conditioning technique. Site 3 produced a much higher seeding percentage of oysters and was therefore a better site to condition oysters pre-operatively (site selection has the single most significant effect.). Increased density increases seedability, but a critical limit was not determined. Fortnightly byssus cutting produced the same results as weekly cutting. Generally the small conditioning basket (B) produced better results, but the large conditioning basket (P) divided horizontally (top-bottom) may have a site dependent positive effect. Longer conditioning periods increased seedability, but a finite limit was not determined.

Data from the Pre-Operative Conditioning Trial showed that measurement of a single parameter - weight, width or height - will give the best prediction of nucleus size. Data analysis work has shown that it is possible to use a predictive model to indicate the proportion of oysters to be seeded with each nucleus size as a function of weight.

It should be noted that experiments looking at interactions between the conditioning effects were not conducted. Seeding of oysters at the completion of the Pre-Operative Conditioning Trial showed that heavier oysters (generally larger) were seeded with larger nuclei, which is to be expected, given that technicians try to maximise the individual value of each pearl. Analysis of seeding data showed that technicians tended to increase the size of nuclei used over time, during a particular seeding session.

It is interesting to note that as a result of trials conducted in NSW, O'Connor et al., (2003) found that Akoya oysters could be seeded successfully when they had achieved a shell weight of approximately 30g or around 60mm in shell height. The current trial indicated similar results, with seeded oysters averaging 27.60g in weight and 59.87mm in shell height.

The 2008 year was marginally warmer in summer than 2009. There is some uncertainty about 2010 due to missing data; however it appears similar to 2008. Temperature differences between sites, years and depths were on a similar scale. Some decreases (0.25 - 0.31°C) in water temperature from 1.7m to 7m were seen at Sites 2, 3, 3a, 4, 4a. This suggests that water mixing at all sites is good, with some stratification apparently occurring at Site 4.

The summer of 2011 was remarkably warm with temperatures exceeding typical levels by 3-4 degrees. These trends were observed consistently across sites and depths. Sites 2 and 3a were the hottest with temperatures up to 1.2 degrees warmer than at other sites. Furthermore, Chlorophyll was significantly lower at Sites 2, 3 and 3a than elsewhere. The remaining factors did not vary significantly between sites. Salinity ranged from 34.07 to 34.98ppt; this is within the optimum range for Akoya oysters. While monitoring of DO is a useful tool especially during critical stages of pearl culture, it appears to have limited value as an environmental monitoring and management tool for industry.

In the current project, the best 3 culture sites (Sites 1, 4 and 5) also had the best survival rates over the monitoring period. Hence for the growout of oysters for pearl culture, Sites 1, 4 and 5 appear to be preferable. Additionally, as a source for broodstock, Sites 1 and 5 were likely to be the most productive. Alternatively, Site 3 produced a much higher seeding percentage of oysters (than Site 4) at the completion of pre-operative conditioning; and was therefore a better site for this aspect of pearl culture. As general oyster body condition is reduced during this process, it is indicative of a poorer site for oyster culture.

The conversion rate (pearl production rate/success rate) of seeded oysters into pearls was highest at Sites 1 and 5 and lowest at Site 3 (due to the mass mortality event). The size of the pearls was largest at Site 5, followed by Sites 1 and 3. Pearl production combined with pearl size resulted in the largest revenue per seeded oyster at Sites 1 and 5.

Nucleus size, oyster growth and site variations had equal impact on pearl size. Furthermore, the size of the seeded nucleus did not affect any aspect of oyster survival and pearl formation. Revenue of $8.78 per harvested pearl was obtained in the current project, with seeding costing directly $1/oyster. The recovery rate of pearls from seeded oysters was 35.2%. This pearl production rate is within an acceptable range of production returns and compares favourably to similar industries overseas. Revenue yielded from pearls cultured during this trial is not quite as high as anticipated, however it is also within an acceptable range and necessarily includes a wide range of oysters providing both negative and positive feedback on culture data. While this project has not attempted to analyse detailed operating costs and projected retail revenue, it is important to realise that commercial scale Akoya culture generally involves rearing large volumes of oysters. In this way, only sufficiently large oysters would be conditioned prior to seeding, with an established and reliable technique. Other smaller oysters would be discarded, hence the resultant pearl production percentages, pearl size and quality would all increase substantially. As the project data has shown, the success rate for seeded oysters producing pearls can be increased by up to 15% through appropriate site choice, timing of seeding operations and better seeding operations. This translates directly to the same percentage increase in oyster revenue.

Overall, combining the commercial scale approach outlined above and the potential improvements that can be implemented as a result of this project, revenue per pearl is expected to increase significantly, to a level that is much more acceptable to pearl farmers and to financial analysts.

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10. APPENDICES

10.1 Appendix 1: Intellectual Property

All information brought into this project or developed during this project is public domain.

10.2 Appendix 2: Staff

Administrative
Erica Starling, Latitude Fisheries Pty Ltd, 1 July 2007 – 10 July 2011.

Roseanne Oliveri, Latitude Fisheries Pty Ltd, 1 July 2007 - 10 July 2011.

Steering Committee
Dr Wayne O'Connor, Principal Research Scientist, Industry & Investment NSW, Port Stephens Fisheries Institute, Department of Primary Industry, NSW.

Dr Brian Jones, Senior Fisheries Pathologist, Fish Health Section, Animal Health Laboratory, Department of Fisheries, WA.

Principal Investigator
Geoff Diver, Diversity Sustainable Management, 1 July 2007 - 29 March 2008.

Derek Cropp, Aquatech Australia Pty Ltd, 1 April 2008 - 10 July 2011.

Co-Investigators
Pia Boschetti, Latitude Fisheries Pty Ltd, 1 July 2007 - 10 July 2011.

Steve Webster, Batavia Coast Maritime Institute, 1 July 2007 - 30 December 2007.

Derek Cropp, Aquatech Australia Pty Ltd, 1 July 2007 - 10 July 2011.

Project Researcher
Luke Neil, 25 August 2007 - 1 April, 2008.

Craig Koltasz, 1 February 2008 - 10 July 2011.

Industry
Pia Boschetti, Latitude Fisheries Pty Ltd, 1 July 2007 - 10 July 2011.

Murray Davidson, 1 July 2007 - 10 July 2011.

10.3 Appendix 3

10.3.1 Environmental Monitoring Data

The following tables and graphs provide details of the environmental monitoring and oyster shell databases recorded for this project.

Ongoing Water Quality Stage

Stage Description/Objective			
Measure water quality parameters and variations across all sites			
Start Date	November 2007	**Finish Date**	May 2011
Sites data collection occurring	Site 1-Easter Group, Rat Island, Abrolhos Pearls Site 1a- Easter Group, Rat Island, Abrolhos Pearls Site 2-Easter Group, Rat Island, Radar Holdings Site 3- Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 3a-Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 4- Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 4a-Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 5- Pelsaert Group, Pelsaert Island, Sea Urchin		
Depths data collected at each site	Deep -7.5 m Middle-4.0m Shallow-1.7 m		

Data collected	**Frequency**	**Method of collection**	**Programs used**
i. Site ii. Visibility iii. DO (mg/L) iv. DO % v. Salinity (PPT) vi. Chlorophyll A vii. TDS viii. Temp ix. pH x. Turbidity	Monthly (4- 6 weeks)	YSI/Horiba Meter YSI/Horiba Meter YSI/Horiba Meter Aquafluor YSI/Horiba Meter YSI/Horiba Meter YSI/Horiba Meter YSI/Horiba Meter	Microsoft Excel
Data collected	**Frequency**	**Method of collection**	**Programs used**
Temperatures Light	30 minutes	HOBO Loggers	HOBO ware Pro The following parameters are compiled in a Microsoft Excel graph after each monitoring trip : • Max Temperature • Minimum Temperature Average Temperature Standard Deviation Maximum Lux • Minimum Lux • Average Lux • Standard Deviation Lux

Stage 1- Broodstock Condition Trial

Stage Description/Objective			
Measure broodstock condition in relation to site and environmental variations			
Start Date	November 2007	**Finish Date**	November 2008
Sites data collection occurred	Site 1-Easter Group, Rat Island, Abrolhos Pearls Site 1a- Easter Group, Rat Island, Abrolhos Pearls Site 3- Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 3a-Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 4- Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 4a-Pelsaert Group, Pelsaert Island, Latitude Fisheries		

Data collected	**Frequency**	**Method of collection**	**Programs used**
i. Site ii. Total weight (g) iii. Meat weight (g) iv. Length (DVM) (mm) v. Width (APM) (mm) vi. Thick (WD) (mm) vii. Gonad Value viii. Glycogen Value ix. Akoya host selection criteria value x. Number of Polychaete	Monthly (4- 6 weeks) 30 oysters per site	Project Researcher	Microsoft Excel

Stage 2- Spat Growout

Stage Description/Objective			
Measure spat survival and growth in relation to site and environmental variations			
Start Date	February 08	**Finish Date**	April 10
Sites data collection occurred	Site 1-Easter Group, Rat Island, Abrolhos Pearls Site 1a- Easter Group, Rat Island, Abrolhos Pearls Site 2-Easter Group, Rat Island, Radar Holdings Site 3- Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 3a-Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 4- Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 4a-Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 5- Pelsaert Group, Pelsaert Island, Sea Urchin		

Data collected	**Frequency**	**Method of collection**	**Programs used**
i. Site ii. Total site weight (g) iii. Length (DVM) (mm) iv. Width (APM) (mm) v. Thick (WD) (mm) vi. Shell nacre, colour and lustre^ vii. Gonad Value^ viii. Glycogen Value ^ ix. Akoya host selection criteria value^	Monthly (4- 6 weeks) 30 oysters per site	Project Researcher	Microsoft Excel

Stage 3 - Conditioning Trial – Seeding

Stage Description/Objective			
Measure quality, survival , growth and conditioning development in oyster in relation to site, treatment and environmental variations			
Start Date	March 2010	**Finish Date**	May 2010
Sites data collection occurred	Site 1-Easter Group, Rat Island, Abrolhos Pearls Site 1a- Easter Group, Rat Island, Abrolhos Pearls Site 2-Easter Group, Rat Island, Radar Holdings Site 3- Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 3a-Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 4- Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 4a-Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 5- Pelsaert Group, Pelsaert Island, Sea Urchin		

Data collected	**Frequency**	**Method of collection**	**Programs used**
Site i. Total site weight (g) ii. Length (DVM) (mm) iii. Width (APM) (mm) iv. Thick (WD) (mm) v. Shell nacre, colour and luster^ vi. Gonad Value^ vii. Akoya host selection criteria value^ viii. Able to Open ix. Tough Gonad x. 2 much Byssus xi. 2 much Spawn xii. Seeded (Y/N) xiii. Nuclei Size xiv. Wet Wt	Start of conditioning trial During seeding	Project Researcher	Microsoft Excel

Stage 4 - Growout Seeded Oysters

Stage Description/Objective			
Measure retention, survival , growth and pearl development in seeded oyster in relation to site and environmental variations			
Start Date	September 09	**Finish Date**	May 2011
Sites data collection occurred	Site 1-Easter Group, Rat Island, Abrolhos Pearls Site 1a- Easter Group, Rat Island, Abrolhos Pearls Site 2-Easter Group, Rat Island, Radar Holdings Site 3- Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 3a-Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 4- Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 4a-Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 5- Pelsaert Group, Pelsaert Island, Sea Urchin		

Data collected	**Frequency**	**Method of collection**	**Programs used**
Site i. Total site weight (g) ii. Length (DVM) (mm) iii. Width (APM) (mm) iv. Thick (WD) (mm) v. Shell nacre, colour and luster^ vi. Gonad Value^ vii. Glycogen Value ^ viii. Akoya host selection criteria value^ ix. Pearl growth	TBA	Project Researcher	Microsoft Excel

Stage 5 - Pearl Harvest and Assessment

Stage Description/Objective			
Measure quality of pearls in relations to site and environmental variations			
Start Date	September 2010	**Finish Date**	May 2011
Sites data collection occurred	Site 1-Easter Group, Rat Island, Abrolhos Pearls Site 1a- Easter Group, Rat Island, Abrolhos Pearls Site 2-Easter Group, Rat Island, Radar Holdings Site 3- Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 3a-Pelsaert Group, Pelsaert Island, Abrolhos Pearls Site 4- Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 4a-Pelsaert Group, Pelsaert Island, Latitude Fisheries Site 5- Pelsaert Group, Pelsaert Island, Sea Urchin		

Data collected	**Frequency**	**Method of collection**	**Programs used**
Site i. Total weight (g) ii. Length (DVM) (mm) iii. Width (APM) (mm) iv. Thick (WD) (mm) v. Pearl nacre, colour and luster^ vi. Pearl growth vii. Pearl quality	TBA	Project Researcher	Microsoft Excel

Example of Data Collected
Hobo Loggers Summary

Sample trip	Site	Number of Samples	First sample Date Time		Last Sample Date	Time	Max Temperature	Minimum Temperature	Average Temperature	Standard Deviation	Maximum Lux	Minimum Lux	Average	Standard Deviation
14	1-d	1531	31/12	1124	1/2	831	23.68	22.24	23.04	0.247	15844.5	0.0	1107.6	1800.9
14	1-m	1532	31/12	1125	1/2	907	23.68	22.14	22.96	0.282	22044.6	0.0	1126.2	2330.0
14	1-s	1532	31/12	1126	1/2	907	24.84	22.05	22.97	0.404	49600.3	0.0	1725.4	4181.9
14	1a-d	1533	31/12	1043	1/2	907	23.58	22.24	22.92	0.233	4994.5	0.0	551.1	785.1
14	1a-m	1533	31/12	1043	1/2	907	23.77	22.14	22.89	0.297	46844.8	0.0	2322.8	5032.7
14	1a-s	1533	31/12	1044	1/2	907	23.97	22.05	22.90	0.361	12400.1	0.0	760.1	1305.3
14	2-d	1528	31/12	1203	1/2	737	24.26	22.24	23.29	0.404	8266.7	0.0	718.6	1072.4
14	2-m	1528	31/12	1203	1/2	737	24.16	21.95	23.15	0.406	11022.3	0.0	834.0	1298.1
14	2-s	1528	31/12	1202	1/2	737	24.84	22.24	23.45	0.460	20666.8	0.0	615.4	1249.8
14	3-d	1497	31/12	901	31/1	1308	23.87	21.86	22.88	0.347	15155.7	0.0	748.6	1356.5
14	3-m	1497	31/12	859	31/1	1308	24.64	21.76	22.94	0.438	24800.2	0.0	691.2	1539.4
14	3-s	1497	31/12	859	31/1	1308	25.61	22.05	23.21	0.540	5166.7	0.0	193.5	321.4
14	3a-d	1497	31/12	728	31/1	1140	25.03	22.43	23.40	0.503	11022.3	0.0	1118.7	1609.9
14	3a-m	1497	31/12	722	31/1	1140	25.71	21.86	23.43	0.638	7577.8	0.0	644.9	962.7
14	3a-s	1497	31/12	723	31/1	1140	26.78	22.43	23.70	0.756	55111.5	0.0	3287.3	6328.4
14	4-d	1526	30/12	1916	31/1	1351	23.48	21.28	22.38	0.392	9300.1	0.0	431.4	780.1
14	4-m	1526	30/12	1916	31/1	1351	24.45	21.47	22.67	0.502	9644.5	0.0	482.1	857.7
14	4-s	1526	30/12	1914	31/1	1351	25.22	21.47	22.76	0.550	46844.8	0.0	728.0	2149.8
14	4a-d	1525	30/12	1938	31/1	1351	24.06	21.47	22.64	0.411	9300.1	0.0	769.9	1198.1
14	4a-m	1525	30/12	1937	31/1	1351	24.35	21.47	22.79	0.494	14466.8	0.0	1229.0	1831.3
14	4a-s	1525	30/12	1937	31/1	1351	25.32	21.47	22.92	0.606	26178.0	0.0	1002.9	1883.5
14	5-d	1494	31/12	800	31/1	1041	23.87	22.43	23.07	0.286	7233.4	0.0	672.5	1152.2
14	5-m	1494	31/12	800	31/1	1041	23.97	22.33	23.00	0.321	5511.1	0.0	388.0	566.7
14	5-s	1494	31/12	800	31/1	1041	23.87	22.43	23.07	0.286	7233.4	0.0	672.5	1152.2

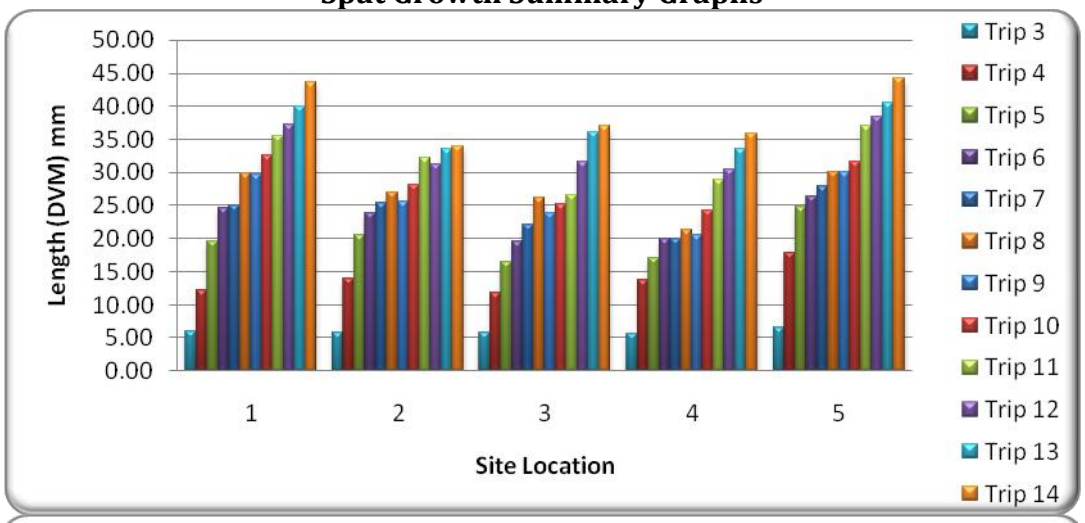
Example of Data Collected

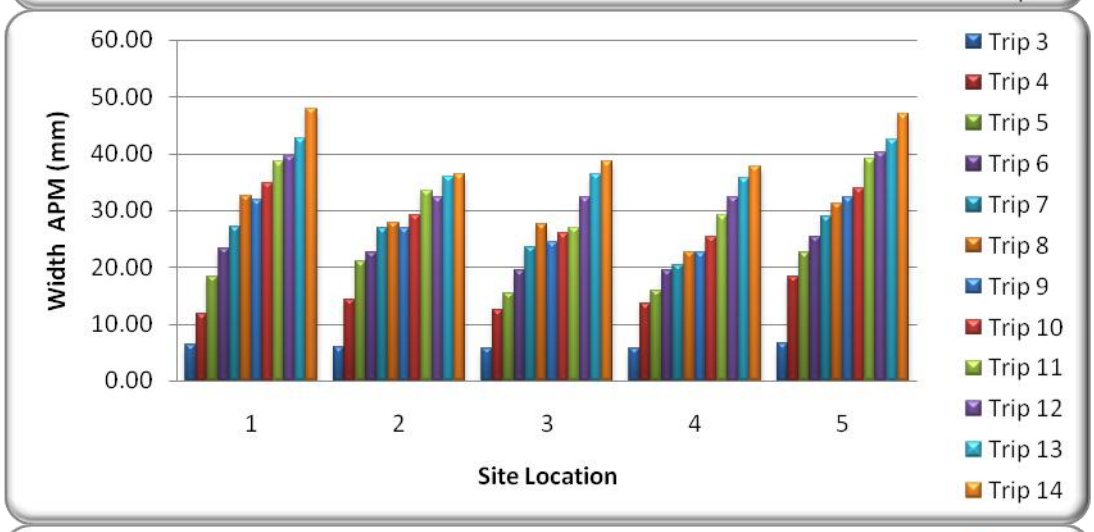
Spat Growth Summary

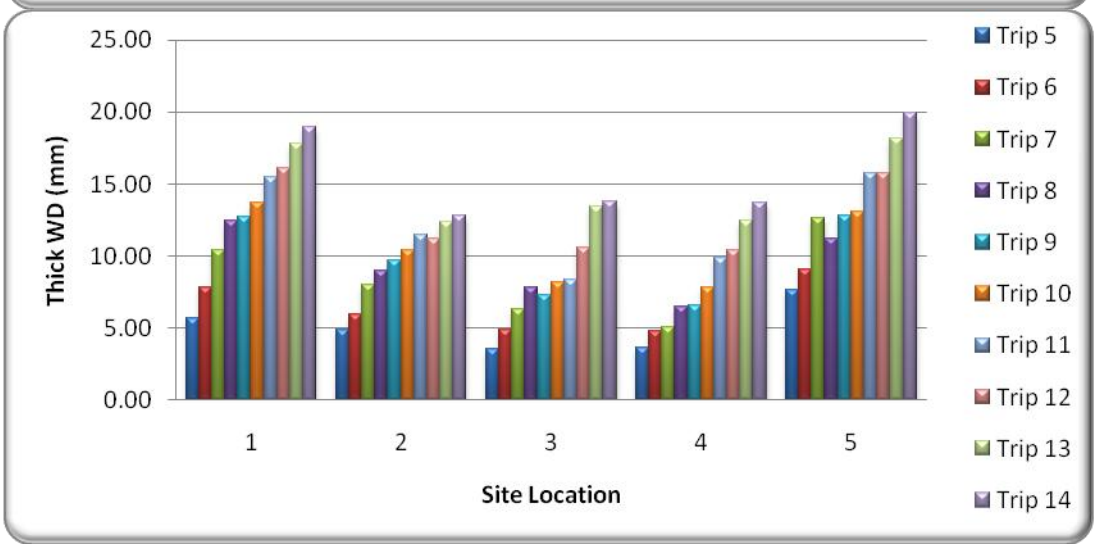
	Site	Trip 3	4	5	6	7	8	9	10	11	12	13	14
Length(DVM)	1	6.00	12.18	19.60	24.63	25.07	29.77	29.73	32.67	35.53	37.13	39.90	43.63
(mm)	2	5.90	13.93	20.63	23.93	25.33	27.00	25.57	28.13	32.13	31.27	33.53	33.87
	3	5.80	11.88	16.47	19.57	22.03	26.17	23.80	25.23	26.60	31.63	36.13	37.03
	4	5.70	13.69	17.13	19.93	20.00	21.37	20.60	24.17	28.90	30.40	33.50	35.87
	5	6.60	17.90	24.77	26.33	27.97	30.03	30.10	31.67	37.03	38.47	40.50	44.20
Width (APM)	1	6.30	11.77	18.37	23.30	27.07	32.63	31.93	34.87	38.53	39.57	42.73	47.80
(mm)	2	6.00	14.33	20.93	22.63	26.93	27.90	26.90	29.20	33.43	32.33	35.97	36.30
	3	5.80	12.40	15.30	19.50	23.40	27.53	24.47	26.00	26.83	32.30	36.33	38.60
	4	5.80	13.49	15.83	19.40	20.40	22.70	22.67	25.30	29.10	32.20	35.67	37.67
	5	6.60	18.39	22.57	25.33	29.00	31.13	32.30	33.87	39.03	40.20	42.37	46.97
Thick (WD)	1			5.67	7.80	10.43	12.47	12.73	13.70	15.50	16.13	17.80	18.97
(mm)	2			4.87	5.93	8.00	8.97	9.73	10.37	11.47	11.17	12.40	12.83
	3			3.57	4.90	6.30	7.80	7.30	8.20	8.37	10.60	13.40	13.77
	4			3.67	4.80	5.07	6.47	6.57	7.87	9.87	10.37	12.43	13.70
	5			7.67	9.03	12.60	11.20	12.80	13.10	15.77	15.77	18.13	19.93
Combined Weight grams	1								172.1	214.8	275.5	327.00	520.00
	2								113.9	150.0	128.5	155.50	240.00
	3								74.9	80.7	143.9	204.60	316.20
	4								71.1	106.5	135.7	215.4	336.3
	5								171.9	251.3	260.5	350.7	580.0

Example of Data Collected

Spat Growth Summary Graphs







Example of Data Collected

Water Quality Summary

Sample Trip	Site	DO (%)	DO (mg/L)	Salinity (PPT)	Chlorophyll A	Cond	Temp	pH
14	1-d	102.5	7.3	35	-0.628	50.39	22.8	8.20
14	1-m	99.2	7.0	35	-0.731	50.40	22.8	8.16
14	1-s	99.5	7.0	35	-0.747	50.43	22.8	8.15
14	1a-d	100.3	7.8	35	-0.770	50.43	22.8	8.21
14	1a-m	99.1	6.8	35	-0.644	50.45	22.8	8.19
14	1a-s	98.6	6.9	35	-0.728	50.50	22.9	8.21
14	2-d	105.1	7.3	35	-0.893	51.18	23.5	7.91
14	2-m	100.7	7.0	35	-0.915	51.19	23.5	8.01
14	2-s	100.1	6.9	35	-0.802	51.20	23.5	8.04
14	3-d	107.1	7.7	35	-0.909	50.23	22.6	8.16
14	3-m	94.2	6.7	35	-0.961	50.30	22.6	8.15
14	3-s	96.4	6.8	35	-0.955	50.56	22.9	8.17
14	3a-d	103.1	7.4	35	-0.867	50.43	22.6	8.13
14	3a-m	103.4	7.3	35	-0.904	50.54	22.8	8.14
14	3a-s	109.8	7.7	35	-0.907	50.99	23.2	8.19
14	4-d	105.8	7.5	35	-0.950	50.12	22.5	8.10
14	4-m	93.7	6.6	35	-0.970	50.37	22.7	8.09
14	4-s	108.2	7.6	35	-0.959	50.86	23.2	8.08
14	4a-d	101	7.1	35	-0.937	50.13	22.5	8.16
14	4a-m	98.2	6.9	35	-0.959	50.50	22.8	8.12
14	4a-s	95.5	6.7	35	-0.962	50.70	23.0	8.11
14	5-d	95.6	6.8	35	-0.827	50.23	22.5	8.12
14	5-m	93.9	6.6	35	-0.829	50.26	22.6	8.13

Example of Data Collected

Broodstock Data

Broodstock Data		Monitoring Trip										
Parameter	**Site Location**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Whole weight (g)	1	75	54	52	69	85	36	62	34	41	21	46
	3	51	64	28	36	61	65	71	81	46	60	58
	4	80	65	21	47	31	76	57	92	66	47	51
Meat weight (g)	1	25	16	15	23	24	11	20	12	15	5	17
	3	12	22	6	10	21	24	24	31	14	17	18
	4	26	13	7	13	9	26	16	30	22	11	14
Length (DVM) (mm)	1	81	74	76	82	86	61	71	57	64	52	63
	3	70	79	61	66	82	77	80	81	71	80	71
	4	87	71	58	70	62	79	73	81	77	68	67
Width (APM) (mm)	1	79	69	74	78	84	60	73	58	68	53	67
	3	69	75	59	63	79	78	81	84	71	81	74
	4	84	68	55	67	61	83	74	83	79	69	69
Thick (WD) (mm)	1	31	27	28	34	31	23	27	22	26	17	27
	3	24	31	21	23	32	29	32	30	25	33	29
	4	32	25	19	27	22	33	27	33	33	26	24
Akoya host selection criteria value	1	0.16	0.16	0.16	0.17	0.15	0.16	0.16	0.16	0.16	0.14	0.17
	3	0.15	0.17	0.15	0.15	0.16	0.16	0.17	0.16	0.15	0.17	0.16
	4	0.16	0.15	0.15	0.17	0.15	0.17	0.16	0.17	0.17	0.16	0.15
Gonad	1	3.3	2.5	2.1	2.2	2.2	3.8	3.7	4.4	4.3	3.3	4.6
	3	1.4	2.2	1.9	1.7	2.5	3.1	3.4	3.1	4.7	3.1	2.8
	4	1.6	1.2	1.8	1.9	2.2	3.2	2.4	2.1	2.3	2.5	2.4
Glycogen	1	2.8	2.2	2.2	2.3	1.5	2.5	1.9	1.6	2.1	1.3	2.7
	3	1	2.1	1.7	1.6	2.1	2.8	1.6	1.4	1.7	1.0	1.3
	4	2.1	1.2	2	1.4	1.6	2.1	1.1	1.0	1.0	1.0	1.0
Polychaete	1	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0

10.3.2 Other Project Reports

A number of reports, plans and programs have been produced during the course of this project.

These are available on request and include the following:

- Akoya Broodstock Program – Selection and Conditioning Plan
- Akoya Spat Monitoring Plan
- Conditioning Protocols
- Seeding Protocols
- Monitoring Program
- Monitoring Trip Data

10.3.3 Photographic Records

A comprehensive set of photographic records has been maintained throughout the term of the project, these include:

1. Equipment
2. Broodstock
3. Hatchery
4. Seeding
5. Environmental
6. Spat Transfer
7. Pearl Harvest

A sample of photographs follows.

Collecting juvenile spat from cages

Collecting juvenile spat from cages

Spat in small mesh pyramid cages

Spat in large mesh pyramid cages

Small mesh cages

Large mesh pyramid cages

Cleaning pyramid cages

Growth on pyramid cages

Condition cage in water

Shell density in lage conditioning cage

Shell density in large conditioning cage

Removing shell from conditioning cage

NSW style conditioning basket

NSW style conditioning basket

WA style conditioning cage

WA style conditioning cage

Pegging shell prior to seeding

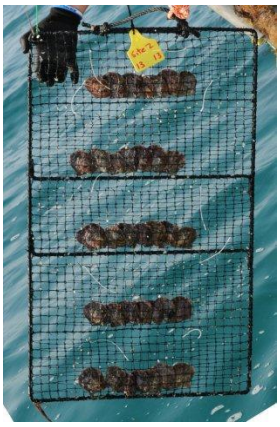
Pegged shell

Seeding oysters

Cutting saibo tissue

Seeding oysters

Placing oyster in panels



Placing oysters on farm site

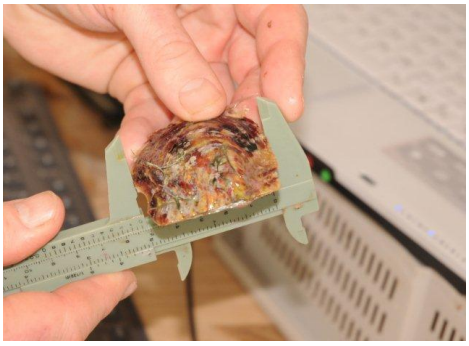
Placing oysters on farm site

Placing oysters on farm site

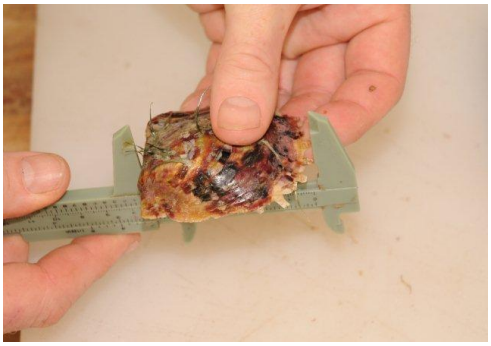
Placing oysters on farm site

Tagging system

Data collection method



Measuring shell



Measuring shell

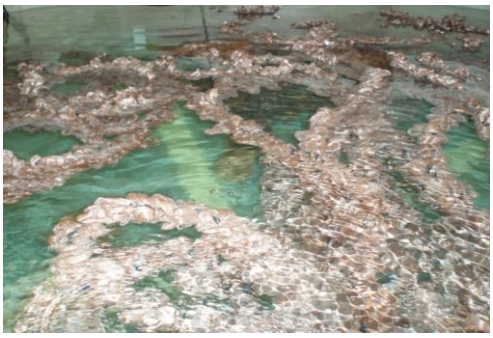
Measuring shell

Spat growth images

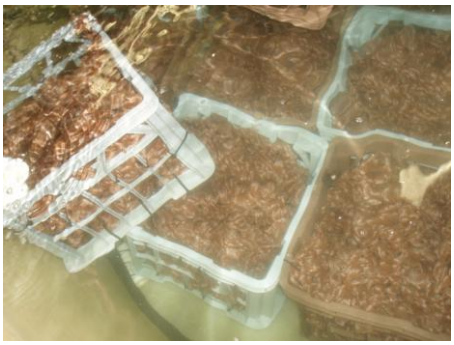
Measuring shell

Collecting water samples

Blue Lagoon Pearl hatchery



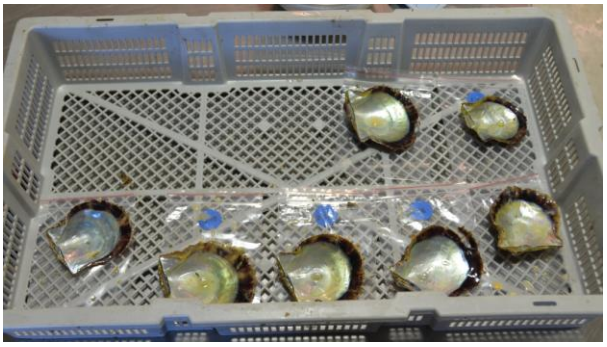
Blue Lagoon Pearl hatchery

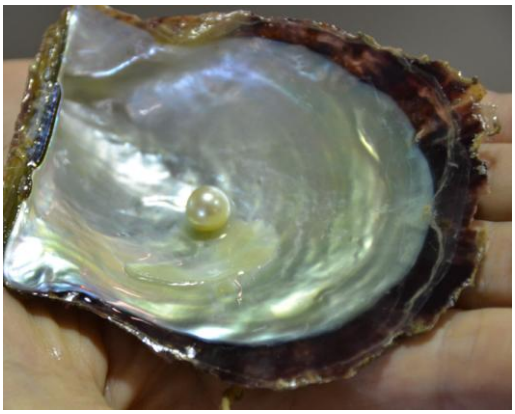


Blue Lagoon Pearl hatchery

Blue Lagoon Pearl hatchery

Final pearl harvest
May 2011





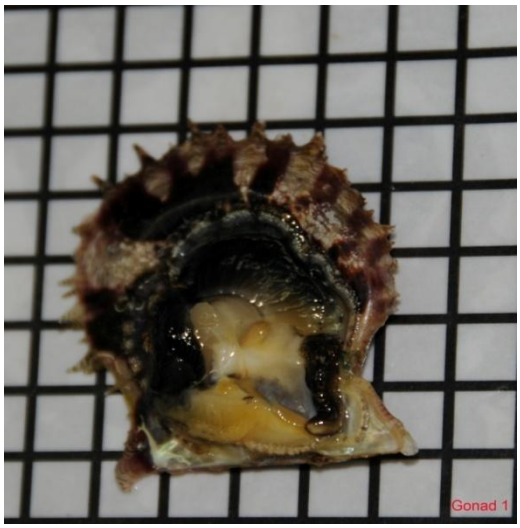
10.3.4 Photo Series

- Gonad Scale 1 – 5
- Gonad Condition Scale 1 – 3
- Glycogen Scale 1 – 5
- Byssus Scale 1 – 5
- Byssus Condition Scale 1 - 3
- Colour Scale

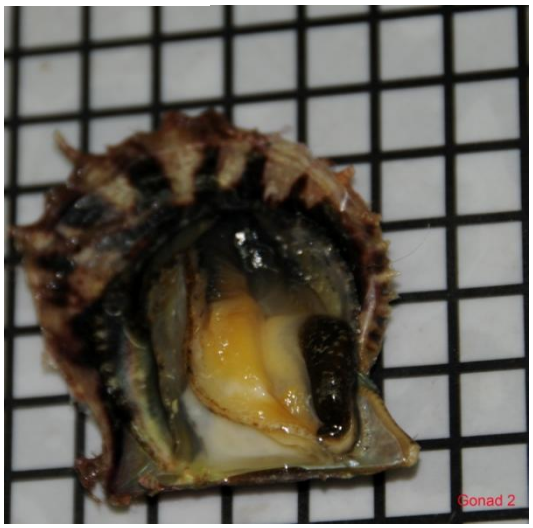
Gonad Scale 1 – 5

.

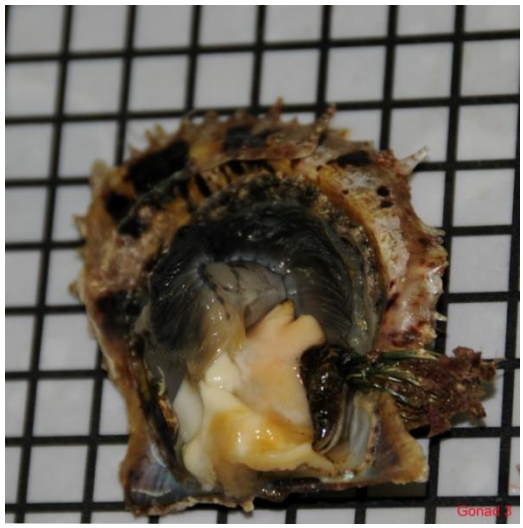
Rating 1



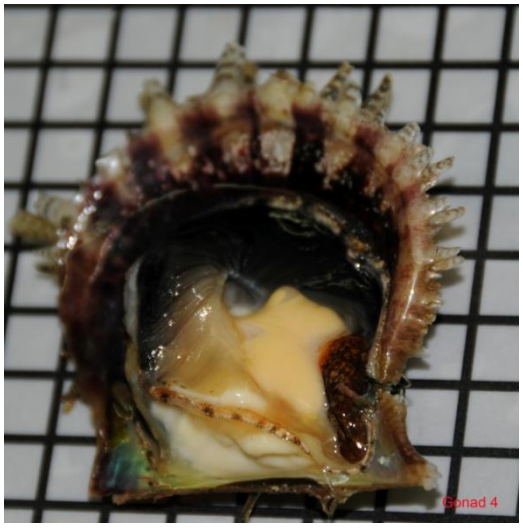
Rating 2



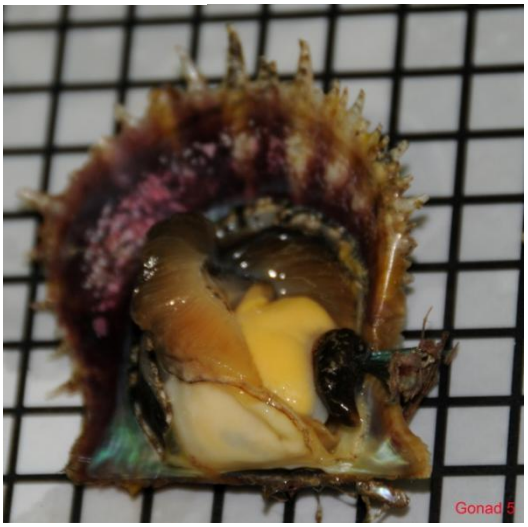
Rating 3



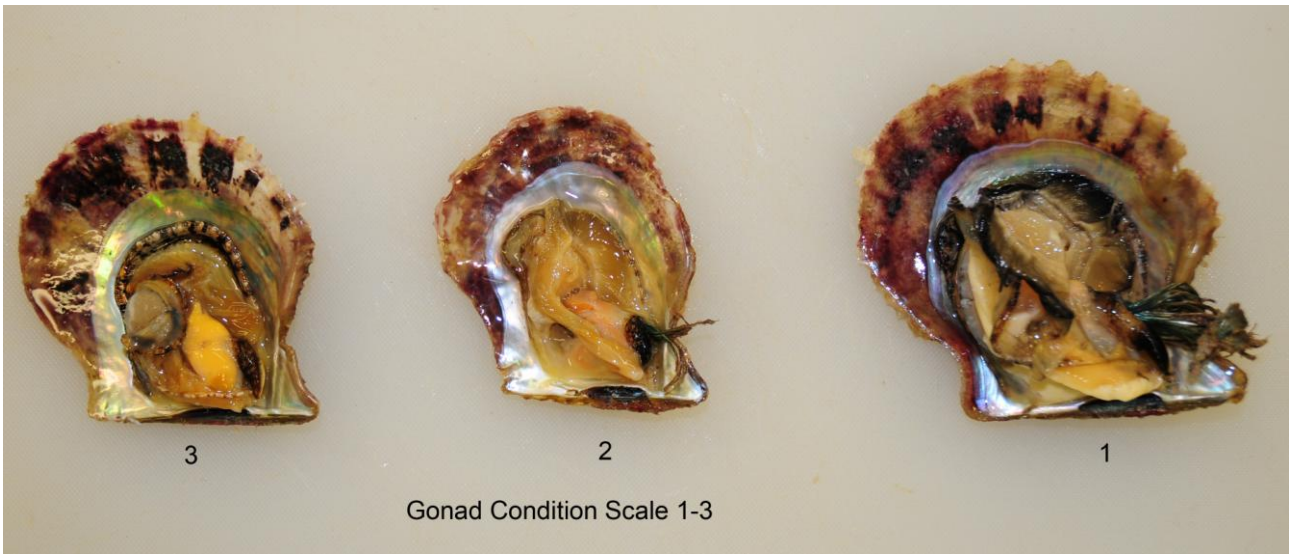
Rating 4



Rating 5

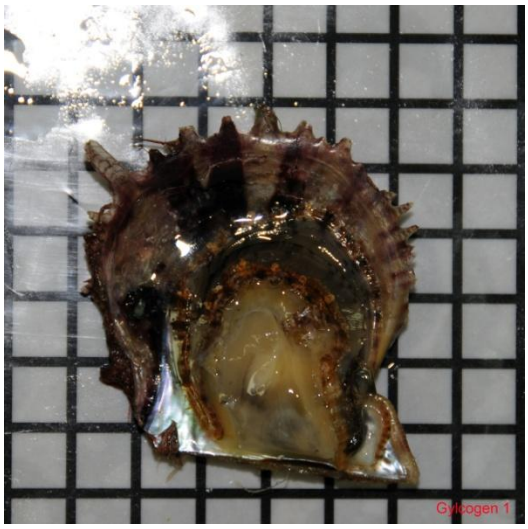


Gonad Condition Scale 1 – 3

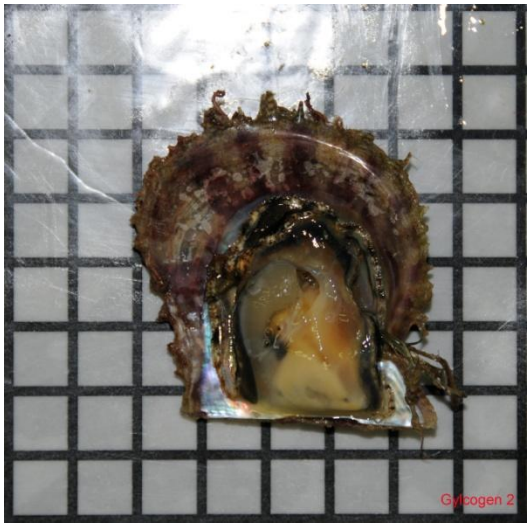


Glycogen Scale 1 - 5

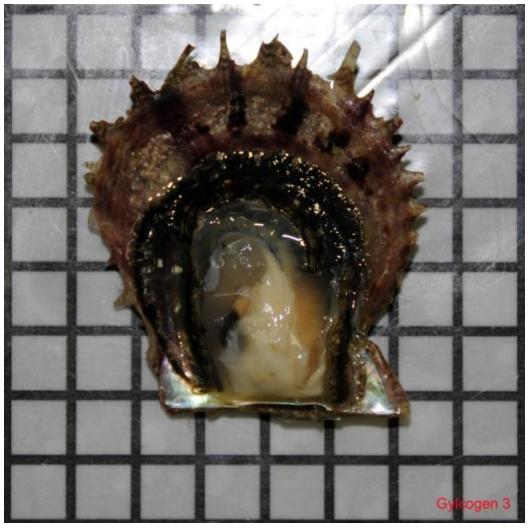
Rating 1



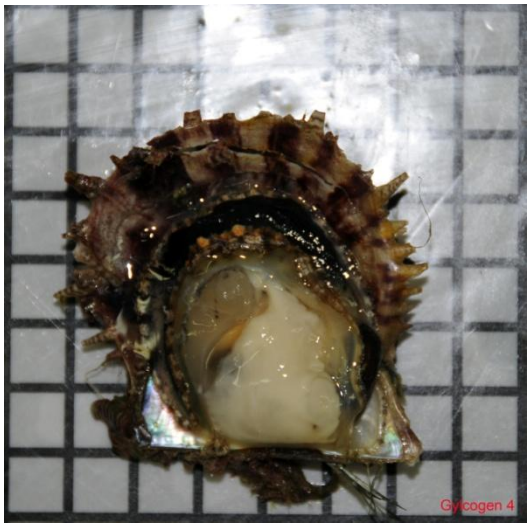
Rating 2



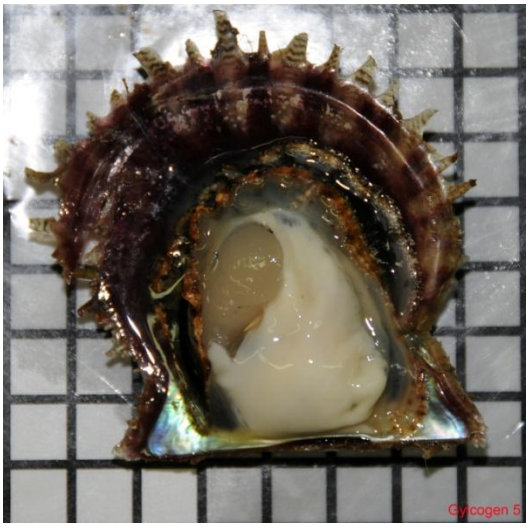
Rating 3



Rating 4

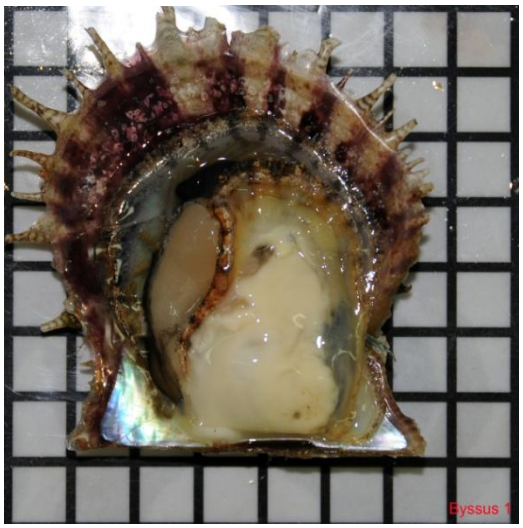


Rating 5

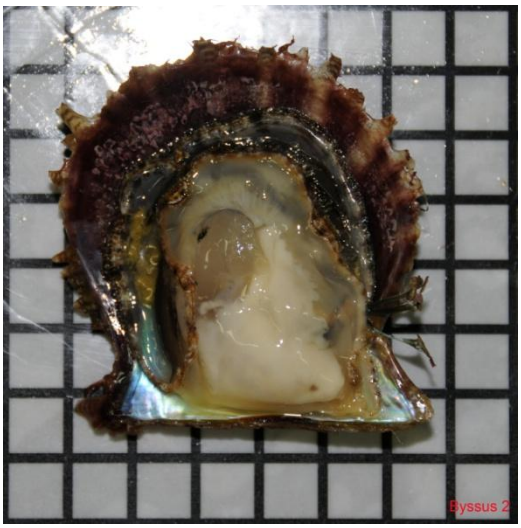


Byssus Scale 1 - 5

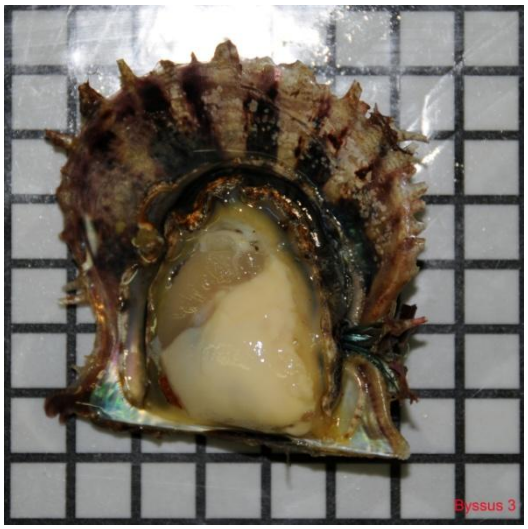
Rating 1



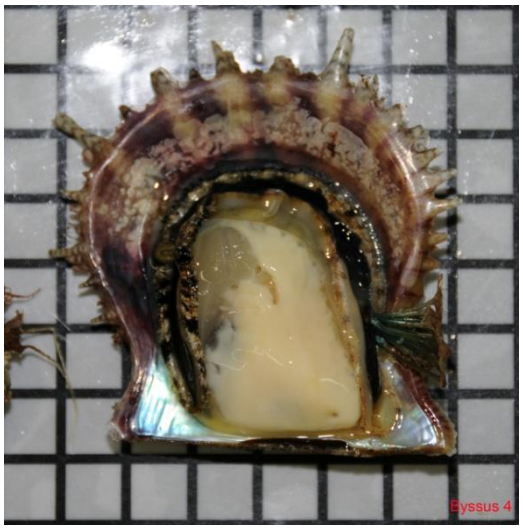
Rating 2



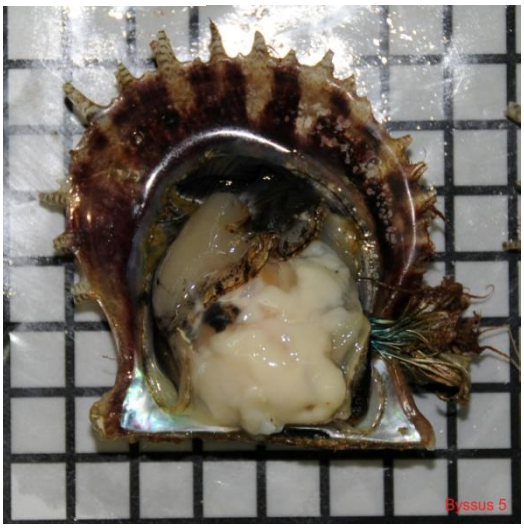
Rating 3



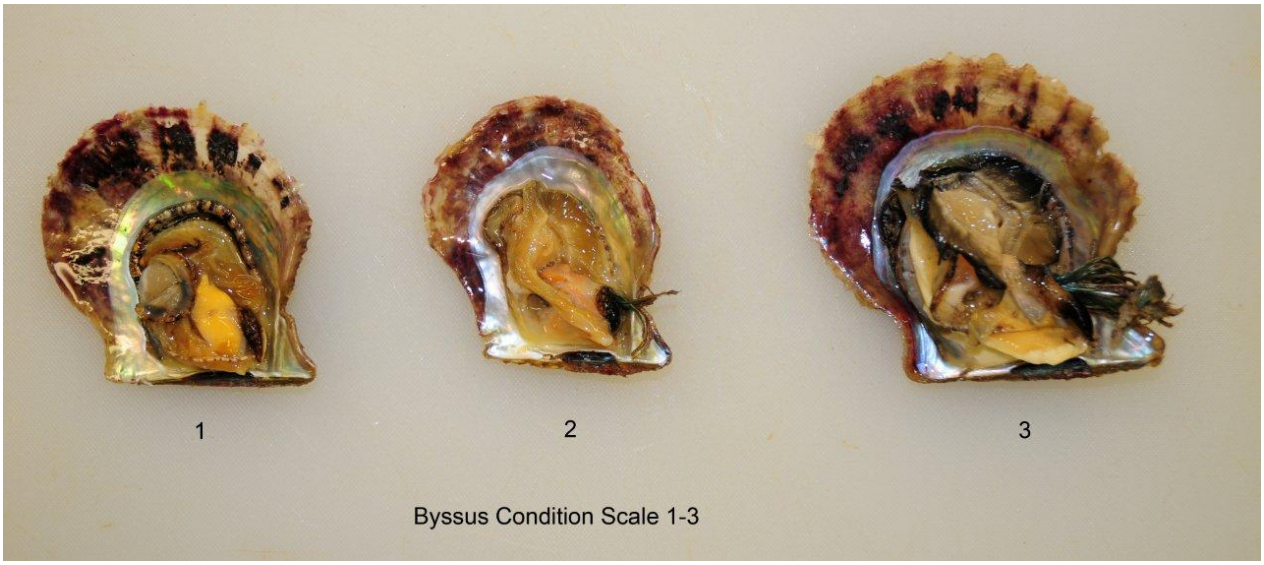
Rating 4



Rating 5



Byssus Condition Scale 1 – 3



Colour Scale