Australian Government

Australian Centre for International Agricultural Research

Final report

project	**Building bivalve hatchery production capacity in Vietnam and Australia**
project number	FIS/2005/114
date published	November 2012
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final report number	FR2012-25
ISBN	978 1 922137 14 2
published by	ACIAR GPO Box 1571 Canberra ACT 2601 Australia

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1 Acknowledgments

We extend our appreciation to the staff of the Ministry of Agriculture and Rural Development, Research Institute for Aquaculture No 1, particularly those based in Hai Phong and Cat Ba working at the National Marine Broodstock Centre. Their dedication and enthusiasm was central to all that was achieved within this project.

We gratefully acknowledge the support of the following colleagues and agencies;

- Dr Mr Barney Smith, Ms Helena Heasman and Dr Geoff Allan, ACIAR/NSW DPI - Australia
- Mr Geoff Morris, Ms Thi Thanh An Nguyen, Ms Lan Phuong Nguyen, Hau Vu, ACIAR – Vietnam. Australian Embassy, 8 Dao Tan Street, Ba Dinh District, Hanoi
- Ms Janine Pierce Uni SA

We thank Shellfish Culture Pty Ltd for provision of triploid Pacific oysters for field trials.

We also acknowledge the staff of the NSW DPI, Port Stephens Fisheries Institute for their involvement in this project: Ms Debra Doolan, for assistance with triploidy induction trials and Ms Lynne Foulkes, Mr Ben Finn, Mr Kyle Johnston, Mr Brandt Archer, Mr Andrew Parnell for hatchery assistance and involvement during training exercises for Vietnamese staff.

2 Executive summary

Vietnam trails its Asian neighbours in bivalve production. Despite approximately 3,260 km of coastline and many native clams, mussels and oysters with excellent production potential, Vietnam produces only about 190,000 t/annum, with only one third coming from aquaculture. Contrast this with its neighbour China, which produces more than ten times this quantity of molluscs per km of coastline with over 90% from aquaculture. Production in Vietnam is limited by seed supply as the bivalve hatchery sector remains largely undeveloped. Most farmers, particularly in poorer northern Vietnam, are reliant on a limited supply of wild caught seed from the south. Attempts to establish hatcheries to increase seed supply had been largely unsuccessful due to limited expertise and poor design. This collaborative project between Research Institute for Aquaculture No1 (RIA1) and NSW Department of Primary Industries (DPI) aimed to assist the development of bivalve hatchery technology in both Vietnam and Australia to promote bivalve production.

As the direct outcome of this project an oyster industry has now developed in Northern Vietnam. From modest outputs of 20 million oyster seed in our first year (2007/08), production at the RIA1, National Marine Broodstock Centre, Mollusc Hatchery has risen to approximately 100 million seed annually since 2009. To support industry seed demand, 3 additional commercial facilities have now commenced production.

In accordance with the development of oyster seed supply, oyster production has also grown rapidly. In the project's first year approximately 100 t of oysters were produced. In 2008/09 production grew to 1000 t, and within 4 years, production exceeded the annual oyster production of NSW (ca. 4000 t). Today production has reached approx 7000 t or the equivalent of 60% of Australia's total production.

A combination of larger producers and an increasing number of small farmers are now growing oysters for local markets. Restaurants in the coastal tourist areas of Quang Ninh and Hai Phong provinces have enthusiastically accepted "milky oysters" (Hau Sua) and are promoting the new product. Over 1500 people are fully employed in the oyster industry and poor coastal communities are seeing the benefits of oyster faming and enthusiastically embracing the opportunity.

The scientific and management capacity to develop mollusc industries in Vietnam has expanded greatly. Seven RIA1 staff were trained in Australian hatcheries, 25 undergraduate students and 3 MSc students were involved directly on the program. Two RIA staff were successful in acquiring John Allwright fellowships to undertake PhD studies in molluscan biology in Australia.

This project also benefited Australian hatcheries. Our understanding of triploidy induction, assessment techniques and its physiological impacts on oysters has expanded and new techniques for the production of alternative aquaculture species, such as pipis, have been developed

The future for a hatchery led expansion of bivalve production in Vietnam is bright. Domestic demand exceeds supply and significant export markets exist. The oyster industry is planning further expansion and the hatchery expertise developed for oysters is now being directed to other species. In Australia, further work has begun to build on the knowledge gained, in particular to expand our knowledge of the biology of species such as pipis, develop reliable hatchery techniques and assess their culture potential.

3 Background

Aquaculture is the world's fastest growing food-producing sector, which now accounts for almost 50% of world's food fish. With projected population growth over next 20 years, estimates predict at least an additional $40x10^6$ tonnes of aquaculture food will be required by 2030 without any increase in per capita consumption (Allan 2005). Mollusc culture is well placed to contribute to this rising demand. The world's mollusc industries now produce over 12 million tonnes annually and production is dominated by bivalves. Oysters are the greatest contributor in terms of tonnage produced, although clams have the greatest commercial value. Other significant contributors to production figures include mussels and scallops, while pearl oysters, although few in number, are commercially of great value. Mollusc production has grown steadily over the last decade and is expected to continue growing as world demand for seafood increases.

Most of the world's mollusc production comes from Asia, with China alone accounting for more than 80% of production. Vietnam however has been unable to follow the example set by China or its other Asian neighbours, producing less than 8% of the equivalent production achieved per km of coastline in China. Vietnam's overall mollusc production of 190,000 t is comparatively small given its coastal resources. Further, Vietnamese bivalve production lacks diversity and is focussed almost entirely on clams. This singular focus limits mollusc industry expansion, limits its market opportunities and predisposes the mollusc industry to the impacts that can arise from such dependence. Calamities such as poor natural spat fall; the accidental introduction or outbreak of significant clam disease; a food poisoning outbreak or a pollutant scare with clams could cause considerable industry and social hardship.

The major constraint to the further development of bivalve culture is the availability of seed. Northern Vietnam is reliant on the collection of wild clam seed, mostly from southern estuaries, and this resource is now fully exploited. While it may be possible to use the available seed more efficiently, the most effective and sustainable path to expansion is the artificial propagation of seed in hatcheries. Artificial propagation would offer the advantages of increased supply, greater assurance of supply and greater variety in the species available for culture. Farmers have recognised this and several attempts have been made to build commercial hatcheries in the north, however these hatcheries have failed to produce significant quantities of seed, largely because of poor initial design and a lack of appropriately trained staff. This program seeks to fill the key knowledge gaps required for the development of a bivalve hatchery sector in northern Vietnam, to commence ongoing research programs that will provide a knowledge base for the development of the industry and to foster the extension of the techniques to commercial hatcheries, nurseries and farms.

In contrast to Vietnam, Australian mollusc production is diverse, well established and considerably more technologically advanced. Molluscs already contribute over 40% of the value of Australian aquaculture. Two of Australia's four most important aquaculture industries are pearls and edible oysters and edible oysters are the fastest growing aquaculture sector in Australia at this time. Hatcheries have been a feature of the progress in mollusc culture in Australia and are at the heart of the recent increase in oyster industry value. The edible oyster industry currently derives more than 60% of its seed requirements (>200 million/annum) from hatcheries. Smaller industries such as abalone, flat oysters and akoya oysters are entirely hatchery dependent.

The development of mollusc hatcheries in Australia has been occurring for more than 25 years and although initially arising from a need to guarantee spat supply, increasingly industries are turning to hatcheries to access the advantages of genetic techniques to improve seed quality. An example is “triploidy induction” and without exception, the major Australian mollusc industries have experimentally evaluated the advantages this procedure. Triploidy is a hatchery dependent process in which molluscs are induced to hold an additional set of chromosomes, which implies a number of practical advantages:

- Triploid bivalves grow up to 30% faster than their “normal” diploid siblings, which in the case of Sydney rock oysters has been shown to reduce time to market by at least a year.
- Triploids can maintain a marketable meat condition for longer and through periods when diploids generally have very poor meat condition which increases their availability and can increase total sales.
- Triploids are in some instances more resistant to certain diseases (Winter Mortality in Sydney rock oysters).
- Triploids are functionally sterile, allowing them to be introduced to new environments with a significantly reduced risk of establishing self supporting populations (Nell, 2002) and offering some protection of IP in selective breeding programs.

A contemporary example of the value of triploidy can be seen following the recent outbreak of QX disease in Sydney rock oysters in the Hawkesbury River in 2004. Within a year of the onset of disease, all Sydney rock oyster farming in the river ceased, 8% of the state’s oyster production was lost and the number of farmers fell from approximately 50 to 1. In response, legislative changes were made to permit farming triploid Pacific oysters and within 12 months farming had recommenced, the number of farmers increased to 15 with new investors purchasing farms and predicting the recovery of the Hawkesbury industry. Triploid Pacific oyster culture is now one of the fastest growing sectors within the NSW industry and prompted renewed calls for the production of triploid Sydney rock oysters.

The major constraint to the wider practical application of triploidy has been that despite their advantages, triploid shellfish are rarely available from commercial hatcheries. Even in the case of Pacific oysters, supply has been erratic and at times failed to meet industry demand. The reasons for lack of supply vary, and in the instance of Pacific oysters have been exacerbated by the fact that the process used is patented by an American company that has granted sole licence to a single Australian hatchery. Regardless, this is not the only limitation. Most commonly, triploidy has been induced through the use of toxic (mutagenic, teratogenic & carcinogenic) chemicals applied very early in the oyster’s development. While this poses no threat to oyster consumers, resistance to the use of such chemicals in association with food products could develop and hatchery workers face the risks associated with handling such potentially toxic chemicals. Accordingly new or refined techniques for triploid induction are required. Alternative procedures using relatively safe methods based on heat and pressure are possible, but need to be evaluated to allow more hatcheries to begin to supply triploid shellfish to farmers and for farmers gain the economic advantages that ensue.

The impetus for the development of this program followed a visit by the Vietnamese Minister for Fisheries. The minister gained an insight into the importance of mollusc industries for NSW and saw first hand the gains in productivity that could be made through genetic selection and triploidy. This visit was in turn followed by a study tour of bivalve culture facilities and farms in Northern Vietnam by Drs Allan and O’Connor in December 2005. The study tour concluded that there were significant opportunities for bivalve industry development and made a number of recommendations. Those recommendations have been adopted in this application to develop a program that will foster the continued development of small-scale agro-enterprises in rural Vietnam that will promote profitable and environmentally sustainable use of coastal and near shore areas.

4 Objectives

The aim of this project is to overcome constraints to the development and diversity of small scale bivalve culture businesses through the establishment of reliable hatchery-based seed production capacity. The specific objectives are:

- To foster the development of the bivalve hatchery facility under construction at Cat Ba Island:
- To establish the knowledge base required for the selection of suitable species and their hatchery production:
- To assist the establishment of nursery facilities to bridge the gap between hatchery production and the provision of suitable sized seed to farmers, and
- To assess chemical-free methods for the production of triploid shellfish in Australia and their use in Vietnam.

5 Methodology

This project was undertaken at two locations, the National Marine Broodstock Center (NMBC), Vietnam and the Port Stephens Fisheries Institute (PSFI), Australia. The bulk of research focussed on aspects of oyster biology, but two key clam species were also involved. Central to the project was a program of hands on training, initially in Australia, but also on-site in Vietnam.

National Marine Broodstock Center

The Ministry of Agriculture and Rural Development's (MARD) Research Institute for Aquaculture (RIA) operate a range of marine and freshwater facilities in northern Vietnam. Among these facilities is the NMBC (Fig.5.1) on the Island of Cat Ba to the west of Ha Long Bay. The Center was established as a combination marine and brackish water research station and is responsible for the development of breeding and growout techniques for mariculture species. The centre acts to preserve marine broodstock and as a source of broodstock for commercial fish hatcheries. The Centre is also the key source for seed used in aquaculture in the northern Vietnamese provinces.

The NMBC was under construction at the outset of this project, but now has fish, crustacean and mollusc hatchery facilities as well as nearby coastal farms and broodstock holding facilities.

Figure 5.1 National Marine Broodstock Center, Cat Ba, Vietnam (photo courtesy RIA1)

Port Stephens Fisheries Institute

Port Stephens Fisheries Institute (PFSI) is located in NSW about 2½ hours north of Sydney and was established in the early 1970's as an aquaculture research facility. The PSFI hosts approximately 100 staff working within five divisions including Aquaculture Research and Ecosystems Research. The aquaculture research group develops technology for new or existing aquaculture industries. Key facilities used for this research at the PSFI include a mollusc hatchery, a quarantine mollusc hatchery, marine fish broodstock centre, marine fish hatchery, marine fish nursery facilities and grow-out tanks and ponds. Facilities for commercial and pilot-scale research are available as well as replicated, small-scale facilities for applied research.

Because of the importance of oysters in NSW, molluscs are central to the PSFI research focus. Key molluscan research programs include:

- the development and improvement of hatchery and nursery techniques for Sydney rock, Pacific, pearl and flat oysters, as well as other molluscs (eg. pipis),
- genetically improving Sydney rock oysters (eg for disease resistance, faster growth and improved condition), and

- investigation of the impacts of human activities and climate change on oysters.

Figure 5.2 Port Stephens Fisheries Institute, NSW, Australia

Species

From the outset, this project sought to transfer skills and technology that would be applicable to a range of mollusc species, and to highlight methodologies that could be used to assess the performance of whichever species were selected. While several species were investigated, the greatest effort was devoted to *Crassostrea angulata*, known as Hau Sua in Vietnam (literally “milky oysters”) and Portuguese oysters elsewhere in the world (Fig 5.3A). Within the hatchery, some effort was also devoted to the otter clam (*Lutaria rhinchaena,* Fig 5.3B).

A)  B) 

Figure 5.3. Key species produced at the NMBC: A) “milky oyster” (*Crassostrea angulata*) and B) “Tu Hai” (*Lutaria rhinchaena*).

In Australia, research initially focussed on the Pacific oyster (*Crassostrea gigas*, Fig. 5.4A) before turning attention to the pipi (*Donax deltoides*, Fig. 5.4b)

A)  B) 

Figure 5.4. Key species produced at the PSFI: A) triploid Pacific oysters (*Crassostrea gigas*) produced at the PSFI during the project and B) pipi (*Donax deltoides*).

Hatchery technology transfer

Three, 2 week, hands-on training modules were conducted for Vietnamese staff within the mollusc hatchery at the PSFI. The initial modules were held in October 2007 and March 2008, and focused on algal culture techniques, early larval rearing methods and nursery culture. On each occasion, two staff from RIA No1 were provided with practical experience in the various procedures and protocols. To reinforce the outcomes of these training exercises, Two staff from NSW DPI spent a total of 4 weeks at Cat Ba working with Vietnamese researchers and advising on procedures and protocols.

In May 2009 a third training module devoted largely to triploid induction was undertaken at the PSFI. In a two week training exercise, three staff from RIA No1 were introduced to practical techniques used in ploidy induction (specifically anaesthetization, spawning and handling techniques) and were shown chemical, pressure and temperature based techniques for manipulating ploidy. These staff were also directly involved in strip spawning (physical removal of gametes from oysters) for all experiments, experimental design to evaluate effectiveness of heat and pressure treatments for triploid induction, gamete quality assessment and quantification, and larval development assessments.

Farms and cultivations techniques

Field evaluations in Vietnam were held either directly on small multipurpose marine farms (Fig 5.5 A) or, as culture developed, on oyster specific rafts (Fig 5.5 B). The cultivation technologies used varied and a number of “Australian” style baskets and cages were assessed; however, the vast majority of cultivation used oyster seed settled directly on shells that were later spaced at intervals on monofilament strings (Fig 5.6 A).

Key trials on oyster growth and survival were undertaken at two sites near Ban Sen Island in Bai Tu long Bay about 10 km from the mainland (Fig 5.7). At each location three experimental rafts typical of those used commercially were constructed (Fig. 5.5 B). Each raft covered approximately 80 m^2 in area and held 550 strings of oysters on cultch (Fig 5.6 A).

A)  B) 

Figure 5.5. Marine farm sites used during the project: A) small scale fish farms and B) oyster specific raft culture

A)  B)  C) 

Figure 5.6. Key culture methods for oysters: A) strings, B) cages and C) baskets.

Figure 5.7. Bai Tu Long Bay, Northern Vietnam showing the two experimental sites: Site A and Site B.

General environmental monitoring

To assist in the interpretation of the outcomes from farm based trials and to inform the project about factors likely to impact on oyster performance and quality, a monitoring program was undertaken (Table 5.1). Monthly sampling occurred at the test sites in Bai Tu Long Bay (Fig. 5.7) for three years (2008-2010).

Variable	***Notes***
Temperature, Salinity, pH and oxygen	*Sampling at two farm sites at two depths (surface & 2 m). Five samples from each location, pooled for analysis.*
BOD, COD, NO_2, NO_3, NH_4 Total N, alkalinity, turbidity	*Sampling from two farm sites*
Petroleum	*Sampling from two farm sites*
Microbiological	*Bacteriological testing of oyster meat and water samples.*
Phytoplankton	*Cell numbers and species composition. Monthly samples from each site: 1 litre water sample for quantitative measures and 500ml sample for a 10 micron mesh plankton net for qualitative assessment.*

Table 5.1 Key water quality variables monitored during this study. Water quality data are appended to this report in Tables 11.1 to 11.7 in Section 11.1.

Ploidy evaluation methods

Trials were conducted at the Port Stephens Fisheries Institute (PSFI) using wild broodstock sourced from NSW and Tasmania. Broodstock were strip spawned at the PSFI mollusc hatchery and gametes were rinsed and screened through a 100µm sieve to remove shell fragments. The sperm was then placed immediately on ice and the eggs were left to water harden for a minimum of 1 h. Egg suspensions were fertilised with 10^6 sperm ml^{-1}.

After each trial, a subsample (50%) of larvae (24 h post fertilisation [HPF]) from each replicate was transferred to a piece of 20µm nylon mesh and packaged in a 10 ml vial which contained only tissue paper dampened with saltwater. The vials were then packed on ice and sent to a laboratory for ploidy testing the following day (48 HPF) using flow cytometry (FCM). The remaining larvae (50%) were incubated until 48 HPF and were then preserved with formalin and assessed for the proportion of abnormalities at the D-veliger stage.

Temperature shock trials were conducted in an aluminium block heated at one end by recirculating heated water and cooled at the opposite end by recirculating chilled water, the apparatus is described by Dove and O'Connor (2007). Sample jars (120 ml) containing 30 ml of filtered seawater were inserted into the block to equilibrate seawater to each of the treatment temperatures before the trial began. Embryos (10,000) were stocked into the treatment vials 15 minutes post fertilisation (MPF), and after 20 min of treatment time the vials were removed from the aluminium block and filled with 25°C filtered seawater to immediately reduce the temperature of the treatment water. Experimental jars were then transferred to an incubator and maintained at 25°C.

Pressure trials were conducted using a 600 ml hydrostatic pressure chamber (Aquatic Ecosystems, Inc. Florida, USA). Embryos (15 MPF) were placed in a 70 ml sample jar filled with filtered seawater (25°C), which was fitted with a 20µm mesh lid to prevent

embryos from escaping while allowing water to move freely in and out of the sample jar. The sample jar was then submerged into the chamber filled with filtered seawater. The desired pressure was applied to the chamber using a 12,000 kg hydraulic shop press for 10-20 minutes. Sample jars were then removed from the chamber, embryos were transferred to a 120ml sample jar, topped up with filtered seawater and incubated at 25°C.

Triploid performance evaluation (Vietnam)

Through out this project a number of growth and performance trials were undertaken in Vietnam (see Section 7); too many to describe individually in this report. The triploid performance evaluation was one of the larger exercises and is briefly described here as an example of the methods used.

A total of 200,000 triploid oyster spat were sent to Vietnam. To minimise the risks associated with international molluscan movements, these oysters were sourced from Tasmanian hatchery populations that are regularly monitored for health under a state certified program. The seed was reared and remained within a commercially hatchery using 1 micron filtered water for their entire lives prior to translocation (not exposed to natural waters). The particular batch of spat used were then health assessed in NSW at the Elizabeth Macarthur Agriculture Institute.

When the seed arrived in Vietnam they were acclimated in isolation at the NMBC for several days following air travel to ensure recovery before being deployed in the bags provided at the NMBC farm. The bags were then carefully monitored and washed regularly to prevent fouling accumulation and mesh occlusion.

Initially spat were graded using screens every 2 weeks and sorted according to size. Oysters were graded using hand-held plastic tubs that have circular holes drilled into their base. A series of tubs with hole apertures ranging from 6 mm to 20 mm were prepared. When the oysters were retained on 8 mm diameter holes they were be placed in hanging baskets with a mesh size less than 3 mm and with suitable protection from fish (mesh covers).

As the oysters grew their density was reduced. For triploids, slightly lower stocking densities were used than those normally applied to diploid oysters, to account for their potentially faster growth rates and minimise overcrowding.

Suggested stocking densities were as follows:

- 1mm mesh bags = ~ 150 ml of triploid Pacific oysters (2.5-3 mm retained)
- 3 or 6 mm baskets = ~ 600 ml of triploid Pacific oysters

Oysters were held at five locations. At each location a minimum of four batches of 100 oysters were monitored for growth and survival.

Every month 30 oysters were taken at random from across the four experimental batches at each site. Shell length, width, depth and weight of each oyster was recorded. The oysters were then photographed. Every month an additional 30 oysters from the general population of oysters (not the experimental groups) was collected and fixed in formalin to be held until the trial ended in case of disease or unexpected loss.

6 Achievements against activities and outputs/milestones

Objective 1: To foster the development of the bivalve hatchery facility under construction at Cat Ba Island through:

no.	activity	outputs/ milestones	completion date	comments
1.1	Review construction of the bivalve hatchery at Cat Ba Island to ensure the facilities are likely to be appropriate for the species of interest to shellfish producers.	Report to RIA N^{o}1 on Cat Ba Hatchery suitability for large scale mollusc production and mollusc research.	March 2008	The rapid pace of development in Vietnam saw the hatchery constructed prior to project approval. In response, the staff training schedule was accelerated and retrofitting of the hatchery to use new culture technology was adopted. New fibreglass tank systems were purchased for assessment to rear and set oyster larvae and for algal production. An algal facility with temperature controlled rooms was constructed and equipped with common bivalve hatchery marine algal species. Downwelling settlement systems for single seed oysters were constructed at the NMBC hatchery following the training in Australia related to chemical settlement induction. These modifications of hatchery facilities at NMBC enabled production of single seed oysters using chemical induction procedures. Existing concrete tanks were used for culturing and settling oysters on to cultch and hard clams and tu hai in sediments. Additional filtration systems were added to the sea water delivery network to improve water quality to the algal lab and larval rearing tanks.

1.2	Provide training for staff from Cat Ba Island in hatchery techniques.		May 2009	Stage 1 Algal training (Oct 07), Stage 2 larval training completed (March 08) and Stage 3 triploid induction training completed (May 2009). Stage 3 training also included instruction on experimental methods and design for evaluation of the triploid induction techniques used. Over the course of this project, Australian staff visited Cat Ba on nine occasions and spent approximately 3 months on site working with RIA1 technicians and scientists. The skills and techniques taught and demonstrated during the Australian training modules were assessed at NMBC on program visits. Where Australian techniques did not suit conditions at the NMBC hatchery, modifications were devised to adapt to the identified constraints.
1.3	Expand the range of algal species to be cultured at Cat Ba Island to reflect current knowledge of bivalve nutritional needs.	Expanded algal culture collection at Cat Ba Island.	March 2008	Following algal training, isolates of 5 algal species were provided to RIA No1 staff and have been established in culture. With the exception of *Pavlova lutheri* (susceptible to elevated temperature) all species are growing well in mass production. Fresh cultures were supplied in May 2009 and again in June 2011. This algal facility now underpins all of the bivalve research and production at NMBC. It also plays a key function for other finfish and shrimp research programs conducted at NMBC through provision of high quality marine algae.

PC = partner country, A = Australia

Objective 2: To establishing the knowledge base required for the selection of suitable species and their successful production through:

no.	activity	outputs/ milestones	completion date	comments
2.1	A desk top audit with emphasis on "grey" literature reports to accumulate all available information of the species of interest.	Data base of current and potential commercial bivalves incorporating a synopsis of relevant hatchery and culture information.	April 2008	Dr Dove visited Vietnam in March 2008 to agree on species of initial interest and establish guidelines for database construction. Data collection commenced and summaries of key outputs (hatchery performance and reproductive timing for oysters and tu hai (clams)) were included in hatchery manual. Experiments were designed with ACIAR project staff to monitor growth and survival of oysters at the field growout sites used. Demonstration of oyster measurement techniques for growth and survival were conducted.
2.2	Reproductive monitoring of the commercially important bivalves	Written reports on reproductive behaviour in selected species (to be incorporated in to data base).	Jan 2009	Milky oysters were selected as the initial candidate for research on their reproductive cycle. Staff were trained at the NMBC to assess reproductive condition using condition index, gonadal somatic index and histological examination. Instruction on how to measure oyster shell dimensions, whole oyster weight, shell weight and soft tissue weight was provided. Staff were also trained in oyster dissection to obtain gonad samples and methods to chemically fix oyster tissues. Spat produced at the NMBC hatchery were taken to experimental farms and grown to a size at which they were sexually mature. Oysters were sampled periodically and their reproductive cycle was determined.. An example of the monitoring outcomes is provided in Section 7. This work was important for hatchery operation and timing of production runs. Knowing the reproductive cycle of broodstock allows the hatchery to time production when ripe oysters are available. This alleviates hatchery conditioning of broodstock. The data gathered from this study also assists farmers to understand the temporal variations in oyster meat quality and the best time to market their oysters.

<table>
<tr>
<td>2.3</td>
<td>Develop species specific techniques for the culture of commercially important bivalves</td>
<td>Reports on culture attempts of selected species (to be incorporated into data base).</td>
<td>June 2010</td>
<td>Following the first successful season with oysters and clams in 2008, culture methodology was described in a hatchery Manual.

The manual was regularly updated and then published in Vietnamese. This manual is one of the projects major outputs.

Due to rapid development of the oyster industry in northern Vietnam, seed supply to industry can not always be met by the NMBC. The Vietnamese version of the hatchery manual has assisted vertically integrated oyster companies establish hatcheries to supply seed to their farms.

Extension of hatchery techniques contained in the manual was achieved through a workshop held in Quang Ninh in March 2010 which included RIA staff from the three institutes, scientists as well as commercial growers.

Approximate figures on oyster production at the NMBC Hatchery Cat Ba:

<table>
<tr><th>Year</th><th>Larvae (million)</th><th>Spat (million)</th></tr>
<tr><td>2008</td><td>300</td><td>10</td></tr>
<tr><td>2009</td><td>1,514</td><td>44</td></tr>
<tr><td>2010</td><td>1,370</td><td>81</td></tr>
<tr><td>2011*</td><td>950</td><td>31</td></tr>
</table>
* in 2011 hatchery production was impacted by Typhoons.</td>
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<tr>
<td>2.4</td>
<td>Establishing surveys to monitor the health of bivalve species, in particular to investigate the causes for the loss of Pacific oysters delivered under an earlier program.</td>
<td>Establish protocols for routine health monitoring of farmed bivalves.

Establish causes for the loss of previous batch of Pacific oysters.</td>
<td>June 2008</td>
<td>Health monitoring protocols were established and regular oyster sampling commenced. Samples were collected routinely in both the hatchery and the field.

Losses of oysters experienced in previous ACIAR program did not recur in the first commercial production season. The only exception to this were the poor performing triploid oysters, which were examined but found to be free of any detectable disease agent.

Baseline data on diploid oyster growth and survival rates were periodically collected at selected growout sites in northern Vietnam to establish growout duration.</td>
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PC = partner country, A = Australia

Objective 3: To assist the establishment of nursery facilities to bridge the gap between hatchery production and the provision of suitable sized seed to farmers

no.	activity	outputs/ milestones	completion date	comments
3.1	Provide training to Cat Ba/commercial hatchery staff in nursery techniques for clams, oysters and byssal attachers (pearl oysters, scallops and mussels).	Two RIA No1 staff trained annually in bivalve larval and nursery technology.	March 2010	Three 2 week training courses were provided for RIA1 staff at the PSFI. Stage 2 larval training dealt specifically with settlement and nursery techniques. Hands on instruction during commercial oyster production was provided and tours of commercial nursery facilities were conducted. This project identified a strong need to develop nursery rearing techniques for single seed oysters to bridge the gap between hatchery and farm growout. It was important that the techniques used did not require a large cultured food input and labour component. In March 2010, a hatchery workshop was held in Quang Ninh. Staff from each of the three Research Institutes for Aquaculture and NSW Department of Primary Industries (DPI) presented information on aspects of mollusc culture to hatchery operators and farmers from the province.

PC = partner country, A = Australia

Objective 4: To assess chemical free methods for the production of shellfish in Australia and their use in Vietnam

no.	activity	outputs/ milestones	completion date	Comments
4.1	To assess alternative non-chemically based techniques for the production of triploid oysters.	Practical user-friendly technique for the production of triploid oysters.	Feb 2009	Temperature and pressure triploid induction trails were completed. These trials indicated that these stressors used individually and synergistically failed to improve triploid percentages beyond those achieved previously with chemical induction techniques. This research provided the scientists and technicians at PSFI skills in triploid induction and assessment techniques. These skills were also used at PSFI to undertake commercial-scale triploid hatchery production.

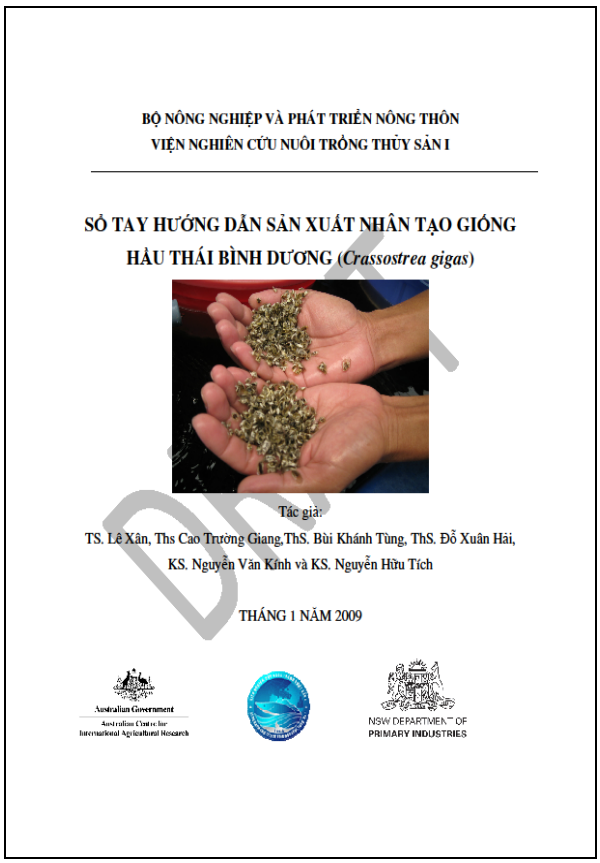
4.2	To assess the comparative performance of diploid & triploid Pacific oysters in Vietnam	An evaluation of diploid & triploid performance on Vietnamese farms	Dec 2011	Triploid induction training to RIA No1 staff was provided in May 2009 and experimental production trials began in Vietnam. Successful batches of triploid larvae were produced at NMBC, however, only small numbers of oysters were settled for assessments. To progress assessments a shipment of 200,000 health tested triploid oysters was sent from Australia to Vietnam for trials.
4.3	To assess the potential for hatchery production of pipis	Rear pipis through the larval phase to settlement		Two batches of pipi were successfully produced though to settlement and the first reports of the early ontogeny in the species were produced.

PC = partner country, A = Australia

7 Key results and discussion

7.1 Vietnam

The key objective for this program in Vietnam was to identify a suitable mollusc species and then develop the capacity to produce commercial quantities of seed. Two species were selected by RIA1 staff (Milky oysters and Tu Hai) and the hatchery methods required to produce these species were collated and published in a Vietnamese hatchery manual. This manual was distributed to industry and formed the basis of an industry workshop held in Quang Ninh in 2010.


BỘ NÔNG NGHIỆP VÀ PHÁT TRIỂN NÔNG THÔN
VIỆN NGHIÊN CỨU NUÔI TRỒNG THỦY SẢN I

SỔ TAY HƯỚNG DẪN SẢN XUẤT NHÂN TẠO GIỐNG
HÀU THÁI BÌNH DƯƠNG (*Crassostrea gigas*)

Tác giả:
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THÁNG 1 NĂM 2009

Australian Government
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Figure 7.1. A manual for the production of Pacific Oysters. The techniques contained in this manual are directly transferable to production of Milky oysters (*Crassostrea angulata*).

Stemming from the ability to reliably produce commercial quantities of oysters, the project then had the opportunity undertake a series of trials fundamental to the successful establishment of an oyster industry. Information was required to specifically answer questions such as:

- What is the optimal density of oysters on collectors when distributed to farmers?
- At what size should oysters be deployed to the field?
- Is oyster growth and survival commercially viable?
- Which cultivation techniques are best applied?
- When are oysters suitable for harvest?
- Are triploid Pacific oysters suitable for cultivation in Northern Vietnam?

Optimising oyster density for string oyster cultivation in Northern Vietnam

Hatchery technology was developed that allowed oyster seed to be produced both as single individual oysters (single seed) or as juvenile oysters attached to an oyster shell (cultch set). The latter, cultch set, being particularly useful in Vietnam because of its ease of adoption by inexperienced farmers. One of the keys however to cultch set is having a the optimal number of oysters settled on each shell. This trial evaluated survival and growth of cultch set oysters at four different densities. The farming method used was sub-tidal raft cultivation using spat set on cultch at different densities. Site A (Fig. 5.7) was used as the experimental site and strings were placed over four bamboo rafts (9m x 9m, Fig. 5.5 B). Four culture densities were compared: 15, 20, 25 and 30 individuals per shell. 300 lines at each culture density were randomly deployed across the four rafts. Shell height, shell length, whole weight and percentage survival were measured. Oysters were monitored at field sites from September 2008 until May 2009.

After 8 months of cultivation oysters stocked at the lowest density (15 oysters per shell) were larger and heavier than the other densities, with strings stocked at 30 oysters per shell having the lowest growth (Figs. 7.2 and 7.3). The highest survival at the end of the trial was also recorded in oysters stocked at 15 oysters per shell (74.4%) (Fig. 7.4). The survival of oysters stocked at 20, 25 and 30 at the same time was 72.5%, 68.1% and 61.3%, respectively.

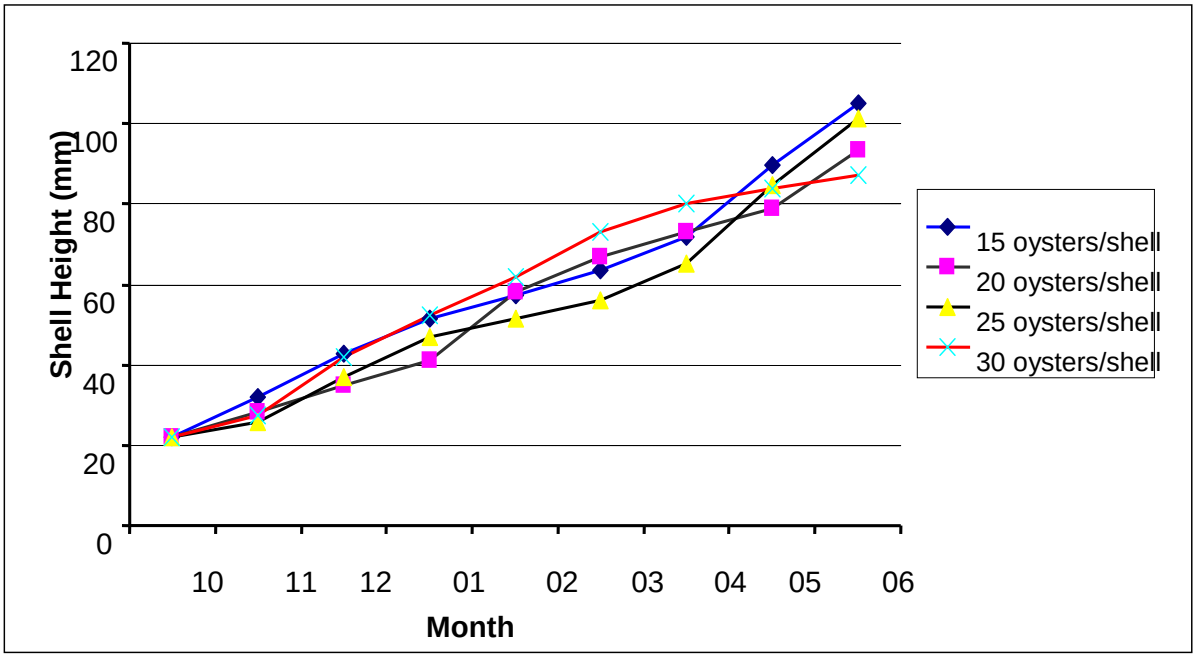

Figure 7.2. Shell height of oysters at Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam stocked at either 15, 20, 25 or 30 oysters on each shell cultch

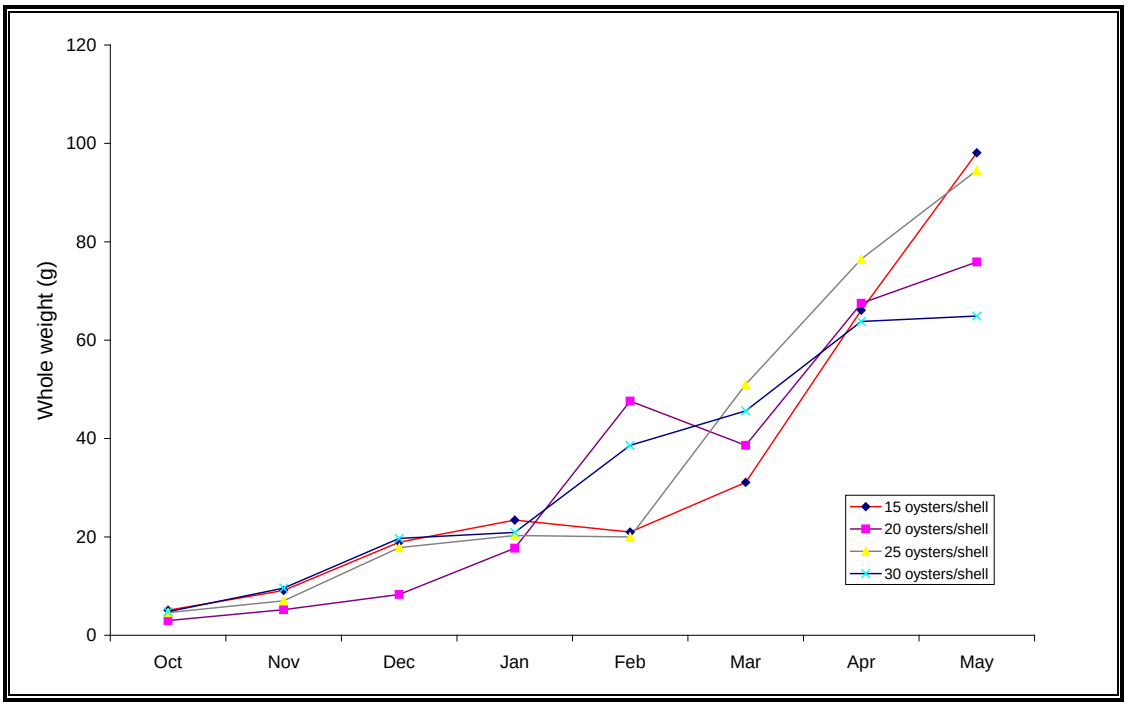

Figure 7.3. Whole weight of oysters at Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam stocked at either 15, 20, 25 or 30 oysters on each shell cultch.

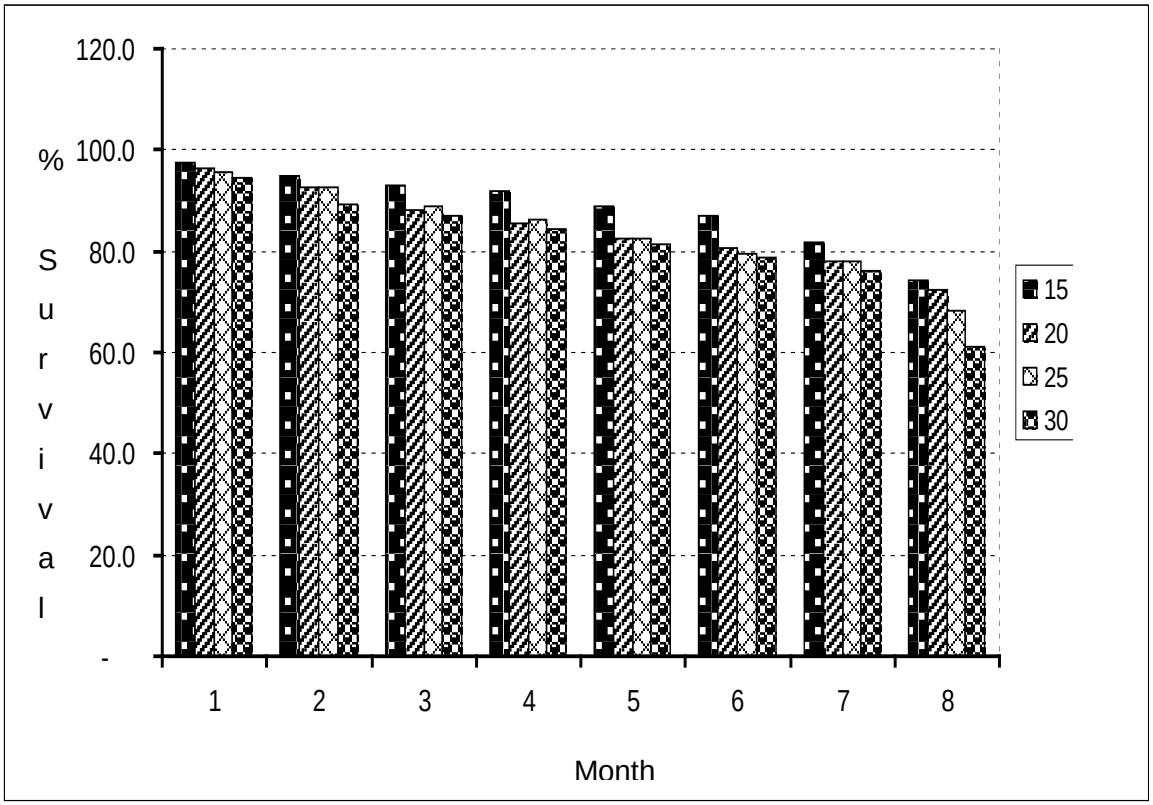

Figure 7.4. Percentage survival of oysters deployed at Ban Sen Island, Bai Tu Long Bay, Vietnam stocked at either 15, 20, 25 or 30 oysters on each shell cultch.

Early deployment of cultch set seed to field sites

Optimising the time at which oyster seed are removed from the hatchery is an important parameter to efficient industry operation. Maintaining spat in the hatchery is expensive as food (algae) needs to be produced to support their growth and in many instances growth in the hatchery is not as rapid as would be observed in the field. However, small oysters seed can be less tolerant of translocation/transport and can be more susceptible to predators in the field. This trial tracked the performance of two sizes of oyster seed (15mm and 25mm) following deployment to the field to determine the best time to deploy cultch set oysters from the hatchery. The two size classes of oyster seed were cultch set onto 300 lines for each treatment were taken to field Site A (Fig. 5.7) and randomly allocated to two commercial rafts. The growth and survival of spat were measured monthly from August 2008 until June 2009.

Stocking oysters at the smaller size did not impact their subsequent performance at Site A in terms of shell height and whole weight (Figs. 7.5 and 7.6). In fact, these oysters out performed the batch that were deployed to the site at 25 mm shell height. The 15 mm size class oysters also had better survival when measured each month and had an average survival after 10 months of 72.5% compared with 70.6% for the oysters deployed at 25 mm. This finding indicates that stocking commercial oyster farms with the 15 mm size class benefits their performance and reduces the time that spat are reliant on hatchery resources. Future trials will investigate the potential for even earlier deployment.

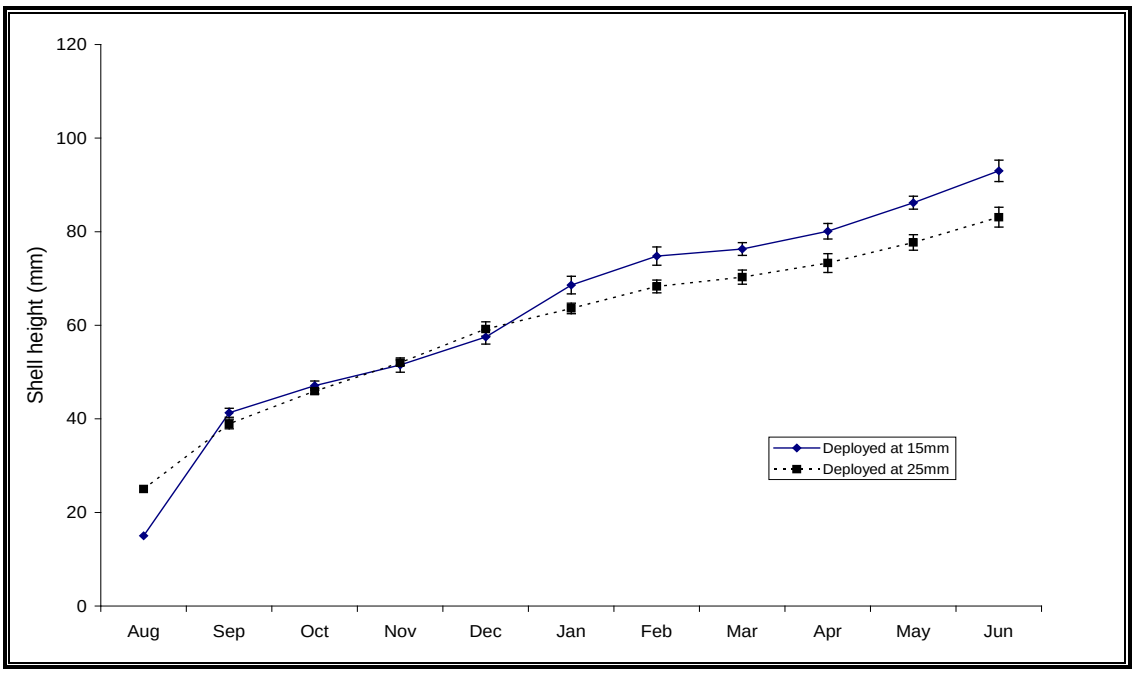

Figure 7.5. Shell height of oysters at Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam deployed at a size of 15 mm shell height or 20 mm shell height.

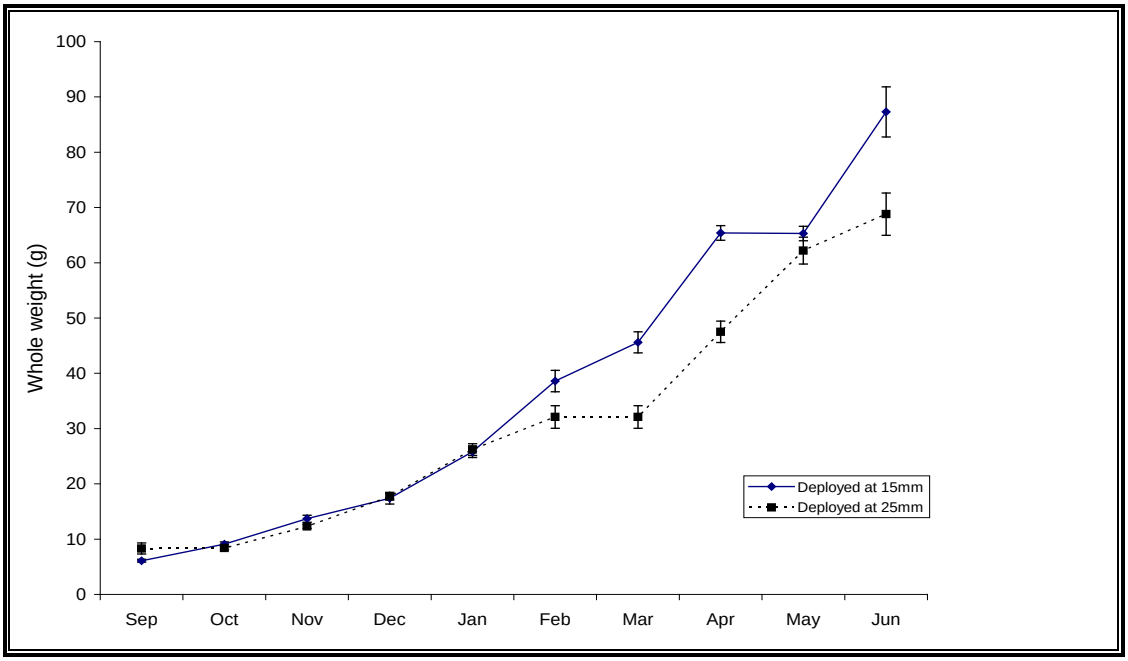

Figure 7.6. Whole weight of oysters at Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam deployed at a size of 15 mm shell height or 20 mm shell height.

Survival and growth of oysters at Ban Sen Island and Bai To Long Bay

The suitability of the area of Ban Sen Island, Bai Tu Long (BTL) Bay for oyster culture was investigated. Experimental oysters were taken to two sites (Site A and Site B, Fig. 5.7) in September 2009 to assess growth and survival and to provide baseline cultivation data. Environmental conditions in this area were measured concurrently and are listed in Appendix 1.

Thirty individuals were randomly selected from each site monthly. Measures of whole oyster weight, meat weight and shell dimensions (height, length and width) were taken on each oyster sampled. Survival was estimated by counting the number of live oysters on each line expressed as a percentage of the number of oysters that were originally on that line at the start of the experiment. Survival was counted on 30 lines at each site on each sampling date. The duration of the experiment was 8 months.

Oysters had a shell height of 20 mm and a whole weight of 3 g when deployed. Oysters at Site A grew slightly faster than oysters at Site B with oysters at Site A having a shell height of 92.4 mm at the end of the experiment (Fig 7.7). Oysters at Site A also weighed more in terms of whole weight and meat weight on each sampling date (Figs. 7.8 and 7.9). Overall growth rates were impressive with oysters reaching a minimum marketable size (> 40g) after 6 months. This experiment was conducted through the winter months and growth rates are likely to be quicker through summer months when water temperatures are higher. Losses of oysters occurred at a steady rate over the course of the experiment at both sites with approximately 74.7% and 67.5% of the oysters surviving at Site A and B, respectively (Figure 7.10).

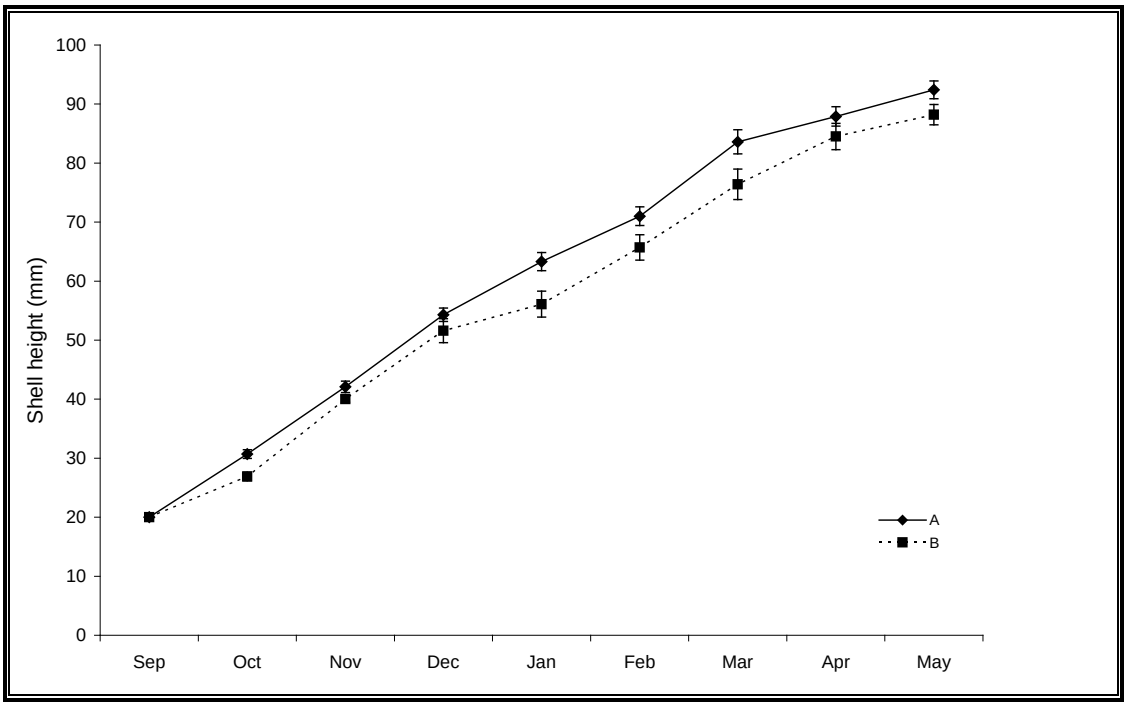

Figure 7.7. Shell height of oysters at Site A and Site B, Ban Sen Island, Bai Tu Long Bay, Vietnam.

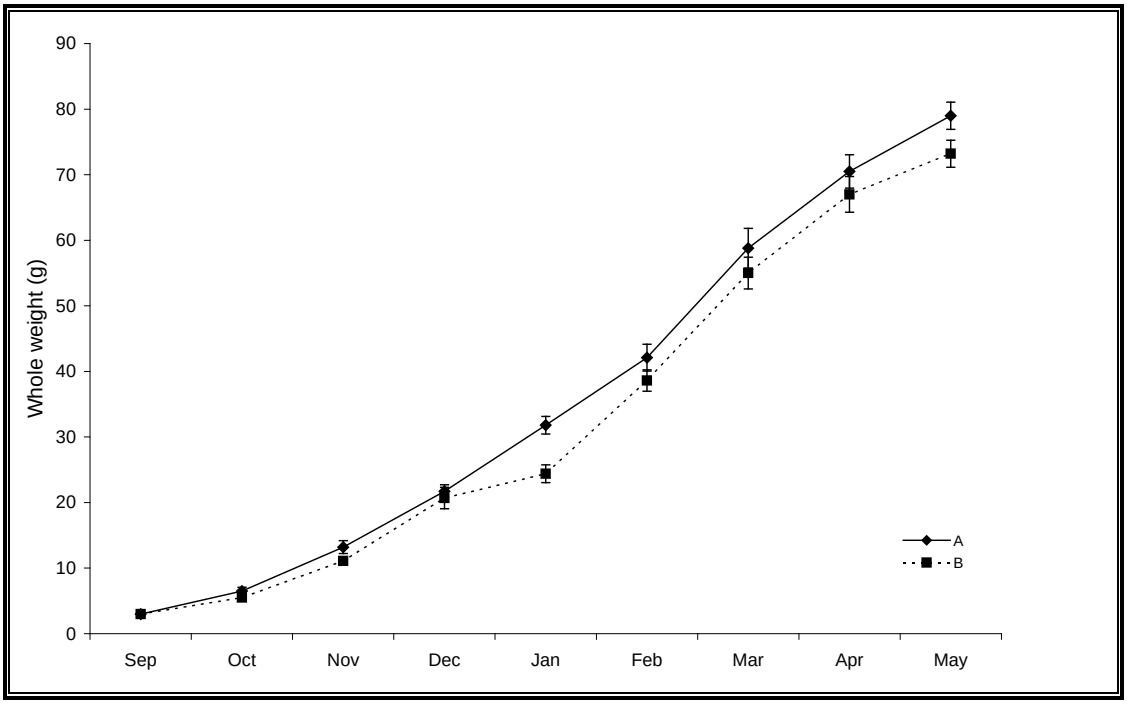

Figure 7.8. Whole weight of oysters at Site A and Site B, Ban Sen Island, Bai Tu Long Bay, Vietnam.

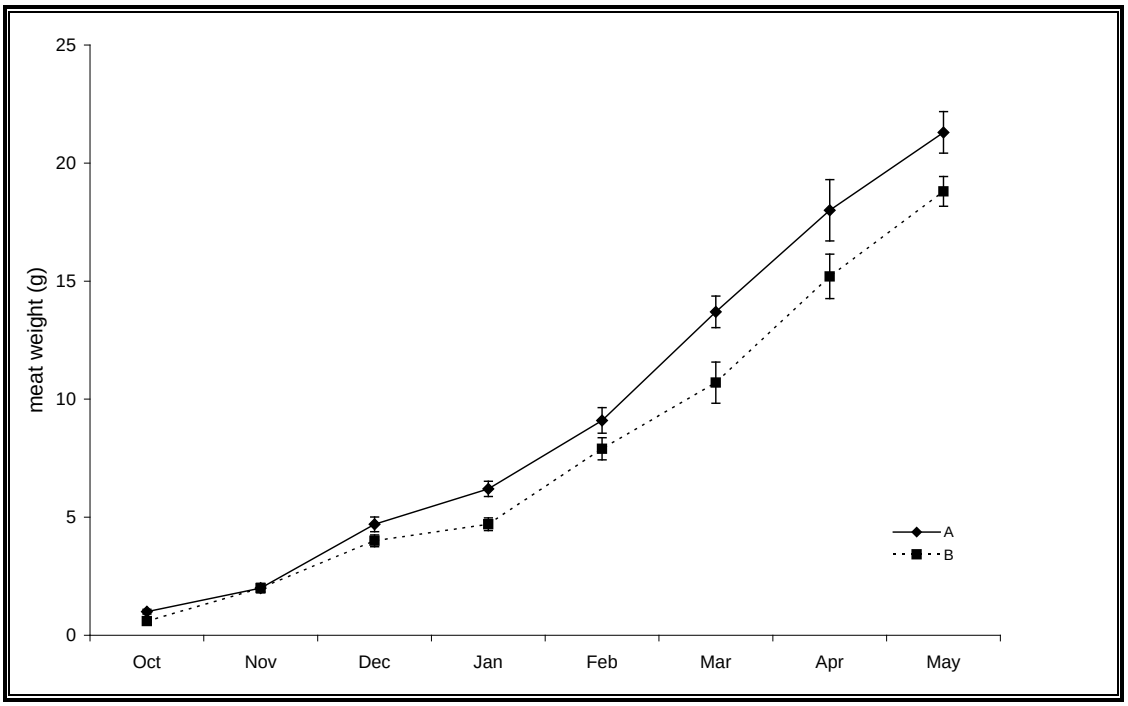

Figure 7.9. Meat weight of oysters at Site A and Site B, Ban Sen Island, Bai Tu Long Bay, Vietnam.

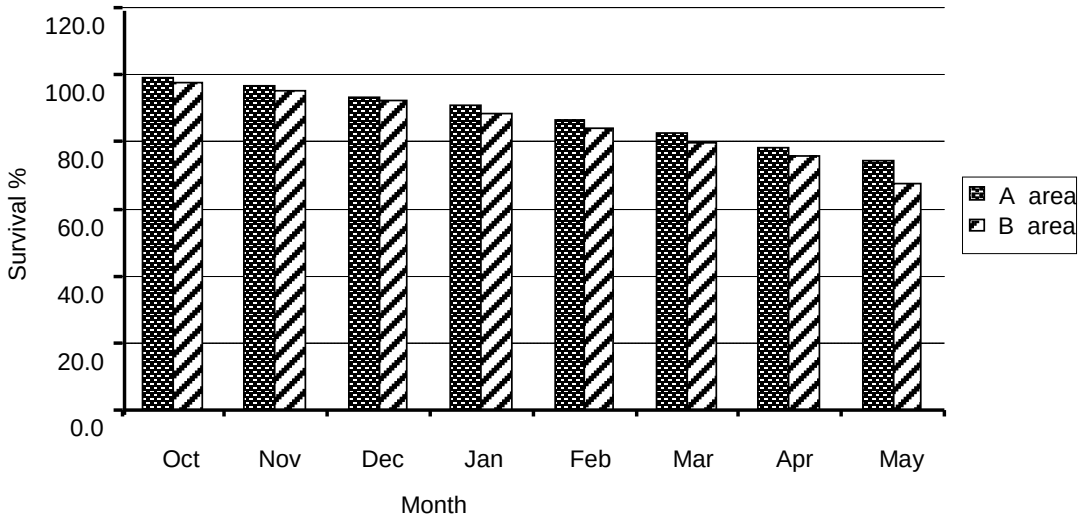

Figure 7.10. Percentage survival of oysters deployed at Site A and Site B, Ban Sen Island, Bai Tu Long Bay, Vietnam.

Assessment of three oyster culture techniques in Northern Vietnam

Large scale commercial oyster cultivation in Vietnam is still in its infancy. Methods of commercial growout of oysters have evolved around existing structures from which oysters are suspended in the water (i.e. sea cages) and available materials that are not excessively costly. Different methods of commercial oyster culture offer both advantages

and disadvantages and is primarily governed by cost of equipment, labour required and the type and price of oyster seed. Lines suspended from bamboo rafts have become the most dominant form of oyster culture. This method uses about 8 adult oyster shells as cultch which are attached to line (usually 2 m in length) that is tied off on bamboo cross members of a floating raft (Fig. 5.5 B).

Two other common forms of oyster cultivation use trays attached to racking or baskets either attached to racking or longlines. Tray and basket oyster culture is generally used by more advanced oyster producers that receive stock for their farms as single seed oysters either from a hatchery or the wild. Single seed systems allow farmers to individually grow small oysters (5 mm shell height) and to take advantage of breeding programs run by hatcheries. Tray and basket systems assist with stock management through their growout cycle as they make it easy to: undertake regular grading, clean fouling from oysters and equipment and to mechanise the farming process. The disadvantage of trays and baskets are that you require baskets and trays with a range of mesh sizes and the equipment is comparatively expensive to purchase initially. However, the quality of stock produced from single seed culture systems is superior to cultch set methods and is able to achieve higher market prices.

This trial assessed three different growing techniques which were specifically adapted to oyster culture in Vietnam. The three techniques assessed were: cultch set lines, tray and basket culture. All three systems were trialled as sub-tidal culture suspended from bamboo rafts. Growth and survival was measured for each type of culture system. For cultch set oysters, the density was set at 20 individuals per shell and there were 8 shells on each line. 500 lines were deployed to three rafts. Trays were stocked at a density of 440 individuals per tray and were 120 x 60 x 15 cm in size. 200 trays were hung sub-tidally from three rafts. 200 plastic baskets were deployed sub-tidally from three rafts. Each basket had a diameter of 40 cm, 10 layers with 15 cm separation between layers and stocked with 440 single seed oysters. Sampling was done monthly and 30 individual oysters from each culture type were removed for measurements. This experiment started in September 2009 and monitoring continued until July 2010.

Oysters settled on cultch had the highest shell height on each date that the oysters were sampled (Fig. 7.11). However, these oysters started the experiment at a larger size than the oysters in baskets. A consistent difference was measured between the three culture methods on each date apart from December 2009 when tray oysters were larger than cultch set oysters (Fig. 7.11). At the end of the experiment oysters that were set on cultch weighed more than oysters in trays and in baskets (Fig. 7.12). Whole weight of oysters in July 2010 for cultch set, tray culture and basket culture were 107 ± 1.72 g, 100.4 ± 1.31 g and 86.2 ± 1.57 g respectively (Fig. 7.12). Figure 7.13 shows survival throughout this experiment for cultch set, tray and basket culture. Best survival (72.5 ± 1.54%) was measured in oysters farmed using the cultch set method, tray culture followed with 70 ± 1.43% survival and lowest survival was recorded in baskets at a level of 61.3 ± 1.4% (Fig. 7.13).

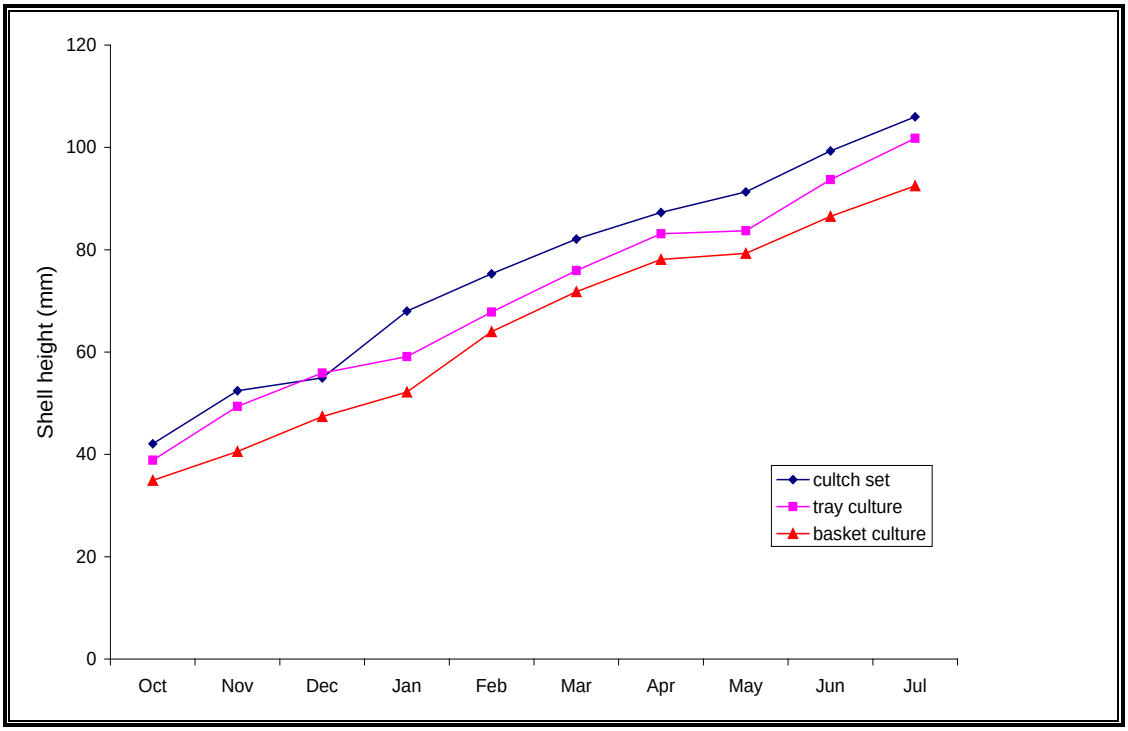

Figure 7.11. Shell height of oysters at Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam deployed as cultch set spat, in trays or in baskets suspended sub-tidally from rafts.

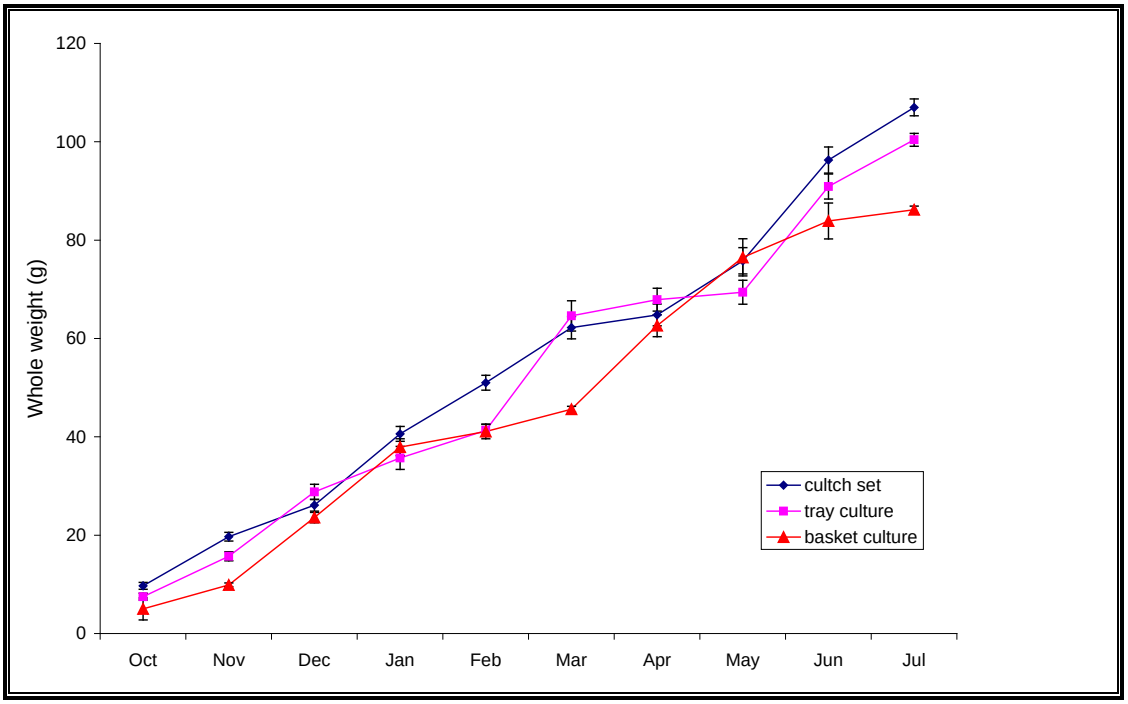

Figure 7.12. Whole weight of oysters at Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam deployed as cultch set spat, in trays or in baskets suspended sub-tidally from rafts.

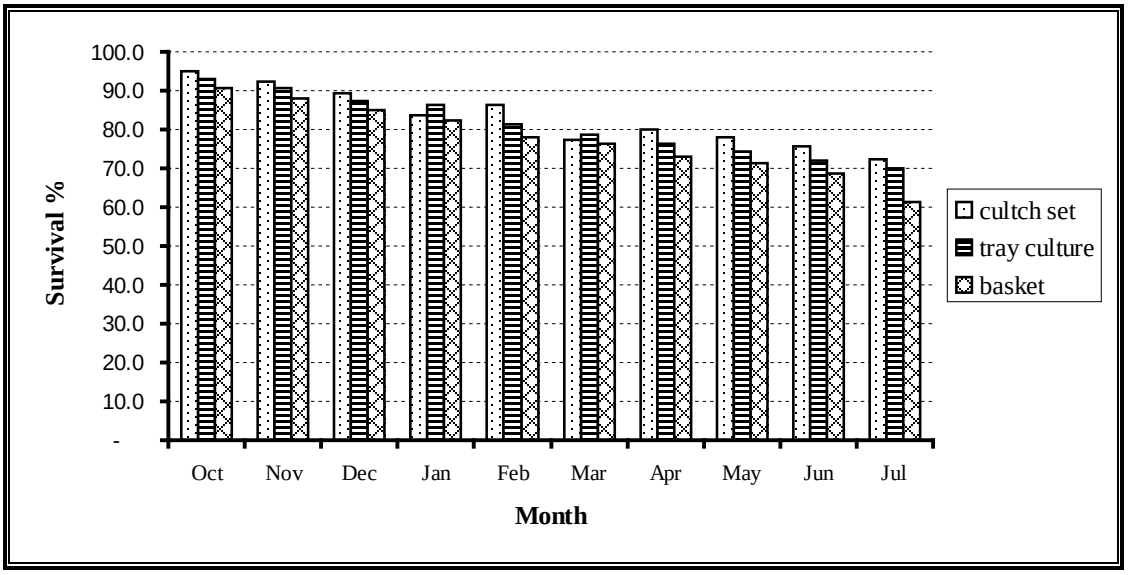

Figure 7.13. Survival of oysters at Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam deployed as cultch set spat, in trays or in baskets suspended sub-tidally from rafts.

The reproductive cycle ratio of cultivated oysters in Northern Vietnam

Understanding the reproductive cycle of commercially important oysters is central to hatchery production and research as well as harvesting and marketing. There are a number of endogenous and exogenous factors that affect the reproductive cycle of oysters. Knowing when broodstock are in a ripening phase, fully ripe or spent assists hatchery planning as larval runs can be timed to utilise oysters that are in peak reproductive condition. Good quality broodstock will produce good quality gametes which can dramatically improve hatchery performance. The ability to collect fecund oysters from farms or the wild can avoid hatchery broodstock conditioning which is costly with respect to the algae required and is a time consuming process. Knowing about the biology of the species under cultivation also assists farmers wishing to catch wild seed in the environment. Information on sex ratio greatly assists hatchery operations and planning, particularly when conducting breeding programs, as it allows better estimation of the number of broodstock required for commercial and breeding hatchery runs.

Oysters are generally consumed when meat condition is of a high quality. It is important for farmers to understand temporal meat condition cycles so that they can harvest crops at opportune times. This means that the oysters sold have good market acceptance, achieve a premium price and consumers enjoy the product.

The reproductive cycle of oysters was measured for this study because of the reasons provided above. After oysters were spawned in the hatchery they were taken to Ban Sen Island, Bai Tu Long Bay (Fig. 5.7) in trays for monthly gonadal assessment. Oysters were stocked into 60 x 40 x 15 cm trays at a density of 40 individuals per tray. Thirty oysters were removed randomly from trays every 14 to 18 days starting on 25 March 2009 and finishing on 26 July 2009. After size measurements were taken oysters were shucked and a transverse tissue cross-section was cut from the anterior of the oyster through the gonad, intestine, digestive diverticula, stomach and labial palps. A second cut was made 3 mm below the first providing a piece of gonadal tissue which was then fixed in 10% formalin and seawater solution. Paraffin embedded 5 µm sections were cut and mounted on acid washed glass slides and stained with haematoxylin and eosin (H&E). Slides were examined with a compound stereo microscope.

Oyster sex was determined and stages of gonadal development were classified into 5 reproductive phases. The first category (0) was assigned to oysters in an indistinguishable regressive phase where gonial cells are indifferent. The second category (I) was assigned to oysters in a very early ripening phase where oocytes and spermatocytes had formed but connective tissues were still dominant in the gonads. The third category (II) was the early development phase. The gonads were ripening and were well developed with gametes having well developed nuclei and occupying a large volume in the gonad glands. The fourth category (III) was the late development phase where oysters were fully ripe and characterised by oocytes and spermatocytes reaching maximal size and spermatozoa were active. The fifth category (IV) was spawned development and gonad regression. This phase occurs post spawning and follicles had reduced in volume due to release of gametes. Some oocytes and spermatocytes were present as well as phagocytes in the gonad in this phase.

The number of males sampled outnumbered females in all size classes except for > 100 mm shell height where the male to female ratio was 0.96 (Fig. 7.14). The ratio of males to females decreased as size class increased. There were 58.3% males and 25% females in the < 70 mm shell height size class with 17% of oysters in an undeterminable state. In all oysters sampled 55.2% were males, 43.8% were females and 1% were indeterminable (Fig. 7.14).

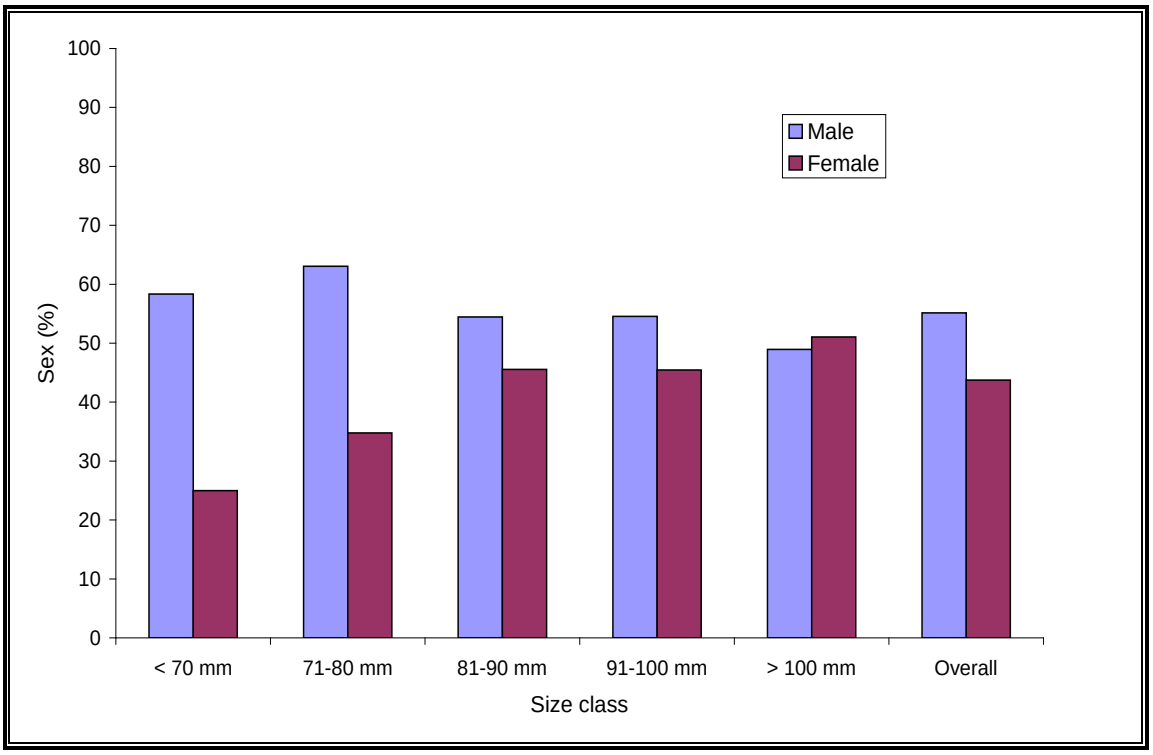

Figure 7.14. Sex ratio of oysters at 5 size classes based on shell height sampled from Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam in 2009.

The reproductive cycle of oysters in northern Vietnam was measured through the late spring and early summer period and is displayed in Figure 7.15. In March and April, most oysters were either in an early or late reproductive development stage. Oysters commenced spawning in late April and the largest percentage of spawning oysters (23.3%) was sampled on the 8th of May (Fig. 7.15). Between late May and late June, 10% or less of the oysters sampled had spawned. Oysters in a fully ripe state were sampled on every occasion. Greater than 56% of oysters sampled were in a fully ripe phase on the 8th April, 25th April, 26th June and 26th of July. The latter date was when the maximum number of oysters sampled were in a fully ripe state (63.3%) which corresponds to the middle of the summer season.

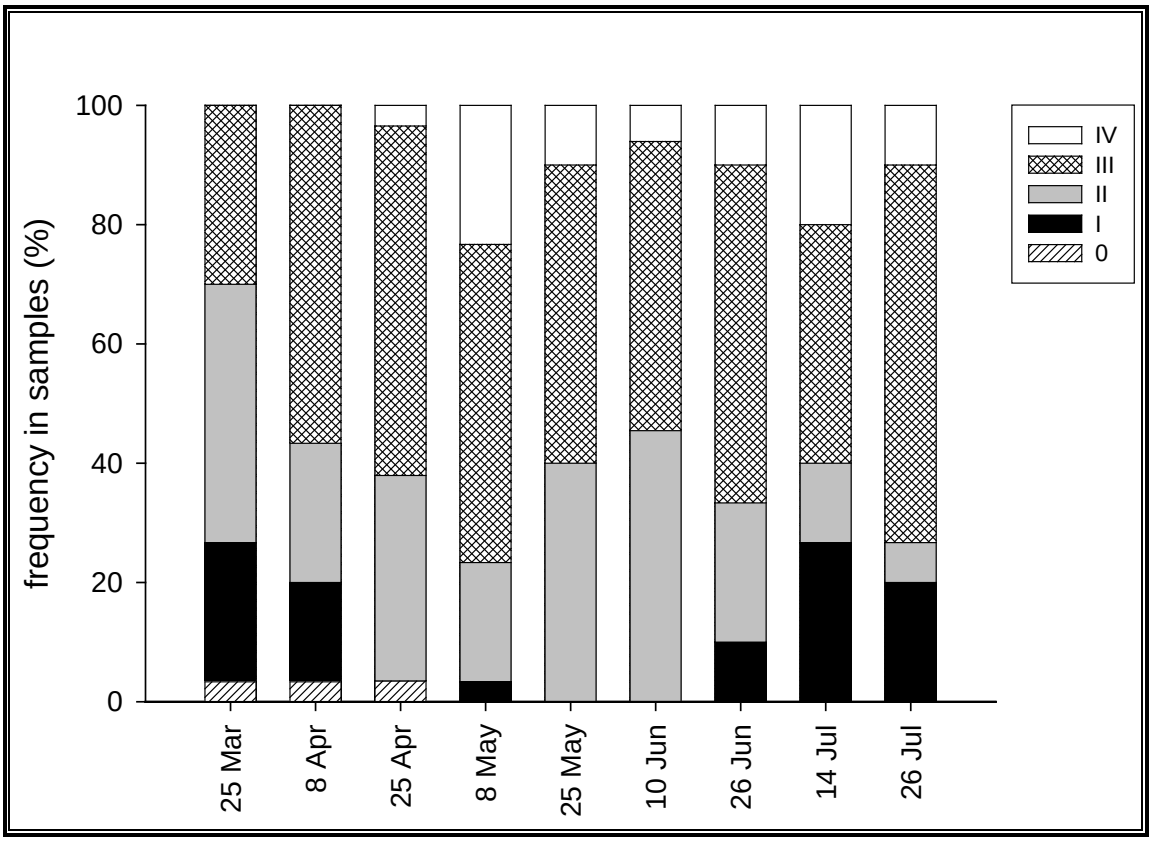

Figure 7.15**.** Frequency of gonadial phases in 30 oysters sampled from Site A, Ban Sen Island, Bai Tu Long Bay, Vietnam, each 14-18 days from 25 Mar 2009 to 26 July 2009. Gonadial phases are: 0 = indistinguishable phase; I = reduced development phase; II = early development phase; III = late development phase; IV = spawned development phase.

Triploid field evaluation

In November 2010, 130,000 triploid Pacific oyster spat were transferred from the hatchery at PSFI to the NMBC. These oysters were acclimated at the Cat Ba hatchery before transfer to five field sites. Some post transport mortality was observed, but this stabilised quickly and the spat were spread to 5 experimental locations including an experimental farm, a large commercial enterprise and three smaller commercial farms (locations chosen on the basis of previous encouraging growth with diploid stock).

Trials underway with triploid Pacific oysters have been disappointing. Growth performance has been comparatively poor and recent mortalities (May/June) have been high. Flat worms and mudworm caused persistent problems, but are unlikely to explain the losses. Inspection of the remaining stock suggested mortalities will continue. The surviving oysters are in poor physical condition: emaciated, weak adductor muscles, pale gut colour and little crystalline style.

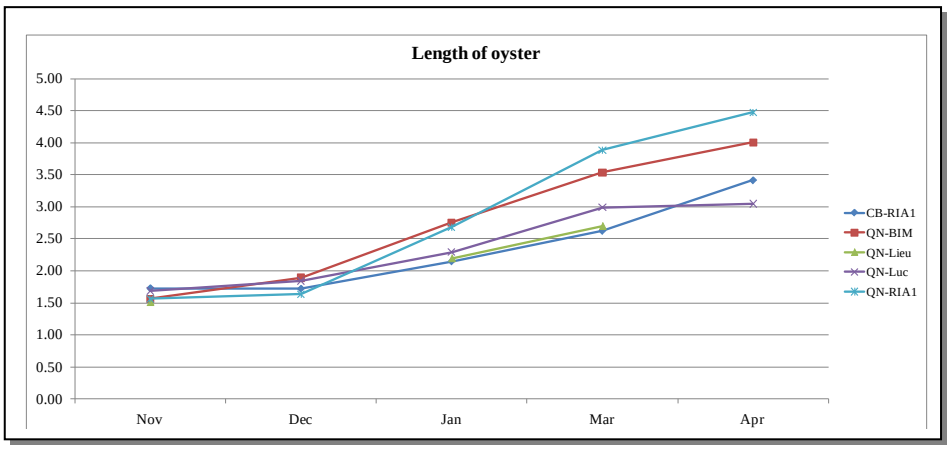

Figure 7.16. Growth of triploid Pacific oysters at 5 sites in Northern Vietnam (data courtesy Dr Le Xan)

From the outset of this program an emphasis has been placed on continual sample collection and fixation for potential disease diagnosis. This sample collection has also been a feature of the triploid assessment and those samples will be processed for histopathology in the hope of better understanding the losses.

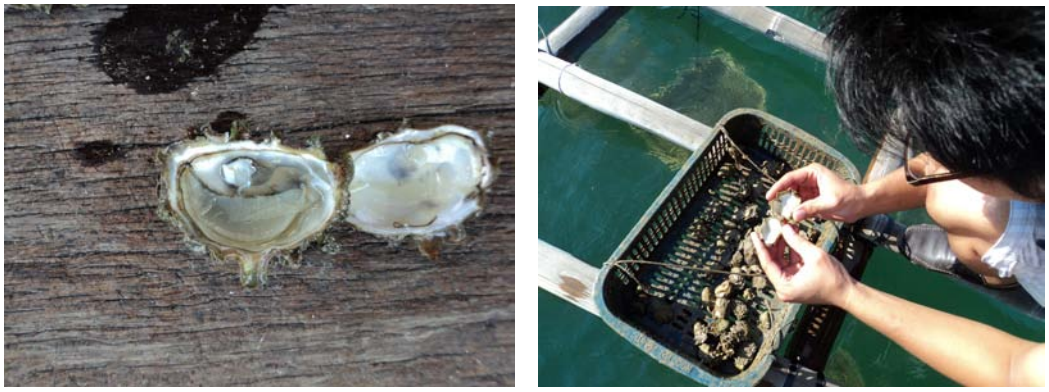

Figure 7.17. Surviving triploid Pacific oysters at the RIA 1 experimental farm, Bai Tu Long Bay (June 2011)

7.2 Australia

The Australian component of this research focussed on two areas: 1) application of alternate methods for triploid induction in Pacific oysters and 2) hatchery production of the pipi (*Donax deltoides*). The first description of the early developmental stages of pips has been published in the journal Molluscan Research (O'Connor and O'Connor 2011).

Triploid induction and evaluation

The induction of triploidy (3n) in edible shellfish has become a popular tool to increase growth rates and the marketing season of stock (Nell, 2002). Additional benefits in disease resistance have been observed, including reduced losses in *S. glomerata* caused by the disease winter mortality (Hand et al., 1998). Triploid oysters can be produced by inhibiting meiosis II using a chemical or physical stress (Nell, 2002). The chemicals

cytochalasin B (CB) and 6-dimethylaminopurine (6-DMAP) have been the primary agents used to inhibit meiosis in oyster eggs and while they continue to be successful, the toxicity of CB in particular has raised health concerns (Guo et al., 1994). It is considered that a physical stress such as heat sock or hydrostatic pressure offers a more user friendly method of triploidy induction and would be more suitable for commercial hatchery use.

Heat shock and hydrostatic pressure have both been employed to inhibit polar body extrusion in a range of shellfish species with varying success. Beaumont and Fairbrother (1991) summarised ploidy manipulation in shellfish using physical shock and reported that temperatures ranging from 25-38°C were successful for triploidy induction, while hydrostatic pressure levels ranging from 6000-8000 psi produced the best results.

The success of triploid inductions is most easily determined using flow-cytometry (FCM). Access to a flow-cytometer can sometimes be limited which creates a need for suitable preservation techniques of larval samples. Yang et al., (2000a) outlined a method for preserving Pacific oyster gill tissue, which included a pre-treatment in a hypotonic solution (0.075M KCL solution) followed by fixation in 70% ethanol, however attempts to preserve SRO larvae for FCM using this technique have so far been unsuccessful. This study aims to investigate the success of a range of temperature shocks on triploid induction in the Pacific oyster (*Crassostrea gigas*).

Pacific oyster temperature Trial 1

Trial 1 was designed as a 3-factor experiment to determine the effects of temperature shock (29-46.5°C) on triploid induction in the Pacific oyster. The three factors included exposure temperature (29, 31, 33.5, 36, 38 or 46.5°C), time of exposure (10, 15 or 20 MPF) and the duration of exposure (10, 15 or 20 min). The experiment was replicated using three mating pairs (fertilised at 24°C).

Triploid Pacific oysters were detected by flow cytometry in oyster samples exposed to heated seawater of 29-36°C (Fig 7.1). Embryos exposed to seawater of 29 and 31°C produced a significantly greater proportion of triploid samples compared to those exposed to seawater of 36°C. No triploid oysters were detected when embryos were exposed to seawater of 38 or 46.5°C.

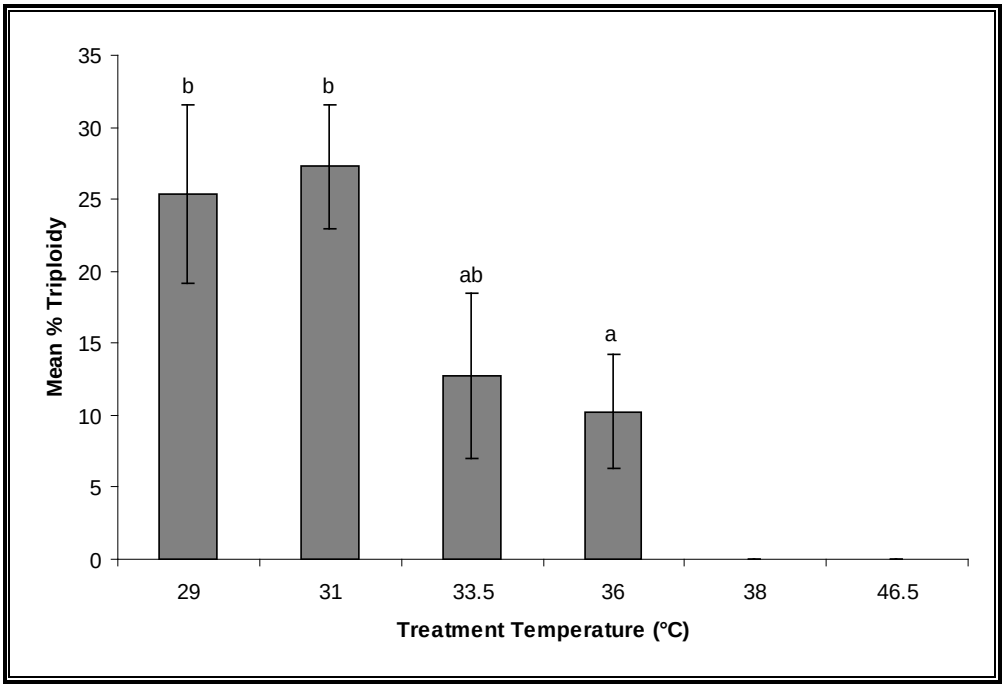

Figure 7.18. The Percent (mean ± SEM; n=6) of triploid Pacific oyster larvae exposed to a heat shock of 29, 31, 33.5, 36, 38 or 46.5°C. Different letters indicate significant differences between treatment temperatures (P<0.05; SNK test).

Pacific oyster temperature Trial 2

Trial 2 was intended to confirm the results of temperature Trial 1 and was designed as a 3-factor experiment to determine the effects of temperature shock (28.5-39.8°C) on triploid induction in the Pacific oyster. The three factors included exposure temperature (28.5, 29.5, 31.1, 32.4, 34, 35.1, 36.8, 38.2 or 39.8°C), time of exposure (5, 10, 20, 23 or 25 MPF) and the duration of exposure (5, 7, 10 min). The experiment was replicated using three mating pairs (fertilised at 24°C).

The experimental design was not orthogonal and the variation in data made statistical analysis unreliable. Therefore, the data were only plotted for visual comparisons (Fig. 7.2). It appears that temperatures between 31 and 35°C were appropriate for triploid induction in this trial. The samples exposed to temperatures of 38.2 and 39.8°C did not produce any triploid peaks.

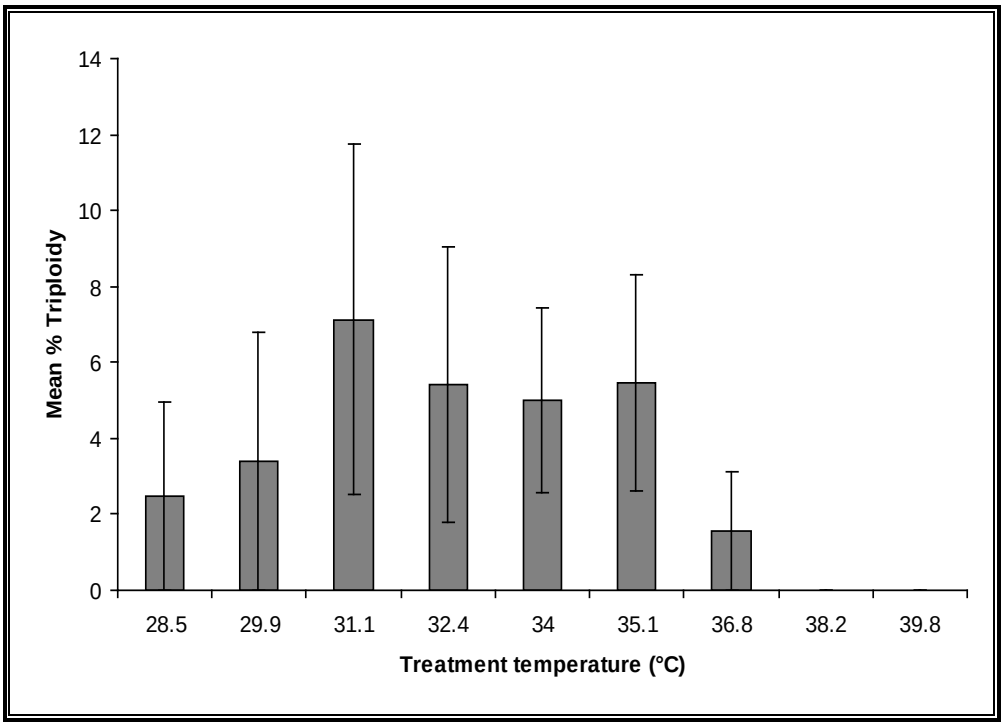

Figure 7.19. The percent (mean ± SEM, n=6) of triploid Pacific oyster larvae after exposure (15 MPF) to a heat shock of 28.5, 29.9, 31.1, 32.4, 34, 35.1, 36.8, 38.2, 39.8°C.

Pacific oyster temperature Trial 3

Trial 3 was intended to further investigate the results of previous temperature trials. A 2-factor experiment was designed to investigate three exposure temperatures (34, 36 or 38 °C) and two exposure times (20 or 25 MPF) on the success of triploid induction in the Pacific oyster.

Triploid Pacific oysters were detected consistently across the treatment temperatures (34, 36, 38°C) (Table 7.1). No significant differences were detected across the treatments for % triploidy or development to D-veliger.

Treatment temperature °C	Mean % triploidy	Development (% D-veligers)
34	41.5 ± 6.1	12.6 ± 2.0
36	47.3 ± 5.0	12.2 ± 2.1
38	46.0 ± 5.2	12.3 ± 2.6

Table 7.1. The percent triploidy and survival to D-veliger (mean ± SEM; n=18) across three temperatures 34, 36, and 38°C.

This study has determined that triploid induction in the Pacific oyster can be achieved using temperature shock and while the data has been variable the results have been promising. Triploid peaks have been detected between 29-31°C (Trial 1), between 31-35°C (Trial 2) and between 34-38°C (Trial 3). The available data also suggests that development to the D-veliger stage is not affected by temperature induction treatments. Temperature tolerances of Pacific oyster larvae have been investigated by Helm and Millican (1977) and it was reported that growth was improved as temperatures increased to 28°C, but at 32°C growth was poor. As the upper temperature tolerance limit of Pacific oysters may be less than the SRO it is understandable that lower temperatures (29-31°C, Trial 1) will provide the necessary shock required to inhibit meiosis.

Future experiments aim to determine the temperature, from 29-38°C, which will produce the greatest percentage of triploid oysters combined with acceptable development rates. Experiments will also be conducted on the hydrostatic pressure levels that will induce triploidy in Pacific oysters, and the induction of tetraploidy using temperature and hydrostatic pressure.

8 Impacts

8.1 Scientific impacts – now and in 5 years

First and foremost RIA 1 now have a first class mollusc hatchery (with algal production facility) that is capable of a broad range of molluscan research. The scientific capacity required to deal with new challenges has also developed. During the course of this project, seven RIA1 staff were trained in Australian hatcheries, 25 undergraduate students and 3 MSc students were involved directly on the program. In particular, two RIA staff were successful in acquiring John Allwright fellowships to undertake PhD studies in molluscan biology in Australia.

The culture techniques developed during this project have been published as a resource to both industry and other researchers in Vietnam. Collaboration amongst the various research institutes in Vietnam was encouraged and representatives of both RIA 2 (Southern Vietnam) and RIA 3 (Central Vietnam) were participants at a specialist shellfish workshop in Quang Ninh.

Figure 8.1. NMBC algal culture unit and hatchery facilities

This project has established the basis of ongoing collaboration between Australian scientists and MARD. An additional follow up project has been funded by ACIAR (FIS-2011-073) and will entail a detailed review of mollusc health diagnostic capacity and mollusc biosecurity in Vietnam to support industry expansion. Further collaboration has also been proposed.

The successful establishment of an oyster industry in Vietnam and its rapid expansion have brought with it notoriety that has encouraged increased international interest. Other research programs targeting oysters are now developing and have greater impetus than in the past. It is now becoming clearer to the international scientific community that Vietnam has an increased molluscan production capacity and that research outputs can be readily adopted. In acknowledgement of recent progress, the World Oyster Society will hold the 5th International Oyster Symposium In Ho Chi Minh City in December 2013.

ACIAR involvement helps develop the communication and extension ability of the Australian scientists involved. It has exposed our researchers to new species and challenges and has assisted in the development of international profiles for those involved – increasing the opportunities for collaborative research in the future.

While investigations of the applications of alternate methodologies for triploid induction failed to provide effective options to current methodologies. This research broadened our understanding of the triploid induction process, provided results that would suggest options for further development of existing protocols (not previously involving factors such as temperature), and has allowed future efforts to be focussed in other areas.

The work on the pipi (*Donax deltiodes*) has provided valuable new information on a species of both economic and ecological interest in NSW. Some previous perceptions on the duration of pipi larval life have been dispelled and for the first time the early development of the species has been described. In undertaking this work the first record of aporocotylids (fish blood flukes) in pipis was made, which has now become the subject of further CSIRO investigation.

8.2 Capacity impacts – now and in 5 years

Mollusc production capacity in northern Vietnam has been significantly enhanced.

- A commercial hatchery facility capable of producing a range of bivalves species has been established.
- Nursery and growout systems have developed that have paved the way for the establishment of a commercial oyster industry and form a platform for further work to assist industry to advance.
- The NMBC has a significantly enhanced capacity to deal with molluscan research programs.
- MARD capacity to manage molluscan industry development has increased and they have actively (independent of this program), begun to formulate the legislative framework for industry control and expansion.
- Local farmers have rapidly adapted simple culture technologies for large scale, low cost oyster production.
- The supply chain required to transport, process and sell up to 7.000 tonnes of oysters (molluscs) has developed.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

The economic impact of the development of an oyster industry in northern Vietnam has been significant. Rising from no commercial output in 2006–07 to 7,000 t in 2010–11, production is continuing to expand. At current output, industry value lies between A$7-9.8 million. If sufficient oyster spat is available to allow expansion, a further 1000 rafts are expected to be constructed in the coming 12 months. On the basis of current production values this would imply a 45% increase in industry value ($3.1-4.4 million).

Table 8.1 Current and potential economic benefit from increased oyster production.

Scenario	**Output (t)**	**Rafts**	**Value (@A$1-1.40/kg)**	**staff**
Current oyster production	7000	2200	A$7.0 - 9.8 million	1500
Additional 1000 rafts	3181	1000	A$3.1 – 4.4 million	681

Figure 8.2. Marketing and distribution of oysters in Vietnam (June 2011)

8.3.2 Social impacts

Over 1500 people are now fully employed in the oyster industry and poor coastal communities are seeing the benefits of oyster faming and enthusiastically embracing the opportunity.

A novel social impact assessment process that had been developed in oyster communities in Australia was applied during this project. The “photovoice” method uses photos and diary comments from farmers, which were then mapped through a five capitals approach (including social capital), which provided a structured assessment of the impact of oyster farming. This method identified a series of positive social impacts including:

- employment (and the retention of the young within the commune),
- increased income and opportunities for diversity
- increased cohesion and community spirit,
- improved sanitation and drinking water,

8.3.3 Environmental impacts

Central to this program was the development of hatchery production techniques for bivalves to allow industry development while concurrently reducing pressure on wild stocks. This is particularly so for a clam industry such as Tu Hai, which was largely

dependent on wild harvest. Now farmers can access hatchery propagated stocks to sustain cultivation.

In the case of oysters, which were not previously cultivated, the wide spread adoption of culture has effectively reduced pressure on wild stocks of other species as producers move to one that is readily and reliably available.

In transferring hatchery techniques, an emphasis was placed on developing the capacity to produce a range of species, through demonstrating processes that have been adapted elsewhere to successfully produce many different bivalves. In the future the maintenance of biodiversity may well be assisted through the propagation of threatened or endangered species in hatcheries.

Oyster cultivation has brought with it an enhanced environmental awareness among framers. During the social impacts surveys, farmers spoke of the maintenance of a clean oyster growing environment, a desire to build oyster farming infrastructure from available natural resources and improvement of the sanitation practices of community members to ensure product safety.

Large scale bivalve production can have both positive and negative environmental impacts. Oysters and clams are filter feeders that perform a number of critical ecosystem services (Ruesnik et al., 2005) to the extent that they are commonly keystone species in the maintenance of ecosystems in coastal and estuarine areas. They can “clean” seawater by stripping organic material from the water column and have been shown to be capable of reducing the impacts of other activities such as fish farming which introduces nutrients.

Poorly managed, bivalve production can pose a variety of threats. The surrounding ecosystem can be affected through a loss of genetic integrity among wild stocks due to interbreeding with hatchery stocks. The movements of cultured stock can introduce or transfer diseases. The culture infrastructure can impede water flows and navigation, and in areas of significant tourist interest (such as Ha Long Bay and Bia Tu Long Bay) can reduce aesthetic appeal. These factors are being considered and dealt with where possible. Protocols for the maintenance of stock genetic diversity are in place and an assessment of oyster broodstock genetic variability is planned to prevent inbreeding and to ensure hatchery stocks are representative of those in the wild. A Health and Biosecurity review has begun to both protect the industry and wild stocks, and the Vietnamese Government is controlling industry development.

8.4 Communication and dissemination activities

Scientific communication

Outcomes from the project have been published in peer reviewed journals and project partners are working on 3 additional manuscripts describing aspects of mollusc reproduction and growth in Vietnam (Section 10.2).

Broader international interaction was fostered through RIA 1 staff involvement in the 4th International Oyster Symposium (Hobart, September 2011). On the basis of RIA 1 staff presentations at the conference and the progress in the oyster industry in Vietnam, the World Oyster Society has invited Vietnam to host the 5th International Oyster Symposium in Ho Chi Minh in 2013. This conference will show case development and attract additional scientific and commercial interest.

Industry communication

In March 2010, a hatchery workshop was held in Quang Ninh. Staff from each of the three Research Institutes for Aquaculture and NSW DPI presented information on aspects of mollusc culture to hatchery operators and farmers from the province.

Within Australia, industry were informed of the project via the NSW Aquaculture Research Advisory Committee and updates on progress were provided during the annual "Shed Talks" farmer communications days, held at various locations throughout NSW.

Progress from this project in both Australia and Vietnam was presented at "Shellfish Futures" (the Tasmanian Pacific Oyster Industry annual meeting) in Hobart.

Media communication

A number of general articles were prepared during the course of this project publicising outcomes:

- Wayne O'Connor, Le Thanh Luu, 2009. Bivalve hatchery production. ACIAR in Vietnam, Feb, 8.
- Le Xan, 2010. Oyster and tu-hai culture in Quang Ninh and Hai Phong provinces. Partners Magazine, 22-23.
- Wayne O'Connor, 2010. Oysters cultivate bilateral industry lift. Fish 18-4.
- Janine Pierce, 2011. Interview with a farmer on oyster farming. Partners Magazine, 22-23.
- Janine Pierce, 2011. Interview with a farmer on oyster farming. ACIAR in Vietnam, 22-23.

The outcomes of the project were featured by ABC's Land Line program and the story was subsequently translated into Vietnamese and shown nationally in Vietnam.

("Spat's what friends are for" August 2010, 15 min 30 sec Http://www.abc.net.au/landline/content/2010/s2990040.htm)

9 Conclusions and recommendations

9.1 Conclusions

- Existing hatchery and nursery technology can be successfully applied in Vietnam to produce both the milky oyster and the otter snout clam in commercial quantities.
- The Vietnamese aquaculture industry (researchers, regulators and producers) has clearly demonstrated the capacity to quickly adapt and adopt culture technology as needed to develop a new mollusc industry.
- The milky oyster offers significant opportunity for diversification and expansion of mollusc production in the future. Local markets have readily accepted the product and export markets have been tested and show promise.
- Heat shock is capable of inducing triploidy (and tetraploidy) in Pacific oysters, however we were unable to achieve the percentage triploidy produced using other methods.
- The pipi (*Donax deltiodes*) can be produced through simple adaptation of existing hatchery facilities and technologies. Its larval life cycle is significantly shorter than previously suggested and is similar to that of a number of other currently produced molluscan species. While spat were produced, nursery technology for this species was clearly identified as a bottleneck to commercial scale production.

9.2 Recommendations

The rate of development within the oyster industry in northern Vietnam and the scope bivalve industry growth is phenomenal. Domestic oyster demand has been estimated at >20,000 t and strong export potential exists to the north in China. However, to increase capacity and further develop markets, hatchery reliability and output must be increased, seed quality needs improvement and the quality of the harvested crop must be improved to exploit increasingly discerning markets. To build on the success of the partnership that established the oyster industry and increased clam seed supply in northern Vietnam, RIA No1 has requested NSW DPI's assistance to consolidate the existing project and address new challenges, both with oysters and clams.

A number of specific research questions need to be addressed, key among these questions are:

- Is the genetic diversity present in the imported oyster broodstock currently being used sufficient to avoid inbreeding and form the basis for a breeding program?
- What culture condition changes (static or flow-through, stocking densities, feed rates) are required to permit small scale, highly replicated larval rearing technology to allow a mollusc breeding program to be undertaken?
- What traits should form the basis of a hard clam breeding program and what potential genetic gains could be made through selective breeding?
- What techniques are required to allow "single seed" culture technology to be used in oyster production to improve production reliability and seed quality prior to distribution to farmers, and which nursery systems and operating protocols are best suited to rearing single seed oysters under local environmental conditions?

- What are the key legislative, operational and biological threats to the expansion of bivalve production. What disease threats exist and how can these be prevented or ameliorated. How should oyster quality be defined and what needs to be done to establish quality assurance standards?

Within Australia a similar project of developmental research is needed to capture potential for new or emerging bivalve species. Specifically, industry interest in flat oysters (*Ostrea angasi*), pipis (*Donax deltoides*) and razor clams (*Pinna bicolor*) has developed. Each species faces particular challenges that can be overcome through targeted research. The research proposed here will complement existing data sets/specimen collections or on-going research projects to maximise cost benefits.

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11 Appendixes

11.1 Appendix 1: Environmental water quality data from Bai Tu Long Bay

Table 11.1. Water temperature (°C) measured in the surface and bottom waters at Site A and Site B (Fig. 5.7) oyster grow-out sites.

Year	2008				2009				2010			
	Site A		Site B		Site A		Site B		Site A		Site B	
Month	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
1	22.1	21.5	22.5	21.2	24.8	24.3	24.5	24	22.1	21.7	21.9	21.3
2	28.2	27.1	27.2	26.6	26	25.5	25.3	25.3	27.2	27.1	27.2	26.6
3	22.1	21.5	22.5	21.2	24.8	24.3	24.5	24	22.1	21.7	21.9	21.3
4	28.2	27.1	27.2	26.6	26	25.5	25.3	25.3	27.2	27.1	27.2	26.6
5	30.3	30.5	30.5	30	28.8	28.5	28.8	28.8	30.5	30.1	30.4	30.1
6	31.2	31.3	30.6	30.5	29	28.7	29.2	29	30.8	30.3	30.6	30.4
7	31.1	30.9	30	29.7	29.7	29.5	29.7	29.7	30.2	29.9	30	29.7
8	30.3	30.5	30.5	30	28.7	28.5	28.7	28.7	28	28.1	28.7	28.7
9	31.2	30	30.1	29.6	29	28.5	28.2	28.2	29.5	28.5	28.1	27.2
10	25.1	24	24.9	24.2	27.7	27.2	27.5	27	28.7	27	27.5	27.5
11	22	22.3	22.2	22	25.2	24.5	25.5	24.7	25.2	24.5	25.5	24.7
12	19	20.2	20.1	20.4	25	24.5	25.2	24.5	25	25.5	24.5	24.5
Min	19	20.2	20.1	20.4	24.8	24.3	24.5	24	22.1	21.7	21.9	21.3
Max	31.2	31.3	30.6	30.5	29.7	29.5	29.7	29.7	30.8	30.3	30.6	30.4

Table 11.2. Salinity (ppt) measured in the surface (Sur) and bottom (Bot) waters at Site A and Site B (Fig. 5.7) oyster grow-out sites.

Year	2008				2009				2010			
	Site A		Site B		Site A		Site B		Site A		Site B	
Month	Sur	Bot	Sur	Bot	Sur	Bot	Sur	Bot	Sur	Bot	Sur	Bot
1	24	25.6	24	25.1	32.9	33.4	32.8	33.1	29	29.4	29.6	29.4
2	23.1	25.6	23.8	25.1	32.4	32.6	32.4	32.5	29	29.9	30.2	30.2
3	23.6	23.6	24.3	24.3	32.9	33.2	33.2	33.2	29	30	30.3	29.9
4	22	22.4	20.9	22.2	29.9	30.2	29.7	29.8	25.7	28.8	28.9	28.3
5	25	25.4	25.7	25.7	31.8	31.9	31.3	31.3	28.5	29	29.3	29.1
6	30	31	30	31	21.2	23	21.2	22.5	30	30.8	30.2	30.5
7	30	30	30	30	20.5	23	21.2	22.5	29.7	30	29.7	29.9
8	30	31	31	31	21	21	21.7	21.7	30.4	30.6	30.6	30.7
9	30	31	30	30	22.5	23	21.5	22.75	30.5	30.7	30.3	30.4
10	32	32	32	32	23	23	23.2	23.25	32.3	32.5	31.8	31.9
11	33	33	33	33	24.7	24.5	24.5	24.25	32.6	32.9	32.7	32.6
12	33	33	33	33	24.7	24	24.7	24.5	33.2	33.3	33.1	33.4
Min	22	22.4	20.9	22.2	20.5	21	21.2	21.7	25.7	28.8	28.9	28.3
Max	33	33	33	33	32.9	33.4	33.2	33.2	33.2	33.3	33.1	33.4

Table 11.3. pH measured at Site A and Site B (Fig. 5.7) oyster grow-out sites.

Year	2008		2009		2010	
Month	Site A	Site B	Site A	Site B	Site A	Site B
1	7.63	7.62	8.47	8.27	7.43	7.42
2	7.61	7.64	7.84	8.22	7.41	7.44
3	7.66	8.29	7.69	8.17	7.46	7.47
4	7.65	7.67	7.89	7.69	7.45	7.47
5	7.7	7.65	7.99	8.14	8.02	7.45
6	7.89	7.88	8.15	7.95	7.69	7.68
7	7.87	7.9	8.1	7.9	7.67	7.7
8	7.92	7.93	7.95	7.85	7.72	7.73
9	7.35	7.38	7.58	7.96	7.67	7.18
10	7.4	8.55	7.43	7.91	7.2	7.21
11	7.39	7.41	7.63	7.43	7.71	7.21
12	7.9	7.89	8.25	8	7.7	7.69
Min	7.35	7.38	7.43	7.43	7.2	7.18
Max	7.92	8.55	8.47	8.27	8.02	7.73

Table 11.4. Oxygen concentration (mg/L) and turbidity (secchi disk, m) measured at Site A and Site B (Fig. 5.7) oyster grow-out sites.

Year	**2008**				**2009**				**2010**			
	Site A		**Site B**		**Site A**		**Site B**		**Site A**		**Site B**	
Month	**Oxy**	**Tur**	**Oxy**	**Tur**	**Oxy**	**Tur**	**Oxy**	**Tur**	**Oxy**	**Tur**	**Oxy**	**Tur**
1	6.07	1.53	6.01	1.46	6.04	1.69	6.34	1.59	5.87	1.33	6.06	1.23
2	6.17	1.41	6.06	1.49	6.24	1.69	6.34	1.89	5.97	1.21	5.97	1.23
3	6.23	1.46	6.05	1.39	5.99	1.64	6.34	1.64	6.03	1.26	6	1.15
4	6.28	1.55	6.1	1.46	5.99	1.89	6.09	1.54	6.08	1.35	6.02	1.22
5	6.2	1.49	6.1	1.52	5.94	1.59	5.99	1.54	6.05	1.29	5.98	1.27
6	6.24	1.57	6.23	1.83	6.44	1.77	7.89	1.8	6.25	1.9	6.6	1.9
7	6.13	1.59	6.32	1.49	6.33	1.79	7.87	1.72	6.3	1.95	6.6	1.85
8	6.23	1.47	6.23	1.49	6.43	1.67	7.92	1.75	6.5	1.95	6.6	2.15
9	6.29	1.52	6.26	1.41	6.49	1.72	7.91	1.65	6.25	1.9	6.6	1.9
10	6.34	1.61	6.28	1.48	6.54	1.81	7.96	1.72	6.25	2.15	6.35	1.8
11	6.26	1.55	6.24	1.53	6.46	1.75	7.96	1.78	6.2	1.85	6.25	1.8
12	6.2	1.64	6.29	1.53	6.4	1.84	7.9	1.9	6.25	1.9	6.5	1.85
Min	**6.07**	**1.41**	**6.01**	**1.39**	**5.94**	**1.59**	**5.99**	**1.54**	**5.87**	**1.21**	**5.97**	**1.15**
Max	**6.34**	**1.64**	**6.32**	**1.83**	**6.54**	**1.89**	**7.96**	**1.9**	**6.5**	**2.15**	**6.6**	**2.15**

Table 11.5. Environmental water quality data measured at Site A and Site B (Fig. 5.7) oyster grow-out sites in 2008.

Sample (month)	Location	BOD_5 (ppm)	COD (ppm)	NO_2-(ppb)	NO3-(ppb)	NH4+ (ppb)	Total nitrogen (ppm)	Alkality (ppm)	Petroleum (ppm)
1	Site A	1.23	3.1	16.2	124	97	0.835	85	0.14
	Site B	1.03	3.95	30.2	106	183	0.682	90	0.154
2	Site A	1.4	3.39	20.2	103	227	1.169	100	0.364
	Site B	1.68	3.76	22.2	65	137	1.582	93	0.185
3	Site A	1	3.46	17.2	91	174	0.982	81	0.158
	Site B	1.26	2.79	22.2	61	226	1.453	102	0.165
4	Site A	1.37	3.48	36.2	102	158	1.124	89	0.273
	Site B	1.24	3.25	24.2	72	206	1.274	85	0.064
5	Site A	0.77	3.77	13.2	92	105	1.352	87	0.029
	Site B	1.65	2.61	55.2	61	285	1.137	121	0.177
6	Site A	1.11	2.98	15	115	86	0.535	79	0.128
	Site B	0.91	3.83	29	97	172	0.382	84	0.142
7	Site A	1.28	3.27	19	94	216	0.869	94	0.352
	Site B	1.56	3.64	21	56	126	1.282	87	0.173
8	Site A	0.88	3.34	16	82	163	0.682	75	0.146
	Site B	1.14	2.67	21	52	215	1.153	96	0.153
9	Site A	1.25	3.36	35	93	147	0.824	83	0.261
	Site B	1.12	3.13	23	63	195	0.974	79	0.052
10	Site A	0.65	3.65	12	83	94	1.052	81	0.017
	Site B	1.53	2.49	54	52	274	0.837	115	0.165
11	Site A	1.03	3.55	10	72	164	1.063	85	0.026
	Site B	0.97	3.57	15	104	193	1.158	78	0.158
12	Site A	0.73	3.16	18	95	263	1.066	82	0.14
	Site B	1.35	2.97	36	86	115	0.949	92	0.114
Min		**0.65**	**2.49**	**10**	**52**	**86**	**0.382**	**75**	**0.017**
Max		**1.68**	**3.95**	**55.2**	**124**	**285**	**1.582**	**121**	**0.364**
TCVN 5943-1995									0
ASEAN standard				55	60	70			0.14

Table 11.6. Environmental water quality data measured at Site A and Site B (Fig. 5.7) oyster grow-out sites in 2009.

Sample (month)	Location	BOD_5 (ppm)	COD (ppm)	NO_2-(ppb)	NO3-(ppb)	NH4+ (ppb)	Total nitrogen (ppm)	Alkality (ppm)	petroleum (ppm)
1	Site A	1.49	2.95	26.2	103	111	1.219	94	0.204
	Site B	1.18	3.88	28.2	65	73	1.632	92	0.037
2	Site A	1.29	2.85	34.2	100	108	1.100	103	0.151
	Site B	1.1	2.84	43.2	88	96	0.875	96	0.414
3	Site A	1.18	2.95	23.2	91	99	1.032	89	0.235
	Site B	1.08	3.64	28.2	61	69	1.503	101	0.267
4	Site A	1.37	2.2	42.2	102	110	1.174	89	0.215
	Site B	1.05	3.32	30.2	72	80	1.324	88	0.16
5	Site A	1.84	3.25	19.2	92	100	1.402	98	0.114
	Site B	1.08	3.16	61.2	61	69	1.187	100	0.216
6	Site A	1.27	3.08	73	133	92	0.585	110	0.173
	Site B	1.08	4.36	80	173	172	0.432	92	0.275
7	Site A	1.37	2.83	25	94	100	0.919	88	0.192
	Site B	1.06	3.76	27	56	62	1.332	86	0.025
8	Site A	1.06	2.83	22	82	88	0.732	83	0.223
	Site B	0.96	3.52	27	52	58	1.203	95	0.255
9	Site A	1.25	2.08	41	93	99	0.874	83	0.203
	Site B	0.93	3.2	29	63	69	1.024	82	0.148
10	Site A	1.72	3.13	18	83	89	1.102	92	0.102
	Site B	0.96	3.04	60	52	58	0.887	94	0.204
11	Site A	1.32	3.29	16	72	78	0.863	100	0.887
	Site B	1.15	3.46	15	104	110	0.638	94	0.023
12	Site A	2.64	2.06	18	95	101	1.066	105	0.383
	Site B	1.35	2.17	36	86	92	0.949	92	0.167
Min		**0.93**	**2.06**	**15**	**52**	**58**	**0.432**	**82**	**0.023**
Max		**2.64**	**4.36**	**80**	**173**	**172**	**1.632**	**110**	**0.887**
TCVN 5943-1995		<10							0
ASEAN standard				55	60	70			0.14

Table 11.7. Environmental water quality data measured at Site A and Site B (Fig. 5.7) oyster grow-out sites in 2010.

Sample (month)	Location	BOD_5 (ppm)	COD (ppm)	NO_{2-} (ppb)	NO3- (ppb)	NH4+ (ppb)	Total nitrogen (ppm)	Alkality (ppm)	Petroleum (ppm)
	Site A	0.38	2.08	20	8	98	0.127	103	0.072
1	Site B	0.97	2.67	10	8	64	0.177	110	0.03
	Site A	0.98	2.63	35	10	47	0.088	107	0
2	Site B	0.69	1.07	13	11	56	0.201	110	0
	Site A	1.08	3.07	36	16	53	0.352	108	0.008
3	Site B	0.95	2.57	18	8	276	0.183	102	0
	Site A	1.05	2.07	10	10	94	0.083	117	0.139
4	Site B	1.18	2.08	26	15	114	0.247	110	0
	Site A	1.03	3.07	22	31	33	0.053	109	0.107
5	Site B	0.69	1.98	13	9	96	0.263	102	0.122
	Site A	0.62	2.14	14	15	105	0.196	110	0.112
6	Site B	1.06	2.98	11	12	32	0.063	115	0.087
	Site A	1.05	2.74	63	10	57	0.283	102	0.098
7	Site B	0.95	3.97	75	15	65	0.585	122	0.128
	Site A	1.24	3.76	28	9	47	0.864	90	0.173
8	Site B	0.73	2.86	37	15	46	0.522	102	0.136
	Site A	1.76	3.07	63	11	67	0.254	113	0.133
9	Site B	0.84	3.51	38	16	66	0.256	106	0.078
	Site A	1.35	2.64	57	13	77	0.874	126	0.103
10	Site B	0.97	2.75	74	9	85	0.876	105	0.108
	Site A	1.65	2.87	25	10	63	0.865	135	0.272
11	Site B	1.37	2.98	87	8	67	0.964	104	0.087
	Site A	1.03	1.98	37	5	43	0.074	107	0
12	Site B	0.97	2.07	16	9	65	0.107	113	0.083
Min		**0.38**	**1.07**	**10**	**5**	**32**	**0.053**	**90**	**0**
Max		**1.76**	**3.97**	**87**	**31**	**276**	**0.964**	**135**	**0.272**

Appendix 11.2. Executive summary from Pierce 2011

Pierce, J., 2011. The impact of oyster Farming in Vietnam (Van Don District), Quang Ninh province: Perspectives of oyster farmers. Final report To NSW DPI and ACIAR. 86 pp.

" **Executive Summary:** This research project was conducted at the request of the Department of Industry and Investment NSW, Aquaculture Section in collaboration with ACIAR and Janine Pierce from the University of South Australia. The project utilises a qualitative research technique called Photovoice. Data was gathered by cameras and diaries that were given to 10 selected oyster farmers from Ban Sen Commune in the Van Don district. Each farmer was asked to record their story regarding the impact of oyster farming on their lives. The research was carried out in late October and early November 2011. In less than one week the study was explained to farmers, the nature of their involvement was outlined, photos and diary entries were captured, and the cameras and diaries were collected. Brief interviews on a few themes were also conducted with farmers. These yielded a range of demographic data and perceptions on oyster farming impact. Data from the photos and diaries was then coded into a five capitals framework (environmental, human, social, institutional and produced). Results indicate that oyster farming has had a predominantly positive effect on lives of farmers and those of their families and communities. Money from oyster farming has enabled diversification into other types of farming, provided jobs including casual seasonal jobs, and opportunities for younger people. The photos also captured some of the impact of the recent typhoon on oyster farms. So great was the impact of the typhoon on individual farmers and their community that a discrete section has been dedicated in this report to examine the gathered photo data (Section 3.6). Issues relating to parasites, seed quality, oyster prices, farmers not always being clear on designated oyster areas and insufficient access to bank loans to expand were highlighted by farmers. Overall, the impact of oyster farming has been mostly positive, with many farmers expressing a desire that the next generation may stay in the area, in oyster farming and that farms may expand.".