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Australian Centre for International Agricultural Research

Final report

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2 Executive summary

This is the fourth in a series of projects seeking to build mollusc industries in Vietnam and support existing industries in NSW. Primarily we have sought to underpin the rapid development of the Vietnamese oyster industry established in earlier work, through increasing oyster supply, opening opportunities for selective breeding and increasing the understanding of oyster quality and quality assurance. In NSW, we focussed on enhancing opportunities for new and emerging bivalve species.

In Vietnam oyster spat supply has increased and oyster production has more than doubled over the life of the program. Farming is now spread across 28 provinces and production has been conservatively estimated to have exceeded 15,000 tonnes/annum (some estimates place production at twice this level). Community involvement has increased with an estimated 2500 coastal-dwelling families now benefitting from the oyster industry. The processing and marketing sectors are also expanding. New processing facilities have been constructed and investment in the industry continues.

Fundamental to ensuring the future of oyster farming in Vietnam, we have confirmed the identity of the species of major interest as the Portuguese oyster (Crassostrea angulata). We have demonstrated that the oyster stocks present in Vietnam are sufficiently genetically diverse to form the basis of a pedigreed oyster breeding program. The highly-replicated rearing systems and production protocols needed to produce oyster family lines have been established and three generations of more than 450 oyster families have now been produced. These families have been assessed for key performance traits including growth and survival and the stock now being produced is acknowledged by farmers as superior to that from any other source, in particular imported stocks. Critical to the future of the breeding program, we have ensured that RIA1 staff have the requisite skills necessary to maintain the breeding program that has been established.

To provide a framework of support for the developing industry, a series of audits of mollusc biosecurity, mollusc health diagnostic capacity and oyster quality assurance were undertaken by independent experts and the outcomes of those assessments were used to direct ongoing research and guide training exercises. Considerable focus was placed on mollusc health management, with over 20 staff from various government institutions receiving training. To detect and manage threats to the industry a water quality monitoring program has been established to regularly collect environmental data on farms that include physico-chemical parameters, nutrients, metals and bacterial contaminants. These criteria were then reviewed and modified following an oyster quality assurance audit. The potential for harmful algal blooms and contaminated wastewater to affect both oyster and human health was acknowledged and we have worked to develop algal sampling and identification skills as well as identifying potential pollution sources through shoreline surveys.

Within Australia, research has made a number of significant contributions. Our understanding of the reproductive cycle of the flat oyster has significantly increased. As a result of hatchery research the output of flat oyster seed from each batch has doubled and has directly supported more than $1 million in oyster production in NSW. The technology has been disseminated to hatcheries in other states and has facilitated assessments of commercial flat oyster production in southern Australia. The possibility of farming pipis is now closer to reality with larval rearing and settlement techniques having been demonstrated for the first time. Razor clams were spawned and descriptions of the early larval stages were made, although more work is required to complete the larval life cycle. Meanwhile, in an effort involving both RIA1 and DPI staff, the development of the improved molecular tools for the assessment of genetic diversity in Sydney rock oysters has led to the most significant change in the Sydney rock oyster (SRO) breeding program in its 25 year history. The mass selected breeding lines that once were the basis for breeding were replaced with a pedigreed, family-based breeding program using among-

family selection with over 200 families, which was used as a model for the Vietnamese oyster breeding program.

3 Background

The impetus for this project began in 2005, following a visit by the Vietnamese Minister for Fisheries to New South Wales (NSW) and a subsequent study tour of bivalve culture facilities and farms in northern Vietnam by Australian researchers. That tour highlighted significant opportunities for bivalve industry development in Vietnam and made a number of recommendations. Those recommendations were adopted in ACIAR Project No. FIS/2005/114 ('Building bivalve hatchery production capacity in Vietnam and Australia'), which established the basis for this project. Here we have used the experience gained in developing and disseminating bivalve culture knowledge in Vietnam by the Research Institute for Aquaculture No. 1 (RIA1) and the NSW Department of Primary Industries (NSW DPI) to continue to develop small-scale agro-enterprises in rural Vietnam that will promote profitable and environmentally sustainable use of coastal and near-shore resources.

Prior to the commencement of this project a further small research project (FIS/2011/073) was undertaken to reinforce the technological progress made within FIS/2005/114 through increased institutional biosecurity and veterinary capacity that is essential for sustained bivalve fishery productivity growth and market-driven improvement in rural livelihoods.

In designing FIS/2010/100 we ensured our objectives were consistent with both the priorities expressed by ACIAR for Vietnam and NSW DPI's strategic plans for NSW Aquaculture at the time. We sought to '*lift productivity*' and '*improve rural livelihoods*' in Vietnam through the promotion of '*high value aquaculture industries*'. In NSW, we sought to achieve the '*Development of environmentally appropriate technology for aquaculture that will support industry expansion*'. The "new" candidate aquaculture species targeted were chosen to contribute to *"Improved performance of key industry* sectors (i.e. aquaculture) *leading to growth in State Product".* The project was also consistent with Vietnamese fisheries and aquaculture development policy calling for the need to: "*strongly promote aquaculture in freshwater, brackish water and marine environment, especially shrimp farming following, improved methods, efficiency and environmental sustainability. … and enhance capacity for post harvest handling to meet domestic and international markets".*

3.1 Vietnam

Most of the world's mollusc production comes from Asia, with China alone accounting for more than 80% of production. Vietnam has only begun to follow the example set by its Asian neighbours. Vietnam's mollusc production of 190,000 t/annum (comprised mostly of clams) is comparatively small given its coastal resources. However, recently there has been remarkable initial growth in the oyster industry, but oyster farming is in its infancy, with a very limited skill base. The singular focus on clams and inexperience with oysters limited market opportunities and predisposed the mollusc industry to the impacts that can arise from such dependence. Calamities such as poor natural spat fall (settlement of larvae onto a suitable substrate), typhoons, hatchery production failures or shortfalls, the accidental introduction or outbreak of significant disease, food poisoning or a pollutant scare could cause or already has caused considerable industry and social hardship.

A major constraint to the further development of bivalve culture in Vietnam at the time (2009) remained the availability of seed. Despite the success of the National Marine Broodstock Centre (NMBC) in producing up to 100 million oyster seed/annum, demand was so great that hatchery outputs needed to increase. Additionally, there is now a realisation that access to some markets will require substantial product quality improvements. The existing grow-out technology was based on oyster spat that were settled on oyster shells ('cultch') that were then hung on strings from rafts. This is simple and readily adopted by farmers due to its low cost, but could be improved if there was a call for greater product quality. Increased production of 'single-seed' oysters (cultch-free) is an effective way to improve product quality and to highlight the characteristics some markets require. Hatchery techniques for the production of single-seed oysters were successfully transferred to Vietnam. However, limitations in nursery capacity and technology made it difficult to rear high quality single-seed juvenile oysters to a size that growers in Vietnam can effectively farm and grow-out to market size. Additionally, raft cultivation techniques restricted growth performance of single seed oysters compared with cultch set spat.

With the refinement of hatchery technology, the development of single-seed methods and the establishment of nurseries, the development of sophisticated breeding programs become considerably more achievable. A breeding program can address many of the challenges facing the Vietnamese industry, including oyster growth, survival and quality and can discourage the use of imported Chinese seed, hence reduce biosecurity risks. Accordingly the development of systems and protocols for selective breeding were the highest priority in Vietnam and were core to the goals of this program.

The risks to the continued development of the hatchery-based bivalve industry are diverse, but are not unique and have been addressed elsewhere internationally. Through capacity enhancement, many risks can be averted, reduced or effectively managed in Vietnam. The continued development of hatchery technology and its ongoing dissemination to newly developing private hatcheries will increase reliability of seed supply and establish multiple independent suppliers. The development of site-specific grow-out technologies will increase productivity and product quality. The development of food-quality standards and practices will improve marketability, consumer acceptance and export potential, while the enhancement of biosecurity and mollusc health management will better prepare the industry for the pest, parasite and disease challenges already beginning to emerge.

3.2 Australia

In contrast to Vietnam, Australian mollusc production was already diverse, well established and more technologically advanced. Molluscs were contributing >40% of the value of Australian aquaculture and was one of our fastest growing aquaculture sectors. That vibrancy had led the mollusc sector to introduce new species, such as flat oysters, and to call for research into opportunities to exploit others that are not yet cultured, such as pipis and razor clams. Disease threats, for example ostreid herpesvirus in Pacific oysters and QX disease in Sydney rock oysters has caused the Australian oyster industry to look to alternate species for diversification. Further, Australian farmers are looking for opportunities to invest in the Asian industry. To date, as a direct result of the previous ACIAR program, three delegations of Australian oyster farmers have visited Vietnam to look at the developing oyster industry and have provided oyster seed for evaluation. This proposal sought to continue to encourage and support these interactions and introduce Vietnamese farmers to a broader range of culture equipment and technology from Australian manufacturers.

4 Objectives

The overarching aim of this project was to increase hatchery-based bivalve production in Vietnam and New South Wales to expand opportunities for coastal communities to rear bivalve molluscs. Our objectives were to:

1. improve hatchery reliability (in government and private facilities) for the production of oysters and clams and increase seed outputs,
2. improve broodstock management and establish the basis for bivalve breeding programs to improve seed quality,
3. develop the basis for cultivation systems designed to increase oyster marketability
4. develop a bivalve health and environmental management program,
5. develop the capacity for researchers, technicians, managers and farmers to safely and sustainably regulate the development of the bivalve industry,
6. extend the scientific, social and economic benefits of improved bivalve culture technology to other areas of Vietnam and,
7. investigate three species (flat oysters, pipis and razor clams) that show potential for successful aquaculture.

5 Methodology

Research methods

Research undertaken in this project in many cases comprised a range of standard scientific studies investigating fundamental biological questions. Many of these studies have been published in peer reviewed journals (see 10.2.1 & 10.2.2) and the precise methodology for each of those investigations is described in those publications.

Marketability and Quality Criteria

Domestic demand for oysters in Vietnam is increasing and new markets and supply chains are evolving for oyster products. The future viability of the Vietnamese oyster industry is contingent on oysters that meet a high quality standard and do not pose a threat to human health. Oyster marketability and quality characteristics fall into two key areas that are interrelated. The term quality and marketability can refer to oysters that are safe for human consumption. This term also refers to oysters that have morphological, visual and sensory characteristics that increase their market price and acceptance. An objective of this project was to define marketability and quality criteria. This was delivered through a quality assurance audit of northern Vietnam, a workshop focused on food safety for oysters and incorporation of selection indices into the program that allows oyster quality traits to be monitored and also improved upon.

Quality Assurance

An objective of the current ACIAR project FIS/2010/100 is to develop capacity for researchers, technicians, managers and farmers to safely and sustainably regulate the development of the bivalve industry (Objective 5). A component of this objective is to improve the quality of oysters to safeguard consumers and industry from impacts caused by food poisoning outbreaks. This can be achieved through development and implementation of an oyster industry quality assurance program in northern Vietnam. Regulation and management capacity is required to safeguard consumers. Therefore an audit of the classifications systems, environmental and microbiological monitoring and post-harvest handling techniques was undertaken as the initial step and a training workshop was conducted for staff in key institutions.

Quality Assurance Audit:

NSW DPI representatives travelled to northern Vietnam to undertake a quality assurance audit of their oyster industry in October 2016. The primary objective of this audit was to investigate oyster industry practices and identify where improvements can be implemented that reduce risks to human health.

Three specific areas were reviewed to address this objective:

1. Classification systems used in oyster harvest areas
2. Environmental and microbiological monitoring currently undertaken for FIS/2010/100
3. Post-harvest handling of oysters in the supply chain

The duration of this audit was from the 8th to the 14th October 2016 and involved visits and discussions with:

- The National Agro Forestry Fisheries Quality Assurance Department – Branch 1, Hai Phong.
- The RIA No1 National Marine Broodstock Centre (NMBC) and bivalve hatchery, Cat Ba.

- Outlets where oysters are sold wholesale and to consumers in Hai Phong, Cat Ba, Ha Long City, Van Don and Ha Noi.
- The BIM Oyster Company oyster processing plant in Van Don.
- The BIM, RIA1 and other oyster farm grow-out sites at Ban Sen and Dao Hoang Tan.
- The Australian Embassy, Hanoi for a meeting with Nguyen Thi Thanh An (ACIAR Country Manager, Vietnam).

In addition to the visits listed above, two university lectures on oyster quality assurance were presented at the Faculty of Fisheries, Vietnam National University of Agriculture, Ha Noi, and the People's Committee of Quang Ninh Ha Long University.

Quality Assurance Workshop:

University of Technology Sydney and NSW DPI representatives travelled to northern Vietnam to conduct a four day workshop on risks to oyster consumers from wastewater, poor water quality, viruses and biotoxins. The workshop was held at Cat Ba from the 4th to the 7th June, 2018. The workshop was attended by representatives from: Environment Department, Ha Long University; RIA2; Research Institute for Marine Fisheries; and, RIA1. The topics covered were as follows:

- Wastewater and water quality – theory session (Day 1)
- Virus management in shellfish harvest areas – theory session (Day 1)
- Oyster biology – theory session (Day 1)
- Phytoplankton sampling techniques – theory and practical session (Day 1)
- Phytoplankton background – theory and practical session (Day 1)
- Basic microscope techniques – theory and practical session (Day 1)
- National Marine Broodstock Centre Shellfish hatchery visit (Day 2)
- Shellfish harvest area classification (Day 2)
- International requirements for bivalve shellfish safety (Day 2)
- Harmful algal blooms: causes and impacts – theory session (Day 2)
- Biotoxin limits – theory session (Day 2)
- Biotoxin-related illnesses – theory session (Day 2)
- Shoreline survey of potential pollution sources at oyster leases – practical session (Day 3)
- Field sampling for phytoplankton at oyster leases – practical session (Day 3)
- Cat Ba Island aquaculture village boat tour (Day 3)
- Counting phytoplankton – theory and practical session (Day 4)
- Oil immersion lens: microscope techniques – theory and practical session (Day 4)
- Isolation of single phytoplankton cells: microscope techniques – theory and practical session (Day 4)

A key focus area of this workshop was on harmful algal blooms. A series of PowerPoint lectures on phytoplankton identification and enumeration, water quality and phytoplankton monitoring, harmful algal blooms, biotoxin regulatory limits and symptoms of shellfish toxin-related illnesses were delivered to participants. The practical components of the workshop included demonstration of sampling techniques, light microscopy techniques to identify potentially harmful species and enumeration of phytoplankton cells.

Phytoplankton monitoring practical demonstrations in Cat Ba harbour

Market Quality and Acceptance

The main focus of the *Crassostrea angulata* breeding program is to increase industry profitability. Faster growth and improving survival are the primary traits of the *Crassostrea angulata* breeding program. Measurements of shell dimensions and meat quality are also performed to calculate meat quality and shell shape indices. Genetic correlations can be determined for primary (growth and survival) and secondary traits (meat condition and shell shape) under selection. Therefore informed decisions can be made that improve key traits and understand the impacts on the secondary traits which relate to marketability of oysters. Information for primary traits (growth and survival) and methods related to selection of broodstock candidates is provided in Section 7. Meat condition of families was calculated by measuring whole weight, shell weight and wet tissue weight of individual oysters and using the following formula:

Condition index = soft tissue weight (g) * 1000 / internal shell cavity capacity (g)

Where the internal shell cavity is shell weight subtracted from whole weight (Lawrence and Scott, 1982). The shell length, width and depth of individual oysters from each family are also recorded. From these measurements a width index and depth index can be calculated to track changes to oyster shell shape attributable to breeding. Width index is calculated by dividing shell width by the shell length and, in a similar way, the depth index is calculated by dividing shell depth by the shell length.

The design of the *Crassostrea angulata* breeding program enables genetic parameters including heritabilities and genetic correlations of growth-related traits (whole body weight at harvest, shell length, shell width, shell depth, shell weight) and meat quality traits (condition index and soft tissue weight) to be calculated. This information informs selection decisions and facilitates effective multi-trait breeding.

Capacity Building

A major component of this project involved training staff in the fundamental skills required for the production and management of molluscs. Key skills included, algal culture, mollusc spawning methods, larval rearing and settlement technologies and hatchery management. “These are highly specific technical skills that were all judged necessary to achieving the project’s objective of reducing the cost of delivering spat to farmers. The growth in the industry over the course of the project is evidence that these skills were developed.” (Mullens et al. 2017). To pass on the knowledge required to undertake these tasks we used a combination of embedding staff in Australian facilities and providing regular review of processes and outcomes within the hatchery facilities at Cat Ba in Vietnam. Much of this information was captured in a revised hatchery production manual (in Vietnamese), which is available on the RIA1 website (Appendix 11.1).

Technology transfer was seen as essential for industry development. This was achieved through a series of specific laboratory placements and commissioned specialist workshops.

In order to manage and develop the industry into the future we engaged strongly with the John Allwright Fellowship program and supported a number of molluscan focused post-graduate candidates in Australia. A lasting legacy of this project will be the mollusc breeding program, but this legacy is contingent upon staff with the appropriate genetic skills remaining in Vietnam to continue the work. In this regard we supported three RIA1 staff to undertake PhDs in quantitative and molecular genetics, two of these projects working specifically with the oyster stocks in Vietnam. More broadly we have additionally supported 4 Vietnamese students (3 from RIA1) to undertake PhD studies investigating:

1) Microbial communities around oysters and their association with disease,

2) Controlling microbial communities on oysters with phages,

3) Physiological mechanisms involved in oyster reproduction, and

4) Molluscs as biomonitors of pollution

International exposure for RIA1 staff (and others) to other molluscan research programs was encouraged by permitting conference attendance opportunities. At the outset of this project we organised for the 5th international Oyster Symposium to be held in Vietnam and coordinated the scientific program. This provided all those interested in oysters (Industry and research) with a chance to meet and hear from many of the world’s leading oyster researchers. Project Staff RIA1 staff also attended two International Conferences for Molluscan Shellfish Safety and a World Aquaculture Society conference.

Impact Assessment

Assessing the impact of this research in Vietnam was undertaken by independent experts, either formally as a part of this program or through external assessments initiated by ACAIR.

As part of FIS/2005/114, we commissioned Dr Janine Pierce from the University of South Australia to undertake social impacts review. That review used a qualitative research technique called Photovoice (see Pierce 2011, Pierce and O’Connor 2014). Data was gathered from 10 selected oyster farmers from Ban Sen Commune in the Van Don district by providing them with cameras and diaries to record 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. The process was then followed up with brief interviews on themes raised by farmers to clarify or flesh out ideas. 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) to ascertain overall sustainability, risks, positives and negatives, and oyster farmer perceptions gleaned from a range of open ended research questions. The Photovoice approach was particularly useful in gaining a better understanding of farmer perceptions (and misconceptions) that were considered when formulating the current project.

Accordingly, we included a second Photovoice survey within the current project with the intention of looking at changes in perceptions over time and as the industry evolved and grew. The second survey targeted the same community and included many of the farmers initially interviewed. The survey questions were expanded to accommodate those being interviewed for a second time and included two new research questions relating to gender and climate change to address key focus areas of ACIAR.

Photovoice questions:

- How has oyster farming impacted on you?
- What does oyster farming mean to you?
- How is your community more/less sustainable since oyster farming began?
- What is good about oyster farming in your community?
- What is bad about oyster fish farming in your community?
- What is the extent of gender equality (types of jobs, roles, level of worker) in oyster farming in your community.
- What does womens' empowerment (or not) look like in oyster farming in your community?
- Climate change: any impact and concerns (or not) on oyster farming?
- Over time what has changed (if anything) about oyster farming since you took part in the study in 2011? (This question only to be answered by those who took part in the study in 2011).

6 Achievements against activities and outputs/milestones

Objective 1: Improve hatchery reliability (Algal and larval systems in government and private facilities) for the production of oysters and clams and increase seed outputs

No	Activity	Outputs/ milestones	Due date of output/ milestone	Comments
1.1	Improve hatchery production technology	NMBC hatchery seed output doubled	Dec 2017	Regular visits are made by project staff to refine both general production techniques and those used for the family line production. These have been included in a revised hatchery manual (Appendix 11.1). While capacity is now more than twice that originally possible, the focus has moved to family line production as additional commercial hatcheries have come on line that have more than doubled seed availability to industry (Appendix 11.3).
1.2	Produce single seed	Ten million single seed available for commercial assessment	Jun 2016	The techniques to produce single seed are now well established and have been used for the production of family lines for performance assessment. More than 10 million single seed have been produced, however, performance of single seed under current commercial conditions needs improvement and there is limited interest in single seed from the commercial sector (Section 7, Key Results and Discussion). Single seed does however continue to be produced for family lines.
1.3	Encourage private hatchery technology adoption	Five private hatcheries to have adopted technology and produced seed	Dec 2019	Contact with companies has occurred. Three general industry workshops have been held (Activity 6.2) and this has been followed by the preparation of a hatchery production manual in Vietnamese that is readily available to the industry (Appendix 11.1). There are now a large number of commercial hatcheries producing *C. angulata*, including a hatchery solely for the largest commercial company (BIM)

PC = partner country, A = Australia

Objective 2: Improve oyster broodstock management and establish the basis for bivalve breeding programs to improve seed quality

No	Activity	Outputs/ milestones	Due date of output/ milestone	Comments
2.1	Assess genetic diversity in NMBC oyster stocks	Diversity assessed and management plan developed if deficiencies occur	Dec 2014	Genetic diversity assessment has occurred based on a range of stock currently available in Vietnam. This work indicated sufficient diversity is available for the development of a breeding program without the need for further stock introductions (In et al. 2016)
2.2	Construct replicated larval rearing systems	System constructed and commissioned	April 2015	Completed. Replicated rearing systems with the capacity to produce 100-125 families have been constructed.
2.3	Produce family lines	Fifty pair-mated oyster family lines produced for field evaluation	Dec 2015	Completed. In 2014, 117 families were produced. Additional breeding runs were performed in 2015 (109 families), 2016 (95 families) and in 2017 (139 families). Progeny from all these breeding runs have undergone field evaluation. An additional 70 family lines were produced 2018 and have been deployed to the field.
2.4	Develop breeding database	System established for collection of family line performance data	Dec 2016	Data collections systems have been constructed and data is being processed for each generation (example Appendix 11.2).

PC = partner country, A = Australia

Objective 3: Develop the basis for cultivation systems designed to increase oyster marketability

No	Activity	Outputs/ milestones	Due date of output/ milestone	Comments
3.1	Establish oyster single-seed nursery systems	Systems constructed and tested	Jun 2015	Single seed spat being produced for breeding line assessment.
3.2	Undertake oyster single-seed grow-out trials	Growth and quality of single-seed vs cultch-set oysters compared	Jun 2016	Comparisons of the performance of single seed and cultch set systems has been undertaken indicating better performance under raft culture conditions for cultch set seed. (See 7. Key Results and Discussion).
3.3	Investigate optimal oyster grow-out technology	Alternative grow-out equipment compared	Reviewed annually	The cultch set spat are presently out-performing single seed oysters. Despite assessment of various cage culture techniques, there has been a reluctance to move from culch. Industry continues to test new approaches, intertidal culture, longline culture and poly culture (with prawns).

Objective 4: Develop a bivalve health and environmental management program

No	Activity	Outputs/ milestones	Due date of output/ milestone	Comments
4.1	Identify environmental monitoring criteria	Monitoring report produced	Dec 2015	Criteria identified and monitoring commenced. Following 12 months, criteria were reviewed and revised (See Section 7)
4.2	Develop laboratory quality-assurance system	Lab manuals written Lab reference materials acquired	Dec 2016	Lab manuals in Vietnamese have been prepared. Histological examples have been provided and lab reference materials have been purchased. As of 2016, the RIA1 CEDMA is accredited to ISO/IEC 17025:2005 standard for the detection of a range of bacterial and viral diseases and nutrient assessments (N&P).
4.3	Assess diagnostic competence	Competency report produced with training needs identified	Jun 2014	Completed (Hardy-Smith & Humphrey Report)
4.4	Conduct diagnostic training	Four collaborator training courses held	Reviewed annually	Diagnostic and Histopathology workshop (Panaquatic, March 2013: 5 staff including 1 from RIA3) 2 RIA1 staff were trained in molecular biology in Australia (Peter Kirkland EMAI) 2 staff trained in electron microscopy in Australia (Alex Hyatt CSIRO) 6 staff trained in Food Quality Assurance and Testing (Cat Ba) 8 staff trained in harmful algal bloom (HAB) monitoring and identification (Cat Ba).
4.5	Conduct histopathology training	Four collaborator training courses held	Reviewed annually	Training class in histopathology in Vietnam (Panaquatic, March 2013: 5 staff including 1 from RIA3) 2 staff trained in histopathology (EMAI) 2 Staff provided PCR training (EMAI) 2 Staff TEM / Tomography trained Histopathological changes due to HABs covered in HABs training.

Objective 5: Develop the capacity for researchers, technicians, managers and farmers to safely and sustainably regulate the development of the bivalve industry

No	Activity	Outputs/ milestones	Due date of output/ milestone	Comments
5.1	Identify biosecurity training needs	Training needs and schedule devised	Dec 2014	Completed. Training needs plan (Hardy-Smith & Humphrey Report). Schedule devised and used to establish diagnostic and histopathology training.
5.2	Complete quality-assurance audit	Quality assurance report produced	Jun 2015	Food quality assurance assessment conducted and report prepared Dec 2016.
5.3	Identify marketability/quality criteria	Marketability and quality criteria defined	Jun 2015	Major producers and marketers were surveyed for key production traits (see methods) from which market criteria were determined for ongoing family line performance monitoring (See Section 7).

Objective 6: Extend the scientific, social and economic benefits of improved bivalve culture technology to other areas of Vietnam

No	Activity	Outputs/ milestones	Due date of output/ milestone	Comments
6.1	Conduct annual RIA workshop	Project progress reported at workshop	Annually	RIA 1 workshops have been held on at least an annual basis since 2013 with a final combined RIA1 and Industry to be held before June 2019. Dates and locations of Annual RIA workshops: 18 December 2013, Cat Ba; 23 March 2015, Cat Ba; 23 August 2015 Bac Ninh; 11 October 2016, Cat Ba; 28 August 2017 Bac Ninh: 4 June 2018, Cat Ba.
6.2	Conduct industry workshop	Workshop held and report produced	Annually	Two industry workshops held in conjunction with annual workshops, with a third final workshop to be held in 2019. 1. Industry Workshop held 14-16 December 2015, RIA No1 Bac Ninh. The workshop was attended by 25 delegates comprising: oyster farmers; RIA No.1, No.2 and No.3 staff; BIM Seafood staff; Vietnam National University of Agriculture staff as well as RDS Partners, ACIAR and NSW DPI. Further information (topics, presentations, delegate details) provided in the NSW DPI internal report: ACIAR Vietnam Trip Report 12-18 December 2015 (M. Dove). 2. Harmful Algal Bloom Workshop held 4-7 June 2018, National Marine Broodstock Centre, Cat Ba. The workshop was attended by 11 delegates from RIA No.1, RIA No.2, People's Committee of Quang Ninh Ha Long University and Research Institute of Marine Fisheries, Hai Phong. Further information (topics, presentations, delegate details) provided in the NSW DPI internal report: Harmful Algal Bloom Workshop 2018 (Farrell, Murray, Zammit, Dove and O'Connor).
6.3	Assess economic and social impact	Impact assessment report written	Dec 2015	Completed by RDS partners Dec 2015 Completed by Mullen et al. May 2016 Completed by Pierce June 2018
6.4	Review project performance	Project progress report written	Dec 2015	Mid program review suspended in the light of significant independent assessment of program performance (6.3)

Objective 7: Extend the scientific, social and economic benefits of improved bivalve culture technology to other areas of Vietnam

No	Activity	Outputs/ milestones	Due date of output/ milestone	Comments

7.1	Evaluate alternate nursery technology for pipis	Documented production protocols	June 2015	Substrate nursery systems assessed
7.2	Lease based assessment of clam/cockle production	Stock performance report	Nov 2016	Pipi stocks supplied to 7 farms Hawkesbury River (nil survival, see Section 7)
7.3	Close the life cycle of razor clams (Pinna sp.)	Documented early ontogeny for Pinna sp.	June 2017	Five attempts to produce Pinna larvae have been undertaken with variable success. Spawning has been achieved, but larvae have only been reared for a maximum of 7 days and only early ontogeny has been described.
7.4	Reproductive timing for flat oysters in NSW	Documented reproductive cycle for *O. angasi* in NSW	Dec 2015	Completed (draft publication prepared).
7.5	Pair mating for flat oysters	Documented protocols	Dec 2016	Systems have been refined for flat oyster bloodstock maturation. This provides the opportunity to cohabit adults to achieve single pair crosses, but is currently of limited feasibility.

7 Key results and discussion

7.1 Vietnam

There were six primary objectives of the Vietnamese component of FIS/2010/100 (Section 4), which can broadly be ascribed to either a research or capacity building component. Here we will address key results for each of these objectives

Objectives 1&6: Increased Production and Industry Growth

The first and last of these objectives of this program were to improve hatchery reliability for the production of oysters and clams and increase seed outputs, and to extend the benefits of improved bivalve culture technology to other areas of Vietnam. Demonstrably this has occurred. Production has more than doubled to have conservatively reached 15,000 tonnes/annum and farming has now spread across 28 provinces. Community involvement has increased with an estimated 2500 coastal families now benefitting from the oyster industry. Overall the benefits from this program have been significant and wide ranging (Pierce and O'Connor, 2011, Mullen et al. 2017, Hiruy et al. 2018, Pierce 2018).

Oysters being marketed in Ha Long City

Objectives 2&3 Production and Breeding

A great deal of emphasis was placed on efforts to improve broodstock management and establish the basis for bivalve breeding programs. This work began with confirmation of the identity of the species of interest, *Crassostrea angulata*, in Vietnam (a world first), followed by the development of the technology for highly replicated small scale family line production. This then led to the production of three generations of selectively bred oysters.

Species identity

During FIS/2005/114 researchers and the Vietnamese government became increasingly concerned over the identity of the species being used for cultivation. The original broodstock had been sourced from Taiwan, where several morphologically similar species could be obtained. In particular, both Pacific oysters (*Crassostrea gigas*), Portuguese oysters (*Crassostrea angulata*) were present, and at that time there was no definitive method to determine which was which, or indeed whether they were hybrids thereof. Without this knowledge any attempts to establish a breeding program were fraught with difficulty. We therefore sought to identify which oyster or oysters are most commonly cultured in Vietnam and, additionally, once the species identity was resolved, to assess three farmed Vietnamese stocks for levels of genetic variation and suitability for captive breeding programs. To resolve the taxonomy issues, we searched for nucleotide differences (characteristic attributes) in published mitochondrial DNA cytochrome c oxidase subunit 1 (COI) sequences that, for the first time, would categorically separate and distinguish in particular *C. angulata* from *C. gigas*. On review of 300 published haplotypes of *C. angulata* and *C. gigas* based on a 293 bp nucleotide-fragment of published COI sequences, we found that there were five distinct nucleotides that are categorically different between *C. angulata* and *C. gigas* and that could be considered as diagnostic nucleotides. Using these five diagnostic nucleotides, we confirmed that the samples from northern Vietnam are *C. angulata*, not *C. gigas*. Similarly, we identified other oyster species in Vietnam from Nha Trang as *Crassostrea sikamea* and *Crassostrea madrasensis*. DNA microsatellite data (following) can also support understanding of the taxonomy, directly by comparing allele types and frequencies between putative species, but also indirectly because as nuclear DNA, microsatellite genotypes may reveal if hybridization is occurring (as evidenced by deviations from Hardy-Weinberg equilibrium). No evidence, considering Hardy-Weinberg deviations, for interspecific hybridization was found. To address the diversity issues, three hatchery bred populations of *C. angulata* were screened for allelic variation at nine DNA microsatellite loci. All three lines had high allelic diversity, moderate effective population sizes (Ne), and little evidence of kinship, which, by precedent with other hatchery bred highly fecund oyster species, is a little unexpected. It is speculated that local hatchery practises may involve sharing stock among hatcheries which then may contribute to the maintenance of moderate to high levels of diversity during hatchery reproduction of this highly fecund species.

Small scale rearing systems

In order to produce the many families required for a modern pedigreed oyster breeding program it is essential to develop small scale highly replicated rearing systems, a task that has taken considerable effort in Australia. Miniaturisation can be problematic as mollusc larval production generally becomes increasingly difficult as culture volumes decrease. It is also necessary to simplify rearing protocols so that the many tasks associated with feeding, rearing and monitoring larval batches can be achieved within the time and with the skilled staff available. This is further complicated by the need for strict biosecurity to ensure the integrity of each individual family being produced is maintained – that there is no exchange of larvae between tanks. A system has been established in Vietnam that has now demonstrated the capacity to concurrently produced 100 pair-mated family lines and has now done so for three generations. This is a significant achievement given that the largest mollusc breeding effort undertaken in Australia only attempts to produce 70 families concurrently.


Rearing systems used for the production of Crassostrea angulata families at the RIA No1 National Marine Broodstock Centre, Cat Ba.

Genetic diversity

Having established the means, project staff then set about producing *C. angulata* families and assessing their performance. One of the first challenges assessed was increasing our knowledge of strain additive genetic effect and heterosis expression in different culture environments as this is not well understood in shellfish species. A study was undertaken to estimate the strain additive genetic and heterotic effects on harvest body weight in a 3 x 3 complete diallel cross involving three populations of *C. angulata*, two populations from Cat Ba (RIA1) and Nam Dinh, northern Vietnam, and an imported stock from China. Strip spawning was applied to produce full- and half-sib families simultaneously of nine cross-combinations for performance testing at two locations (Cat Ba Island, Hai Phong city and Van Don Island, Quang Ninh province, Vietnam) using two culture systems ('single seed' and 'cultch set'). A total of 7269 individual oysters were examined over a grow-out period of 270 days. A linear mixed model was used to estimate strain additive genetic and heterotic effects for body weight at harvest. Ranking of strains based on their additive genetic effects did not differ between the locations and culture systems. The non-additive genetic (heterotic) effects were low and not significantly different from zero for the traits studied among culture systems. There were no significant differences in levels of heterosis for harvest body weight between the two test environments or culture systems. This information is critical because it showed that, based on the non-significant heterotic and large additive genetic effects among strains used for this study, a breeding program for *C. angulata* could be based simply on choice among different strains based on their performance.

Selective Breeding

The Portuguese oyster (*C. angulata*) breeding program was established at the National Broodstock Centre for Mariculture, RIA1, Cat Ba. The base population was established in 2015 from three hatchery strains, including two populations from north Vietnam (Cat Ba – Hai Phong City and Nam Dinh province) and a population imported from China (V. V. In, Sang, et al., 2017). Individual oysters that were greater than 50 g whole weight were selected from a complete 3 × 3 diallel cross population involving the three strains to form a base population for genetic selection (Nam Dinh, RIA1 and Van Don) in 2014.

Strip-spawning was used to produce all full and half-sib families. Estimated breeding values (EBVs) for whole weight at harvest were used to select broodstock candidates to produce subsequent generations (2015, 2016 and 2017 year classes). The selection method was a combination of within and among families. Genetically unrelated individuals were selected to mate with each other based on their genetic relationship in the pedigree and ranking of their EBVs. The procedures on selection and mating remained consistent over all the year classes produced. Generally, elite breeders were chosen from the highest ranked 40 to 60 families over the generations. The selection proportions over generations G1 to G3 were 4.0% for females and 1.6% for males representing an average selection intensity of 2.15 in females and 2.5 in males, respectively. In total, there were 27,036 offspring generated by 349 sires and 428 dams over a 4-year period from 2015 to 2018. The breeding objective is to create high quality offspring with superior growth rates and high survival to increase profitability for the oyster industry in northern Vietnam.

The design of this breeding program allows estimates of genetic parameters including heritabilities and genetic correlations of growth-related traits (whole weight at harvest, shell length, shell width, shell depth, shell weight) and meat quality traits (condition index and soft tissue weight) to support enable multi trait selection. The heritabilities (h^2) estimated for body traits were moderate, ranging from 0.11 to 0.18 (Table 1). The estimated heritabilities for traits such as whole body weight, width, depth, and shell weight were similar (0.18) but h^2 for length trait (h^2 = 0.11). Estimated heritabilities are reasonably low at 0.08 and 0.05 for soft tissue and condition index, respectively. The fraction of variance due to maternal and common environmental effects (c^2) for the body traits was from 4.0 to 13.0% whereas lower c^2 values of 4% and 6% were obtained for soft tissue and depth trait, respectively.

Table 1. Variance components, genetic (σ^2_A), common full-sibs (σ^2_C) and residual (σ^2_C) variance heritability (h^2 ± standard errors) and common environmental effects (c^2) for traits studied

Traits	σ^2_A	σ^2_C	σ^2_E	h^2 ± se	c^2
Weight	40.68	23.59	164.92	0.18±0.04	0.10±0.02
Length	0.11	0.13	0.82	0.11±0.03	0.12±0.02
Width	6.03E-02	4.97E-03	2.39E-01	0.18±0.04	0.10±0.02
Depth	4.75E-02	1.65E-02	1.99E-01	0.18±0.03	0.06±0.01
Shell weight	2.22E+01	1.61E+01	8.16E+01	0.18±0.05	0.13±0.03
Soft tissue	1.35E-02	7.43E-03	1.57E-01	0.08±0.04	0.04±0.02
Condition Index	3.56	7.24	58.85	0.05±0.03	0.11±0.02

Genetic gain (selection response) is calculated by the formula (($R_{Generation} = (i_{male} + i_{female}) * \sigma_P * h^2/2$)) where i is selection intensity, R is selection response, σ_P is phenotypic standard deviation, h^2 is estimated heritability. Genetic gain in the present study for the whole weight trait at harvest is 6.3% per generation. (Genetic gain is calculated from (2.502 for selection intensity of male, 2.154 for selection intensity of female, σ_P= 15.13, h^2 = 018).

Single seed vs cultch

In Australia, hatchery production has led to the availability of “single seed” oysters. These are stocks of oysters that are treated to prevent them from settling on a substrate. In this form they are readily handled, counted and graded and they are free to grow naturally without distorting in shape to conform to the surface on which they have settled. Grading promotes greater growth, while being free of a substrate increases uniformity in shell shape, which improves their marketability. Increasingly single seed is becoming the bench mark for oyster quality and is critical to maximise the price obtained certain markets. To ensure this option was available to the Vietnamese industry, we worked to establish the techniques for single seed production (Included in the Vietnamese Hatchery Manual Appendix 11.1) and sought to test its utility on Vietnamese farms.

The first year class of oysters saw 112 pair-mated families produced and spat from each of these families set on cultch and set as single seed (without cultch). The cultch set stocks were then deployed at Cat Ba (20.673°N, 107.062°E) and Van Don (20.954°N, 107.478°E) and the single seed were deployed in hanging baskets at Van Don. The comparative performance of these stocks was assessed in August 2015 when the cultch set oysters had reached a commercially saleable size (Table 7.1). At this time, cultch set oysters were twice as large as single seed and survival was over five times higher. These results were typical of those experienced for single seed in Vietnam and were in part responsible for reduced enthusiasm for single seed production at this time. While we feel that further development of the technology could be achieved and that the disadvantages encountered could be reduced, the fact is that cultch set is simpler and easier to manage for small scale farming. Additionally, cultch set oysters are more suitable for transfer straight from the hatchery to oyster farms at a small size, which circumvents the field nursery phase in the growout cycle. Furthermore, with the vast majority of oysters marketed within Vietnam as meat only, there is no great disincentive to produce cutch set stock at this time.

Table 7.1 Growth and survival of cultch-set and single seed Crassostrea angulata spat held at two locations in northern Vietnam.

		Traits (Average ± SD)					
Site	**Settlment Type**	**Survival (%)**	**Whole weight (g)**	**Shell height (mm)**	**Width index[1]**	**Depth Index[2]**	**Condition index[3] (%)**
Cat Ba	Cultch	12.1 ± 6.2	50.7 ± 15.0	65.7 ± 8.7	0.7 ± 0.1	0.4 ± 0.1	-
Van Don	Cultch	29.2 ± 9.3	47.0 ± 14.9	70.4 ± 9.6	0.6 ± 0.9	0.4 ± 0.5	17.4 ± 14.6
Van Don	Single seed	5.3 ± 5.8	21.9 ± 8.1	54.3 ± 8.5	0.6 ± 0.1	0.4 ± 0.1	-

[1] Shell width / Shell height

[2] Shell depth / Shell height

[3] Wet Meat Weight divided by Whole Weight * 100

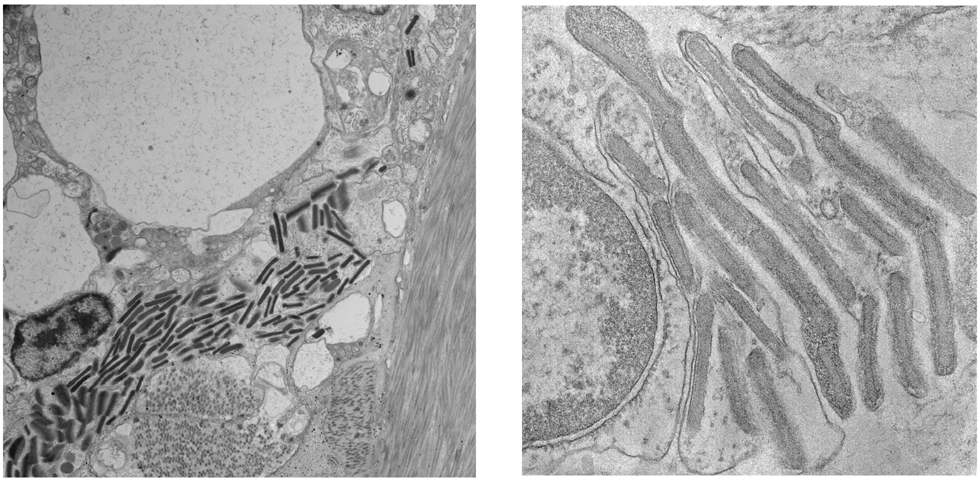
Objective 4 & 5 Bivalve Health and Environmental, Management and Regulation

All oysters industries around the world have periodically faced challenges arising from the threat of disease and environmental contamination. The resultant damage to industry however can often be ameliorated by well trained staff with appropriate facilities and monitoring programs. To facilitate this development we have supported RIA1 in developing their molluscan monitoring, management and regulation capacity.

Health management

Efforts to improve molluscan health management were based on a distillation of the 14 recommendations of Hardy-Smith and Humphrey (2012). Several of these recommendations (7, 8, 9 & 11) related to increased training for hatchery and diagnostic staff and this is discussed elsewhere in this report. These authors then suggested “a, system of quality assurance and quality control based on known and unknown samples should be introduced into diagnostic laboratories, especially in regard to bacteriology, histopathology and molecular diagnostics. Such a system should at a minimum include (1) the drafting of laboratory manuals for each laboratory and the accessions area to ensure consistency of operations and a smooth handover in the event of staff changes, and (2) the acquisition of reference material for quality control purposes”. In accordance a lab manual has been developed “Diagnostic laboratory manual for aquatic animal disease” that addresses parasitological, bacteriological, histophathological and molecular techniques (PCR) for disease diagnosis. The manual includes general diagnostic process, diagnostics at farm level, lab diagnostic protocols, Machine/equipment management in labs (Recommendation 5) and diagnostic submission forms (Recommendation 1). To ensure the quality of its molluscan health service, RIA1's Center for Environment and Disease Monitoring in Aquaculture (CEDMA) has received ISO/IEC 17025 accreditation.

During the Hardy-Smith and Humphrey review they noted disease was already an issue for molluscs industries and suggested (Recommendation 2) " The cause or causes of the losses in oysters, hard clams and otter clams requires urgent investigation utilising the principles of veterinary clinical investigation, supported by comprehensive epidemiological, histopathological and bacteriological analysis". We used this recommendation to provide a basis for training for RIA1 staff in Australia. In FIS 2005/114 we had worked with Vietnamese staff to develop the hatchery production technology for otter clams (*Lutaria phillipinensis*), a local high value clam species, which in turn promoted rapid development of that industry. Unfortunately, after a short period of rapid growth, the industry collapsed due to "Swollen Siphon Disease". Observations by CEDMA staff suggested the aetiological agent was a "virus like particle" and found that the transmission of fluids from one individual to another could transmit the disease. Staff at the Elizabeth MacArthur Agricultural Institute (EMAI) arranged the necessary permits for samples to be brought from Vietnam that were used during training exercises. This training developed CEDMA staff skills in histopathology, molecular disease detection, ultrathin sectioning, negative contrast staining, tomography and x-ray microanalysis. While it was concluded that the ultrastructure of the VLPS did not match that of any known animal virus, it was suggested that they were most-likely protein aggregations and may have arisen as a result of viral insult or the over production of a viral protein. Critically, the two CEDMA staff spent 5 days involved in active diagnostic work at EMAI with world leading scientists addressing health challenges for molluscs of direct relevance to them.



Transmission electron micrographs of "Virus like Particles" from otter clams, Lutaria phillipinensis. (Courtesy Alex Hyatt).

In addition to its work on mollusk health, the CEDMA has also extended its ISO/IEC 17025 accreditation to seawater chemistry and in particular methods for monitoring of nutrient enrichment (nitrogen and phosphorus). This has formed the basis of water quality monitoring programs for both hatchery facilities and oyster farms. Seawater at the National Marine Broodstock Center (the location of the RIA1 Cat Ba Mollusc Hatchery) is now monitored routinely for: Temperature, pH, DO, salinity, alkalinity, Nutrients (N- NH_4^+, N -NO_2^-, N - NO_3^-, P - PO_4^{3-}) and metals (As, Hg, Cu, Pb, Zn). During larval production and nursery rearing, culture water is monitored daily for temp, pH, DO, salinity, alkalinity nutrients in incoming seawater are assessed every 3 days. Growout sites are monitored similarly for temperature, pH; DO; S‰; Alkalinity; nutrient (N- NH_4^+, N -NO_2^-, N - NO_3^-, P - PO_4^{3-}) and are also subject to monthly assessments of heavy metals concentration (As, Hg, Cu, Pb, Zn) in seawater and oyster meats.). Initially growout monitoring was also augmented with twice monthly bacterial monitoring of seawater water (Salmonella, Vibrio and E. coli), but following the quality assurance audit (discussed below) sampling of oyster meats has been included.

Quality Assurance

The October 2016 Quality Assurance Audit by NSW Department of Primary Industries reviewed:

a) the classification systems used in oyster harvest areas in Vietnam,
b) the environmental and microbiological monitoring currently undertaken for this project, and
c) post-harvest handling of oysters in the supply chain.

The classification of shellfish harvest areas in Vietnam is limited to the areas classified by NAFIQAD for the purpose of facilitating export to the European Union (EU). NAFIQAD follow the EU system of classification.

Shellfish samples are collected by the local authority and sent to NFIQAD for analysis using ISO method 16649-3. The initial shoreline survey is undertaken by NAFIQAD and is used to set the sample sites for the area. Subsequent sanitary survey reports are completed by the local authority. On-going monitoring samples are collected by the local authority and sent to NAFIQAD for analysis. The results are reported to the local authority who are responsible for deciding what action to undertake at the harvest area level including the development of any harvest area management plans. The local authority perform annual reviews and submit them to NAFIQAD, a copy of a review or sanitary survey report could not be made available.

Harvest areas are classified according to the EU A, B or C classification. At the time of the visit there were mostly B classified areas, some C classified areas and no A classified areas. Van Don was previously A classified but was downgraded to B as a result of a dam in the catchment being damaged. The dam previously held back upstream runoff/contamination.

The harvesting area operated by RIA 1/BIM has not been subject to a formal sanitary survey or a detailed shoreline survey. The brief visit to the farm conducted as part of this audit indicates that the area is well situated away from major pollution sources. A more detailed survey of potential pollution sources in the area is needed to confirm this. The main source of microbiological contamination risk identified is effluent from farm workers on the rafts. It is recommended that a toilet facility be provided where effluent can be contained and transported offsite for disposal. A “portaloo” similar to that used for camping/caravans would suffice. It is recommended that a key staff member of RIA1 visit NSW to learn more about the process of completing a sanitary survey to support formal classification of a shellfish harvest area.

The NAFIQAD laboratory has the ability to analyse shellfish samples for norovirus and hepatitis A via PCR. They follow the described EU method and have participated in the CEFAS ring trials. A copy of the CEFAS report was not available but it was reported that the laboratory received an “A” rating. This capacity will prove useful should a norovirus or hepatitis A outbreak be linked to Vietnamese oysters.

Monthly phytoplankton sampling has been undertaken at the RIA1/BIM harvest area by the RIA staff in the period March to December for 2014, 2015 and 2016. Some limited data was provided for the last sample run. Sampling appears to be ad hoc and not consistent. Analysis of phytoplankton samples is undertaken at RIA1 by the single employee running the algal production facility in the RIA1 hatchery. It was noted that *Alexandrium* sp. have not been detected in any samples collected to date. This is not surprising given that the staff undertaking the analysis has not received HAB identification training and is unable to liaise with anyone with expertise in this field. Staff at the facility reported that they did not know how to calculate total phytoplankton counts utilising the Sedgwick rafter counting chamber. This capability gap is impacting on the efficiency and efficacy of the oyster hatchery operations. This gap also raises doubts about the ability of staff to undertake more complex analysis of environmental samples.

To ensure that the analysis of samples is undertaken accurately and to support the efficient operation of oyster hatchery it is recommended that a comprehensive training course on marine phytoplankton identification and enumeration is undertaken as a matter of urgency. The course must be undertaken by someone with expertise in the identification of a wide range of harmful marine phytoplankton.

The identification of marine phytoplankton to species requires specialised microscopic equipment. Practitioners who undertake this work regularly detect unusual species that are difficult to identify or novel to the region sampled. The ability to take high resolution photos of phytoplankton and email them to colleagues is imperative. A suitable microscope coupled with a high resolution digital camera should be provided to RIA1 prior to the phytoplankton identification course being held. A suitable setup can be purchased in Australia for ~AU$7,000. As well as enhancing the ability of the local scientist to monitor phytoplankton populations the provision of this equipment and training will enhance the ability of RIA1 to collaborate electronically with international experts in hatchery technology and animal disease diagnosis. This is a valuable capability that will enhance the ability of the RIA1 to respond to future industry challenges promptly and effectively.

Regular microbiological water samples are being collected from the shellfish growing area and analysed for total coliforms, *E. coli*, Salmonella and *Vibrio parahaemolyticus*. The water sampling results are informative, but as most microbiological food safety standards are based on bacterial levels in shellfish it is difficult to compare the results to the established standards. Analysis of shellfish for *E. coli*, Vibrio and salmonella would be more informative. It is recommended that shellfish samples are analysed for these parameters instead of water. Sampling should be undertaken at least monthly during the harvest season (year round if there is no defined harvest season), additional samples should also be collected following rainfall events. Salinity and temperature should continue to be collected for all sampling occasions.

The 2016 data set for water bacterial monitoring appears to indicate moderate to high levels of contamination in the harvest area. Of concern is that the contamination levels do not appear to be linked to environmental variables such as salinity. This precludes the development of a predictive management plan to manage food safety risk in the harvest area. However, the data set available for analysis was limited (only 14 samples collected during 2016). The RIA staff agreed to provide the reviewer with additional data to facilitate further analysis.

It was difficult to ascertain whether the equipment used to measure salinity and temperature is periodically calibrated, a calibration needs to be undertaken to verify the accuracy of the equipment. This can be undertaken in conjunction with the phytoplankton training workshop.

Oysters are landed and loaded onto trucks at Ha Long port. Oysters were not subject to any temperature control on the harvest vessels or on the transport vehicles. Oysters are sold at traditional food markets. A site visit to the Ha Long local food market provided an opportunity to observe storage conditions at the end of the supply chain. Oysters being sold at the local market had a different shell shape to oysters marketed through supermarkets. At local markets and at street side stalls in Van Don, oyster sellers were all women providing an opportunity for these women to support their families. While it is good to see the industry grow and provide income for low socio-economic group families there are significant questions about the microbiological safety of oysters sold in this way. There is no temperature control and the time between harvest and sale cannot be verified due to an absence of labelling or documentation.

The growth of pathogenic bacteria is a significant concern, an outbreak of Vibriosis could have a significant impact on the health of the local community. *Vibrio parahaemolyticus* populations fluctuate significantly both seasonally and from year to year depending on large scale climatic conditions. As well as changes in total Vp populations changes in the virulence of the dominant strain in the local population can fluctuate significantly. If poor post-harvest handling is combined with extended post-harvest storage times (time from harvest to consumption) and a high prevalence of a virulent and toxigenic strains a large outbreak is possible leading to a high number of illnesses and deaths.

To better understand the microbiological status of oysters sold through traditional markets a market place survey should be undertaken. The survey should include sampling of oysters throughout a 12-18 month period targeting the summer months. The samples should be analysed for TPC, *E. coli*, Salmonella, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. If the budget allows it would be useful to analyse and Vp positive samples to determine the prevalence of TDH positive Vp in the population.

Further Observations

From visual inspection of the sewerage system servicing the Cat Ba township, it appeared that the system is a direct gravity feed into the harbour. This was confirmed by the RIA1 staff upon inquiry. Most restaurants in Cat Ba hold live seafood, including bivalve shellfish, in water sourced directly from the harbour. There are also several swimming beaches nearby that are a very popular tourist attraction for the town. The disposal of raw sewage into the harbour represents a significant public health risk and threatens the future attractiveness of Cat Ba to international tourists. It is likely that illnesses are occurring among tourist and local population but are not being attributed to this issue. This problem is likely to grow as tourism numbers swell following the completion of the bridge to Cat Ba. The provision of a sewage treatment plant should be considered, the plant should be a simple design that accounts for the regular power outages. It is acknowledged that raw sewage discharges are likely to still occur during extended power outages and wet weather, but a treatment plant will significantly reduce the pathogen load entering the harbour reducing illness rates amongst both tourists and locals.

Recommendations

To significantly improve oyster quality making this food product safer for consumers and, at the same time, providing assurance for oyster industry growers and marketers it is recommended that:

1. Toilet facilities be installed on oyster rafts where effluent can be contained and transported offsite for disposal.
2. RIA1 staff member(s) visit the NSW Shellfish Program to learn about the sanitary survey process.
3. A comprehensive training course on marine phytoplankton identification and enumeration is delivered to the staff at RIA1 as well as other institutions involved in monitoring and managing food quality.
4. A suitable microscope coupled with a high resolution digital camera should be provided to RIA1 prior to the phytoplankton identification course being held.
5. The microbiological monitoring program switch to analysis of *E.coli,* Vibrio and Salmonella in shellfish (as opposed to analysis of water) to facilitate assessment against international standards
6. Water microbiological samples should be analysed for Faecal coliform bacteria to facilitate assessment against international standards.
7. A microbiological survey of oysters in the market place be undertaken specifically testing for TPC, *E.coli, Salmonella, Vibrio parahaemolyticus and Vibrio vulnificus*

Quality Assurance Workshop

This workshop was held at the National Marine Broodstock Center, Cat Ba from the 4th to the 7th June, 2018. University of Technology Sydney and NSW DPI representatives presented lectures and practical exercises on risks to oyster consumers from wastewater, poor water quality, viruses and biotoxins.

All participants demonstrated a high level of competency in the techniques demonstrated and readily contributed to all practical components of the course. Phytoplankton identification requires a high level of skill and requires several years of practice and learning to establish capacity to manage food safety risks from potentially harmful species. This knowledge should be developed to manage the risk of potentially harmful phytoplankton in Vietnamese waters.

All participants exhibited aptitude for phytoplankton sampling and identification. Given that several harmful genera (*Dinophysis* spp. – diarrhetic shellfish toxin producing species, *Pseudo-nitzschia* spp. – potential amnesic shellfish toxin producing species and *Alexandrium* spp. - potential paralytic shellfish toxin producing species) were identified in wild samples collected from water adjacent to oyster leases during the workshop, an ongoing baseline monitoring (initially weekly) should be established with further training in identification to support the analyst.

Practical microscopy session identifying harmful algal species during Quality Assurance Workshop

To continue development, collaboration and capacity building in assessing risks to oyster consumers the following recommendations were provided:

- Purchase equipment and literature resources essential to establishing baseline phytoplankton monitoring capacity.
- Establish baseline monitoring for potentially harmful and indicator phytoplankton species in oyster production zones.
- Align current water quality sampling of oyster producing areas (Ban Sen and Cat Ba) with algal monitoring.
- Collaboration across regions within Vietnam to initiate a countrywide approach to creating phytoplankton baseline dataset.
- Collaboration with Australian research group to build capacity for phytoplankton identification via light microscopy, electron microscopy and genetic tools.
- Publish the baseline data set in collaboration with Australian research group to highlight potential issues that may negatively impact the shellfish industry in Vietnam.

- Build capacity for harmful algal bloom identification via the IOC training course on harmful algae.
- Increase network in the HAB community by attending the International Conference on Harmful Algae (https://www.icha2018.com/).

7.2 Australia

The objective of the Australian component of this research was to enhance existing breeding and hatchery technology for mollusc species. In particular, to investigate aspects of the biology of three species (flat oysters, pipis and razor clams) that show potential for successful aquaculture. To engage Vietnamese staff in cutting edge molecular genetic technologies to enhance breeding programs, we also took the opportunity to investigate inbreeding in Sydney rock oyster families, which in turn led to the most significant refocussing of breeding to occur in the 25 year history of the NSW breeding program.

Flat oysters (Ostrea angasi)

Efforts to improve culture technology for flat oysters was a major target for research in the NSW component of this project. The flat oyster is found along the southern coast of Australia from Fremantle in Western Australia, through the southern states of Australia to Southern Queensland. This species is found sub-tidally to depths of 40 m, attached to hard substrate or free on the sea floor. Successful hatchery production of the flat oyster has occurred in Australia since the early 1970s, but has been hampered by high mortalities during larval settlement and metamorphosis. Initially we sought to better understand the reproductive cycle of flat oysters so that reproductively mature stock could be reliably obtained from the wild. We subsequently undertook a range of trials to improve larval growth and survival, before investigating techniques to enhance larval settlement.

Increased interest in hatchery production of flat oysters for farming and reef restoration has highlighted the need for a greater understanding of reproductive behaviour of this species. Flat oysters retain or brood their larvae on their gills for approximately half the larval phase. For hatchery rearing, larvae are collected from brooding oysters. To determine the best times to obtain brooding oysters for larval collection, flat oysters were collected over a 12 month period from oyster farms located in four estuaries in NSW; Camden Haven River (Laurieton), Bermagui River (Bermagui), Wagonga Inlet (Narooma) and Merimbula Lake (Merimbula). The shell length of oysters sampled ranged from 49 to 118 mm with the shell size of brooding oysters ranging from 53mm to 95mm. The occurrence of oysters brooding larvae did not differ between the most northern estuary, Camden Haven and the most southern estuary, Merimbula. Across all estuaries examined, oysters were found brooding larvae for 11 months of the year and were found brooding larvae at water temperatures between 12.2^{o}C and 26^{o}C. Histological examination of oysters sampled at each location revealed between 30 to 40% was male, 20 to 30% female, 10 to 20% hermaphrodite, 10 to 15% spent and 10 to 30% non-defined. Changes in water temperature or salinity were not correlated with changes in oyster condition index. There was no relationship found between changes in water temperature, salinity or condition index and the gametogenic phases determined with histology, fecundity or spawning events.

The diet used to rear bivalve larvae is critical for larval growth and survival and establishes the energy reserves required for successful metamorphosis into the juvenile form. A review of the literature showed that diets used previously for flat oyster production had largely been based on those used for other mollusc species and had not received great attention. We adopted a systematic approach to look at the influence of algal diet on survival, growth and development of hatchery reared flat oysters. This was achieved by evaluating uni, binary and ternary algal diet trials. Early stage larvae (140–230 µm shell length) generally grew faster than late-stage larvae (230–340 µm shell length) when fed unialgal diets. Of the 24 algal diets evaluated, larvae fed solely *Tisochrysis lutea* (T. Iso), *Nannochloropsis oculata*, *Tetraselmis chuii* or *Diacronema lutheri*; a binary diet of T. chuii+T. Iso; or ternary diets of T. chuii+T. Iso combined with *D. lutheri* or *N. oculata* had the greatest larval growth, survival, development and metamorphosis, in the respective trials. The correlation between growth rate and spat produced in late-stage larvae was stronger when fed unialgal diets (r = 0.75) than when larvae were fed either binary or ternary diets (r = 0.44 and r = 0.45 respectively). Marked differences in proportion of spat produced (24 h post metamorphosis) were evident among diets producing similar growth rates. For hatchery production of flat oyster larvae, ternary diets of T. chuii+T. Iso combined with either *D. lutheri* or *N. oculata* were the best diets to maximize larval growth rate, development and survival 24 h post metamorphosis.

A need to improve larval rearing techniques led to the development of protocols for catecholamine induced settlement of flat oyster larvae. To further refine these techniques and optimize settlement percentages, the influence of salinity or temperature on development of flat oyster larvae was assessed using epinephrine-induced metamorphosis. Larvae were reared between salinities of 15–35 and temperatures between 14.5 and 31°C. The greatest percentage survival, growth, development occurred when larvae were reared between 26 and 29°C and between salinities of 30 and 35. Larvae reared outside this salinity and temperature range exhibited reduced growth, survival and/or delayed development. Short-term (1 h) reduction in larval rearing temperature from 26°C to 23.5°C significantly increased larval metamorphosis without affecting larval survival. Short term (1 h) increase in larval rearing temperature from 26°C to 29 and 31°C decreased larval survival and metamorphosis. To ensure repeatability in outcomes, tests showed that larvae sourced from different estuaries did not vary significantly in their metamorphic response to short-term temperature manipulation and epinephrine-induced metamorphosis.

Collectively the work undertaken on flat oysters has significantly increased our understanding of flat oyster reproductive patterns and larval biology. The seasonal availability of larvae and the main environmental factors purported to influence reproduction of this flat oyster have been examined. The environmental factors that can be easily and economically adjusted for greater larval rearing success have been refined. Commercially, the cumulative improvements in larval rearing technology described have increased the percentage of “black sick” (advanced larvae) larvae that successfully metamorphose by 50% from an historical average of 23% to 36%. Additionally, this work adds to the broader understanding of the acquisition of competency and larval metamorphosis in bivalve molluscs. Practically, the spat produced over the many trials conducted were supplied to the NSW oyster industry and have underpinned the production of more than $1 million of flat oyster sales (farm gate).

Pipis (Donax deltoides)

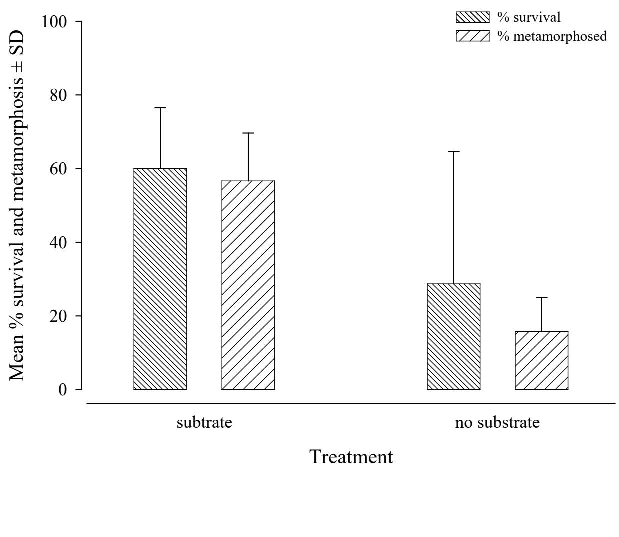
The ‘pipi’, *Donax (Plebidonax) deltoides*, is a common bivalve found on open beaches throughout south eastern Australia with major pipi fisheries being found in New South Wales (NSW) and South Australia. For over a decade in NSW, the pipi fishery has harvested between 200 and 400 t annum^{-1}, valued in excess of US$1.5 million. However, in 2009 reductions in commercial harvest have raised interest in reseeding juveniles into affected areas to promote the recovery of wild stocks and increase the potential for pipi mariculture. Both these activities rely on hatchery propagation of pipi juveniles. However, while attention has been paid to the early ontogeny of members of the genus *Donax* overseas there is little information on the genus in Australia, and none on the early ontogeny of *D. deltoides*.

Two consecutive batches of pipi larvae were produced by stripping gametes from the gonads. Fertilised eggs averaged 75 µm in diameter. When incubated at 23°C, first and second polar bodies were evident after 45-50 min and 95-100 min, respectively, while first cleavage occurred within 155 min of fertilisation. Embryos reached trochophore stage within 24 h and developed to D-veliger stage (112 ± 0.5 µm antero-posterior measurement (APM)) within 48 h. The first pediveligers (318 µm, APM) were observed after 14 days and settled spat (320 µm APM) were present one day later (Day 15). This study confirmed that the production of pipi spat is possible, but highlighted the need for further research into spawning, fertilization and larval nursery techniques for this species (See O’Connor and O’Connor, 2011).

Attempts to produce pipis have highlighted two challenges: success in spawning pipis has been variable and the techniques used for nursery rearing other bivalves have been of limited success. To improve spawning induction we examined a range of commonly used spawning techniques. These have included, thermal stimulation (raising and lower the water temperature), thermal stimulation and salinity adjustment to 24 ppt, emersion (12h exposure to 23°C) and thermal stimulation, chilling (exposure to 4°C) for 2 to 4 h and thermal stimulation and short term exposure to sunlight (1 to 4 h at 42°C) and thermal stimulation. These generally were unsuccessful. Chemical induction using serotonin (5HT) was also assessed using a range of concentrations varying from 2 mM to 0.002 µM 5HT at various temporal frequencies (a single injection or injections repeated hourly or daily), and again these have been largely unsuccessful. It is likely, variable reproductive condition of stocks is confounding some of these observations, but to date hatchery conditioning and strip spawning has been the best approach.

Our first attempts at settling pipis were based on screen systems used for oysters and were successful in that metamorphosis did occur; however, settlement percentages were low, progressive losses occurred and observations of juvenile behaviour suggested that the addition of a substrate could improve outcomes. In work being prepared for publication comparisons of set were made using bare set screens and screens with a bed of various size grade sand substrates. The presence of a substrate could increase percentage survival from 29% to 60% and percentage metamorphosis from 16% to 56% at 3 days post settlement.

Mean percentage survival and metamophosis of hatchery reared *Donax deltoides* larvae with or without settlement substrate.



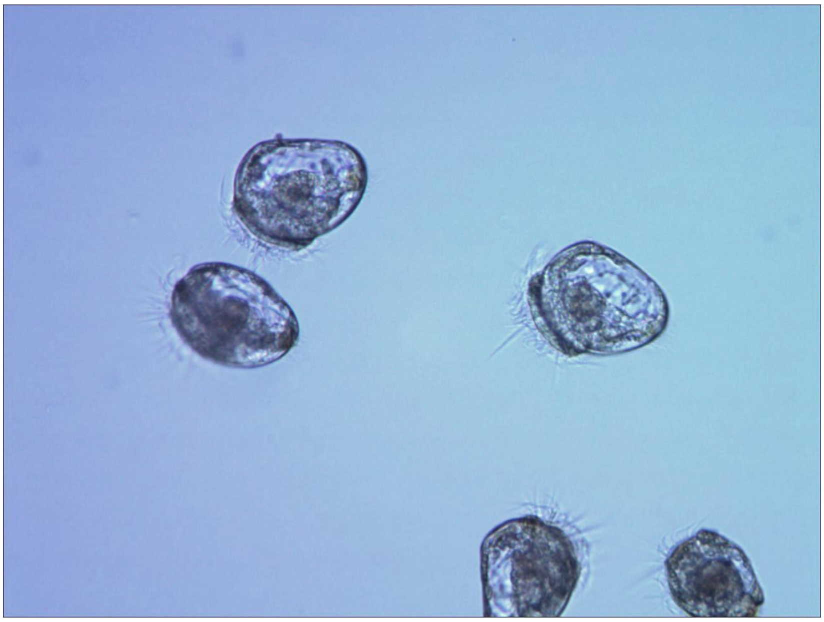
Following the outbreak of Ostreid Herpesvirus in NSW, oyster farmers were looking to diversify their businesses by assessing the performance of alternate bivalve species that were unlikely to be affected by the virus. This provided the opportunity to place pipis in baskets at a number of locations in the Hawkesbury River and assess performance. While we had previously been able to rear clams (*Katelysia* and *Tapes*) on oyster leases, this was not the case for pipis, which appear to require more oceanic conditions as opposed to water quality conditions experienced in estuaries. We are currently looking at opportunities to access more suitable sites for farming and potentially ranching pipis.

While following the reproductive condition of pipis from Stockton Beach in order to develop culture techniques, we observed the presence of a parasite within the gonad. This parasite is most likely a member of the fish blood fluke family, the Aporocotylidae, which characteristically have two hosts and have been described in molluscs before; although, this is the first aporocotylid reported from a marine mollusc from Australian waters. By comparing 28S rDNA with other known species, the parasite from pipis was most closely related to *Chimaerohemecus trondheimensis* van der Land, 1967, the only aporocotylid that has been described from a holocephalan: a subclass of the chondrichthyans, the group that includes sharks and rays. Thus we predict that the bivalve-infecting species we have observed is also a parasite of sharks and rays. Further work is continuing to determine the proportion of the pipi population affected by the parasite and the potential impact on the fishery (See Cribb et al. 2016)

Razor clams (Pinna bicolor)

Over the course of this project, razor clam brood stock have been collected and held at PSFI on 5 separate occasions. Adults were cleaned of fouling organisms, placed in filtered seawater at 23°C and fed a mixture of *Tisochrysis lutea, Diacronema lutheri* and *Chaetoceros muelleri* Lemmermann at a rate of approximately 1×10^{10} cells/adult/day. Attempts to spawn adults have included gonad stripping (with and without ammonia activation of gametes) and temperature shocks (4 – 5°C above ambient), without success. On two occasions, excess broodstock, not used in stripping or temperature shock trials, were left in the holding tanks where, after the period of one week, they spawned naturally. Small numbers of eggs (approx. 1 million) were collected from the tanks and stocked into larval rearing tanks.

The techniques tested to rear razor clam larvae were similar to those described for other bivalves. Zygotes were stocked at a density ≈ 5 ml^{-1} into a 1000-L aerated, polyethylene tank of seawater held at 23 ± 1°C. All seawater (34 g kg^{-1} salinity) used was filtered with 1 μm (nominal) cartridge filters. After 48h, the tank was drained and D-veliger larvae were collected on a 35 μm sieve. A total of 1.0×10^5 larvae were then stocked into fresh seawater in a second 1000-L tank. Larvae were fed a mixture of *Tisochrysis lutea, Diacronema lutheri* and *Chaetoceros muelleri* Lemmermann on an equal dry weight basis in accordance with the feed curve described by O'Connor *et al.* (2008). On the most successful of the two attempts larvae were reared for seven days. This confirmed that the adults were capable of producing viable larvae and allowed initial observations of early ontogeny.



Three day old razor clam (Pinna bicolor) larvae

Sydney rock oysters

Since 1990, a mass selection program has been undertaken in NSW to improve growth and survival in Sydney rock oysters (SRO). This program was particularly successful and after five generations of selection for fast growth, SRO lines were developed that could reach market size almost a year faster and that were significant more tolerant of the two major diseases that affect SRO (QX and Winter Mortality). In order to achieve these gains there was a requirement in mass selection to “close” the population, and stop adding wild oysters to the breeding nucleus. This practice of closing the nucleus, conducting selection over generations and not controlling the crosses at fertilisation leads to inbreeding, especially when there are high selection intensities e.g. for fast growth or disease resistance. The genetic diversity of broodstock used for breeding can be restricted due to selecting individuals from just a few outstanding families, or simply due to chance sampling of a few families where the species are highly fecund, and where one or two females can contribute the majority of oocytes for the next generation. Oysters may be particularly vulnerable to loss of families and consequent inbreeding when no pedigree is maintained as a single oyster female can release millions of eggs in one spawning event (O'Connor et al., 2008).

To test the extent to which inbreeding may have occurred, we assessed the genetic diversity of four different hatchery bred lines of SRO after three, five and seven generations of mass selection using microsatellite DNA markers and mitochondrial cytochrome C oxidase subunit 1 sequences (COX1). This was achieved using seven newly developed microsatellite markers, along with three published loci. Considering the same number of samples in each line, the number of different alleles in each of the four hatchery lines ranged from 49 to 70 alleles from 10 loci, which was approximately 50% less than the 120 alleles found in the samples from the wild population. However, if all four hatchery lines are pooled together, then their total number of alleles was not significantly different from the wild population. Similar to the patterns found for the DNA microsatellite alleles, mtDNA haplotype numbers were considerably lower in each of the hatchery lines than in the samples from the wild population. Again, pooling the hatchery lines gave a total haplotype number not statistically significantly different from that in the samples from the wild. Considering together the DNA microsatellite alleles and the mtDNA haplotypes, we conclude that there was a substantial loss of genetic diversity within all lines separately over generations of mass selection, and that such mass selection was not sustainable in the long term. However, importantly, these data indicate that for oysters, if multiple independent mass selection lines are kept, then their combined genetic diversity may approach that of the ancestral line or wild population even after many generations of mass selection. These results for oysters, finding preservation of diversity among lines yet loss within them, are similar to recent findings for multiple banana shrimp lines; considering both species together it suggests that one relatively simple option suitable for some farms and industry to maintain genetic diversity during mass selection over many generations of selection is to subdivide their breeding nucleus and keep multiple different and independent lines over generations. It remains to be tested if this approach is general across many aquaculture species and circumstances.

While mass selection can improve given traits quickly, the selection of individuals on the basis of best performance of certain traits without regard to pedigree information can result in inbreeding and loss of potentially valuable alleles and net additive genetic variation. Inbreeding can adversely affect genetic breeding programs in a number of ways. The rate of selection response is expected to slow over generations in inbred lines and mating with close relatives unmasks recessive deleterious alleles resulting in inbreeding depression. In hatchery populations, inbreeding often causes significant negative effects on stock performance and production traits. For example, there was significant inbreeding depression for yield after selection for individual growth and survival in Pacific oysters.

These findings contributed to a fundamental shift in Sydney rock oyster breeding where traditional mass selection breeding was replaced with methods to facilitate production of single-pair mated families and to use among-family selection. The parentage of individuals used as broodstock is recorded so that genetic correlations and genotype-by-environment interactions can now be evaluated. This has enabled the breeding program to address impacts to meat condition caused by intense selection for faster growing oysters over many generations. The focus of the Sydney rock oyster family-based program is to develop a breeding strategy that incorporates disease resistance, growth and meat condition for industry.

8 Impacts

8.1 Scientific impacts – now and in 5 years

Since the inception of oyster production research in Vietnam, questions have persisted regarding the identity of the majority of the oyster stocks being farmed. A survey of three key stocks has now confirmed that the species being produced by RIA1 and farmed in Vietnam is *Crassostrea angulata*. This confirmation (and the molecular genetic capacity to make it) is critical for ongoing oyster research in Vietnam. This permits a more targeted use of the existing scientific literature regarding the species and provides the basis on which larger stock surveys can be undertaken. This survey additionally determined that sufficient genetic diversity is present within existing stocks to establish a breeding program, which eliminates the need to import more broodstock and reduces the associated biosecurity risks.

The successful development of an oyster breeding program will not only underpin future development of the oyster industry, but has also provided the infrastructure, training and understanding required for the development of similar breeding programs for other species. The families themselves are a resource for further research that can be used to look at the adaptive capacity of *C. angulata* cultured and natural populations to deal with threats. For example, the adaptive capacity to cope with climate change variables, such as ocean acidification and emerging diseases.

Within Australia, the development of the improved molecular tools for the assessment of genetic diversity in Sydney rock oysters has led to the most significant change in the Sydney rock oyster (SRO) breeding program in its 25 year history. The breeding program was originally based on mass selected breeding lines, in which we were unable to effectively measure or control inbreeding. Research undertaken allowed us to determine the extent of inbreeding and to develop interim strategies to restore diversity while a new approach was adopted. The mass selected lines have now all but gone, rolled into a pedigreed breeding program comprising over 200 families, which has been commercialised in Australia and used as a model for the Vietnamese program described previously.

An archive of flat oyster samples collected from four locations across NSW was processed to provide greater insight into reproductive behaviour in the species at the northern extent of its natural range. This work confirmed an extended breeding season for flat oysters in NSW that better suits commercial production timelines. The results of this assessment have been submitted for publication and have been presented at an FRDC Flat Oyster Workshop held in Adelaide (22 June 2017).

Routine monitoring of pipi populations for ACIAR program research discovered a hitherto unknown Aporocotylid parasite in the gonad. This parasite is the first aporocotylid reported from a marine mollusc from Australian waters. By comparing 28S rDNA with other known species, the parasite from pipis was most closely related to Chimaerohemecus trondheimensis van der Land, 1967, the only aporocotylid that has been described from a holocephalan: a subclass that includes the chimaeras. Thus we predict that the bivalve-infecting species we have observed is also a parasite of sharks and rays. Further work is continuing to determine the proportion of the pipi population affected by the parasite and the potential impact on the fishery.

Program Publications (2017/18)

1. Vu VI, Vu VS, Phan TV, O'Connor WA, Knibb WK 2017. Resolution of the controversial relationship between Pacific and Portuguese oysters internationally and in Vietnam. Aquaculture 472, 389-99.
2. Cribb TH, Bullard SA, Chick RC, O'Connor WA, O'Connor SJ, Johnson D, Cutmore S. (2017) Phylogenetic position of a fish blood fluke (Trematoda:

Aporocotylidae) infecting a bivalve: implications for cophyly. Evidence that blood flukes (Trematoda: Aporocotylidae) of chondrichthyans infect bivalves as first intermediate hosts. Int. J. Parasitol.

3. Dove, M, O'Connor W 2018. History, status and future of oyster culture in Australia. International Oyster Symposium, Rushan China

8.2 Capacity impacts – now and in 5 years

A project "Recognising the Contribution of Capacity Building in ACIAR Bilateral Projects: Case studies from Three IAS Reports" (Mullen et al. 2017) was commissioned in 2017 to deal specifically with this topic and FIS/2010/100 was among the three selected for evaluation. Within that assessment, it was estimated that 24% of the overall budget was attributed to capacity building, but the estimates of the of the importance of capacity building relative to knowledge discovery were 70:30 for Australian researchers in comparison to 50:50 for key Vietnamese staff. It was suggested that this could reflect the fact that the greater proportion of knowledge was new to Vietnamese scientists, nonetheless, it highlights the importance of capacity building now and in the immediate future.

Central to our efforts has been human capacity building. In the projects that preceded FIS 2010/100 the emphasis had been on a range of "practical" skills required for hatchery operation. These skills underpinned progress in this project, but increasingly as the complexity of the research increased, greater academic skills were required. The growing importance of breeding in ensuring the future of the oyster industry necessitated the establishment of an ongoing genetics capability within RIA1 staff.

Three RIA1 staff members involved with the ACIAR project have been accepted as John Allwright fellows (JAF) to study in Australia. The first, Dr Vu Van In, has now completed his PhD studies on oyster genetics at the University of the Sunshine Coast and has returned to Vietnam to take control of the National Marine Broodstock Centre at Cat Ba. Dr Vu Van In now oversees the oyster breeding program. The second JAF, Cao Truong Giang has completed his PhD studies at the University of the Sunshine Coast on assessments of the capacity of selective breeding to develop a genetically improved strain of Pacific white leg shrimp *(Litopenaeus vannamei).* He has now returned and work for the National Marine Broodstock Center – RIA1 as head of Crustacean and mollusc research division. A third JAF, Vu Van Sang, has commenced a PhD program at the University of the Sunshine Coast to investigate selective breeding in the Portuguese oyster, *Crassostrea angulata*.

A member of the oyster breeding team in Vietnam, Mr Nguyen Viet Khue has a PhD program at the University of Technology Sydney, under the supervision of Drs Dove and O'Connor. The program involves the molecular assessments of changes in bacterial communities in and around oysters during disease events and has now completed the first season of sampling including assessments of the microbiome variability within Sydney rock oyster family lines. A further RIA1 staff member, Mr Le Tuan Son, has commenced a PhD investigating "Bacteriophage control of pathogenic *Vibrio sp.* resulting in mortality of larval oysters in hatchery production" at the University of the Sunshine Coast under the supervision of Dr O'Connor. Initial trials establishing experimental models and investigating Vibrio virulence have been completed.

More broadly, Drs Dove and O'Connor have provided significant intellectual and material support to other Vietnamese students studying in Australia. Ms Tham Nguyen from RIA1 is completing a PhD studying the role in insulin-like growth factors in growth and reproduction in oysters at the University of Newcastle. Tham has sourced all her experimental stock from DPI. Ms Thi Lien Ha Bui has commenced a PhD under the supervision of Dr O'Connor investigating the use of freshwater mussels to monitor pollutants.

The following publications are from RIA1 students under the supervision of Drs O'Connor and Dove:

Tran TKA, MacFarlane GR, Kong RYC, O'Connor WA, Yu RMK 2016. Mechanistic insights into induction of vitellogenin gene expression by estrogens in Sydney rock oysters, *Saccostrea glomerata.* Aquatic Toxicol. 174, 146-158.

Tran TKA, MacFarlane GR, Kong RYC, O'Connor WA Yu RMK 2016. Potential mechanisms underlying estrogen-induced expression of the molluscan estrogen receptor (ER) gene. Aquat. Toxicol. 179,82-94 .

Tran TKA, MacFarlane GR, Kong RYC, O'Connor WA, Yu RMK 2017. The constitutively active estrogen receptor (ER) binds and activates the promoter of vitellogenin (VtG) gene in the Sydney rock oyster, *Saccostrea glomerata*. Mar. Poll. Bull. 1-2, 118, 397-402.

Le TS, Southgate P, O'Connor W, Poole S, Kurtboke I 2017. Bacteriophages as biological control agents of enteric bacteria contaminating edible oysters. Current Microbiol. 75; 611-619.

Tran TKA, Yu RMK, Islam R, Nguyen THT, Bui TLH, Kong RYC, O'Connor WA, Leusch FDL, Andrew- Priestley M, MacFarlane GR 2019 The utility of vitellogenin as a biomarker of estrogenic endocrine disrupting chemicals in molluscs. Env. Poll.

King WL, Siboni N, Williams N Kahlke T, Nguyen KV, Jenkins C, Dove M, O'Connor W, Seymour J, Labbate M Variability in the composition of Pacific Oyster microbiomes across oyster family lines exhibiting different levels of susceptibility to Pacific Oyster Mortality Syndrome.

Nguyen THT, Macfarlane GR, O'Connor WA, Yu R 2018. Utilisation of single nucleotide polymorphisms in the insulin-like growth factor binding protein-5 (IGFBP-5) gene as potential genetic markers associated with growth traits in the Sydney rock oyster *Saccostrea glomerata.* International Symposium of Genetics in Aquaculture, 15-20 July, Cairns, Australia.

Over the life of the project, 25 RIA1 & RIA3 staff were provided with training opportunities in workshops and placements in Australian Laboratories provided by international experts.

Establishing the *C. angulata* breeding program increases the capacity of the northern Vietnamese oyster industry to quickly respond to an emerging disease threat. Additionally the breeding program provides industry with the capacity to assess new traits for addition to the program that will increase industry profitability

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

Following the rapid growth in oyster production initiated in 2007 by the predecessor ACIAR program (FIS 2005/114), the continued supply of hatchery oyster seed has permitted continued growth in the oyster industry in northern Vietnam. Farming is now spread across 28 provinces and production has been estimated to have exceeded 15,000 tonnes/annum, now exceeding Australia's total production of 12,000 tonnes/annum. Prospects for continued growth are strong with negotiations underway with a second large (industrial scale) oyster producer that has accessed new lease sites near Van Don. This producer has indicated an interest in single seed oyster production with a view to the production of higher value products with greater export potential.

Oysters are now well established as regular items on local restaurant menus both in the coastal towns and in Hanoi. Freshly shucked oyster meat is now also available in large city supermarkets at a cost of VND 26,333 for 100 g. Improved processing, handling and distribution systems are being developed, creating further socioeconomic opportunity.

While the vast majority of production is consumed locally and the estimates for potential growth in local markets indicate far greater supply is needed, international companies have shown an interest in export opportunities. The most recent of these, a Dutch company (Lenger) has begun export to EU and claims to be able to produce 300 tonnes of oyster a day.

As a result of flat oyster research in NSW, over 3.8 million flat oyster seed have been produced in experimental trails and distributed to industry. In total, the ultimate farm gate of value of that seed has exceeded $1million over the life of the project. The technology developed for flat oyster production has also been disseminated to hatcheries in southern Australia, which has facilitated the production of seed for both oyster reef restoration projects in Tasmania, Victoria and South Australia, and commercial production trials designed to promote oyster industry diversification in South Australia.

8.3.2 Social impacts

Mollusc production in northern Vietnam continues to provide incomes for a large number of families in small impoverished rural/coastal communities. In 2011, production was estimated to have reached 7000 tonnes and employing over 1500 people. Current estimates suggest production has at least doubled and oysters are now produced over a much larger geographical area. Given that oyster farming in Vietnam is labour intensive it is reasonable to expect there has been concomitant increase in employment as production has grown. In addition to this oyster farming has created a processing sector and distribution network that formed and has grown over the course of this project, therefore proportionally more employment has been created in the associated businesses. Furthermore, regional tourism is another industry that benefits from oyster production in this region. Local and international visitors travelling to eat oysters and visit aquaculture facilities would then, in turn directly support the oyster industry in northern Vietnam.

The ongoing community impacts of the oyster program in Vietnam were assessed by Pierce and O'Connor (2014) and have more recently undergone review by Hiruy (2018) as part of a broader Fisheries program impact study. "The exponential increase in the oyster industry has brought changes to the livelihood of surrounding communities in many ways. The three traditional communities of Ha Long Bay have long been used to drawing their living from its waters. The growing numbers of bamboo rafts used by the oyster farmers laid out in neat formation are an extension of what has always been. The rafts are a familiar sight, even if the catch is new. Visually striking and culturally congruent with this dramatic landscape, the oyster farms have even become a local tourist attraction. Local farmers have been more than willing to try a new product, the Portuguese oyster, which does not require expensive feed to grow and fetches high prices" (Hiruy 2018).

"As the yield grew, so did the services needed to get the goods to market. Oysters needed to be cleaned, graded, packed in the shells in which they had grown or shucked, boiled and bagged ready for sale to supermarkets or restaurants. The oyster supply chain opened up opportunities across the community. One such example is in the sale, collection and resale of the cultch shells used both in the hatcheries to grow the oysters from seed and in restaurants to serve them on ice, western-style. Importantly, the women from the community assumed great significance in the area of processing and packaging, supplementing family incomes and increasing their participation in the formal economy." (Hiruy 2018).

A more recent review has been undertaken, again by Pierce (2018), which found "oyster farming, as in the 2011 study continues to have a predominantly positive effect on lives of farmers and those of their families and commune. Money from oyster farming continues to enable diversification into other types of farming, provided jobs including casual seasonal jobs, and opportunities for younger people both in oyster farming jobs within the commune, and also for supporting advanced further education elsewhere for their children."

Gender equity was included in the most recent survey and oyster farming was viewed as equitable and providing opportunities for both men and women. "Overall impact of oyster farming continues to be positive and central to family and commune life in this area of Vietnam. The industry appears to have plateaued somewhat but still is sustaining a better life than before oysters, with economic benefits often profiled as positively impacting on lives." The oyster farming story in this area of Vietnam appears to be now embedded into how people in this study define themselves, both as individuals and as a commune, taking pride in their oyster ventures, assisting in supporting their families in income and jobs and in planning sustainable futures in their communities."

This survey did however highlight a number of concerns including an increase in unpredictable weather and crop failures attributed in part to increases in "parasites" and declines in water quality. These concerns align with project staff observations and form the basis for our recommendations.

8.3.3 Environmental impacts

Genetic and environmental monitoring studies to underpin oyster industry development are providing useful insights to better manage the environment and risks posed by aquaculture development. The potential translocation of pests and diseases through stock introductions has been a major concern. The confirmation that the primary commercial species in Vietnam is *C. angulata* reduces this risk by discouraging the importation of extraneous species (eg *Crassostrea gigas*), which are readily available from China. Our work to demonstrate sufficient diversity exists within local stocks of *C. angulata* to establish a breeding program will further negate the need for stock imports. While seed produced from RIA1 is already acknowledged as superior, the progression of a breeding program will further increase the quality of stock available with an additional economic disincentive to import. Collectively, these measures will reduce the biosecurity risks associated with unregulated oyster imports. Environmental conditions and oyster diseases are often correlated. Oyster disease (for example ostreid herpesvirus) is a looming risk for this oyster industry. Extensive environmental monitoring provides useful information on the conditions when disease outbreaks occur and the breeding program allows greater understanding of the disease itself and whether selective breeding can be used as a potential solution.

The water quality monitoring regime that has been established is now providing valuable environmental data, including metal levels and phytoplankton diversity. Following the first year of assessment the program has been refined to better target potential threats to the industry. This increased monitoring of oyster farming sites will serve to provide additional "state of the environment" information for Ha Long Bay and Bai Tu Long Bay world heritage areas.

Within Australia concerns have been raised regarding the decline of pipi stocks in certain locations along the eastern Australian seaboard. The work undertaken on parasites in pipis has elucidated some of the threats to pipi populations, while the improved knowledge of hatchery production has moved us further toward the potential for stock enhancement of depleted populations.

8.4 Communication and dissemination activities

Scientific communication

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

RIA1 staff now have adjunct positions with the Vietnamese National University of Agriculture where they are responsible for courses on Mollusc Aquaculture.

Broader international scientific interaction was fostered through RIA 1 staff involvement in conferences.

- 2013, two RIA 1 staff attended 5th International Oyster Symposium in Ho Chi Minh and the concurrent Asia Pacific Aquaculture 2013 Conference.
- 2013, one RIA1 staff member attended the International Conference for Molluscan shellfish safety, Sydney, Australia.
- 2017 two RIA1 staff sent to Int. Conference for Molluscan Shellfish Safety, Galway, Ireland.

Australian project staff provided progress updates at a range of conferences and workshops (see 10.2.2). Australian staff were responsible for attracting the 5th International Oyster symposium to Vietnam and were the program organiser for the conference.

Industry communication

Within Vietnam, RIA1 has enabled direct industry dissemination of project knowledge by conducting field trials in two key commercial areas; Cat Ba and Van Don. At Cat Ba Ria1 also operate their own commercial oyster farm, which serves demonstrate both the quality of the stock and the latest production methodologies being trialled. RIA1 are also a commercial scale seed supplier and work with farmers operating at all scales, including the largest of the oyster companies (BIM). To further encourage industry communication, two major industry workshops were held to disseminate project information and a third and final workshop is planned for April.

Publications were provided on the RIA1 website (1) and regular updates were made available through the RIA1 Newsletter (2):

1) http://ria1.org/Ria1/Defaults.aspx?ctl=newsdetail&LangID=1&p=&aID=2385&stID=197

2) http://www.ria1.org/ria1/Defaults.aspx?ctl=news&mID=334&stID=334&tab=62&LangID=2

Within Australia, industry have been informed of project progress on a regular basis via the NSW Aquaculture Research Advisory Committee and updates on progress are provided during the annual "Shed Talks" farmer communications days, held at various locations throughout NSW. Project progress has also been profiled at national oyster workshops such as the FRDC Flat Oyster Workshop in Adelaide in 2016 and the FRDC Tropical Oyster Workshop in Darwin in 2018.

Media communication

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

FRDC Fish Magazine 22(1) Oyster health on international agenda.

FRDC Fish Magazine 25(2) Fisheries in global push for sustainable development.

Partners Magazine 2017 (4) Oysters – feeding a new industry.

In 2016, project progress was included in a VTV2 interview with Vu Van In (Allwright fellow and now Director, Northern National Broodstock Center for Mariculture).

In 2106 our work was covered in the “New Seed” section of Vietnamese Agriculture newspaper and that story was mirrored on RIA1 website.

The program received considerable media attention when Dr O’Connor was awarded the Vietnamese Medal for Agriculture and Rural Development in July 2018. Vietnamese television interviews were provided by Dr O’Connor and Dr Vu Van In to VTV and VTC channels on the night. Articles appeared in the Newcastle Herald, the Telegraph and radio interviews were conducted with 2SM.

A) *The Australian Minister for Agriculture (The Hon. David Littleproud) with the Australian Ambassador to Vietnam (His Excellency Craig Chittick) and the ACIAR CEO (Prof. Andrew Campbell) tasting oysters in at the stall in Vietnam (Hanoi 2018).*

B) *Local staff (Dr Vu Van In) interviewed by national media on the success of the oyster program (Hanoi 2018)*

NSW DPI promoted the program with press releases: https://www.dpi.nsw.gov.au/about-us/media-centre/releases/2019/nsw-research-shows-vietnam-the-world-is-your-oyster and on Facebook: www.facebook.com/watch/?v=796718084004856

9 Conclusions and recommendations

9.1 Conclusions

ACIAR has over the long term committed to the development of mollusc industries in Vietnam and has had significant impact. Oyster production continues to grow, investment remains strong and new markets are being developed. To support this development and to protect the industry from emerging and inevitable challenges this program has:

- Promoted increased mollusc production,
- Established an oyster breeding program, and
- Significantly enhanced the molluscan scientific and management capacity

This project has also been of significant value within Australia. Flat oyster research has had an immediate financial impact for industry through the seed supply. The possibility of farming pipis is now closer to reality and we continue to work on razor clams as an option for industry diversification. Meanwhile the use of the Sydney rock oyster breeding program as a model for similar development in Vietnam has in turn led to improvements in the way our program is designed and managed.

9.2 Recommendations

ACIAR and the Vietnamese Government have invested considerable resources in the development of both the oyster and clam industries in Vietnam. Through a series of programs we have worked to establish reliable hatchery production techniques for molluscs that have seen the distribution of oyster and otter clam seed to farmers and have been instrumental in the dissemsinaton of those techniques to other hatchery producers. Based on this work, the oyster industry in Vietnam (based on *Crassostrea angulata*) has grown rapidly to the extent that approximately 2500 families are currently involved in the production of over 15,000 tonnes of oysters. The industry is profitable and continues to grow, however stock survival, in comparison to the Australian industry, is poor: (10% vs >50%). The causes are varied but predominantly arise from the impacts of pests and disease.

The Vietnamese otter clam (tu hai) industry has also experienced strong growth. Otter clams were first cultured in Northern Vietnam using spat from the wild in 2001, when there were about 40 households farming approximately 50 tons per anum. However, following the success of the first ACIAR program which trained hatchery staff at the RIA1 National Marine Broodtock Center in otter clam seed production, the industry expanded quickly to the point that in 2010 it involved over 1050 households and annual production reached 5500 tons, valued at over $A50 million. In early 2011 the first outbreaks of clam siphon disease were observed with mortalities exceeding 80%. By 2013 the disease spread to all growing areas and the industry collapsed. Interest remains strong as prices for otter clams have climbed to A$25 per kg at the farm gate, but production has dropped back to levels well below the pre-hatchery levels of 50 tonnes per annum.

There are approximately 3500 families deriving an income from oyster and clam farming in Vietnam, as well as those involved in marketing and distribution of the product. To support these industries there is a need to address the fundamental disease challenges facing the ongoing development of mollusc production in Vietnam. We believe this can be achieved by:

- expanding the current oyster breeding program and applying cutting edge molecular genetic techniques to permit better identification of the specific causes of stock loss and the quantification of the potential for selective breeding to address those causes,
- determining estimated breeding values (EBVs) for key oyster performance traits and apply these within the current breeding program,
- gaining an improved understanding the impact of known oyster pest species (Stylochid flatworms and spionid polychaetes) in Vietnam to permit practical, on-farm control methods to be developed along with the protocols (including timing and frequency) for their use.
- establishing the techniques required to permit individual spawning of otter clams to permit the production of pair-mated family lines as the basis of a pedigreed breeding program.
- miniaturising production systems for otter clams to permit concurrent, highly replicated breeding of family lines, and
- producing otter clam family lines to test for the capacity to selectively breed for resistance to siphon disease.

More broadly than Vietnam, mollusc industries are found throughout SE Asia and the Indo-Pacific and we believe there are opportunities for improved mollusc hatchery production technologies to provide benefits at a number of scales. Whether it was promoting the development of tropical oyster industries elsewhere in SE Asia or targeting small scale, enhancement of artisanal mollusc fisheries in the South Pacific, significant improvements could be made. Further, with the skills the Vietnamese have developed in this area, they could also become involved in multilateral knowledge sharing programs.

Within Australia, the drivers for species diversification remain and flat oysters, pipis and razor clams all continue to attract commercial interest, but research needs for these species are diverging. Flat oysters are now readily produced in hatcheries, but the impact of Bonamiasis (a parasitic disease) on the industry in the southern states of Australia is focussing efforts on breeding. The market for pipis continues to grow while natural catches have fluctuated. We now have the techniques to produce pipis but research is required to find suitable growout technologies and locations. Ranching (release into the environment for later harvest) appears to be an economically viable possibility for pipis, but this needs to be confirmed in field trails. Finally, we still have to refine hatchery technology for razor clams and our next steps will be to focus on broodstock maintenance and spawning.

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10.2 List of project publications

10.2.1 Publications:

O'Connor S, Moltschaniwskyj N, Bolch, CJS, **O'Connor WA**, 2013 Dietary influence on growth and development of flat oysters, *Ostrea angasi* (Sowerby,1871), larvae. Aq. Res. 43, 1317-1327.

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- Program staff in bold

11 Appendices

11.1 Hatchery Manual

This document is available at:
http://ria1.org/Ria1/Uploads/Doc/Vn%20Hatch%20Manual%20So%20tay%20san%20xuat%20giong%20nhuyen%20the%202%20manh%20vo%20RIA1%20-%202018.pdf

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

SỔ TAY HƯỚNG DẪN SẢN XUẤT GIỐNG NHÂN TẠO MỘT SỐ LOÀI ĐỘNG VẬT THÂN MỀM 2 MẢNH VỎ (Hàu Thái Bình Dương: *Crassostrea gigas;* Tu hài: *Lutraria philipinarum; ...*)

Tác giả:
TS. Lê Xân, Th.S. Cao Trường Giang, Th.S. Đỗ Xuân Hải, Th.S Vũ Văn Sáng và TS. Vũ Văn In

HIỆU ĐÍNH THÁNG 12 NĂM 2018

Australian Government
Australian Centre for International Agricultural Research

NSW DEPARTMENT OF PRIMARY INDUSTRIES

11.2 Hatchery data base example:

A	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
INDIV_ID (yyyy-family-sample)	FAMILY_ID		cross	Year	SITE 1=Van-don 2=Catba	ASSESS 1=Aug 2014	Rep 1= repli 2=repli 2 3=repli 3)	Type (1=Single; 2=Clutch)	Shell Length (mm)	Shell width (mm)	Shell Depth (mm)	W_Index	D-Index	Whole Weight (g)	Soft Tissue (g)	Shell Weight (g)	CI_TW	Shell color (N=Normal)	Sex	Gonad/Fatness (N=Normal G=Good, B=Bad)	External shape (G=good, N=normal, B=bad)	Inner shape (G=GoodN =normal B=bad)	Desease (ill = illusion occurs in the gonad)	Survival (%)	Note
3	1			6	7	8	9	10	11	12	13			14	15	16		17	18	19	20	21	22	23.00	24
2014001-1	2014001	001	CC	2014	1	1	1	2	85	51	32	0.6	0.376470588	100	12.0	69	12	N	M	3	3	3	None	33.14	**Site**
2014001-2	2014001	001	CC	2014	1	1	1	2	82	50	35	0.609756098	0.426829268	75	13.0	48	17.33333333	N	F	3	3	3	None		
2014001-3	2014001	001	CC	2014	1	1	1	2	83	49	28	0.590361446	0.337349398	85	10.0	58	11.76470588	N	F	3	3	3	None		B=Bad
2014001-4	2014001	001	CC	2014	1	1	1	2	78	52	29	0.666666667	0.371794872	66	9.0	42	13.63636364	N	F	3	3	2	None		**Disease**
2014001-5	2014001	001	CC	2014	1	1	1	2	75	42	26	0.56	0.346666667	56	9.0	35	16.07142857	N	M	3	3	2	None		None= no disease
2014001-6	2014001	001	CC	2014	1	1	1	2	87	40	27	0.459770115	0.310344828	38	6.0	25	15.78947368	N	F	3	2	2	None		ill= plenty illusion b
2014001-7	2014001	001	CC	2014	1	1	1	2	70	55	43	0.785714286	0.614285714	95	11.0	69	11.57894737	N	F	3	3	3	None		
2014001-8	2014001	001	CC	2014	1	1	1	2	83	47	26	0.56626506	0.313253012	52	9.0	28	17.30769231	N	M	3	2	3	None		
2014001-9	2014001	001	CC	2014	1	1	1	2	98	52	33	0.530612245	0.336734694	85	10.0	60	11.76470588	N	F	3	3	3	None		
2014001-10	2014001	001	CC	2014	1	1	2	2	87	54	37	0.620689655	0.425287356	75	10.0	52	13.33333333	N	M	3	3	3	None		"1"=Van Don
2014001-11	2014001	001	CC	2014	1	1	2	2	78	43	24	0.551282051	0.307692308	55	8.0	42	14.54545455	N	F	2	2	2	None		"2"=Cat Ba
2014001-12	2014001	001	CC	2014	1	1	2	2	75	41	29	0.546666667	0.386666667	55	9.0	40	16.36363636	N	F	3	2	2	None		
2014001-13	2014001	001	CC	2014	1	1	2	2	75	45	27	0.6	0.36	65	9.0	45	13.84615385	N	M	3	3	3	None		**Broodstocks**
2014001-14	2014001	001	CC	2014	1	1	2	2	77	50	29	0.649350649	0.376623377	57	10.0	39	17.54385965	N	M	3	2	3	None		"C"=China
2014001-15	2014001	001	CC	2014	1	1	2	2	91	48	29	0.527472527	0.318681319	85	12.0	60	14.11764706	N	F	3	3	3	None		"N"=Nam Dinh
2014001-16	2014001	001	CC	2014	1	1	2	2	70	42	27	0.6	0.385714286	50	8.0	47	16	N	M	3	3	3	None		"V"=Ria1
2014001-17	2014001	001	CC	2014	1	1	2	2	62	40	26	0.64516129	0.419354839	32	6.0	22	18.75	N	F	2	2	2	None		
2014001-18	2014001	001	CC	2014	1	1	2	2	74	51	31	0.689189189	0.418918919	65	8.0	53	12.30769231	N	M	3	3	3	None		**Assess**
2014001-19	2014001	001	CC	2014	1	1	2	2	72	42	23	0.583333333	0.319444444	37	11.0	21	29.72972973	N	M	3	2	2	None		"1"=08.2015
2014001-20	2014001	001	CC	2014	1	1	2	2	75	49	28	0.653333333	0.373333333	70	8.0	35	11.42857143	N	M	3	3	3	None		**Shell color**
2014001-21	2014001	001	CC	2014	1	1	3	2	64	41	33	0.640625	0.515625	49	9.0	38	18.36734694	N	F	2	2	2	None		N=Normal
2014001-22	2014001	001	CC	2014	1	1	3	2	72	43	32	0.597222222	0.444444444	70	11.0	51	15.71428571	N	F	2	3	3	None		
2014001-23	2014001	001	CC	2014	1	1	3	2	83	50	37	0.602409639	0.445783133	75	11.0	43	14.66666667	N	F	3	3	3	None		**Sex**
2014001-24	2014001	001	CC	2014	1	1	3	2	68	48	34	0.705882353	0.5	65	9.0	30	13.84615385	N	M	3	3	3	None		F=Female
2014001-25	2014001	001	CC	2014	1	1	3	2	62	45	29	0.725806452	0.467741935	45	8.0	30	17.77777778	N	M	3	2	3	None		M=Male
2014001-26	2014001	001	CC	2014	1	1	3	2	75	54	37	0.72	0.493333333	90	15.0	60	16.66666667	N	M	3	3	3	None		
2014001-27	2014001	001	CC	2014	1	1	3	2	76	43	22	0.565789474	0.289473684	38	8.0	30	21.05263158	N	M	3	2	2	None		**Gonad, Fatness,**
2014001-28	2014001	001	CC	2014	1	1	3	2	71	47	29	0.661971831	0.408450704	60	10.0	41	16.66666667	N	M	3	3	3	None		G=Good

11.3 Hatchery Production estimates:

Spat production at the National Marine Broodstock Center, Cat Ba			Commercial hatchery production in Quang Ninh and Nam Dinh provinces	
	Single seed (Million)	Cultch spat (Million)	Single seed (Million)	Cultch spat (Million)
2014	0	15	0	200
2015	7	15	0	300
2016	2	20	0	350
2017	0	30	0	500
2018	0	35	0	500

Comprehensive information on hatchery spat production in Vietnam is unavailable. Estimates here are based on the observations of RIA1 Staff made during their regular visits to key facilities that have sought technical support