

Final report for Mini-project MS0401:

**Confirmatory testing of the viral status of
Penaeus monodon (Black Tiger shrimp)
populations in the Fiji Islands**



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Executive summary

As a growing multi-billion dollar industry, the world-wide shrimp industry has encountered shrimp diseases, especially viral outbreaks, as one of the major stumbling blocks to its progress. In the South Pacific, shrimp industries are currently operating in New Caledonia, French Polynesia, Solomon Islands, Vanuatu and Fiji (*Penaeus stylirostris*). In Fiji alone there is a big and growing demand. The high potential of *Penaeus monodon* or Black Tiger shrimp, as a major source of revenue and its natural presence in Fiji waters has led to the initiation of this study. The objective of this study is to explore the possibilities of culturing *P. monodon* in Fiji by first determining the viral disease status of local *P. monodon* wild broodstock.

P. monodon shrimp samples were brought from Fiji to the CSIRO Aquaculture Health Laboratory, Live-stock Industries, in Brisbane, Australia for analysis, as part of the Sustainable aquaculture development in the Pacific region and northern Australia project and funded by the Australian Centre for International Agricultural Research (ACIAR). A total of 247 shrimp (from five different sites within Fiji) were screened for eight different shrimp viruses namely, Hepatopancreatic Parvovirus (HPV), Monodonbaculovirus (MBV), Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), White Spot Syndrome Virus (WSSV), Mourilyan Virus (MoV), Taura Syndrome Virus (TSV), Gill Associated Virus (GAV) and Yellow Head Virus (YHV). All samples were tested in pools or groups of 10.

Polymerase Chain Reaction (PCR) and Histology were the two diagnostic methods used. The PCR method was further divided into three different formats Conventional, Real Time and Real Time in Conventional. The Fiji shrimp viral DNA was also sequenced to determine the presence of any unique viral DNA strains. The PCR results of the study revealed HPV, MBV, IHHNV, MoV and GAV to be present in all samples except for MBV absent in one of the five sites. Haemotoxylin and Eosin (H & E) stains and *In situ* hybridisation of selected samples that were positive for certain viruses, also confirmed the PCR results. Of the four viral DNA sequences studied (HPV, MBV, MoV and MBV) HPV from Fiji showed a significant 20% difference to the Australian HPV strain. Fiji GAV and MoV were 1% to 2% different, when also compared to the Australian strain. Fiji MBV however, was 5% different to an Indian strain.

This study has shown that although Fiji harbours Penaeid shrimp viruses, it **does not** however harbour shrimp disease. Lightner (1988) states that a shrimp disease is evident in the presence of three components: the host (shrimp), the agent (virus) and an environment that would favour the development of a disease (for e.g high pond stocking densities). The last component is not present in Fiji, however studies have shown that it is still possible to farm shrimp and produce a harvest with the presence of viruses (Flegel *et al*, 2004; Tsai *et al*, 1999). Therefore a disease outbreak can be avoided if the environment that favours it is avoided through the establishment and the sustainability of optimum hatchery and pond conditions.

The DNA viral sequencing implies that Fiji GAV and MoV may have originated from Australia. On the other hand, the HPV and MBV sequence suggests that they could have been in Fiji shrimp all along and not introduced or that they may have been acquired from another local Penaeid species. Further research would need to be carried out to confirm the origins of these viruses.

The implications of this study are positive for Fiji's potential shrimp industry, capacity- building for the University of the South Pacific and the South Pacific region as a whole. This study has been presented in two local workshops and has also been used by CSIRO, Brisbane to study Penaeid genetic markers and various strains of shrimp viruses.

Introduction

Economic significance and demand

The growing shrimp industry is a multi-billion dollar industry world-wide, with China being the number one producer of farmed shrimp. World production of farmed shrimp was about 1.6 million metric tons in 2002. The industry has been growing at the rate of 10% to 20% a year over the last few years. Projections are for it to continue growing at the rate of 12% to 15% a year (Chamberlain, 2003). Despite shrimp being a luxury food, demand for it continues to grow. Between 1998-2002, the estimated annual shrimp consumption (in metric tons) in leading shrimp consuming countries are: USA (500,000), Japan (245,000), Spain (120,000), United Kingdom (80,000), France (65,000) and Germany (25,000) (Filose, 2003).

There is also demand locally, with about 600T of shrimp per year for Fiji's hotels and restaurants, of which 400T is imported, 150T comes from the wild fishery and up to 50T is farmed (Naisua, 1999). The Black tiger shrimp is found naturally in Fiji waters and at prices of around \$30/kg, this large volume of shrimp imports represents a significant fiscal leakage or hard currency for Fiji.

Compared to other shrimp species *Penaeus monodon* is the most highly favoured shrimp species for aquaculture because of its fast growth rate, large size at maturity and high meat quality and quantity (Bailey-Brock and Moss, 1992).

Disease threats to the shrimp industry

Shrimp diseases have become major stumbling blocks to leading shrimp producers such as Asia. These industries have been hit hard especially by viral outbreaks. The most renowned shrimp viral-disease disaster struck Taiwan's *Penaeus monodon* industry in 1988, with an outbreak of White Spot Syndrome Virus. As a result there was a drastic drop in production by 70% and export declining to 80%. Approximately US\$376 million was lost. Following the discovery of the Taura Syndrome Virus, Ecuador lost US\$763 million in 1999 (Kautsky, 2000).

At present, there are no known captive viral-free (specific pathogen free or SPF) populations of *Penaeus monodon*. Escape of viral infected shrimp from farms and hatcheries has led to high viral infection rates of wild (ocean) *P. monodon*. It is now nearly impossible to find viral-free *P. monodon* wild shrimp where *P. monodon* are farmed. Without such culture and domestication (isolation) of viral-free *P. monodon* species, it is almost certain that this species will decline further worldwide in favour of *P. vannamei* and or other SPF species (Arlo fast, 2004, pers. comm.).

Thailand has long been the leading world producer of farmed-reared shrimp. Until recently, more than 90% of all farmed shrimp in Thailand were *P. monodon* which was based on ocean-caught broodstock. The escapement of farmed viral-infected shrimp has led to the high viral-infection rates of wild *P. monodon* broodstock. This trend has led to the use of SPF *P. vannamei* in Thailand. During 2003, *P. vannamei* accounted for about 80% of all farmed shrimp production, while *P. monodon* accounted for less than 20% (Arlo fast, 2004, pers. comm.).

The majority of current shrimp aquaculture research is now dedicated to finding, domesticating, rearing and maintaining viral-free shrimp populations in biosecure hatcheries and farms. As stated before, there are still no known viral-free brood stocks of *Penaeus monodon*. The discovery of such a population will be highly valuable.

Current shrimp disease diagnostic methods

Infectious shrimp diseases (caused by living micro-organisms) include viruses, bacteria, fungi and protozoa. Among the infectious diseases of cultured shrimp, certain virus-caused diseases stand out as the most significant. All other infectious diseases are known to be curable and can be treated immediately. Viruses on the other hand, do not have cures and as previously outlined have caused major economic threats and progress to many leading shrimp producers around the world. As a result over the years, many diagnostic methods have been designed to help recognise shrimp disease especially viral infections.

The earliest diagnostic methods developed for these pathogens included the traditional methods of morphological pathology (direct light microscopy, histopathology, and electron microscopy) with enhancement and bioassay methods. Molecular methods, such as the Polymerase Chain Reaction (PCR), have more recently been developed. PCR involves the use of different short synthetic oligonucleotides probes and complementary sequences in a complex mixture of nucleic acids to DNA synthesis using a thermostable DNA polymerase. With multiple cycles of target DNA denaturation, annealing and extension the result is a massive amplification of the target sequence located between the two primers (Cann, 1997). Primers to target viral pathogens such as White Spot Syndrome Virus (WSSV), Monodonbaculovirus (MBV), Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV), Yellow Head Virus (YHV) or Gill Associated Virus (GAV), Taura Syndrome Virus (TSV) and others have been produced and are routinely used in pathological laboratories. Viral DNA sequencing has also determined various strains of such viruses, making it possible to design PCR tests that can detect more than one strain of the same virus. Many studies have been carried out on various Penaeid shrimp viruses and strains (Brun (1991), Brock (1992) and Spann (1995) in Spann *et al*, 1997).

Histology is another diagnostic tool and is considered a classical method, Haemotoxylin and Eosin (H&E) staining is one such histological method. Routine H&E staining, whereby thin tissue sections mounted on microscope slides are dipped into various dyes and stains and later observed microscopically for viral bodies, such as inclusions or occlusions. *In situ* hybridization is another histological method that utilizes digoxigenin (DIG)-labeled gene probes and other stains to highlight specifically targeted viral tissue.

The University of the South Pacific (USP) currently has two labs with the necessary equipment (such as thermocyclers) to carry out PCR tests. However, there are no proper facilities to carry out histological studies and DNA sequencing. Since 2003, the Institute of Marine Resources (IMR) has been carrying out routine shrimp viral tests on local and imported *P.monodon*, with the use of test kits from Taiwan (Magellan Biotechnology). Recently, tests have also been carried out on imports of *Litopenaeus stylirostris* larvae from Brunei.

Shrimp aquaculture in the Pacific

Successful shrimp industries are currently operating in New Caledonia and French Polynesia. Solomon Islands had two successful farms prior to its political crisis and one has now relocated to Vanuatu. Shrimp farming in Fiji is very recent and begun just over twenty years ago.

Shrimp aquaculture in Fiji

Penaeid shrimp are distributed widely in the coastal waters of Fiji and represent a very important species for aquaculture in the region. Known species of *Penaeus* in Fiji are *P. merguensis*, *P. japonicus* and *P. monodon* (Forbes, 2002). Choy (1988) also reported the fishery and biology of *Penaeus canaliculatus* in Laucala Bay - a subsistence fishery very popular amongst the residents of urban Suva.

Shrimp farming

Shrimp farming began as a commercial project and utilized otherwise unused coastal land. The joint effort by the Government and the Food and Agricultural Organisation of the United Nations resulted in the first established shrimp farm in Raviravi, Ba. With the establishment of other shrimp farms, including a Government hatchery, shrimp disease soon became a problem due to the importation

of diseased post-larvae (PL) stock. As a result of this and additional financial management problems, some local shrimp farms collapsed (Naisua, 1999). In 2002, there were four shrimp farms, of which only one was operating - Pacific Prawns Limited (Navua). Importing Australian post-larvae, this farm ceased operations after a study confirmed that most Australian West-coast shrimp hatcheries had larvae that tested positive for Yellow Head and Gill Associated Viruses (Spann et al 1997). Gulf Seafood Fiji Limited is currently the largest operating shrimp farm in Fiji. Recently, this farm imported SPF *P. stylirostris* post-larvae because there are no known viral-free *Penaeus monodon* stocks. For quarantine measures, samples of these shrimp were screened for viral-diseases before and after they arrived in Fiji, at the University of the South Pacific (USP) DNA laboratory, and were found to be negative (Forbes, 2004, pers. comm.). The Raviravi shrimp farm is still under development with its takeover by Taiwanese owners, following its collapse in 2000 (mainly due to the lack of funding). Another farm is currently being developed in Dreketi (Vanua Levu) by Asian investors. There are currently no operational *P.monodon* farms in Fiji.

P. monodon hatcheries

In 2001 the USP (Marine Studies Programme) established a hatchery, but ceased operations in mid-2004 following consecutive years of very low PL survival. The Fiji Government hatchery in Galoa is currently the only known operating hatchery. Despite their continuing efforts, there has not been enough production for a commercial basis, mostly because there is still little information on the availability, seasonality and reproductive traits of shrimp broodstock from various locations around Fiji. However, no matter how fecund shrimp broodstock are, they are of no commercial value unless they come from an SPF population.

As yet there have been no studies carried out on viral-disease presence for Fiji *P. monodon* shrimp. Owing to a history of post-larval importations to Fiji from Australia and elsewhere, it is important to now determine and assess the viral-disease status of naturally-occurring Fiji *P. monodon*. Even if some parts of the Fiji group have viral-disease present, there is a high possibility of the presence of some viral-free shrimp populations due to the isolation and deep water separating many island groups in Fiji. This may create ideal biosecure sites.

This study aims to determine where there are viral-free *P. monodon* natural populations in Fiji, which are worth protecting and investing in for aquaculture. The hypothesis is that there are virus-free natural populations of *P. monodon* in Fiji that have high aquaculture value. This is the first report on the viral-disease status of local Fiji *P. monodon* stocks.

Materials and Methods

Sampling

Shrimp samples for analysis were collected from five selected sites from around the Fiji group, two off the mainland; Kadavu and Vanua Levu, and three on the mainland, Nadi, Tailevu and Navua (Fig. 1). Each of these sites had been recommended by the Government Fisheries Department as potential sources of *P. monodon* broodstock. Fifty shrimp were taken from all sites, except for Kadavu where only 47 were captured.



Figure 1. The five selected sites for *P. monodon* sample collection. Kadavu-1, Vanua Levu-2, Nadi-3, Tailevu-4 and Navua-5.

Preparation of samples for diagnostics

All samples were dissected and the necessary tissues were preserved for molecular and histological examination. Tissues used for molecular diagnostics were gills (2-3 pieces), lymphoid organ and hepatopancreas (2-4 pieces) and were preserved in 95% lab grade ethanol.

In addition to determining the presence or absence of shrimp viruses, this study had also originally aimed to determine the virus prevalence if there was viral presence. Therefore the fifty or so samples collected from each site were to be tested firstly in groups or pools of five (i.e. ten groups of five animals each). A positive result for a group would lead to individual tests for each of the five animals. In this way, time, energy and resources would be saved through not having to test every animal. However, time constraints meant the prevalence study could not be carried out. Results

presented in this study pertain to group samples, not individual shrimp.

Diagnostic techniques

Two methods of diagnostics were carried out in this study, molecular techniques and histology.

(a) Molecular techniques

(i) Polymerase chain reaction (PCR)

DNA and RNA (nucleic acid) extraction was carried out on the three different tissue types (pooled), with the use of the Magellan Pathotech test kits. This extracted nucleic acid would be used for testing by the Polymerase Chain Reaction (PCR) method. The PCR method was the principle molecular technique used for testing for various shrimp viruses. Various sets of primers produced by CSIRO, the Office International des Epizooties (OIE) and the Magellan test kit were used. The use of appropriate primers was critical to these tests. Primers target the viral DNA, which is then amplified to a detectable amount during analysis. There were two types of primer sets used: conventional and Real Time. The first detects the presence or absence of a virus (qualitative) whilst the second detects the virus and quantifies the viral load in the presence of a virus (qualitative and quantitative). Conventional primers were used initially for each sample whereas Real Time primers were only used if the conventional primers failed to detect any virus.

The following viruses were tested for:

- Hepatopancreatic Parvovirus (HPV)
- Monodonbaculovirus (MBV)
- Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)
- White Spot Syndrome Virus (WSSV)
- Mourilyan Virus (MoV)
- Taura Syndrome Virus (TSV)
- Gill Associated Virus (GAV)/ Yellow Head Virus (YHV)

Appropriate concentrations and volumes of reagents were made up to a volume of 25 µl per reaction (per test) in eppendorf tubes. These were then placed in a 96-well BIO RAD *i Cycler* (thermocycler) to undergo PCR under the appropriate cycling conditions for viral DNA amplification. Af-

ter completion, 8µl of each sample (including positive and negative controls) and 5 µl of the DNA ladder were then loaded into a 2% agarose gel for electrophoresis, at 180V for 30 minutes. When viewed under ultra violet light, the gel highlights various bands that may either be indicative of the presence or absence of a virus. Samples that produced "bands" in the same location as the positive control were considered positive, whereas those that did not have bands coinciding with the positive control were considered negative. Different targets or viruses were of different sizes and appeared on different regions of the gel. The size of each target was different and measured in base pairs (bp).

A Real Time PCR was carried out for IHHNV and Ct values were generated. Such values greater than 24 were indicative of a positive result.

The GAV/YHV PCR test was a differential one, in which two bands of different sizes appear in the positive control lane, if both viruses are present.

The gel photos were generated by Quantity One 4.1.0 and a Gel Doc 2000 BIORAD transilluminator.

(iii) DNA sequencing: An analytical tool to studying virus strains and possible origins

Viral presence was further confirmed by viral DNA sequencing. Sequencing also allowed us to determine the possible origins of these viruses by comparing the Fiji viral DNA sequences to other known viral DNA sequences. Two to three Fiji samples that showed very strong positive results for the certain virus tested were selected for DNA sequencing. PCR was carried out on these samples to amplify the target or viral fragment, purified and then sent to the Australian Genome Research Facility (AGRF) for sequencing. Results were received via the CSIRO network and analysed with other known sequences, using *Sequence Editor Version 1.0.3 (SeqEd)* and *MacVector 7*. Due to their larger size, viral DNA that was sequenced were HPV, MBV, GAV and MoV. The remaining viruses (or DNA fragments from these viruses) were too small to observe any changes in the sequence and were therefore not analysed.

The Fiji viral sequences were also compared to other known strains of viruses, through BLAST analysis of nucleotides in the NCBI website (www.ncbi.nlm.nih.gov/BLAST/Blast.cgi).

However a thorough analysis of the comparison of these sequences with the Fiji samples was not carried out, since it was beyond the scope of this study.

**(b) Histological techniques
(confirmatory diagnostic tool)**

Histology is a less sensitive method than PCR and involves the observation of viral bodies in specific tissue sections on stained slides. This method requires a high viral load to detect the presence of a viral body or a positive signal (Jeff Cowley, 2004 pers. comm.). The following histological techniques were only done on the samples that had showed up very strong positive bands during the PCR tests (indicative of high viral loads), which were HPV and MoV. It was decided that it would be irrelevant to do this for all the sites that had such PCR results, but rather on one representative site, Nadi.

(i) Routine Haemotoxylin and Eosin (H & E) staining

Shrimp samples that were PCR positive (viral presence) had complementary microscope slides stained with Haemotoxylin and Eosin (*H & E*), a routine stain used in most histology labs for observing viral body presence. The slides were then observed with the help of a manual on histopathology and shrimp diagnostic procedures (Bell et al, 1988; Lightner, 1996). Photos of slides were taken and edited using *Axio Vision 3.1* and a Zeiss camera.

(ii) In-situ hybridization

In-situ hybridization was another histological tool used. Additional unstained slides were produced from their complementary positive PCR samples where ready made DNA probes, antibodies and non-radioactive labels were applied to the slide. Observations for stained viral bodies were then made with a normal light microscope. Photos of slides were taken and edited using *Axio Vision 3.1* and a Zeiss camera.

Full details of the molecular and histological methods will be discussed in the MSc. thesis "The quality of *Penaeus monodon* (Black Tiger shrimp) broodstock among natural populations in the Fiji Islands: their viral-disease status." which is currently being written up.

Results

PCR

The following gel photos are based from one representative site, Nadi. Following this are the shrimp test results from all sites, in tabulated form.

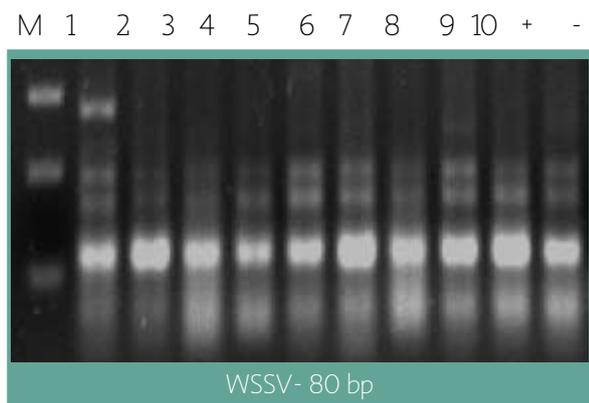
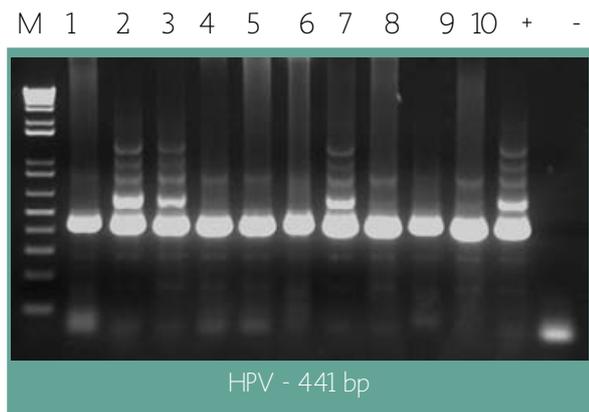
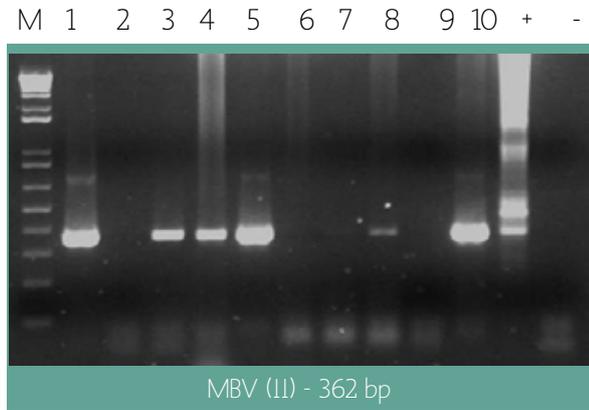
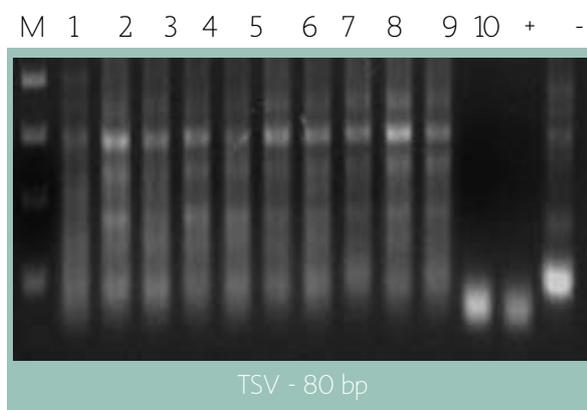
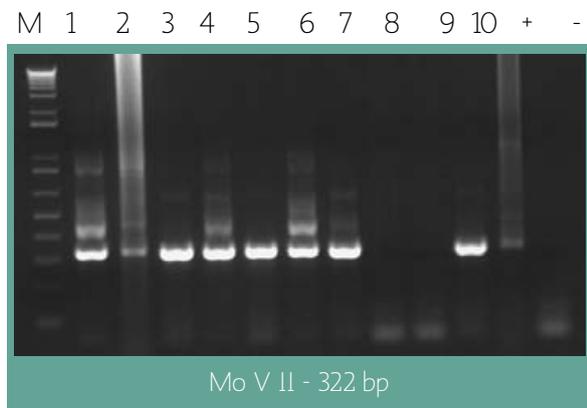
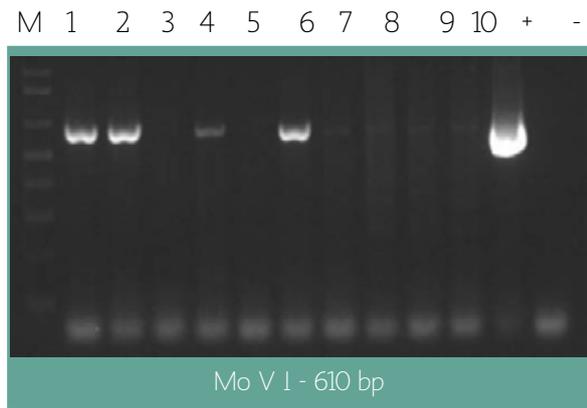
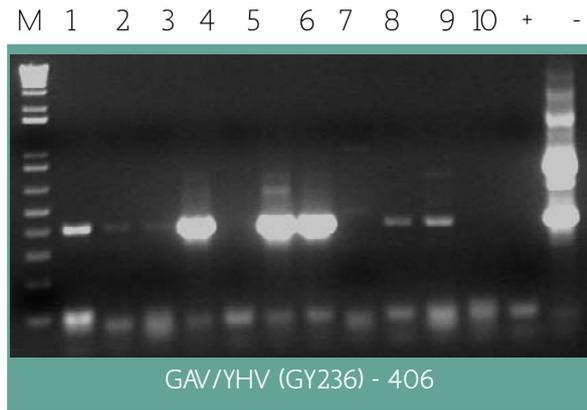


Figure 2. Various PCR results from the Nadi samples. Each PCR gel figure shows the DNA marker in the first lane, followed by the 10 groups of samples and finally followed by the positive and negative controls.



Histology

The following results are representative of one site, Nadi.

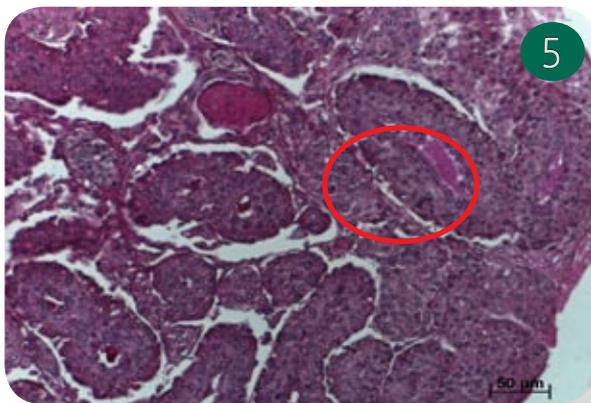
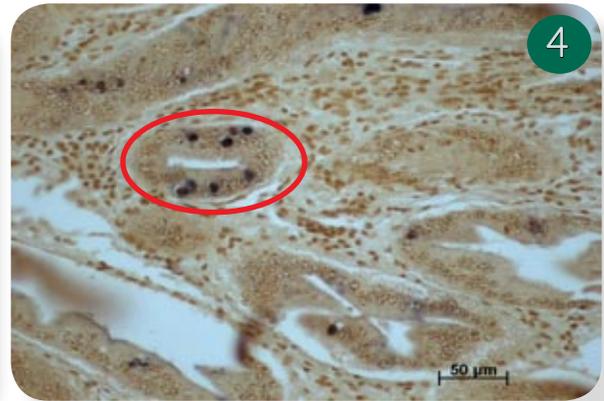
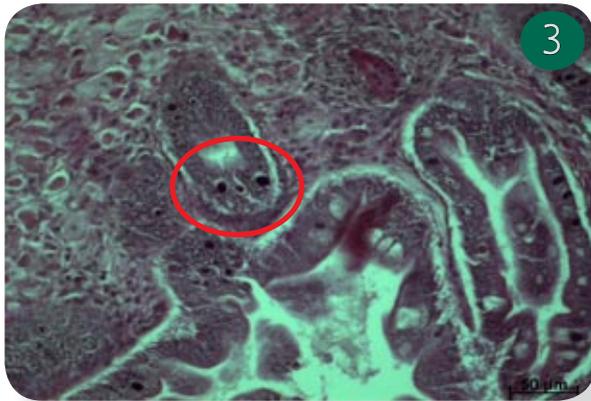


Figure 3. H & E stain showing positive signal of HPV viral bodies in hepatopancreas tissue.

Figure 4. In situ hybridisation confirms HPV viral bodies in hepatopancreas tissue.

Figure 5. H & E stain for Mo V showing lesions in the lymphoid tissue. Despite the absence of Mo V viral bodies, the lesions indicate the presence of foreign bodies

Table 1. Collective results of all shrimp virus PCR tests done and the type of primers used for all five sites. The PCR results were the same for every site, except Navua which did not have MBV.

Site/sample group #	Primer used	Virus tested	Result
Kadavu	Conventional	HPV	Positive
	Conventional	MBV	Positive
	Real Time	IHHNV	Positive (av. Ct value = 31.17)
	Real Time	WSSV	Negative
	Conventional	MoV	Positive
	Real Time	TSV	Negative
	Conventional	GAV	Positive
	Conventional	YHV	Negative
Vanua Levu	Conventional	HPV	Positive
	Conventional	MBV	Positive
	Real Time	IHHNV	Positive (av. Ct value = 28.78)
	Real Time	WSSV	Negative
	Conventional	MoV	Positive
	Real Time	TSV	Negative
	Conventional	GAV	Positive

	Conventional	YHV	Negative
Nadi	Conventional	HPV	Positive
	Conventional	MBV	Positive
	Real Time	IHHNV	Positive (av. Ct value =28.45)
	Real Time	WSSV	Negative
	Conventional	MoV	Positive
	Real Time	TSV	Negative
	Conventional	GAV	Positive
	Conventional	YHV	Negative
Tailevu	Conventional	HPV	Positive
	Conventional	MBV	Positive
	Real Time	IHHNV	Positive (av. Ct value =27.31)
	Real Time	WSSV	Negative
	Conventional	MoV	Positive
	Real Time	TSV	Negative
	Conventional	GAV	Positive
	Conventional	YHV	Negative
Navua	Conventional	HPV	Positive
	Conventional	MBV	Positive
	Real Time	IHHNV	Positive (av. Ct value = 27.10)
	Real Time	WSSV	Negative
	Conventional	MoV	Positive
	Real Time	TSV	Negative
	Conventional	GAV	Positive
	Conventional	YHV	Negative

Viral DNA Sequencing

Tables 2 to 5 shows the comparison of the Fiji shrimp viral DNA sequences and that of Australia and India (positive controls used), as percentage similarities. This data was solely based on the Nadi site, which was treated as a representative site for the sequence data of the study, as there was very little or no differences of sequence between Fiji sites.

The same viral DNA sequence comparisons were also made between sites of Fiji. The results below are the average after comparing sequence data between each site, for each of the four viruses.

Table 2. Average percentage similarities of the HPV viral DNA sequence between, Fiji and Australia, and also within the five sites of Fiji.

HPV	Australia	Fiji
Fiji	80.0	94.0

Table 3. Average percentage similarities of MBV viral DNA sequence between, Fiji and India, and also within the five sites of Fiji.

MBV	India	Fiji
Fiji	95.0	100.0

Table 4. Average percentage similarities of GAV viral DNA sequence between, Fiji and Australia, and also within the five sites of Fiji.

GAV	Australia	Fiji
Fiji	98.0 - 99.0	98.0 - 99.0

Table 5. Average percentage similarities of MoV viral DNA sequence between, Fiji and Australia, and also within the five sites of Fiji.

MoV	Australia	Fiji
Fiji	98.0 - 99.0	100.0

Discussion

Preliminary tests

Before these tests were carried out at CSIRO, preliminary tests had been done on some of the local shrimp, using Pathotech Test Kits (Magellan Biotechnology Group) from Taiwan. This was part of a project for the Institute of Marine Resources. The results were negative for WSSV, YHV, MBV, TSV and IHHNV, which contradict results from the CSIRO analyses, reported here. It is possible that the primers used in these test kits were produced to target the Taiwanese strain of the above viruses, which may have been the reason for negative results for Fiji *P. monodon*.

Fiji *P. monodon* viral-disease status

This study revealed that Fiji *P. monodon* harbour Penaeid shrimp viruses, which are HPV, MBV, MoV, GAV and IHHNV. The tests, however, did not reveal any WSSV, TSV or YHV. Despite this, studies have shown that it is still possible to farm shrimp and produce a harvest with the presence of viruses (Flegel *et al.*, 2004; Tsai *et al.*, 1999). Although there were viruses found in wild Fiji *P. monodon*, there were no gross clinical signs (overall external appearance) of *viral-disease* observed. Lightner (1998) states that a disease outbreak is a result of three components: the host (in this case *P. monodon*), the agent (virus) and an environment that favours the development of a disease (such as poor water quality). This means that Fiji has shrimp viruses but not necessarily viral-disease. Of the three components for disease, Fiji has the first two; the host and agent, however if the conditions of shrimp hatchery and farming practices are not optimum then the chance of a disease outbreak is high. Therefore a disease outbreak can be avoided if the environment that favours it is avoided, meaning the establishment and sustainability of optimum hatchery and pond conditions.

DNA sequencing

The DNA viral sequencing implies that Fiji GAV and MoV may have originated from Australia, potentially due to a local farm that was once importing post larvae from Queensland. The farm later stopped after a GAV outbreak, during the late nineties. On the other hand, the HPV and MBV sequence suggests that they could have been

in Fiji shrimp all along and not introduced or that they may have been acquired from another local Penaeid species. Further research would need to be carried out to confirm the origins of these viruses.

The various Fiji shrimp viral DNA sequences that were obtained were stored in the CSIRO database and used to compare with other known strains especially Australian ones. The Fiji HPV sequences were especially significant and this information will be used by CSIRO and USP, to publish a journal article on Australian, Fijian and possibly Indian HPV sequences. In addition to this, some of these Fiji samples (along with others from other countries) are currently being used by another Aquaculture group at CSIRO, Brisbane for a study on Penaeid shrimp genetic markers.

Implications for the industry

Fiji's shrimp industry: Although the Fiji shrimp industry is still in its developing stages, it is now in a better position to make decisions concerning shrimp viral diseases for farming *P. monodon*. At a recent workshop for shrimp stakeholders (8th - 11th March, 2005) the Fiji Quarantine services were informed of the results and are now aware of the importance of protecting our local *P. monodon* stocks from the introduction of major shrimp viruses, such as Yellow Head and White Spot Syndrome Virus, possibly from imported shrimp. The workshop also highlighted the importance of maintaining optimum hatchery and pond conditions to minimize the chances of viral outbreaks.

Fiji's shrimp future lies in providing first for the local demand before any possibilities of exporting to the region and beyond. In terms of quarantine, a Biosecurity Plan can be set up to protect our current *P. monodon* stocks from the major viruses that haven't been detected in Fiji *P. monodon* (WSSV and YHV). This study can also be used as a model to determine and/or monitor animal diseases that hold aquacultural and agricultural potential in the region.

Capacity-building impacts

Institute of Marine Resources: The Institute has also benefited from this since IMR operations also include testing local and imported shrimp for various viruses. The skills that have been acquired

will help us improve the current techniques we are using. Capacity building is also a major benefit and puts IMR in a better position to advise shrimp stakeholders on shrimp disease.

University of the South Pacific: The skills that have been acquired are generally the same for any diagnostic lab and any other that utilizes molecular techniques such as PCR. Therefore the basics of this skill can be taught in some courses that involve molecular work, especially since there is already a PCR lab at the Marine Studies Programme. It would also be good to incorporate it in any courses that involve agriculture or aquaculture, to reinforce the importance of monitoring any livestock for disease and quarantine purposes. Besides capacity building, this study has also helped establish and strengthen ties between CSIRO and USP, for the benefit of other students who want to pursue training attachments.

South Pacific region: Capacity building for the region in an area which is vital for aquaculture and agricultural purposes. This study can also be used as a model to determine and/or monitor animal diseases that hold aquaculture and agricultural potential in the region.

Personal benefit: I feel I've greatly benefited from this attachment in terms of the skills that I have acquired in molecular diagnostic techniques such as PCR and Histology.

Not only did I learn the skills needed to carry out such techniques, but also how PCR and Histology labs should be set up. Working alongside PhD. students (University of Queensland) and Post-Doctorate staff at CSIRO has also helped me organise my work more efficiently and provided me with skills to undertake higher-degree studies. Being exposed to the way CSIRO go about their research has also taught me more about the way research should be carried out and what to keep in mind when putting together a project.

The above results were presented at the following workshops:

- Strategic Development Planning Workshop for Shrimp and Seaweed held at the Tradewinds, Lami on the 8th - 11th March, 2005. A paper on "Research Constraints in Development of a shrimp aquaculture industry in Fiji" was produced (see below).

- A brief Training Workshop on *P. monodon* Biology and Fisheries for the community of Moala village (a major potential site for brooders) on 22nd -23rd March, 2005. A vernacular presentation was done here.

Constraints

There were two major problems encountered during this attachment.

Firstly, the quality of the Fiji shrimp nucleic acid (DNA and RNA) was not the best for molecular purposes. This was because the tissues (gills, hepatopancreas and lymphoid organ) from which the nucleic acid were extracted, were preserved in lab-grade ethanol instead of analytical grade. The use of such a grade of preservative increases the chances of nucleic acid degradation within the tissue over time, before the actual extraction. Nucleic acid degradation causes DNA fragments to break up into smaller and smaller pieces over time, until they become too small for identification by PCR. This problem was only apparent during my first week at CSIRO. Fortunately, this was not such a hindrance to the study as the nucleic acid had not degraded to such a point of not being able to be detected. In addition to this, the various PCR formats that were used during the screening, especially Real Time PCR, were able to detect varying sizes of shrimp viral DNA.

Secondly, there were no alterations made to the timing I had set for myself to complete the attachment at CSIRO. I had initially decided on 10 weeks to complete all the diagnostic screening and analysis of any Fiji shrimp viral DNA, however it actually took me close to eight months to complete. This problem gave rise to two other problems: financial and immigration matters. The timing was crucial in applying for the right visa and for estimating the amount of money I would need during my stay in Brisbane, for food and accommodation. Based on the ten-week period, I was issued a Temporary Business Visa (Sub Class 456), which allowed a stay of up to three months only. Extending the Visa would have only been possible by applying for it outside Australia (condition 8503). However, I overcame the problem by applying for the waving of condition 8503. Various letters from all my relevant superiors from CSIRO Brisbane, ACIAR and Fiji were submitted along with

my form of application. Two weeks later I was waived the condition and was now allowed to apply for another visa within Australia. A week later, with the assistance of ACIAR, I was approved a Temporary Resident Visa allowing me to stay up to March 2005.

In relation to my financial problems, ACIAR had given me money to last me up to ten weeks only. At the time I was also a holder of the USP Graduate Assistant Scholarship in which I received a monthly allowance. After my ten weeks had passed I arranged for this allowance to be sent to me every month. The difference in currency rates meant losing a bit of money, but after arriving in Brisbane, I found out I had relations there and staying with them alleviated my accommodation costs greatly.

The problem of timing would have been avoided if I had set more time for any unforeseen problems.

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Research constraints in development of a shrimp aquaculture industry in Fiji

Marine Studies Programme
Institute of Marine Resources

The main constraint in shrimp aquaculture in Fiji is seed (post-larvae) for stocking ponds.

There are two operating shrimp hatcheries in Fiji, (the Government hatchery in Galoa, and the USP hatchery at the Marine Studies Programme), however there has not been enough post-larval production to support a commercial industry. USP in 2003 developed a research strategy to address constraints in the development of a industry based on monodon shrimp, with the following steps to be followed in sequence:

1. Establish what is the current virus-disease status of Fiji monodon. Broodstock and PL are of little commercial value if they contract a viral-disease;
2. Find out the availability and seasonality of monodon broodstock from various locations around Fiji, to improve broodstock supply and lessen dependence on only two collection locations;
3. Establish the quality (reproductive characteristics) of Fiji monodon broodstock in comparison with Australian and Asian broodstock; and
4. Achieve domestication and artificial selection of broodstock based on reproductive quality.

Step 1 is almost completed. During early 2004, monodon shrimp samples were collected from five different sites around Fiji (Nadi, Kadavu, Vanua Levu, Tailevu and Navua) and tested by PCR at CSIRO, Brisbane for presence of various shrimp viruses.

Questionnaires and interviews were also carried out at these collection sites to determine traditional knowledge on capture techniques and seasonality/availability of monodon broodstock.

Step 1 results:

- Fiji *P. monodon* contained the following viruses at all collection sites (except no MBV in Navua samples):
 1. Hepatopancreatic parvovirus (HPV)
 2. Monodonbaculovirus (MBV)

3. Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)
4. Gill Associated Virus (GAV)
5. Mourilyan Virus (MoV)

- Fiji *P. monodon* samples collected did not contain the following viruses:
 1. White Spot Syndrome Virus (WSSV)
 2. Yellow Head Virus (YHV)
 3. Taura Syndrome Virus (TSV).

Implications for policy

Does this mean that Fiji *P. monodon* have shrimp disease?

No. Fiji Black Tiger shrimp have viruses, but do not necessarily have disease. They may develop a disease if exposed to stressful conditions (e.g. high stocking density).

Could Fiji be a supplier of SPF *monodon* PL to other countries, and if so then how could this be achieved?

Yes. They can be exported as SPF free for WSSV, TSV and YHV, but they will not be free of other viruses previously outlined above.

Does Fiji have any kind of competitive advantage in monodon grow-out, e.g. because WSSV and YHV are not yet here?

Yes. WSSV outbreaks presently overshadows all other disease agents as the leading cause of production losses in Asia.

Virus-positive shrimp can still be farmed without a disease breakout, provided stressful conditions are minimized as much as possible.

This advantage is put at risk, however, by continued imports of WSSV and YHV positive frozen shrimp to supermarkets in Fiji.

Biosecurity and quarantine/border-protection recommendations.

Continue to carry out PCR tests to monitor the viral status of Fiji shrimp, especially on live shrimp imports to avoid the introduction of WSSV and YHV.

Attempt to restrict the import of frozen shrimp

Draft and implement a Biosecurity Plan for shrimp imports

Where did these viruses come from?

This will need more research on Fiji shrimp. Some may have been here all the time, others may possibly have been imported.

Summary

Fiji's shrimp future seems promising in farming its own local shrimp (*monodon*) and supplying to domestic and regional demands. Depending on the success at this level, Fiji can then move into exporting to other markets overseas. Despite this new information about the viral status of Fiji shrimp, it is still possible to farm *monodon* shrimp and produce a good harvest.

Other Penaeid species?

Litopenaeus stylirostris appears to have good prospects for Fiji, and is easier to domesticate than *monodon*. It has recently been imported on two

occasions, however neither importation has led to domestication of *styli* in Fiji. Every importation carries risks, and these risks need to be minimized by avoiding unnecessary translocations. The New Caledonia industry of 2000T per annum was established on the basis of a single importation, and only now after 20 years are they considering a second importation.

A vital role for government in Fiji is to coordinate all stakeholders, if necessary via import permit conditions, to ensure that no opportunity to domesticate *styli* from a reputable source into Fiji is missed.