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Final report

project

Pearl industry development in the western Pacific

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Contents

1 Acknowledgments

We acknowledge the support, collaboration and various research inputs of all partner institutions, collaborating farmers, communities, and other stakeholders to this project.

2 Executive summary

Research outputs in Tonga have substantially improved the production capacity of the half-pearl (mabé) industry. A key component of this research was to develop routine and regular hatchery production of juveniles (spat) on which pearl farmers rely. Research resulted in continuing improvements in hatchery efficiency, increased production and simplification of hatchery processes. The latter included the exclusive use of commercially available microalgae concentrates as a larval food source to replace the technically demanding use of on-site, live microalgae culture. Significant advances were also made in nursery culture procedures, with combined results achieving a substantial increase in the numbers of oysters available to pearl farmers. Since start of the project, the number of active pearl farms in Tonga has more than doubled quadrupled – from 4 to 17. Expansion includes transfer of pearl farming from the traditional farming area of Vava'u to four new community demonstration farms around Tongatapu that have already harvested high quality pearls and have further expanded their pearling operations. Successful production at these sites has confirmed the potential for expansion of the Tongan pearl industry to include new island groups. New demonstration farms also provide training venues for new and prospective pearl farmers that facilitate their gradual handover to local communities. Another three community demonstration farms were established in the remote and impoverished Ha'apai island group where they have achieved good initial oyster growth and survival. 'Volunteer' positions funded through the Australian and New Zealand aid programs provide additional expertise relating to development of the demonstration pearl farms at Tongatapu and Ha'apai, and in the increasingly important area of pearl business development and marketing.

Research in Fiji focused on developing new, high resolution genome-wide SNP markers for *P. margaritifera*, which provide a highly useful resource for investigating genetic structure and population connectivity at both fine and broad spatial scales. Marker sets of several thousand SNPs were tested for Fijian oysters and were subsequently applied to a genetic audit of both farmed and wild populations collected from 11 sites in Fiji. Results have shown that Fijian populations are part of a single, large biological stock, which will make fishery management and aquaculture practices in the country relatively straight-forward. Further research examined genetic structure at the level of the ~18,000 km species distribution and discovered substantial genetic structure for the first time. Three and five genetic stocks have been identified in the Indian and Pacific Oceans, respectively, and recommendations made for regional fishery management, particularly for Pacific basin populations. The taxonomic identity of *P. margaritifera* has also been evaluated using molecular techniques and revealed that instead of being a single discrete species possessing regional sub-species variants, it is highly likely to be a large species complex, sharing similarities with the Akoya pearl oyster species complex. This discovery indicates that the species identity of *P. margaritifera* will need revision, and possible reclassification following further study.

The first half-pearls produced from *Pteria penguin* in PNG were harvested in 2016 and were of high quality. Spat collection activities in the Kavieng lagoon generated relatively small numbers of spat; however, hatchery culture of *P. penguin* at the Nago Island Mariculture and Research Facility was conducted and successfully produced spat. High mortality of hatchery produced pearl oyster spat resulted from predation, and it is likely that appropriate management of predation will require innovative design and improved management of culture systems. A number of capacity related training activities were completed for the women's handicraft community at Nusalik including business training courses delivered by the National Fisheries College, and handicraft skills training programs delivered by an Australian Business Volunteer (ABV) to provide specialised creative design training. The activities of this handicraft community are now linked to the related ACIAR project FIS/2014/060[1]. Consumer surveys conducted during this project and FIS/2014/060 provided

[1] *FIS/2014/060: "Developing pearl industry-based livelihoods in the western Pacific"*

feedback about product range, price of shell handicrafts and preferred products, and have informed skills training needs, product design and marketing strategies.

The project demonstrated successful hatchery production of *Pteria penguin* in Vietnam, and subsequent culture of mabé pearls, provides a good basis for further development of pearl production as a potential mariculture and livelihoods-supporting activity. Such development would be based on existing capacity in mollusc hatchery production at RIA3 and mollusc farming capacity within the local oyster farming community.

3 Background

Fifty years since its introduction to French Polynesia, pearl farming has become the country's second largest export earner (to tourism) with a current value of around US$170 million p.a. A similar industry, currently valued at around US$1-2 million p.a. (Ponia, 2010), was later developed in the neighbouring Cook Islands. Pearl culture has been successful in these countries because it is compatible with traditional lifestyles and provides opportunities for income generation at a number of levels. Individuals may be directly involved in pearl farming or in supply of pearl oysters to pearl farms. Associated handicraft activities also offer considerable opportunity for income generation, particularly for women and younger people. Indeed, pearl culture has been introduced to some atolls in Polynesia to address sociological problems such as depopulation and unemployment (Southgate *et al*., 2008). Pearl culture itself is environmentally 'benign' and the products are ideal as an export commodity for Pacific island countries as they are small, lightweight, non-perishable and of high value.

Based on these characteristics, cultured pearls are the Pacific region's highest ranking and highest priority aquaculture commodity (Secretariat of the Pacific Community, Aquaculture Plan 2007). Successful development of pearl culture in Polynesia has provided impetus for research towards similar developments in other parts of the Pacific. ACIAR has led in this development with, for example, research conducted in Fiji in the 1990s (FIS/1997/031[2] and FIS/1993/720[3]) that resulted directly in the development of today's successful cultured pearl industry. An overview of ACIAR leading role in developing pearl culture methods appropriate to the non-atoll environments of western Pacific countries is shown in Fig. 1.

The fledgling cultured pearl industries in Fiji and Tonga have considerable potential for further development, similar to that in Polynesia. Fiji already has an international reputation for its high-quality round pearls, which are available in a range of colours, many unique to Fiji. Tongan half-pearls, produced from *Pteria penguin*, are a unique product among Pacific island nations. This project supported development of these industries towards this potential, and assessed the potential of half-pearl production in Papua New Guinea and Vietnam.

3.1 Partner country and Australian research and development issues and priorities

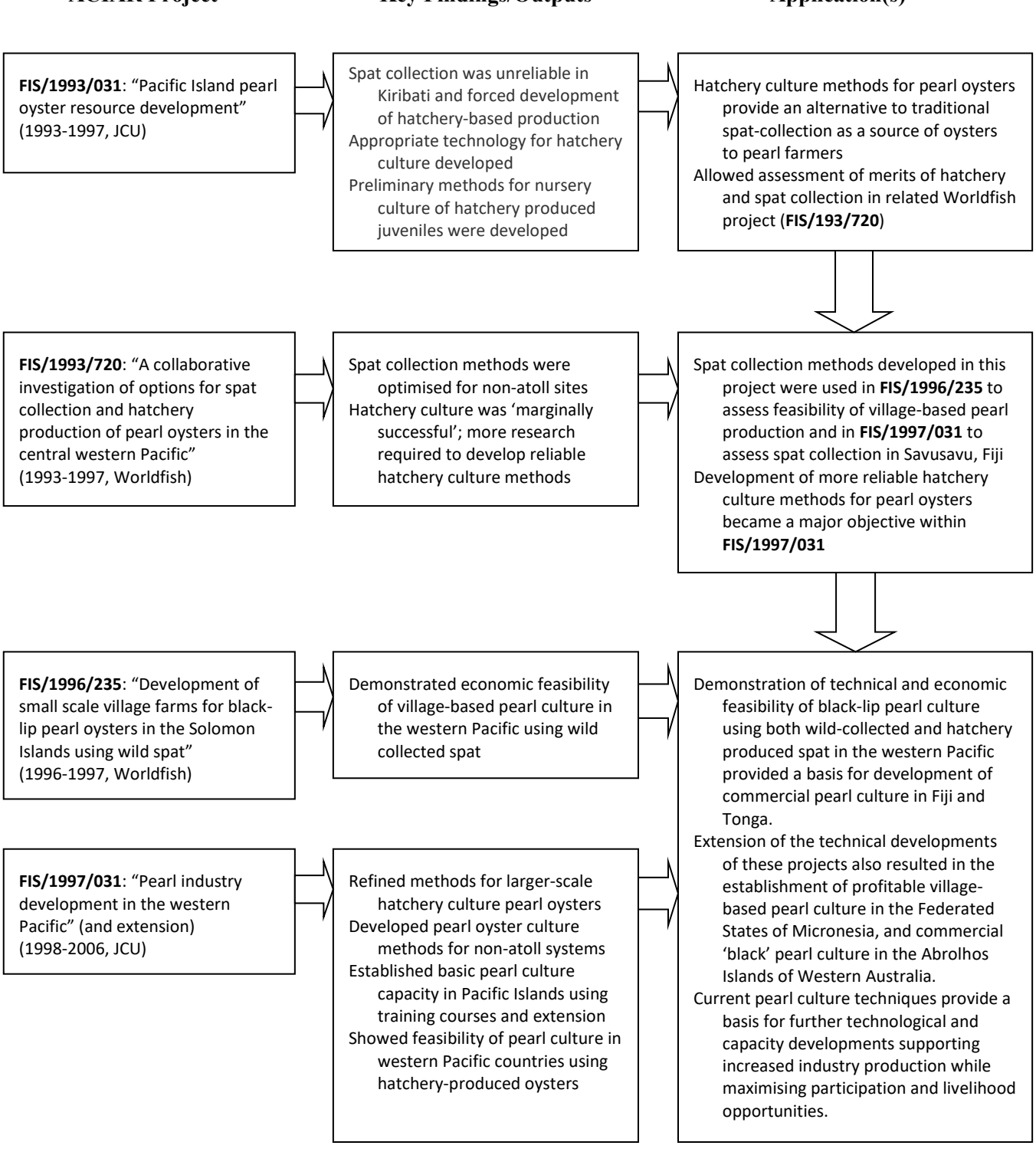
3.1.1 Fiji

The important role of ACIAR-funded research in helping establish a viable cultured pearl industry in Fiji is described in Fig. 2. There are currently ten pearl farms in Fiji, at various stages of production. All focus on round pearl production from the black-lip pearl oyster, *Pinctada margaritifera.* There is one large company (J. Hunter Pearls), which has championed industry development, and three smaller farms which contribute to annual exports. Pearls are sold through Japanese auctions organised by J. Hunter Pearls. Current farmers focus on round pearl production which has a longer investment cycle and better export returns. Although a relatively new export industry, Fiji pearls have rapidly achieved an international reputation for both quality and colour. This provides Fiji with a considerable market advantage, and demand for Fiji pearls is high (Torrey, 2007).

[2] *FIS/1997/031: "Pearl oyster resource development in the Pacific islands" (JCU)*

[3] *FIS/1993/720: "A collaborative investigation of options for spat collection and hatchery production of pearl oysters in the central western Pacific" (Worldfish)*

Fig. 1. The role of ACIAR-funded research Projects in developing pearl culture in the western Pacific.



Expansion and diversification of the cultured pearl industry is a priority of the Fiji government. Two key factors are: (1) expansion of pearl farming activities to other sites in Fiji to extend income generating opportunities; and (2) appropriate supply of juvenile oysters to support expansion of pearl farming activities. Both were addressed in research conducted within the ACIAR PARDI Pearl Project[4], which assisted the Fiji government to extend spat collection activities to support livelihood opportunities and expanded the industry through adequate oyster supply. However, as with other cultured bivalve industries (e.g. oysters and scallops) it is likely that hatchery production will take an increasingly important role as the industry matures. This will be particularly important in Fiji where pearls in 'niche' colours, unique to Fiji, are produced. Hatchery production is used by J. Hunter Pearls to produce a portion of the juveniles used by the industry. However, hatchery production of pearl oysters is unreliable and often unsuccessful, and more research is required to determine optimal conditions for hatchery culture of *Pinctada margaritifera.* Recent results from hatchery culture of the winged pearl oyster (*Pteria penguin)*, have shown that commercially available micro-algae concentrates are highly nutritious for pearl oyster larvae. The use of such pastes for hatchery culture of *Pinctada margaritifera* has not yet been assessed but could simplify production methods and become an important component of developing more efficient hatchery methods for this species.

Expansion of pearl culture activities throughout the Fiji Islands has become a priority of the Fijian government to provide resilience to natural disasters such as cyclone Tomas, which destroyed many small pearl farms in 2010. However, given the range of pearl colours produced in Fiji, it is imperative that this expansion is managed to retain genetic diversity within Fijian pearl oyster stocks. This is particularly important given the potential for transfer of spat between different areas of Fiji and the resulting impacts on the genetic makeup of local populations. This project conducted a genetic audit of *Pinctada margaritifera* in Fiji to define the resolution of genetic structure and provide a basis for stock management protocols for the Fiji pearl industry. This output was incorporated into the Development Plan for the Fiji pearl industry being developed by the ACIAR PARDI Pearl Project.

The J. Hunter Pearls hatchery in Fiji provided an opportunity to investigate the potential of triploids in pearl oyster culture where benefits may include: (1) quicker growth to pearl producing size; (2) elimination of the need to condition oysters prior to pearl grafting/seeding; (3) more rapid secretion of nacre from triploid donor tissue to produce larger pearls and/or reduce pearl production times; and (4) reduced genetic transfer risks because triploids are generally sterile. Although triploids are widely used in commercial production of other bivalves, such as oysters and scallops, and despite offering considerable potential benefits to cultured pearl production, use of triploids in the pearl industry has so far received little attention.

3.1.2 Tonga

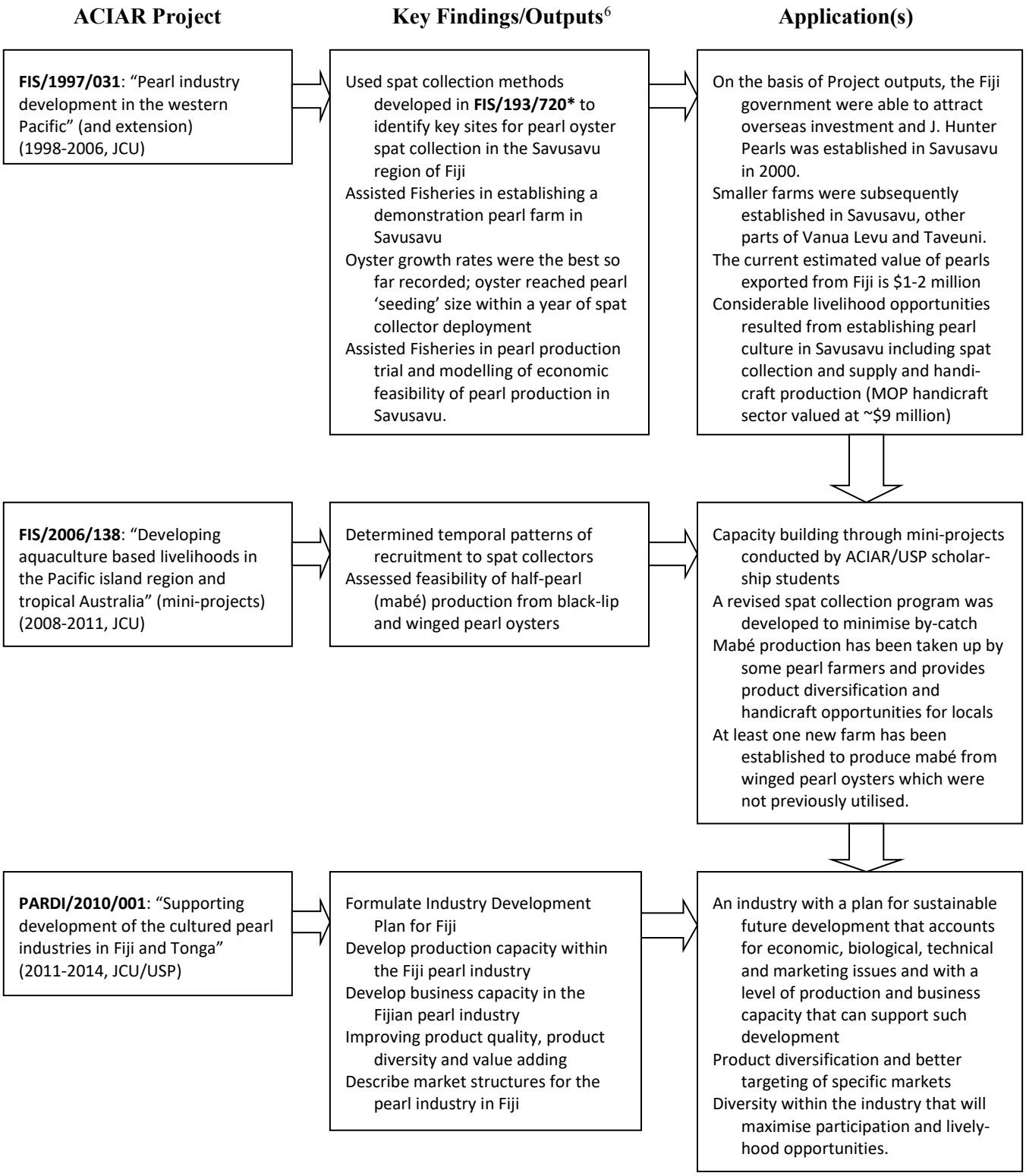
There is a small cultured pearl industry in Tonga that satisfies a local handicraft market. It is based on half-pearl ('mabé') production from the winged pearl oyster, *Pteria penguin*. Finau (2005) pointed out that Japanese scientists in the 1970s estimated a potential annual (export) income of around US$7.5 million for a fully developed Tongan pearl industry. Subsequently, pearl culture was identified as among the highest priority aquaculture commodities by the Tongan government in the recently completed Tonga Aquaculture Commodity Development Plan 2010-2014.

A major bottleneck for the Tongan pearl industry over recent years has been supply of sufficient numbers of juvenile oysters that can be grown to a size appropriate for pearl production. This resulted in a decline in pearl production and in the number of pearl farms in Tonga. These issues were addresses by FIS/2006/172[5] which successfully developed hatchery culture methods for *Pteria penguin*, and was successful in developing routine supply of juvenile oysters to pearl farmers. This

[4] *PARDI/2010/001: "Supporting development of the cultured pearl industries in Fiji and Tonga"*

[5] *FIS/2006/172: "Winged oyster pearl industry development in Tonga"*

Fig. 2. The role of ACIAR-funded research Projects in developing pearl culture in Fiji



[6] *Or anticipated outputs; *FIS/1993/720: "A collaborative investigation of options for spat collection and hatchery production of pearl oysters in the central western Pacific" (1993-1997, Worldfish).*

activity supported some recovery of the Tongan pearl industry with an increase in the number of farms, greater numbers of oysters being cultured and ex-pearl farmers returning to the industry on the basis of reliable oyster supply.

Running concurrently with FIS/2006/172 was an MSc research project conducted within FIS/2006/138[7] by an ACIAR/USP Scholarship holder and member of the aquaculture staff at Tonga Fisheries (Mr. Martin Finau). Mr. Finau's research resulted in more effective nursery culture techniques for hatchery produced juvenile pearl oysters. However, further research was required to fully address the issues that are fundamental to sustainable long-term production, and to provide Tonga Fisheries with the knowledge and skills required to support consolidation and proposed expansion of the Tongan pearl industry. Additionally, research into factors affecting pearl quality (and therefore value) that was planned within FIS/2006/172 could not be attempted because slower than anticipated growth of hatchery produced *Pteria penguin* resulted in limited availability of appropriately sized oysters. However, hatchery oysters produced during FIS/2006/172 were used to assess factors affecting pearl quality in this project.

Research outputs from FIS/2006/172 and FIS/2006/138 showed that the growth rates of hatchery produced *Pteria penguin* spat were as good on the main Tongan island of Tongatapu as at the main pearl culture sites in Vava'u, 500 km to the north. A priority for the Tongan government is to assess the feasibility of transferring pearl culture technology from Vava'u to Tongatapu, to provide income generating opportunities in the most populated area of Tonga. The outputs from ACIAR-funded research supporting development of the Tongan pearl industry are shown in Fig. 3.

3.1.3 PNG

PNG has a vast coastline, with numerous communities primarily dependent on marine resources for their livelihoods. The sea cucumber fishery, which is traditionally the main village-based fishery generating significant export income, was a moratorium at the start of this project and there was an immediate need for social/economic activities, as alternatives to sea cucumber, to support livelihoods in coastal communities in PNG, and the National Fisheries Authority (NFA) was under political pressure in this regard. Development of mariculture opportunities in PNG can now be supported by the recently completed NFA Nago Island marine hatchery and training facility at Kavieng, New Ireland. The role of this facility is to develop marine aquaculture-based livelihood opportunities for PNG and to become a training centre for students from the National Fisheries Centre (NFC).

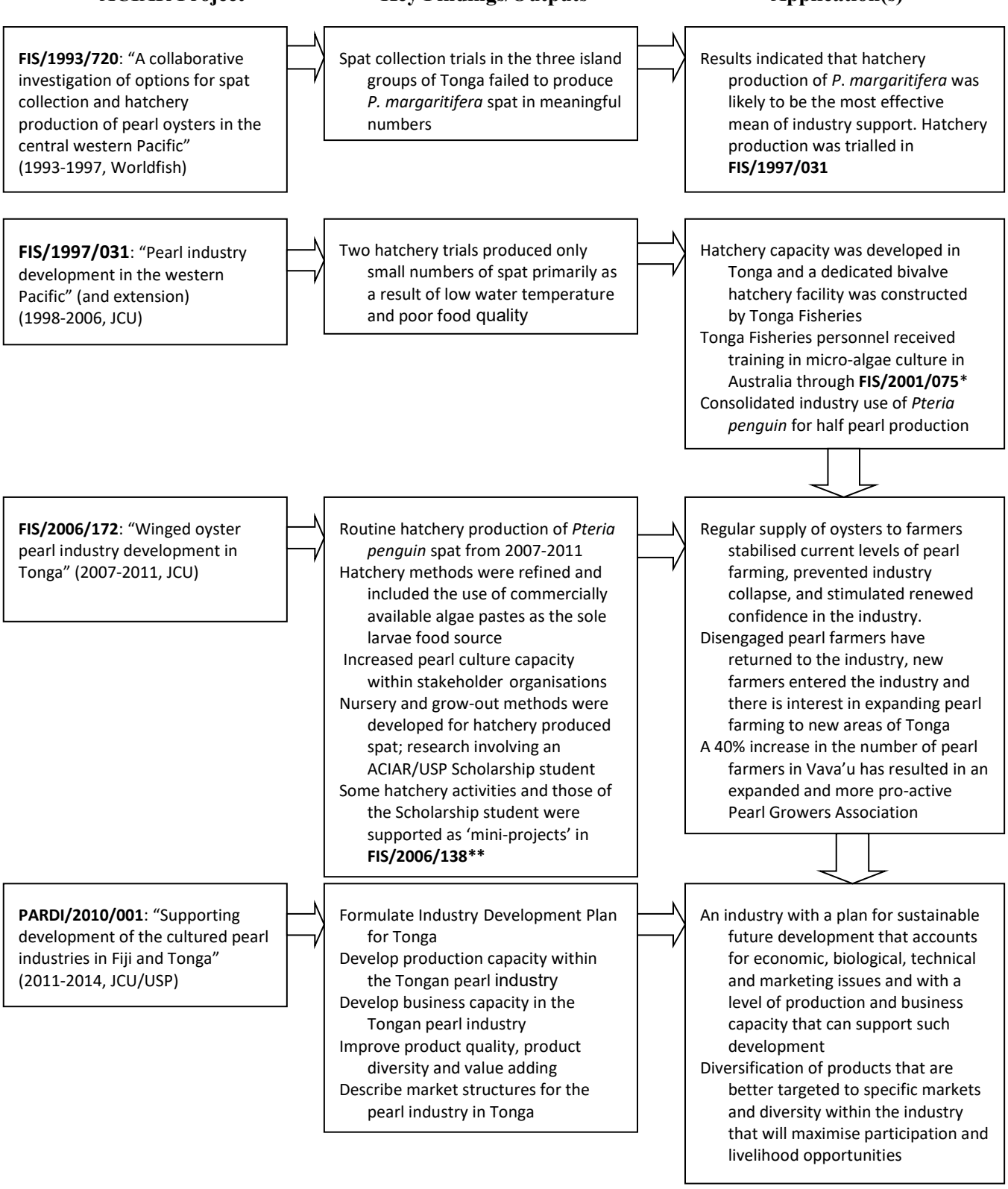
Research conducted during FIS/2006/138 identified pearl oysters (*Pteria penguin*) as a promising commodity for further research and development. This species readily recruits to spat collectors at coastal locations in Kavieng, and assessment of the feasibility of pearl culture to support local livelihoods and generate potential export income, is a priority for NFA (PNG National Aquaculture Development Policy). While there was no current pearl culture activity with *Pteria penguin* in PNG, this can be kick-started by transferring culture techniques developed for this species in Tonga during FIS2006/172 and FIS2006/138. The outputs from ACIAR-funded research supporting development of pearl culture in PNG are shown in Fig. 4.

3.1.4 Australia

The Australian cultured pearl industry is likely to benefit from the results of this project. Diversification within the industry has seen the utilisation of *Pinctada margaritifera* and *Pteria penguin* for pearl and/or meat production in Queensland and Western Australia. As such, the research outputs from this project will have direct relevance to pearl culture in Australia. Similarly, hatchery and nursery culture techniques developed for *Pinctada margaritifera* in FIS/1997/031, were later used to establish culture of this species in the Abrolhos Islands and other parts of Western Australia.

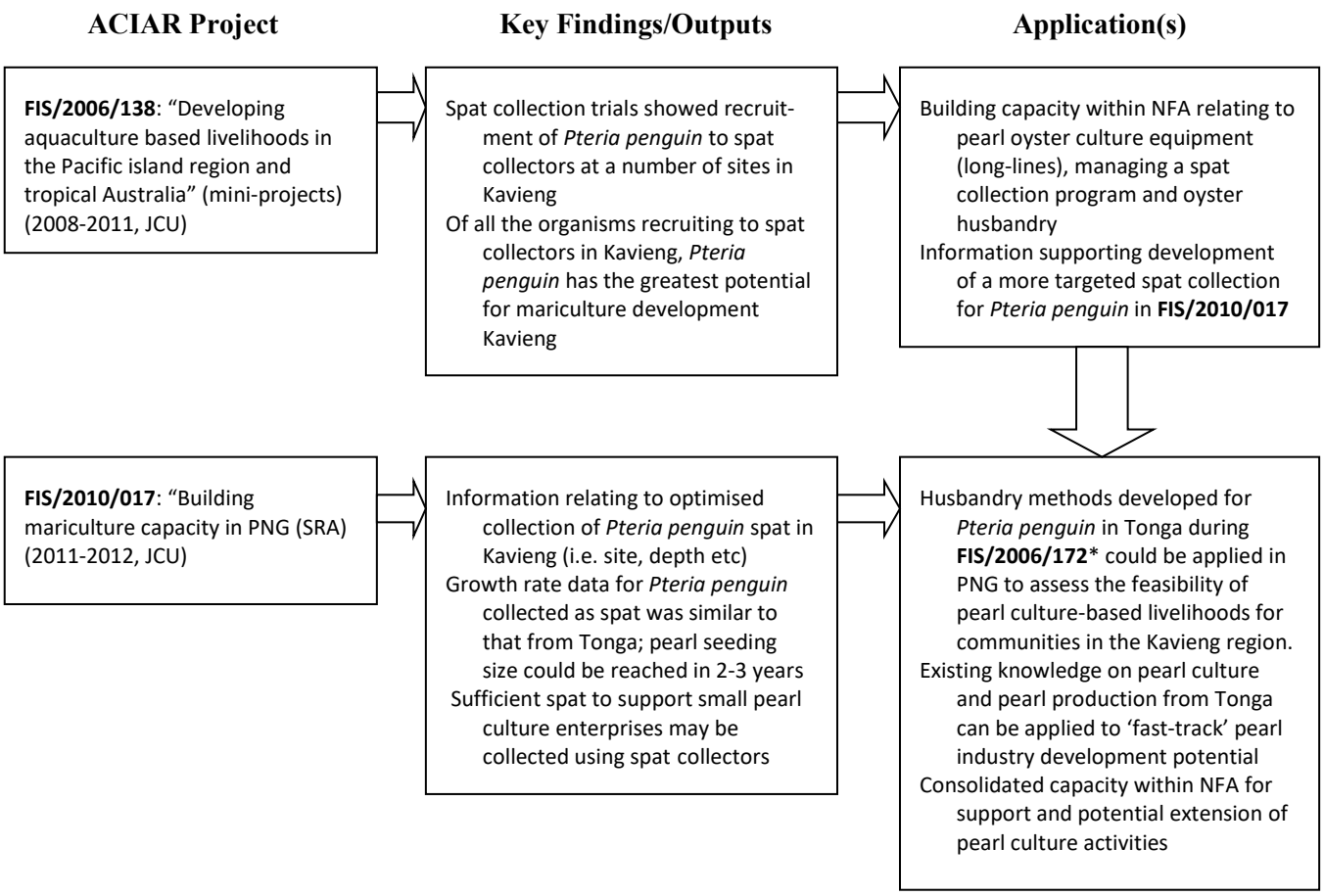
[7] *FIS/2006/138: "Developing aquaculture-based livelihoods in the Pacific islands region and tropical Australia"*

Fig. 3. The role of ACIAR-funded research Projects in developing pearl culture in Tonga



[8] *Or anticipated outputs; *FIS/2001/075: "Sustainable aquaculture development in Pacific Islands region and northern Australia" (2004-2007, QDPI); **FIS/2006/138: "Developing aquaculture-based livelihoods in the Pacific island region and tropical Australia" (2008-2011, JCU).*

Fig.4. The role of ACIAR-funded research Projects in developing pearl culture in PNG



4 Objectives

The overall aim of this project was to provide a sustainable basis for the continued development of cultured pearl industries in the western Pacific. The specific objectives and associated activities were:

Objective 1: To develop more effective hatchery production methods for *Pinctada margaritifera* and *Pteria penguin.*

- Conduct controlled experiments to optimise larval culture conditions.
- Assess feasibility of high-density larval culture for *Pteria penguin*.
- Assess commercially-available microalgae pastes as a food for *P. margaritifera* larvae.
- Refine settlement protocols for *P. penguin* and *Pi. margaritifera* larvae.
- Develop triploid induction method for *P. margaritifera*.
- Assess performance of triploid *P. margaritifera*.

Objective 2: To enhance development of the cultured pearl industries in Fiji and Tonga.

- Develop improved nursery protocols to maximise spat production in Tonga.
- Trial pearl oyster culture at a new community site in Tongatapu (Tonga).
- Assess potential for round pearl production from *Pteria penguin* in Tonga.
- Conduct experiment to optimise the quality of half-pearls produced by *P. penguin* in Tonga
- Genetic analysis of pearl oyster stocks in Fiji.
- Deploy data buoy in Savusavu Bay to improve knowledge of the relationships between physico-chemical characteristics, pearl oyster growth and pearl quality in Fiji.

Objective 3: To undertake baseline studies for the development of half-pearl culture in Kavieng, PNG, and trial half-pearl production in Vietnam.

- Establish a long term spat collection program in Kavieng targeting pearl oysters and optimising spat collection protocol (methods, seasonality, location, handling, etc).
- Conduct grow-out trials for *Pteria penguin* in the Kavieng area.
- Production of half-pearls from wild-collected *P. penguin* in Kavieng to assess quality.
- Trial pearl production in Vietnam.

5 Methodology

This project drew heavily on prior ACIAR research (see *sections 2.1 and 2.2*) which developed generic methods for pearl oyster culture in non-atoll environments of the western Pacific. It fine-tuned these practices to provide a basis for further sustainable development of the pearl industries in Fiji and Tonga. Given similarities in geographic, environmental and social factors between Vava'u (Tonga) and Kavieng (PNG), the Tongan pearl industry provides a useful model for similar development in PNG. Pearl culture was introduced to Kavieng during this project to assess the feasibility of pearl culture in this region. Further sustainable development of pearl culture in all three countries will rely on a greater understanding of the husbandry requirements of pearl oysters and of the influence of environmental parameters and culture methods on productivity.

5.1.1 Spat Collection

Methods for spat collection are well established and have been utilised in a number of recent ACIAR projects including FIS/2006/138 and FIS/2006/172. A long-line is deployed on the surface (or sub-surface) and is supported by floats. It provides a substrate from which spat collectors are attached. Spat collectors are composed of mesh bags containing settlement substrate (e.g. shade cloth, monofilament net) and are deployed for up to 15 months when they are retrieved and inspected for recruits.

5.1.2 Hatchery culture

Prior to the start of FIS/1997/031 and FIS/2006/172, little was known of the development of *Pinctada margaritifera* or *Pteria penguin* larvae or their hatchery culture requirements. Knowledge resulting from FIS/1997/031 (e.g. Southgate and Beer, 1997) proved to be an appropriate basis for hatchery culture of *Pteria penguin* in FIS/2006/172. A significant finding of FIS/2006/172 was that *Pteria penguin* larvae could be reared using commercially available micro-algae concentrates (pastes) as a food source, thus eliminating the need for live micro-algae culture and the associated technical and resource demands. This project further developed hatchery culture methods for both *Pinctada margaritifera* and *Pteria penguin* to standardise larval culture methods for both species. Research addressed optimum larval stocking density with a view to developing high-density culture methods, optimum feed ration at different stages of development, appropriate water quality parameters, and optimised use of micro-algae concentrates as a larval food source. Research also addressed methods for settlement and transfer of spat to the ocean. Husbandry following transfer is important in minimising losses of spat through predation and biofouling etc. and these issues were addressed to optimise husbandry methods.

5.1.3 Nursery culture

Much of our knowledge of nursery culture requirements of pearl oysters results from prior ACIAR projects (e.g. Friedman and Southgate 1998; Southgate and Beer, 2000). These general methods provided a successful basis for nursery culture of *Pteria penguin* in FIS/2006/172 and were refined during FIS/2006/138.

However, under mass culture conditions where commercial and/or logistic issues play a part, production may not be optimal. For example, there have been recent incidents of disease amongst *Pinctada margaritifera* at Savusavu in Fiji, thought to result from over-stocking, deteriorating water quality at certain times of the year and/or limited food availability. In Tonga and Fiji the frequency with which pearl oysters should be inspected and cleaned also needs to be determined to reduce, for example, high mortality resulting from predation by *Cymatium* gastropods.

Our knowledge of the influence of environmental parameters on pearl oyster growth, survival and pearl production is extremely limited for tropical species.

Collaboration with SOPAC to generate further information on the influences of physico-chemical characteristics on pearl oyster performance and pearl quality in Fiji was continued in this project, through deployment of a logging data buoy in Savusavu Bay. The Ministry of Marine Resources (MMR), Cook Islands, with the assistance of the bilateral aid program of the New Zealand

government, purchased and deployed two oceanographic monitoring data buoys in the atolls of Manihiki and Penrhyn (Tongareva) in 2003 to provide on-going information on the environmental stressors that have triggered pearl oyster mortality events in the Cook Islands. The data generated was a key component in assisting the pearl industry to develop standard management and monitoring practices. The data buoys were removed in 2008 for maintenance and a partial refit by SOPAC. This project will fund recalibration of one of the data buoys and its redeployment to Savusavu Bay in Fiji. The other will return to Manihiki Atoll in the Cook Islands.

There was considerable liaison with, and direct involvement of, industry collaborators in this project. This was possible because of existing linkages established through prior (e.g. FIS/2006/172, FIS/2006/138 and PARDI/2010/001) ACIAR projects. Pearl farms in Vava'u (Tonga) and Savusavu, Taveuni and Rakiraki (Fiji) were actively involved in nursery culture experiments and made significant in-kind contributions to the project (labour, long-line space, boat use/fuel etc).

5.1.4 Pearl production

One of the initial objectives of FIS/2006/172[9] was to assess some of the factors influencing the quality of half-pearls produced by *P. penguin* with a view to optimising their quality. However, this aspect of the project was not attempted because of slower than anticipated growth rates of hatchery produced *P. penguin* and limited availability, during the Project, of oysters of an appropriate size for pearl production.

Prior research has shown that the quality and (therefore) value of half-pearls produced by the closely related species, *Pteria sterna,* is influenced by a number of factors including:

(1) duration of pearl production;
(2) position of half-pearl nuclei within the shell (Ruiz-Rubio *et al*. 2006); and
(3) depth/method of oyster culture

This study assessed the impacts of all these factors. The methods used for half-pearl production are now well established (Ruiz-Rubio *et al*. 2006; Taylor & Strack, 2008). Briefly, oysters are selected for half-pearl production on the basis of good nacre colour and quality. They are then anaesthetised using benzocaine or proplene phenoxitol (Acosta-Salmon and Southgate, 2005). Commercially available plastic hemispherical nuclei are then glued to the inside surface of the shells of anaesthetised oysters. Multiple nuclei can be applied to each shell surface (Ruiz-Rubio *et al*., 2006). The oyster is then placed in clean seawater to recover from anaesthesia and, once recovered, is placed into culture nets and removed to the pearl farm. A nacre thickness of around 1 mm is considered commercially acceptable and, while the rate of nacre secretion over the nucleus varies according to culture conditions and water temperature, a period of between 6-10 months is normally required. After this period, half-pearls are drilled from the shell and prepared for sale. Pearl quality was determined using qualitative assessment of colour, lustre and surface blemish, according to standard pearl quality assessment (Matlins, 1996). Nacre thickness will be determined at the base, apex and sides of resulting half-pearls using vernacular callipers following sectioning (Ruiz-Rubio *et al*., 2006).

Round pearls have considerably greater value than half-pearls and are commonly produced from pearl oysters of the genus *Pinctada*. While in the same family (Pteriidae), oysters from the genus *Pteria* differ anatomically and this restricts round pearl production (Xie et al., 2012). Small-scale experimental round pearl production from *P. penguin* has been reported from Hainan Island (Fu et al., 2008).

[9] *FIS/2006/172: "Winged oyster pearl industry development in Tonga"*

This Project also included trials to assess the feasibility of half-pearl (mabé) culture as a means of income generation in Kavieng, PNG. Small long-lines were established at the NFA Nago Island facility in Kavieng, for holding oyster prior to, and after, pearl seeding. Pearl seeding was done with wild collected oysters using well established methods (Ruiz-Rubio *et al*., 2006) and was conducted as a small workshop (e.g. Southgate *et al*., 2006) to train NFA personnel and students from the National Fisheries College (NFC) in Kavieng. A follow-up workshop was held for the same participant to outline pearl harvesting methods, value-adding and handicraft techniques.

This project also included preliminary assessment of the potential for half-pearl production in Vietnam as a linkage activity with FIS/2005/114[10]. Again, pearl seeding was done as a workshop for research staff at RIA3 in Vietnam and followed up with second workshop to harvest the pearls.

5.1.5 Genetic components

Prior research has shown *Pinctada margaritifera* to be genetically fragmented in the Pacific. This project completed a genetic audit of *P. margaritifera* populations in the Fiji Islands. The programme sampled pearl oysters from a large number of sites within the Fiji Islands to establish a genetic database and define resolution of genetic structure for *P. margaritifera*, for which markers are already established. This work was undertaken by Mr. Monal Lal, a Fijian JAF awardee studying at JCU. His research will include sample taking (field-based) and laboratory analysis at both USP and JCU. The information generated was used to inform a protocol for responsible use and transfer of pearl oyster stocks within Fiji.

To determine patterns of genetic diversity and structuring in Fijian *P. margaritifera*, 100+ oysters from a broad variety of sites throughout Fiji were non-invasively sampled for genetic analyses by biopsy of ~ 3 mm^2 of foot, mantle or adductor muscle tissue (Lind *et al*., 2007). Samples were preserved in 70% ethanol. Similar numbers of settling spat were collected from each location to obtain data on the temporal stability of genetic structure, origin of recruits (i.e. local or from common pool) and effective number of breeders contributing to the recruitment pulse. Emphasis was placed on spat collection sites established during the ACIAR-PARDI Pearl project. Genomic DNA will be extracted from tissues using high-throughput Tween-20 protocols developed at JCU for pearl oysters (Lind *et al*., 2007, Kvingedal *et al*., 2010). Oysters were genotyped at up to 20 microsatellite loci and for the mitochondrial COX1 gene (Lind *et al*., 2007). Allele frequencies, genetic diversity and allelic richness was calculated using the software Fstat 2.9.3.2 (Goudet, 1995), while genetic structure was determined using both an analysis of molecular variance approach (AMOVA) and Bayesian STRUCTURE analyses.

Production and use of triploids is common in commercial bivalve culture and is widely and successfully used with rock oysters. Triploids are induced by inhibiting extrusion of the second polar body during embryological development, using chemicals such as 6DMAP or with high pressure/water temperature.

Genetic components of this project were overseen by Dr. Kyall Zenger and A/Prof. Dean Jerry from the Aquaculture Genetics Section at JCU. Both have worked extensively in the field of pearl oyster genetics – both quantitative and population genetics – in a commercial context with the gold/silver lip pearl oyster, *Pinctada maxima*.

[10] *FIS/2005/114:"Building bi-valve hatchery production capacity in Vietnam and Australia"*

6 Achievements against activities and outputs/milestones

Objective 1: To develop more effective hatchery production methods for Pinctada margaritifera and Pteria penguin.

No.	Activity	Outputs/ milestones	What has been achieved?	Comments
6.1.1	Conduct controlled experiments to optimise larval culture conditions	Complete one multi-factorial experiment per year addressing larval culture parameters.	Milestone completed. A number of multi-factorial experiments along with many smaller less formal trials were conducted alongside hatchery production runs conducted in Tonga. Experiments assessed important husbandry components including feeding ration (using micro-algae concentrates), larval density, water temperature, use of probiotics and settlement substrates. Outputs provided a basis for development of improved procedures for successful large-scale medium density larval culture. These hatchery procedures supported a tripling of spat output from the Tongan hatchery compared to the prior low density larval culture system (see 6.2.1).	The refined hatchery process has been documented as a practical working manual. The "*Hatchery and early nursery production manual"* is now available and in use. Experimental outcomes have resulted in two journal publications (Wassnig and Southgate 2016; Southgate et al., 2016). Another detailing recommended hatchery and early nursery culture methods for *Pteria penguin* is nearing submission for publication (Wingfield et al., see Publications List) The manual and the papers are novel in that they describe successful hatchery production without use of live micro-algae. This is a first for any bivalve culture industry and supports expansion of hatchery culture in the region through simplified culture methods that are much cheaper and do not require micro-algae culture expertise. We are aware of the incorporation of micro-algae concentrates into hatchery culture protocols at commercial hatcheries for pearl oysters (Indonesia and Australia) and other bivalves (oysters and scallops) in USA, UK and Chile as a direct result of this project's findings. Our results also provided a basis for successful use of micro-algae concentrates for sandfish larvae (in FIS/2010/042, FIS/2010/054), which has also resulted in simplified hatchery procedures for sea cucumbers (Militz *et al.,* 2018)

6.1.2	Assess feasibility of high density larval culture for Pteria penguin	Complete high-density larval culture trials to determine optimal larval density, feeding rate and water flow rate using commercially available microalgae pastes	Milestone completed. This research component was addressed by a USP/ACIAR Master's scholarship student Jerome Taoi. The research demonstrated that although high-density larval culture has some merit when conducted on an experimental scale, there are still significant practical issues that limit the application of such systems for large-scale production.	Research outcomes contributed to the successful development of hatchery culture methods using an intermediate or 'medium' larval density that supports improved hatchery production (see 6.1.1).
6.1.3	Assess commercially-available micro-algae pastes as a food for *Pinctada margaritifera* larvae (Fiji)	Complete small-scale trials to determine the nutritional value of available algae species Complete small-scale trial to determine optimal larval density versus ration for most nutritious species Complete trial to assess use of microalgae paste under commercial scale culture conditions with varying degrees of aeration	Milestone not completed.	Preliminary trials showed that *P. margaritifera* larvae are able to ingest the flagellates 'Pavlova' and 'Isochrysis' from the 'Instant Algae' product range. However, rigorous growth trials to assess these products rely on hatchery availability within production schedules. Growth trials planned at the J. Hunter Pearls hatchery in Fiji did not initially take place because of production problems in the hatchery and a need for repeated attempted commercial runs. The hatchery was then unavailable because of 'troubleshooting' activities and finally the hatchery was lost during TC Winston. No alternative hatchery options were available in Fiji and so this component of the project was not conducted (see 6.1.1).

6.1.4	Refine settlement protocols for Pteria penguin larvae	Complete two trials to assess different settlement substrates and settlement tank set-ups. Determine optimal time (post settlement) for transfer of spat collectors to the ocean.	Milestone completed. Throughout the project various settlement substrates were tested with two specific settlement trials being conducted. Although spat can be successfully settled on a range of substrates in various tank set-ups, the greatest success (for settlement and subsequent nursery culture) is achieved with a configuration of commercial spat collectors secured within Harwood oyster trays. This system facilitates direct transfer of settlement units from settlement tanks to the field. Milestone completed. An experiment assessing the optimal timing for the transfer of spat to the ocean confirmed that both survival and growth are significantly improved when spat are transferred to the ocean nursery lines at an early stage after settlement. Optimal growth and survival are achieved when spat are transferred at between 5 to 10 days post-settlement. This simplifies and quickens the hatchery culture procedure for *Pteria penguin*.	Optimal production outcomes are achieved through the relatively early transfer of larvae from the hatchery to the ocean. This significantly reduces the time and workload associated with completing the hatchery production process. These findings constitute an integral component of the "Hatchery and early nursery production manual" and will be published as part of a journal paper that is currently in preparation: Wingfield et al. "Hatchery and early nursery culture of the winged pearl oyster *Pteria penguin*" (see Publications List)
6.1.5	Develop triploid induction method for *Pinctada. margaritifera*	Determine timing and duration of second polar body extrusion Assess three concentrations of 6-DMAP for proportion of triploid induction	Milestone not completed.	This research was anticipated to be undertaken by a PhD student recruited in the first year of the project. However, a suitable student was not identified and this component of the research was not addressed.

6.1.6	Assess performance of triploid *Pinctada margaritifera*	Complete large-scale larval culture trial including triploid induction of some progeny (Australia) Determine relative growth rates of triploid and diploid juveniles (Australia) Determine relative growth rates, sex and gonad state of triploid and diploid juveniles (Australia) Complete large-scale larval culture trial including triploid induction in Fiji for assessment of relative growth rates, sex and gonad condition under Fijian condition.	Milestone not completed.	The 'Australian' components of this research milestone were not addressed because an appropriate student was not identified to undertake the research (see 6.1.5). The planned 'Fijian' component of this proposed milestone relied on successful outcomes from the 'Australian' component. But it could not have been undertaken anyway because of limited hatchery availability and then loss of the hatchery in TC Winston (see 6.1.3)

Objective 2: To enhance development of the cultured pearl industries in Fiji and Tonga

No.	Activity	Outputs/ milestones	What has been achieved?	Comments

6.2.1	Develop improved nursery protocols to maximise spat production in Tonga	Complete two successful hatchery runs per year using standard 'static' system	Milestone completed. A minimum of two hatchery runs were completed during each year of the project. Their success is demonstrated by the increasing numbers of spat produced. Hatchery procedures have been significantly refined during the project (see 6.1.1) resulting in substantial increases to hatchery output. Sequential numbers of spat transferred from the hatchery to the ocean were: 1) May 2013 spawning = 4,000 spat 2) Dec 2013 spawning = 40,000 spat 3) Feb 2014 spawning = 220,000 spat 4) Mar 2014 spawning = 170,000 spat 5) Nov 2014 spawning = 300,000 spat 6) Jan 2015 spawning = 350,000 spat 7) Jan 2016 spawning = 450,000 spat 8) Nov 2016 spawning = 200,000 spat 9) Mar 2017 spawning = 300,000 spat	Output has increased as production practices have been further refined. Once considered a bottleneck for industry expansion, technical advances in hatchery and nursery production, developed during this project, now support regular supply of oysters to pearl farmers for the first time. This has stimulated an increase in the number and size of pearl farms and expansion of pearl farming to other island groups in Tonga; to the main island of Nuku'alofa where three new community-operated farms were established in 2015, and to Ha'apai where two community farms were established in 2016. Given that each additional oyster used successfully for pearl production generates around $90-$300 of income for pearl farmers, there is the potential for major economic benefits and considerable increase in industry production value. Between 2013 and 2016 the number of families actively involved in pearl farming in Tonga increased from 3 to 17; the number of oysters under culture increased from 1,200 to 32,000; the number of pearls harvested increase from 1,585 to 6,000, and the value of the pearls harvested increase from T$ 149,000 to T$ 564,000. Outcomes have supported significant practical advances in the efficiency of both nursery culture and grow-out that minimise handling and transfer stress and reduce workload. Refinements to the nursery protocols have been incorporated into the "*Hatchery and early nursery manual"*. Many of the findings are also relevant to grow out process and have been incorporated into a separate manual focused on grow-out. Both manuals now constitute essential resource documents for Fisheries staff and industry. A number of manuals have been developed during this project relating to culture of *Pteria penguin*: 1. "Hatchery and early nursery manual" 2. "Husbandry guide to *Pteria penguin* farming" 3. "Grow-out manual"
		Complete two nursery experiments per year.	Milestone completed Two nursery culture experiments, along with several scoping trials, were conducted each year. Some culture factors evaluated were: use and configuration of various protective cages and mesh sizes relative to oyster size, fish predation thresh-hold size, hanging depth, chaplet configuration, cleaning frequency and stocking density, etc.	
		Complete standard nursery culture protocol for *Pteria penguin*	Milestone completed. A standard nursery culture protocol has been successfully developed	

				4. “Suppliers of pearl farming equipment” Other manuals developed during this project (also serving FIS/2014/060) are based on research outcomes to optimise mabé production methods in Tonga: 5. “Mabé pearl seeding manual” 6. “Mabé pearl grading manual” All have been distributed to pearl farmers and artisans where appropriate and provide a basis for fisheries industry extension activities. Consolidation of these manuals into a single publication to be published possibly within the FAO Fisheries Technical Paper series, will be undertaken.

6.2.2	Trial pearl oyster culture at a new community site in Tongatapu (Tonga)	Establish an experimental pearl culture site in Tongatapu	Milestones completed. In 2015 four community demonstration farms were established on three of the small islands adjacent to Tongatapu.	Growth and production results have been encouraging and three of the new farms in Tongatapu have added additional pearl lines (one 4 new lines) as a result of the success of this activity.
		Begin growth trials with hatchery spat at the site	Hatchery reared spat have been grown successfully at each of the above Tongatapu sites.	The process of setting up new farms in Tongatapu and linking these farms with community demonstration activities has proven successful and the process has been replicated in the remote and impoverished Ha'apai island group. Three new community demonstration farms were recently established (2016 and 2017) in Ha'apai. Initial growth and survival results look very promising at all three Ha'apai sites.
		Comparative growth rate data for Tongatapu	An experiment to monitor relative growth and survival at four Tongatapu sites and three sites in Vava'u ran for 18 months and provided important baseline data documenting differences between sites and island groups.	
		Establish half-pearl culture trials	Half-pearl culture trials have been undertaken at three of the Tongatapu sites and in Vava'u. An experiment to assess the rate of nacre deposition and the quality of resulting half-pearls from varying culture periods (6-12 mo.) has been completed.	Establishment of new pearl farms involved considerable training of locals (farm establishment, oyster husbandry, pearl seeding, pearl harvest etc.) and ongoing extension support from project and Fisheries staff.
		Harvest half-pearls for quality assessment	Excellent quality half-pearls have been harvested from these initial trials at all three Tongatapu sites.	Detailed analysis of nacre deposition requires equipment not currently available in Tonga. Samples of mabé from culture trials in Tonga have been taken to Australia for future analysis. Experiments confirmed that pearls require a minimum 11-12 month culture period to maximise quality. This information has been a valuable addition to extension information for pearl farmers (particularly new ones) and has been incorporated into extension and training materials ("Mabé pearl seeding manual" and "Mabé pearl grading manual"; see 6.2.4) Findings confirm that favourable pearl culture conditions exist throughout Tonga and that there is enormous potential for further industry expansion. Further analysis of mabé samples (see above) will allow assessment of seasonal effects on nacre deposition rates and quality and will be used as a basis for further refining pearl culture methods.

6.2.3	Assess potential for round pearl production from *Pteria penguin* in Tonga	Identify suitable Chinese seeding technician and establish appropriate protocol for his/her visit to Tonga Nucleate 200-300 oysters for round pearl production Harvest round pearls Professional assessment of pearl quality and value Economic assessment of the relative merits of round versus half pearl production in Tonga using economic modelling software developed by Bill Johnson of QDPI	Milestone not completed and activity reassessed	Since the start of this project, research in China has shown that commercial round pearl production from *Pteria penguin* is not feasible because of difficulty in seeding for round pearl production in this species (Mao et al., 2004), low survival and low nucleus retention (Liang et al., 2008; Xie *et al*., 2012). On this basis redirection of research resources towards improving the yield and quality of half-pearls was considered a more reasonable and productive approach in this project (see 6.2.2).
6.2.4	Conduct experiment to optimise the quality of half-pearls produced by *P. penguin* in Tonga	Establish half-pearl culture trials at Vava'u Harvest half-pearls for quality assessment Optimised protocol for half pearl culture from *P. penguin* in Tonga	Milestones completed. Pearl culture trials have been undertaken in Vava'u and these are reported in section 6.2.2. Two experiments to assess rates of nacre secretion relative to nuclei position, number of nuclei implanted and nucleus profile began in 2016. Resulting pearls will be harvested in August 2017 and the results will help to further refine and optimise seeding protocols for this species. A major outcome of this project has been development of successful and appropriate protocols across all aspects of pearl culture and production in Tonga. They are reported in two manuals relating to pearl seeding and pearl grading developed during this project (see 6.2.1)	In addition to the specific culture trials, nine new pearl farms have been established in Vava'u and assessed for suitability. Newly developed protocols for pearl culture in Tonga have been produced as a Pearl farming resource kit. This kit includes a number of dedicated manuals listed in 6.2.1.

6.2.5	Genetic analysis of pearl oyster stocks	Complete collection of samples for analysis Complete laboratory analysis at JCU and USP. Complete genetic database and define resolution of genetic structure for *Pinctada margaritifera* in Fiji. Completion of draft protocol for responsible use and transfer of pearl oyster stocks within Fiji. Completion of protocol following consultation with stakeholders	All milestones completed	A study to develop genome-wide SNP markers for *P. margaritifera* is complete and published in *Marine Genomics* (Lal et al., 2016). 5,243 SNPs were used to assess marker suitability for population genomic analyses in 156 Fijian oysters. Results indicated that the marker set was robust, and highly useful for examination of genetic structure, population connectivity as well as gene architecture in this species. The outcomes of this study were used to make recommendations for a genetic stock assessment exercise for Fijian *P. margaritifera* populations. A genetic audit of Fijian wild and farmed oysters involving 427 oysters from 11 sites utilised 4,123 SNP markers. All Fijian populations were shown to constitute a single biological stock, which was supported by particle dispersal modelling to assess larval connectivity between sites. There is no evidence for local adaptation among surveyed sites, supporting country-wide stock translocations for both aquaculture and stock replenishment. Further research examined genetic structure and evolutionary relationships over the Indo-Pacific distribution of *P. margaritifera*. There is a high degree of population connectivity within the Pacific, with Fijian and Tongan populations being part of a single biological stock. Further results indicated that instead of being a discrete species possessing regional sub-species variants, *P. margaritifera* is in fact a very large species complex and further study is required to revisit the species description. This aspect of the project (6.2.5) was conducted by JAF-PhD student Monal Lal. It resulted in four high quality publications in high ranking international journals. Monal successfully completed his PhD in early 2017 and has returned to USP where he contributes significantly to the institution's capacity in molecular biology.

6.2.6	Deploy data buoy in Savusavu Bay to improve knowledge of the relationships between physico-chemical characteristics, pearl oyster growth and pearl quality in Fiji.	Data buoy deployed in Savusavu Bay. Establish growth trials to allow correlation of growth, survival and pearl production with variations in physico-chemical factors. Long-term data set for pearl oyster performance and physico-chemical factors in Savusavu Bay. Investigate correlations between physico-chemical parameters and biological data and publish results.	Milestones completed.	The fully operational buoy was eventually deployed in Aug 2014, but in Feb 2015 problems developed with the buoy's sensors and it was removed from the water. Following recalibrated in the USA the buoy was redeployed in Aug 2015. The buoy was removed from the water again in Feb 2016 because of damage resulting from TC Winston and further repair would be very costly. Multi-data loggers have since been used to support ongoing project research. An ACIAR/USP Scholarship Master's student (John Carreon) conducted growth trials with various ages/sizes of *P. margaritifera* in Savusavu Bay to determine relationships between physio-chemical factors and growth rates, survival and condition of farmed oysters. This research began in June 2015, was completed in late 2016 and will be written up by mid-2018 when John completes his MSc. At least one publication is expected from this student project.

Objective 3: To undertake baseline studies for the development of half-pearl culture in PNG, and trial half-pearl production in Vietnam

No.	Activity	Outputs/ milestones	What has been achieved?	Comments
6.3.1	Establish a long term spat collection program in Kavieng targeting pearl oysters	Identify appropriate sites for long-lines deployment at three sites in Kavieng Source and obtain required equipment for long-lines (ropes, anchors, etc.) Deploy three long lines and establish spat collectors at each Establish spat collection program that will run for the life of the Project	All milestones completed. Long-lines were deployed at a number of sites in the Kavieng Lagoon and a spat collection program was established soon after the start of the project. However, *P. margaritifera* and *Pt. penguin* spat were consistently collected in very low numbers at these sites. A number of trials were conducted throughout the year using different collector materials deployed at different depths in an unsuccessful attempt to collect larger numbers of spat. Recruitment of predators (molluscs and crabs) to spat collectors at a high frequency occurred throughout the year at all sites and no doubt impacted oyster recruitment.	Results indicate that a productive spat collection program targeting pearl oysters is probably not feasible in Kavieng Bay because of low recruitment and high rates of oyster predation. In an effort to overcome this and to provide oysters for subsequent research, hatchery runs were conducted with *Pteria penguin* at the Nago Island Mariculture and Research Facility (NIMRF) in Kavieng in 2015 and again in 2106. But high levels of predation caused significant mortality of resulting spat during ocean culture. Although not initially planned, the hatchery run provided a capacity building exercise for NFA hatchery staff at NIMRF that had previously only worked with sea cucumbers.

6.3.2	Conduct grow-out trials for *P. penguin* in the Kavieng area.	Establish grow-out trials at all 3 sites (see 6.3.1) using spat from spat collection program. Trials will run for the life of the Project	Milestone completed. Given the security issues of remote field sites, within the Kavieng lagoon, only two sites were used in growth trials; a floating timber pontoon adjacent to the Nago Island Mariculture Research Facility (NIMRF) and later at an adjacent site on the mainland.	Growth trials require large numbers of juvenile oysters of the same size/age. However, spat collection and natural recruitment (see 6.3.1) did not provide sufficient oysters to establish a large multi-factorial trial as planned. A small trial was conducted at the NIMRF site using wild collected spat. High mortality of juvenile oysters held on suspended ropes ('chaplets' – used for pearl culture throughout the Pacific) resulted from fish predation. High mortality of adult oyster collected for half-pearl seeding (see 6.3.3) also occurred from fish predation at the NIMRF site. Culture apparatus that minimised predation (mesh cylinders enclosing the chaplets) were developed in response. A hatchery run with *Pteria penguin* was conducted at NIMRF in May 2015 to provide spat for growth trials. However, numbers of resulting spat were very low and another hatchery run was conducted in 2016. Growth trials were conducted with resulting spat at a second mainland site adjacent to Nago Island (a heavily fished site to reduce potential predation). But again, predation resulted primarily from gastropod predation (*Cymatium* spp.) was very high. It is likely that adequate control of predation would require considerable husbandry input that is currently unavailable to the project.

6.3.3	Production of half-pearls using wild-collected *Pteria penguin*	Collect 75-100 adult *Pteria penguin* from the wild Undertake small workshop for NFA mariculture staff to demonstrate half pearl seeding methods and to establish seeded stock Harvest half-pearls in a workshop environment with NFA staff to demonstrate harvesting methods, the tools used and principles of pearl and pearl shell handicrafts. Production of pearl and pearl shell jewellery items Marketing trial of pearl/shell jewellery	All milestones completed.	Eighty ear-hung adult *Pteria penguin* initially collected for pearl production were lost to fish predation. This delayed pearl seeding work and enclosed culture apparatus were developed to prevent subsequent oyster mortality (see 6.3.2). Fifty adult oysters were seeded Sept 2015. Seeding was done in 'workshop' style and involved NFA NIMRF staff. Seeded oyster were housed on the floating timber pontoon adjacent to the NIMRF which provides security but potentially suboptimal culture conditions. Pearls were harvested in June 2016 from the remaining 25 oysters. The pearls produced were of very high quality with excellent colour. A further seeding of 150 oysters occurred in 2016 and will be harvested in July 2017. Pearls and pearl shell pieces (MOP) resulting from the recent harvest were provided to the women's handicraft community at Nusalik. They were fashioned into jewellery and handicraft items as part of further handicraft skills training activities[11] in 2016. Resulting items were used in marketing trials in early 2017. Capacity related activities completed for the women's handicraft community at Nusalik over the past year include: (1) a follow-up design and handicraft training program in May 2016 (via Scope Global); and (2) in June 2016 De'arne Kershler provided updates and market/design training relating to consumer surveys conducted with cruise passengers visiting Milne Bay.

[11] *Associated with FIS/2014/060: 'Developing pearl industry-based livelihoods in the western Pacific'.*

6.3.4	Trial pearl production in Vietnam	Meet with stakeholders and determine site(s) for pearl production trial Hold workshop to demonstrate half-pearl production methods and begin culture trials Hold workshop to demonstrate half-pearl harvest and handicraft production methods Determine resulting pearl quality and conduct small marketing trial of pearl/shell jewellery	Most milestones completed: Oysters were successfully seeded for mabé production and resulting pearls were harvested in Dec/Jan 2017. Because of a delay in obtaining oysters and pearl seeding the planned 'handicraft production methods' workshop and 'marketing trial' of resulting pearls was not undertaken.	A visit to Research Institute for Aquaculture No. 3 in Nha Trang (RIA 3) took place in August 2015. It established Mr. Bay Phung as the counterpart scientist for this work. A program for trial half pearl production was developed following meetings with stakeholders, visits to potential culture sites and negotiation of logistic issues. Pearl seeding took place at RIA 3 in April 2016. A total of 110 oysters were seeded and transferred to a commercial oyster farm site at Nha Phu. Fouling and some initial mortality forced subsequent transfer to a deeper (7-8 m) nearby site two weeks later. Oysters were regularly checked and maintained by RIA 3 staff. A total of 71 pearls were harvested from 28 remaining oysters in December/January 2017. Initial mortality (~20% within 4 days of seeding) of seeded oysters indicates that improvements to oyster handling are required, and that the relatively shallow (2-3 m) oyster farm sites initially used for pearl culture was suboptimal for pearl oysters. Subsequent mortality (20% survival after 6 months) indicates suboptimal culture and husbandry conditions. Oyster survival declined from 54% to 12.5% in November/December as a result of rapid reduction in salinity (to ~ 17-20 ppt) caused by heavy rains. Six RIA 3 staff were trained in pearl seeding, drilling of seeded oysters and preparation of chaplets, and basic pearl oyster husbandry and maintenance.

PC = partner country, A = Australia

7 Key results and discussion

This project was successful in achieving the major goals of:

- supporting recovery and expansion of the Tongan pearl sector;
- providing improved understanding of the genetic makeup of Fijian *Pinctada margaritifera* to inform translocation policy; and
- assessing potential for mabé pearl production in PNG and Vietnam.

Key Results:

Improved hatchery production resulted from application of the outputs of small-scale experiments to large-scale hatchery culture methodology. Over the course of this project hatchery production increased from around 4,000 to >300,000 spat. This supported increased availability of oyster juveniles to farmers, allowing farm size to increase, and further expansion of the Tongan pearl sector through establishment of new farms.

A **novel larval food source** was successfully investigated for hatchery culture of *Pteria penguin*. Use of commercially available micro-algae concentrates was assessed experimentally and its use incorporated into Tongan hatchery culture protocol as a complete replacement for cultured live micro-algae. This development had huge significance in greatly simplifying and reducing the cost of hatchery culture and supporting improved hatchery output. On-site culture of live micro-algae as a larval food source is labour and resource demanding, is often beyond local technical capability and is a bottleneck to hatchery production in developing countries.

New pearl farms were established in Tonga's most populated island (Tongatapu) and its most remote major island group (Ha'apai), supporting sector expansion from its hub (Vava'u) to become a truly national activity. Four farms were established in Tongatapu and three farms in Ha'apai, and new farms were also established in Vava'u, increasing the number of farms from four, at the start of the project, to 17.

Genomics research in Fiji assessed the relatedness of Fijian populations of *P. margaritifera*. This information is important because pearl oyster spat collection has become a national activity, with potential for translocation of spat between distant parts of the country (collection site to farm). Using novel SNP and oceanographic dispersal models, results showed that there was very good mixing of *P. margaritifera* that could be considered a single stock. On this basis there are no major implications for translocation of oysters between pearl farming sites within Fiji.

Spat collection and field-based juvenile culture of pearl oysters in PNG was largely unsuccessful because of the influence of poor recruitment and predation. These results indicate that pearl culture may not be ideally suited to the Kavieng lagoon. Nevertheless, trials showed that **high-quality mabé pearls could be produced** in Kavieng indicating that, should culture problems (i.e. predation) be overcome, mabé pearl culture could become a viable livelihoods activity in the Kavieng area and provide a valuable raw material for the already established shell handicraft production sector in Kavieng.

Trials in Vietnam showed **successful hatchery production** of pearl oysters (*Pteria penguin*) at RIA3 and demonstrated production of **high-quality mabé pearls**. Problems encountered were primarily weather related (cyclone damage and heavy rainfall) and are easily managed. An established rock oyster culture sector in the area around Nha Trang, with experience oyster farmers, provides positivity regarding the potential to establish mabé pearl farming.

8 Impacts

Tonga: Increased hatchery production and improved knowledge of *Pteria penguin* husbandry has supported a more than quadrupling in the number of pearl farms in Tonga, and expansion of pearl farming to Tongatapu and Ha'apai to establish a truly 'national' pearl industry.

Completion of a Development Plan for the mabé pearl sector in Tonga provides a blueprint for industry development and prediction, based on current trajectory, of sector expansion to 29 farms by 2020, and production levels of ~7,000 mabé pearls with a value of ~TOP 850,000 by 2018.

Development and reporting of protocols for the hatchery use of commercially-available micro-algae concentrates in international journals has resulted in simplified hatchery procedures for the global aquaculture industry, particularly those focused on molluscs and other invertebrates.

PNG: Successful production of half-pearls has provided broader income generating opportunities for established handicraft producers and options for production of higher value handicraft items that are better tailored to the local tourist market.

Vietnam: Pearl oysters appear to be well-suited to co-culture with edible oysters within existing culture regimes. Half-pearl culture will provide broader product options and increased income generating opportunities for established oyster farmers.

8.1 Scientific impacts – now and in 5 years

The project has had scientific impacts in the following areas:

Pearl oyster culture: new information on the hatchery culture of *Pteria penguin* which will help simplify and improve culture methods and maximise production of juvenile oysters available to pearl farmers in Tonga; new information on field-based culture of juvenile and adult *P. penguin* which has helped develop improved culture methods and provide growth rate and other biological information for new culture sites (e.g. Tongatapu, Ha'apai, Kavieng); and development of hatchery protocols using commercially available micro-algae pastes to replace live micro-algae is likely to have broad benefits of significance beyond the pearl culture industry. Two journal publications have been produced describing the use of these products for hatchery culture of pearl oysters (see section 11).

Genetics: new information on the genetic structure of black-lip pearl oyster populations throughout Fiji provides a basis for developing industry guidelines and government policy regarding translocation of pearl oysters between culture sites in Fiji; new information on the relatedness of Pacific black-lip pearl oyster populations to more distant Indo-Pacific populations and the relationships between black-lip pearl oyster 'subspecies', and with other species in the Pacific. Four journal publications have resulted from this component of this project.

Postgraduate students: the project involved three postgraduate students; two MSc (Taoi and Adams, USP) and one PhD (Lal, JCU). Involving postgraduate students broadens the relevant outputs from the project, increases the project's contributions to current scientific knowledge and raise the profiles of both ACIAR's Fisheries Program and mariculture research in the region.

Publications: this project developed new methods and techniques and applied existing scientific and culture techniques in novel ways. It also involved postgraduate research students. Project research resulted in a number of publications in international journals, regional newsletters and bulletins and extension materials and manuals (see section 10).

There will be enduring scientific impacts (i.e. 5 years and beyond) from this project as a result of continued use and further development of culture methods developed in this project. The publications and other scientific outputs provide a basis for further international research, such as application of results and genetic analyses methods by global research groups. The post graduate students involved in this project will generate lasting legacy in the region. For example, the JAF student involved in this project (Dr. Monal Lal) is now based back in Fiji at the University of the South Pacific where he continues his research and supervises his own postgraduate research students from the region. Dr Lal

is employed on a new ACIAR project (FIS/2016/122) where he will apply the skills developed and learned during this project and improve capacity in these techniques within partner organisations in the Philippines and Vietnam.

8.2 Capacity impacts – now and in 5 years

Tonga:

- Direct impacts have been made in building capacity within the Tongan Fisheries Division with four new Aquaculture recruits having joined the in 2014 and 2015 and subsequently trained to an operational level in maintaining a productive and functional pearl oyster hatchery and nursery. In 2017 one very capable member of the Aquaculture staff was appointed as a dedicated "Pearl Industry Development Officer" and given responsibility for development oversight. In 2017 three Aquaculture staff members received scholarships to undertake formal studies (part-time) for their BSc (Marine Science at the USP-Tonga Campus)
- To date there have been nine hatchery runs completed by project staff and the output from the hatchery has shown steady and substantial improvement (refer to 2.1) as a result of advances in operational procedures and hatchery capacity.
- During project pearl farm capacity increased from just four operating pearl farms in 2013 to 17 in 2017. Even though the four newer farms are still building up their oyster stocks, the number of oysters being cultured has increased six-fold over this period. There are now 10 operational pearl farms in Vava'u, four in Tongatapu and three recently established community demonstration farms in Ha'apai. Likewise, nursery and broodstock holding capacity has also continued to increase with the deployment of two new Fisheries long-lines at Tongatapu and Ha'apai. There is now a total of five Fisheries oyster lines being utilized and maintained by trained Fisheries staff.
- General pearl farming practices were improved through regular workshops, extension, interaction and advice to farmers from project and Fisheries staff and external experts[12].
- The operational capacity of the peak pearl farming industry body has been significantly enhanced through new membership and greater production. It has been renamed as the Tonga National Pearl Farming Association (TNPFA) and is now registered as an incorporated society.
- The TNPFA has obtained the use of an old Fisheries building in Vava'u to be renovated and developed as a Pearl Information Centre and Workshop. This will provide a point of sale for pearl products and for developing a carver's/handicraft workshop with appropriate tools. The Centre will also function as a training centre and pearl 'museum' facility. This new facility will function as a focal point for industry activities (artisanal, retail and training) and should greatly elevate the TNPFA's capacity to work as a cohesive industry unit. It should substantially lift their market presence and help to standardize product quality and price. Although at an early stage of development this is a great example of industry stakeholders coming together to progress a common goal.
- In 2015 the first comprehensive production survey of all Tongan pearl farms was conducted. It provided detailed baseline data (on production, value, farm area, labor etc) against which future growth of the industry can be gauged. The survey is now an annual activity.

Fiji:

- This project has involved three Fijian post-graduate research students. They were guided by internationally recognized supervisory teams, have undertaken both laboratory and field-based research activities and learned state-of-the-art methodology in their respective fields.

[12] *Including training activities coordinated with those FIS/2014/060: 'Developing pearl industry-based livelihoods in the western Pacific'*

They have also been involved in the day-to-day running and aspects of bigger-picture planning within the project, and with liaison at government, industry and community levels. These experiences are important components in building scientific capacity within the region.

- Hatchery staff at Fiji's only commercial pearl oyster hatchery (J. Hunter Pearls Fiji) worked with project scientists to plan experiments with *P. margaritifera*. Hatchery staff have new knowledge regarding appropriate experimental design. These are valuable capacity impacts for the Fijian pearl industry.
- Pearl farmers and pearl farm staff worked with project scientists in data collection from the multi data logger and interpretation of these data. Given the relationship between water quality and oyster performance, this is a valuable capacity impact for the Fijian pearl industry.

PNG:

- Direct impacts have been made in building capacity within NFA and volunteer staff at the NIMRF relating to hatchery production, field-based aspects of pearl oyster culture, spat collection, maintenance of oysters and culture equipment and half-pearl seeding.
- Capacity impacts at community level have resulted from training in business operation and development, product design and marketing and small power tool operation and use, provided to the women's handicraft community at Nusalik Island. Consumer survey results will inform handicraft production and increase the capacity of artisans to better target their principal markets.

Vietnam:

- Six RIA 3 staff received hands-on training relating to half-pearl seeding, preparation and deployment of chaplets and husbandry of pearl oysters in the field. Three commercial edible oyster farmers received similar training relating to husbandry of pearl oysters.
- There will be enduring capacity impacts (i.e. 5 years and beyond) from this project as a result of new knowledge within partner organizations, industry groups, communities and other stakeholders that will provide a platform for further industry, method and livelihoods development. The major capacity impacts from this project that will be enduring include: increased general pearl production and farming capacity within Tonga Fisheries supporting industry expansion; increased knowledge, improved infrastructure and pearl farming capacity at farmer and community level supporting improved pearl production and income generation; improved organization within the Tongan pearl farmers Association supporting industry development and interaction with government; improved regional capacity for high-end genomics research at USP supporting new regional developments in this field; increased pearl farming capacity within NFA staff in PNG supporting development of this sector in PNG; improved community capacity relating to handicraft design, production and marketing supporting improved income generation. The post graduate students involved in this project will generate lasting capacity in the region. As detailed above, the JAF student involved in this project (Dr. Monal Lal) is now based at the University of the South Pacific (USP) in Fiji where he has brought new research capacity in the field of genomics. Dr. Lal is applying this capacity through supervision of USP research students and within a new ACIAR project (FIS/2016/122) where he will work closely with partner organizations in the Philippines and Vietnam.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

Many of the economic benefits resulting from this project are yet to be realised as there is obviously a time lag between research outputs (or the implementation of new production practices) and realisation of the benefits. In Tonga for example, hatchery production has steadily increased during the project from an initial hatchery run output of 4,000 spat in 2013 to a peak of 450,000 in January 2016, representing a 100-fold+ increase in the number of oysters potentially available to pearl farmers. Given that each additional oyster successfully used for pearl production generates around $90-$300 of income for pearl farmers, and provides additional raw materials for handicraft production, a 100-fold increase in hatchery production supports major potential economic benefits. However, hatchery cultured oysters require 1.5-2 years to reach pearl seeding size and a further 10-12 months for pearl production. Thus, it will be > 2 years before the economic benefits from increased hatchery production will be realised.

Economic benefits for pearl farmers in Tonga have been further advanced through strong links developed between the project and the Ministry of Commerce and Labour (MCL). This link was substantially strengthened when a Pearl Business and Marketing volunteer (NZ-VSA) commenced duties in June 2015. He was aligned to this project but is based within the Trade and Business Development group of MCL.

Essential information on the status of the pearl industry is now being assessed and documented through an annual Pearl Farming Survey, which commenced in 2015. This will provide the necessary data to better assess industry growth and economic impacts. In 2015, 3,510 mabé were sold for a total value (first point of sale) of $TOP 330,100. In 2016 this had increased to 6,000 mabé being sold for $TOP 564,000.

Some economic benefits in PNG have already been realised through improved business and handicraft skills which in turn have contributed to improved livelihood opportunities and increased income by some artisans at Nusalik Island. Consumer surveys will provide feedback that will allow local handicraft people to better target and supply the tourist market with their products.

8.3.2 Social impacts

This project aimed to provide direct benefits to communities in rural and economically depressed areas of partner countries through establishment or expansion of pearl farming activities resulting in employment opportunities, income generation, and associated health, educational and social benefits. Research outcomes have resulted in improved livelihood opportunities through associated activities such as handicraft production. The latter is likely to be of particular benefit to women and younger people. Development of community-based livelihood opportunities has been shown to reduce the migration of people (especially youth) to larger towns and cities, bringing social benefits to the communities involved and to the country as a whole. It is already evident in Tonga for example, that greater numbers of women and younger people are now involved in the pearl handicraft sector as a result of the sector expansion supported by this project. Social and socio-economic impacts resulting from pearl culture development in target countries will be studied and documented in detail as part of FIS/2014/060[13].

8.3.3 Environmental impacts

Pearl culture is an environmentally benign form of aquaculture. It is a form of suspended culture which minimises benthic impacts and it is usually conducted away from coral reefs to minimise predation of farmed oysters. Pearl farming often has positive environmental impacts including: (1) pearl oyster culture equipment may act as fish aggregating devices and can result in increased local availability of food fish; (2) hatchery-produced pearl oysters, if allowed to reach maturity, will breed

[13] FIS/2014/060: "Developing pearl industry-based livelihoods in the western Pacific"

and contribute to local stock; and (3) routine hatchery production of culture stock for pearl farmers is likely to reduce collecting pressure on local wild oysters.

8.4 Communication and dissemination activities

Target audience	Products	Processes
Target communities	Powerpoint presentations, DVD's, radio programs, technical information packs and manuals appropriate for different levels of the community level audience.	Training by local project staff, workshops, presentations in local language with technical backstopping, peer-to-peer learning and capacity development.
Local, provincial and national governments, policy makers	Technical information packs and manuals, policy briefs, fact sheets, best practice documents, website, DVD's	Face-to-face meetings and counterpart training, co-development of extension and training materials, capacity development.
Local NGOs	Information packs and extension materials, radio programs, market information, DVD's, website, social media	Training forums/workshops, meetings, face-to-face training with community groups.
International NGOs and regional bodies, importers and ethical trading organisations	Briefing documents, website, success stories, social media, handicraft products	Presentations, round tables, events, networks, learning forums
International science community	Peer-reviewed journal papers, formal oral presentations, website, social media	Journals, seminars, conferences, social media
ACIAR and other Donors	Project reports, success stories, impact briefs, website, social media	Presentations, round table, events

9 Conclusions and recommendations

9.1 Conclusions

This project was successful in consolidating and supporting significant expansion of the Tongan pearls sector. Improvements to hatchery methods allowed much improved hatchery production which, in turn, has supported significant expansion of the Tonga pearl industry. This is a key sector for the Tonga government with projections for industry growth targeting 29 farms by 2020, producing mabé pearls with a value in excess of TOP 1 million. This target is achievable with continued support. Pearls are one of few farmed commodities in Tonga with significant export potential.

In Fiji, genetic research showed that *P. margaritifera* populations make up the same stock and so there no potential genetic impacts associated with oyster translocation between sites within Fiji. This finding has significant policy implications because the Fijian government has invested in developing a national spat collection program that involves around 27 communities that collect wild oyster spat for sale to pearl farms. This program will provide a basis for planned round pearl industry expansion.

The project was also successful in examining the potential for mabé pearl production in both PNG and Vietnam. In PNG, oyster culture was impeded by poor recruitment of spat to spat collectors and high mortality of cultured oysters resulting from predation. While hatchery production can overcome the former, and was successfully undertaken during this project, management of predation will require innovative design and improved management of culture systems. Mabé pearls and pearl shell would be valuable additions to the raw materials used for handicraft production by local artisans in Kavieng. Demonstration of successful hatchery production of *Pteria penguin* in Vietnam, and subsequent culture of mabé pearls, provides a good basis for further development of pearl production as a potential mariculture and livelihoods-supporting activity. This is particularly worth considering given existing mollusc culture capacity at RIA3 and within the local oyster farming community.

This project was not successful in completing milestones relating to assessment of the potential of triploidy in pearl oyster culture and in assessing micro-algae concentrates as a food source for *P. margaitifera* larvae. This resulted from failure to identify an appropriate student to undertake triploidy research and lack of an available hatchery to support larval nutrition research. Both aspects have considerable potential benefit to pearl culture and should be pursued in future.

9.2 Recommendations

This project was reviewed in July 2017. Presentations from the review meeting, covering the major research outputs in partner countries, are presented in Appendix 1.

The research conducted in this project provided a basis for a subsequent ACIAR project, FIS/2016/126 (Half-pearl industry development in Tonga and Vietnam) that continues research in Tonga, supporting further development of the pearl industry, and in Vietnam where it seeks to develop potential for mabé pearl culture among existing edible oyster farmers.

10 References

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10.2 List of publications produced by project

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Lal, M.M., Southgate P.C., Jerry D.R., Bosserelle C., Zenger K.R (2017). Swept away: ocean currents and seascape features influence genetic structure across the 18,000 km Indo-Pacific distribution of a marine invertebrate, the black-lip pearl oyster *Pinctada margaritifera*. **BMC Biology** 18: 1-21.

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11 Appendixes

11.1 Appendix 1: Presentations made at the Project Review Meeting, July 2017, Suva, Fiji