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1 Acknowledgments

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2 Executive summary

Since 2013 Fiji's cultured pearl industry has been affected by poor condition and mortality of oysters in the main farming areas in Vanua Levu. Overcoming this bottleneck requires an understanding of the problem, before appropriate mitigation measures can be developed and applied (i.e. is it caused by disease or does it result from an environmental agent such as poor nutrition?). This project had two research elements. The first involved sampling to determine oyster condition and oyster tissue analyses (bacteriological, parasitological, histopathological) to diagnose clinical symptoms of 'disease'. It also included assessment of water quality (turbidity, chlorophyll *a*, micro-algae species compositions) at all major nursery and grow out sites used by the industry partner (J. Hunter Pearls). This sampling was overseen by the Mariculture Officer and aquatic animal health specialist of the Secretariat of the Pacific Community (SPC). The second research component of this project is growth trials with pearl oysters in Savusavu Bay, Vanua Levu, supported by water quality data loggers to determine longer-term relationships between oyster performance/health and water quality parameters.

Initial assessment of oyster tissues indicated that affected oysters were diagnosed by poor condition, watery consistency of soft tissues and digestive gland, empty digestive system, limited lipid content, degraded adductor muscle fibres, discoloration of mantle, discoloration of the inner shell and the presence of blisters in the mantle. Oysters at nursery sites were infected by the gram negative bacteria, *Vibrio parahaemolyticus*; an opportunistic pathogen that can cause serious infection. Oysters at the grow-out sites were also infected but to a lesser degree. Water quality analyses indicated appropriate turbidity levels at culture sites, but highly variable levels of chlorophyll *a* that were extremely low at the nursery sites indicating low levels of phytoplankton in the water column. Analysis of micro-algae populations showed medium to low levels of green micro-algae and normal levels of diatoms at grow-out sites, and very low levels of green algae and low levels of diatoms at nursery sites. It was concluded that limited food availability is at least a contributing factor to oysters being infected by a secondary/opportunistic pathogen. On the basis of these findings it was recommended that new nursery sites be identified, strict biosecurity protocols be established to minimize the transfer of pathogenic bacteria from nursery sites to other farming locations, and reduced biosecurity levels be introduced at the hatchery, prior to transfer of juveniles to the sea, to better acclimate them to common gram negative bacteria present in Savusavu Bay. Use of preventive salt baths for oysters, to decrease bacterial load and minimize transfer of pathogenic bacteria from nursery sites to grow-out locations, were also recommended and adopted.

Follow-up sampling four months later showed that oysters from all farming sites were much stronger, healthier and in better condition than those sampled previously and only a few showed limited clinical signs of infectious disease. This observation suggests that either food availability at nursery sites had improved or that the salt bath treatment and transfer of oysters earlier from the nursery to the grow-out sites had improved the general health status of the oysters, or both. The former hypothesis was supported by water quality data showing a 2.7-5.8 times increase in chlorophyll *a* levels across sites between the first and second sampling periods. A series of recommendations were made to improve oyster health status and biosecurity within pearl culture sites including: (1) regular assessment of chlorophyll *a* values and water quality parameters at each farming site in order to obtain baseline data; (2) regular assessment of micro-algae composition and quantity at different farming sites throughout the year to generate a better understand of the influence of available food on oyster growth, survival and health status; (3) regular recording of fitness values from different oyster groups and ages to obtain some baseline information that will allow rapid health assessment that is particularly relevant to oysters implanted for pearl production; (4) routine salt bath treatment of oysters prior to transfer between different farming sites; and

(5) antibiotic treatments carried out prior to and after implanting for pearl production. These practices are now routine at the partner pearl farm.

Following initial diagnosis of oyster disease symptoms and likely causative water quality factors, growth trials were established in Savusavu Bay with oysters of different ages, at different depths. Multi-parameter data loggers and a data buoy were used to log water quality information. Oyster growth rates were highest at a depth of 8 m probably as a result of higher levels of micro-algae (confirmed by significantly higher chlorophyll *a* levels) and higher water temperature at 8 m compared to 20 m. Growth rate was also highest in oysters with DVM of 70-80 mm and declined with increasing DVM. The shallowest depth tested (8 m) also recorded the highest rates of fouling and predators; however, this did not influence oyster mortality during the project because oysters were monitored and cleaned on a monthly basis. Highest rates of oyster mortality were recorded at a depth of 14 m. Poor oyster condition (diagnosed by the symptoms outlined above) was not observed in oysters in any of the size classes tested at any depth.

The results of this study have helped improve husbandry protocols for pearl culture in Fiji. They have provided a basis for new biosecurity protocols relating to transfer of oysters between sites and from the hatchery to the ocean, and a new protocol for treatment of oysters prior to nucleus implantation to improve survival and reduce nucleus rejection.

3 Introduction

Cultured pearls are the Pacific region's most valuable and highest priority aquaculture commodity (SPC Aquaculture Action Plan, 2007) and expansion and diversification of this industry are priorities for the Fiji government. The past decade has seen Fiji become a noted producer of cultured 'black' pearls with an export industry valued at around $6 million per annum. However, continued development of Fiji's cultured pearl industry has stalled because of poor condition and mortality of oysters at the main farming sites around Savusavu, Vanua Levu. This has resulted in a 60-70% decline in production over the past 3-4 years, farm closures, and loss of local jobs and livelihood activities in this economically depressed area of Fiji. Although mortality of cultured pearl oysters in atoll-based pearl farming environments in Polynesia has been linked to poor husbandry practices and environmental factors, these research findings have limited relevance to high-island environments like Fiji because of hydrographic and environmental differences.

Preliminary assessment of pearl oyster health by the Secretariat of the Pacific Community (SPC) in December 2013 collected oyster samples from all farming sites that were analysed for bacteriology, parasitology and histopathology. Water quality, chlorophyll *a* content and micro-algae composition of water from the main farming locations were also determined. Results obtained showed that: (1) most oyster samples from nursery sites were infected with *Vibrio parahaemolyticus*; (2) samples from Naloa were positive for *Perkinsus olseni*; (3) most of the samples from nursery sites showed signs of inflammation, infection and strong degradation of the adductor muscle fibres; (4) chlorophyll content was extremely variable among sites with nursery sites having much lower levels of chlorophyll than grow-out sites; and (5) micro-algae levels at the nursery sites were relatively low, with very low levels of diatoms. The main conclusion of this preliminary assessment was that oysters at the nursery sites were infected by an opportunistic pathogen (*Vibrio parahaemolyticus*) that caused serious infection probably due to low levels of nutrients and an imbalanced micro-algae diet at the nursery sites during certain periods of the year. Oysters at the grow-out sites were also infected, but recovering, probably due to an improvement in their feed supply.

The main aim of this project was to follow-up the preliminary study of SPC to determine the factor(s) causing loss of condition and mortality of black-lip pearl oysters (*Pinctada margaritifera*) in Fiji, and to attempt to determine relationships between biological factors (oyster age, growth rates, survival, health status and condition) and environmental factors (e.g. micro-algae availability and composition, water temperature, turbidity, culture depth) to identify key factors affecting oyster health.

4 Research activities, outputs and application

4.1 Research activities

The major research activities were:

- Bacteriology, histopathology and parasitology analysis of pearl oyster samples from all farming sites;
- Assessment of water quality, chlorophyll content and micro-algae composition at farming sites;
- Implementation, monitor and assess a new protocol for oyster treatment during nucleus implantation; and
- Establish experimental growth trials within Savusavu Bay.

4.2 Research Methods

4.2.1 Oyster health, bacteriology, histopathology and parasitology

Oyster samples: 10-15 oysters were collected form the following farming sites: (1) Matuku (spat collectors); (2) Wailevu 1 (nursery site); (3) Waileu 2 (nursery site); (4) Cousteau (grow-out site); (5) Nawi (grow-out site); and (6) Naloa (wild oysters collected at Naloa – outside Savusavu Bay, and later transferred to grow-out sites in Savusavu Bay).

All oysters were analysed as follows:

- Observation of general behaviour.
- Observation of gross clinic signs: outer shell, inner shell and internal organs.
- Collection of mantle, gills and adductor muscle for histopathology, parasitology and bacteriology.
- Samples for histopathology were fixed in 10% formalin.
- Samples for parasitology were fixed in 95% ethanol.
- A certain number of “healthy” and “relatively sick/unhealthy” oysters from each farming site were weighed to obtain baseline values of fitness values for wet and dry oysters. Average fitness values at different stages, for healthy and unhealthy oysters, are provided below.
- Samples for bacteriology were analyzed in place: culture in blood agar and TCBS agar, and subculture of relevant colonies for further identification.
- Identification of relevant bacterial colonies was carried out through basic biochemical tests: gram, potase reaction, catalase reaction, oxidase reaction, TCBS growth, motility, O/F glucose, sensitivity to the vibrio static 0-129 and Api 20N tests.

Samples were sent to the Ministry of Primary Industries New Zealand, animal health laboratory for histopathological analysis, and to the CSIRO Animal Health Laboratory in Australia for parasitological analysis.

4.2.2 Water quality analysis

Water samples were collected at farming depth (18 m in average) at the following farming sites: (1) Matuku; (2) Wailevu; (3) Cousteau; (4) Nawi; and (5) Raviravi (grow-out site). The following sampling methodology was followed:

- General water quality parameters were assessed using standard laboratory equipment.
- Chlorophyll *a* content was determined using a fluorimeter *Turner Aquaflor* calibrated (in µg/L).

- Samples for large species of micro-algae (>35 µm) were collected using a plankton net.
- Samples for small species of micro-algae (<35 µm) were collected in 1.5 L plastic bottles. The bottles were left to settle for 48 hours and the sediments were fixed with 5% formalin.

Samples to be analysed for micro-algae composition were sent to the University of Perpignan, France, for analysis.

4.2.3 Oyster treatment during nucleus implantation

The following treatments were carried out with healthy oysters ready to be implanted:

- Antibiotic (oxytetracycline, 20 ppm) bath + salt bath 2 days after implant/antibiotic bath same day of implant/antibiotic bath 2 days after implant
- Antibiotic bath + salt bath same day of implant/antibiotic bath 2 days after implant
- Salt bath same day of implant/antibiotic bath 2 days after implant
- Only antibiotic bath at the day of implant
- Control group with no treatment

All oysters accepted the treatments and no mortality was observed during the procedures. It was confirmed that implementing the antibiotic bath and the salt bath in the sea was relatively easy.

4.2.4 Oyster growth trials in Savusavu Bay

Growth trials were established in Savusavu Bay with oysters of different ages, at different depths and at different sites for a one year period. They involved ACIAR/USP Scholarship Master's student John Carreon. Oysters were held in panel nets suspended from a longline (Southgate, 2008) at three depths (8, 14 and 20 m). They were checked monthly for survival and condition and measured to determine changes in dorso-ventral and antero-posterior shell measurements (DVM and APM, respectively). Fouling was cleaned from oysters on a monthly basis and composition of fouling was noted. Multi-parameter data loggers and a data buoy (deployed as part of FIS/2009/057[1]) were deployed to Savusavu Bay to log water quality information.

4.3 Research outputs

4.3.1 Oyster health, bacteriology, histopathology and parasitology

Oysters from Matuku, Cousteau and Naloa showed no clinical sings of disease: oysters were strong and healthy, with a strong adductor muscle. No watery tissue content was observed and lipid content in internal tissue was relatively high. There was no discoloration in the inner shell.

Three oysters from Wailevu 1/2, and two oysters from Nawi showed clinical signs of bacterial infection: high watery content, low lipid content, weakness of adductor muscle/degradation of muscle fibres, discoloration of inner shell, and discoloration and blisters in mantle tissues.

[1] *FIS/2009/057: "Pearl industry development in the western Pacific"*

Blacklip pearl oysters (*Pinctada margaritifera*) showing clinical signs of disease. Both specimens show discolouration of inner shell surface.

Bacteriology:

Oysters from Matuku, Cousteau and Naloa were all negative in blood agar and in TCBS agar. Oysters from Wailevu 1 and 2 were positive in blood agar and TCBS agar.

Wailevu 1 and 2: There was only one bacteria of relevance found in two individuals. These bacteria were identified to the species level and tested for antibiotic sensitivity.

Identification results:

- Gram negative; oxidase positive; potase positive; TCBS positive; 0-129 positive; salinity growth positive; O/F glucose positive; motility positive.
- Api 20E results, code: 7146125, identified as *Vibrio parahaemolyticus*.

Antibiotic sensitivity tests results: Amoxicillin: 0 cm; Flumequine: 2 cm; Erythromycin: 1.5 cm; Oxytetracycline: 2.4 cm; Tetracycline: 1 cm; Chloramphenicol: 1.3 cm

One specimen from Nawi was also positive for bacterial growth in blood agar and TCBS agar. One bacterial colony was identified and antibiotic sensitivity tests were conducted.

Identification results:

- Gram negative; oxidase positive; potase positive; TCBS positive; 0-129 positive; salinity growth positive; O/F glucose positive; motility positive.
- Api 20E results, code: 3100105, identified as *Vibrio vulnificus*.

Antibiotic sensitivity tests results: Amoxicillin: 0 cm; Flumequine: 2.5 cm; Erythromycin: 0 cm; Oxytetracycline: 2.3 cm; Tetracycline: 1.5 cm; Chloramphenicol: 1.4 cm.

Ten oysters from Nawi that were treated with antibiotics (oxytetracycline 20 ppm) prior implanting were also tested for bacteriology after the treatment, to assess the efficacy of the treatment. There was no positive growth in any of them.

Fitness values:

Dry and wet weight values of 14 specimens from grow-out sites were obtained, in order to assess common fitness values/ration shell:flesh for this specific growth stage. The ratio shell:flesh will help technicians to assess whether a certain specimen is healthy and strong enough to be implanted for pearl production or not. The average fitness value for wet healthy oysters was < 4.5 (ø = 4); while it was much higher for unhealthy specimens, >4.5 (ø = 5.1). Detailed information is provided in Appendix 1.

Parasitology:

All samples were negative for *Perkinsus olseni.*

In general, all specimens (from all farming sites) were much stronger, healthier and had improved condition than the specimens sampled during the baseline study in December 2013. Just a few oysters showed limited clinic signs of infectious disease (e.g., watery content, low lipid content, degradation of adductor muscle, discoloration of mantle, discoloration of inner shell, presence of blisters in mantle, etc). This observation indicates that either the nutritional problem/food availability constraint at nursery sites has been resolved or has improved or that the salt bath treatment, introduced following the baseline study in December 2013, and transfer of shells earlier from the nursery to the grow-out sites is improving the general health status of the oysters.

Micro-algae composition:

Chlorophyll *a* levels were highly variable and extremely low at the nursery sites indicating low levels of phytoplankton in the water column. The analysis of micro-algae populations at culture sites showed medium/low level of green micro-algae (Chlorophyceae and Prasinophyceae) and normal level of diatoms (Bacillariophyceae) at grow-out sites, but very low levels of green algae and low level of diatoms at nursery sites. It was concluded that limited food availability is at least a contributing factor to oysters being infected by a secondary/opportunistic pathogen.

Oyster growth trial:

Oyster growth rates were highest at a depth of 8 m probably as a result of higher levels of micro-algae (confirmed by significantly higher chlorophyll *a* levels) and higher water temperature at 8 m compared to 20 m. Growth rate was also highest in oysters with DVM of 70-80 mm and reduced with increasing DVM. The shallower depth tested (8 m) also recorded the highest rates of fouling and predators; however this did not influence oyster mortality during the project because oysters were monitored and cleaned on a monthly basis. Highest rates of oyster mortality were recorded at a depth of 14 m. Oysters showing clinical signs of poor condition (e.g., watery content, degradation of adductor muscle, discoloration of mantle, discoloration of inner shell, presence of blisters in mantle, etc., see above) were not seen in any of the size cohorts tested at any depth during the growth trials.

5 Major Outputs

- Results showed improvement in the health and condition of pearl oysters compared to prior assessment in December 2013. This supports the hypothesis that 'disease' was caused by opportunistic *Vibrio* spp. infection of oysters suffering nutritional stress, and that subsequent improvements in oyster health probably resulted from improved availability or composition of local micro-algae.

- Oysters from all grow-out sites apart Nawi were healthy, strong and free of bacterial infection. Some oysters from Nawi (grow-out) and Wailevu were still infected by *Vibrio* spp. indicating that some of the weaker oysters have yet recovered from the nutritional problem faced in 2013.

- Application of a salt and antibiotic bath prior to nucleus implantation was shown to be useful and simple to implement. Ongoing assessment will confirm if treated oysters have higher survival and improved nucleus retention and pearl quality than non-treated oysters.

- A series of recommendations were made to improve oyster health status and biosecurity within pearl culture sites including: (1) regular assessment of chlorophyll *a* values and water quality parameters at each farming site in order to obtain baseline data; (2) regular assessment of micro-algae composition and quantity at different farming sites throughout the year to generate a better understand of the influence of available food on oyster growth, survival and health status; (3) regular recording of fitness values from different oyster groups and ages to obtain some baseline information that will allow rapid health assessment that is particularly relevant to oysters implanted for pearl production; (4) routine salt bath treatment of oysters prior to transfer between different farming sites; and (5) antibiotic treatments carried out prior to and after implanting for pearl production. These practices are now routine at the partner pearl farm

- Oysters grew more rapidly at a depth of 8 m (compared to lower depths) and this correlates with higher water temperature and increased micro-algae abundance (chlorophyll *a*). This contrast with the usual pearl farming depth in Savusavu Bay of ~18 m and adjustment of culture depth may help mitigate nutritional stress in oysters. Disadvantages of culture at shallower depths include increased fouling and presence of predators which would require increased husbandry input to control.

6 Conclusions and recommendations

6.1 Conclusions

- The results of this study strongly suggest that nutritive stress and secondary infection of oysters by opportunistic bacteria (*Vibrio* spp.) is the major cause of poor condition of pearl oysters (*Pinctada margaritifera*) in Savusavu Bay, Fiji.
- Changes in the abundance and composition of micro-algae are likely to be the major cause of this condition and increased knowledge of these changes, and the conditions that cause them, are required as a basis for mitigation measures.
- Although the timing of this study did not align with an outbreak of oyster 'disease' in Savusavu, it provided an opportunity to generate baseline information on oyster condition, water quality parameters and oyster husbandry that will be helpful in further developing health diagnostic and husbandry measures for *P. margaritifera*.
- This study also provided the opportunity for identification and implementation of basic biosecurity measures that will bring increased sustainability and resilience to the Fiji pearl industry. A series of recommendations were made to improve oyster health status and biosecurity within the partner pearl farm and these practices are now routine.

6.2 Recommendations

- Unfortunately, the relatively short duration of this study did not align with a period of oyster 'disease' in Savusavu Bay and so new information on the potential causes of this condition was limited. However, the growth trial and water samples indicated that micro-algae availability and composition (i.e. nutritive stress) is a possible causative factor. It is recommended that on-going, routine assessment of chlorophyll *a* and micro-algae quantity/composition be carried over the longer term at pearl farms throughout Fiji, in order to better understand the effects of changes in micro-algae composition/availability on oyster health. Such information would assist with farm decision making such as site selection and stocking density.
- Treatment of oysters in an antibiotic bath (chloramphenicol at 10-15 mg/L) for 30 minutes at least three times before and following nucleus implantation is recommended as a routine biosecurity measure.
- On the basis of our findings it is recommended that new nursery sites be identified and that strict biosecurity protocols be established to minimize the transfer of pathogenic bacteria from nursery sites to other farming locations, and reduced biosecurity levels be introduced at the hatchery, prior to transfer of juveniles to the sea, to better acclimate them to common gram-negative bacteria present in Savusavu Bay.
- Use of preventive salt baths for oysters, to decrease bacterial load and minimize transfer of pathogenic bacteria from nursery sites to grow-out locations, were also recommended and adopted.
- Finally, it should be noted that poor oyster condition resulting in an increased proportion of oyster that are unsuitable for nucleus implantation, has again become an issue for Fijian pearl farms in early 2017. The cause(s) of this reoccurrence is unknown although 'climate change' has been implicated (Appendix 2). Further research is required to assess this and the influence of other anthropogenic influences on pearl farming in Savusavu Bay.

7 References

7.1 References cited in report

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8 Appendixes

8.1 Appendix 1: Fitness values for healthy and sick pearl oysters

	WET WEIGHT - HEALTHY OYSTERS				DRY WEIGHT - HEALTHY OYSTERS		
	FLESH	SHELL	RATIO shell:flesh		FLESH	SHELL	RATIO shell:flesh
	35.4	135.2	3.8		46.4	132	2.8
	43	193.1	4.5		46.4	192	4.1
	44	177.4	4.0		46.4	176	3.8
	50.5	172.3	3.4		35.7	171	4.8
	40	165.8	4.1		35.7	162	4.5
	47.7	177.2	3.7		35.7	173	4.8
	28.1	102.8	3.7		35.7	100	2.8
	46.8	184.6	3.9		41.2	183	4.4
	47.8	222	4.6		41.2	220	5.3
	37.1	164	4.4		41.2	162	3.9
	44	204.5	4.6		33.8	201	5.9
	56.2	195.6	3.5		33.8	193	5.7
	67.9	233.6	3.4		33.8	231	6.8
	44.2	178.9	4.0		33.8	176	5.2
AVERAGE	**45.2**	**179.1**	**4.0**	**AVERAGE**	**38.6**	**176.6**	**4.6**

	WET WEIGHT - SICK OYSTERS		
	FLESH	SHELL	RATION Shell:flesh
	33.3	167.9	5.0
	42.1	171.3	4.1
	40.4	177.6	4.4
	33.2	184.7	5.6
	37.2	198.7	5.3
	28	167.9	6.0
	41	155.9	3.8
	36	160.8	4.5
	31	175.8	5.7
	44.1	299	6.8
	36	169.7	4.7
AVERAGE	**36.6**	**184.5**	**5.1**

8.2 Appendix 2: Recent article from the Fiji Times

The Fiji Times ONLINE

Study to determine pearl deaths

Luke Rawalai
Wednesday, May 24, 2017

MAJOR pearl producer J Hunter Pearls is conducting further tests to determine whether deaths of pearls in waters around the Savusavu area were a result of climate change.

The company's nursery manager, Rynae Lanyon, said earlier tests done where they were producing pearls in Savusavu confirmed the pearl deaths did not result from poisoning or the contents of the water in the area.

Ms Lanyon said the owner of the company, Justin Hunter, continued to invest more on research to determine the cause of these pearl deaths.

Responding to questions whether deaths were in any away related to climate change, Ms Lanyon said chances were high.

"However, we have to confirm this yet and it is still early to say whether climate change has a direct effect on these pearl deaths," she said.

"Mr Hunter is implementing studies on the food cycle of the oysters. Initial tests to consider whether the deaths were related to poisoning or water content had been negative."

Ms Lanyon said they were working closely with the Department of Fisheries in the North to carry out research.