An evaluation of the culture performance of two *Macrobrachium* species in Vanuatu: the exotic *M. rosenbergii* and the indigenous *M. lar*

Sompert Gereva

A thesis submitted in fulfilment of the requirement for the degree of Master in Applied Science

School of Earth, Environmental and Biological Sciences
Science and Engineering Faculty
Queensland University of Technology

March 2014

Statement of Original Authorship

The work contained in this thesis has not been previously submitted for a degree of diploma at any other higher education institution. To the best of my knowledge and knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

QUT Verified Signature

Signed... Date, 20th March 2014.....

Acknowledgements:

I would like to express my sincere appreciation to my supervisors, Dr Satya Nandlal, Professor Peter Mather and Dr David Hurwood, Science and Engineering Faculty, Queensland University of Technology (QUT), Brisbane, Australia for their assistance and guidance through the difficult days of carrying out the trials and writing up my thesis. It was a complex journey and I thank Almighty God for all the guidance and knowledge that He has given us to complete this piece of work. It would have been impossible without the help of Dr. Satya; he had a lot of trust in me and encouraged me all the way through to thesis write up.

I would also like to thank Director of Vanuatu Fisheries Department, Mr Moses Amos, and Government of the republic of Vanuatu for financial assistance and materials provided to ensure that my research work was completed successfully. Similarly I would also like to thank AusAID for having the trust in me and for the scholarship that enabled me to study and do this research that I believe will be of great benefit to the people of Vanuatu. I would also like to acknowledge the assistance of Fiji' Ministry of Fisheries (Naduruloulou Research Station) for providing prawn broodstock that were used in my research. It would have been very difficult to achieve good results without the support from Japan International Cooperation Agency (JICA) for providing equipment and vehicles, and the contribution of the Aquaculture Advisor (Mr Robert Jimmy) and other staff of Secretariat of the Pacific Community (SPC), Noumea, New Caledonia for their assistance and support for the supply of tilapia feed and temperature loggers including the use of results reported in Chapter 2.

I would also like to thank all my friends and colleagues for their help through my difficult times and while I cannot name all for their great assistance, I also say a big "TOK TANGIO", to Dr. Satya and his family, Professor Peter Mather and Professor Jane Hughes, Mr. Lency Dick and his family, Mr Pascal Dumas, Mr Andrew William, Mr Rodrick Tatuna, Mr Rocky Kaku, Mrs Nettie and some people whom I have not

mentioned, but not forgetting Mr Jackson and Charlie for security service provided at Tagabe Freshwater Aquaculture Centre.

Finally I would like to thank my wife, Jane Gereva, family and friends who supported me all the way through my two and half years of work. Thank you all for your understanding and assistance.

Abstract

Fish and fisheries continue to play key roles in human nutrition, employment and indigenous culture in the western Pacific region because aquatic species provide the most affordable animal protein source for local people. In Vanuatu, results of an agriculture census in 2006 indicated that 72% of local households engage in fisheries related activities including harvesting for home consumption and for their livelihoods. Thus fish, crustaceans and molluscs are critical to long term sustainability of many communities. In recent times however, wild aquatic resources in many parts of the region have declined due to over-fishing, impacts from growing human populations and habitat modification. Thus human nutrition has suffered because of large increases in local prices for seafood, their reduced availability and limited affordable alternative options. Fish farming, while not a traditional activity in many parts of the Pacific, has been identified by many regional governments as a way of potentially addressing this growing problem. While there are abundant marine species available naturally across the Pacific that can be targeted for development in culture, some freshwater species in general, provide easier culture options and require less intensive production environments to be successful (e.g. tilapia, carps and freshwater prawn species). Unfortunately, freshwater species are depauperate in the island states of the Pacific, so there are limited native species to choose among with appropriate life history traits. While exotic farmed species for which simple and cost effective production systems are available and offer options, often where available, native species are preferred because they are unlikely to cause negative impacts on wild populations should escapes from culture eventuate.

There are many freshwater prawn species (*Macrobrachium* spp.) worldwide, some of which that now support large culture industries (notably the giant freshwater prawn, *M. rosenbergii*), but this is an exotic species in the Pacific (except for Australia and Papua New Guinea). A species of freshwater prawn that is native to many Pacific island states and that can grow to quite large size is *M. lar* and this species also occurs widely in Vanuatu, so it has been identified as a local species that could support a local culture industry if it can be cultured productively in small-scale farming and if its life cycle can

be closed in captivity effectively. Other regional countries (notably Fiji) have already opted for the exotic species (*M. rosenbergii*) as their preferred farming candidate and could provide broodstock for an industry in other Pacific island states e.g. Vanuatu if requested, but an informed decision would best be based on an initial assessment of the relative culture performance of the two candidates under local farming conditions. The current project therefore had two objectives, (1) to evaluate the regional scale at which hatchery produced postlarvae or wild juveniles of the native species *M. lar* could be sourced to farmers in Vanuatu without potentially impacting wild gene pools negatively and (2) to undertake a rigorous evaluation of the relative growth performance and survival of the indigenous *M. lar* and exotic *M. rosenbergii* under similar production conditions. Results were clear, (1) all wild stocks of *M. lar* in Vanuatu constitute a single stock and so could be translocated for culture without any negative impacts on wild gene pools and (2) the relative culture performance and relative productivity of *M. rosenbergii* was far superior to that of *M. lar*. The data are now available so can allow an informed choice about which species offers the best potential for developing a new freshwater prawn culture industry in the country.

Abbreviations and acronyms:

ACIAR	Australian Centre for International Agriculture Research
B	Berried female
BC	Blue-claw male *Macrobrachium rosenbergii*
BrC	Black-claw male *Macrobrachium lar*
FAO	Food and Agriculture Organization
FCR	Food Conversion Ratio
FRP	Fibre Reinforced Plastic
GFP	Giant River Prawn
M. l	*Macrobrachium lar*
M.r	*Macrobrachium rosenbergii*
NRS	Naduruloulou Research Station
OC	Orange Claw male *Macrobrachium rosenbergii*
PICT's	Pacific Island Countries and Territories
PL	Post larvae
PNG	Papua New Guinea
PVC	Poly (vinyl chloride)
QUT	Queensland University of Technology
SM	Small Male prawn
SPC	Secretariat of the Pacific Community
S	shed female prawn
TFAC	Tagabe Freshwater Aquaculture Centre
UN	United Nations
USA	United States of America
VFD	Vanuatu Fisheries Department
VMS	Vanuatu Meteorological Service

Table of Contents

List of Tables:

List of Figures:

List of Appendices:

1.0 General Introduction

1.1. **Current status of modern fisheries and aquaculture**

Fisheries and aquaculture are major source of nutritious food and animal protein for much of the world's population. In addition, the sector provides livelihoods and income, both directly and indirectly, for a significant share of the world's population. Fish and fishery products are also among the most traded food commodities worldwide and this is expected to continue to rise. According to Akpaniteaku *et al*. (2005), the most reliable source of animal protein for the majority of people is fish and as a consequence world fisheries are now a rapidly developing sector in the food industry. Global production from combined capture fisheries and aquaculture reached 148 million mt of fish in 2010 and provided an estimated supply of 18.6 kg *per capita* (live weight equivalent) FAO (2012). Of this, aquaculture accounted for over 46% of total fish production and continues to be the fastest-growing animal-food-producing sector with per capita supply increasing from 0.7 kg in the 1970 to 7.8 kg in 2008, an average annual growth rate of 6.6 percent (FAO 2010). In contrast, annual growth rates for capture fisheries and terrestrial farmed meat production systems were only 1.2% and 2.8%, respectively (FAO 2006). Production levels from capture fisheries however, have remained relatively stable over the past two decades (i.e., since the 1990s) after showing a marked increase during the 1970s and the 1980s while global *per capita* fish consumption has doubled over the same period, increasing from an average of 9.1 kg in 1961 to an estimated 16.3 kg by 2003 Delgado *et al*. (2003); Ahmed & Delgado (2000); FAO (2006), and to a record of 17 kg in 2008, supplying over three billion people with at least 15% of their average animal protein intake (FAO 2010). Consumption trends show an increase in demand for fish products for food partly due to growing purchasing power in some developing countries and changing dietary habits where consumers are becoming increasingly health and diet conscious and see fish as having a positive impact on their health. Based on the current annual human population growth rate, the world population is expected to grow to 9.3 billion by 2050 (UN, 2010). As a result, the demand for food including fish is increasing rapidly, and predictions are that we will need to double fish production to meet projected

demand over the next 25 years. Most forecasts for the future predict a stable or decreasing supply from capture fisheries and an increasing proportion coming from aquaculture. According to Wijkstrom (2003) the demand for aquatic products for human consumption will grow to 121.1 million mt by 2010 and this will exceed total capture fisheries supply. The shortfall in supply must largely be filled from aquaculture to feed an estimated additional 80 to 90 million people each year, most of them in developing countries (Akpaniteaku *et al*. 2005). Overall fisheries and aquaculture support the livelihoods of an estimated 540 million people (FAO 2010). In addition, people are consuming fish and more people than ever are employed in or depend on the sector. Yet many who depend on fish to live are constantly exposed to food shortages and the projected human population increase will lead to increasing shortages of protein and other natural resources, placing increased stress on global ecosystems that are already facing significant stress.

According to recent FAO data, around one-quarter of wild fish stocks around the world were considered to be moderately exploited, while half were fully exploited and were estimated to be close to their maximum sustainable yields. Remaining fish stocks were either overexploited or were experiencing excessive fishing pressure with production from capture fisheries in the Asia-Pacific region predicted to decline over the next 10-20 years unless fishing capacity and fishing effort is greatly reduced as reported by Sugiyama *et al*. (2004). Following from above, the overall percentage of overexploited, depleted or recovering fish stocks in the world's oceans has not dropped and is estimated to be slightly higher than in 2006 (FAO 2010). Taken together, this indicates that the maximum yield from wild capture fisheries in the world's oceans and from major river drainage systems has probably already been reached, and in the long-term, can only be improved by better management, by reducing fishing capacity and effort, and by restoring fish habitat. None of these options however, is easy in practice. Another possible option for selected fish species is to release hatchery-reared juveniles into the wild (stock enhancement) that has been practiced for example, for salmonid fisheries in the United

States of America (USA) and in Japan. Up to 1999, some 90 species of fish and invertebrates have been released to enhance wild stocks (Imamura 1999).

There is clear proof now of widespread overfishing and general overexploitation of all or most harvested fish species suggesting that wild fish resources are declining with the effect of this on market supply having been masked in many countries by emergence of farmed production since the 1970s. According to Naylor *et al*. (2000) the total yield from capture fisheries is not expected to increase greatly and so fish supply from capture fisheries will not be able to meet growing global demand for aquatic food. Globally, the main source of fish production has shifted from developed to developing States and the share from aquaculture has increased substantially (Sugiyama *et al*. 2004). In addition, seafood demand from developing States is expanding rapidly and major shifts in seafood and aquaculture production, trade, and consumption worldwide is expected to continue over the next 10-20 years. There are also reports (e.g., New 1991, 1997, 1999; Hempel 1993; FAO 1997) that have indicated the urgent need to increase the global supply of seafood through the expansion of aquaculture activities leading to recent FAO reports (FAO 2005-2013) suggesting that global aquaculture production will need to reach 80 million mt by 2050. In fact, fisheries officials all over the world have been examining aquaculture as an alternative to harvesting wild stocks with some viewing it as a universal remedy for problems of declining catches from the wild. Thus, expansion of aquaculture has been given high priority both in developed and developing countries (e.g., FAO 1995; CGIAR 2005).

There is significant potential for aquaculture to meet increasing demand for aquatic products in most regions of the world and this sector continues to grow very rapidly and has been promoted as the most likely source of additional seafood production (Sugiyama *et al*. 2004). It appears that the full potential of the aquaculture sector to contribute to aquatic food has yet to be realized, and the sector may require new approaches to realize its goals beyond the 21st century. Prominent will be the need for animal protein that aquatic resources can supply in abundance. Thus the oceans of the world and particularly

inland water systems and brackish water regions offer many prospects for developing new food resources. Rapid expansion of aquaculture however, has created many ecological problems and this is compounded by growing use of exotic species including resources (feed and water, etc.) and ecosystems services (waste assimilation, etc.).

1. 1.1 Current status of aquaculture

In 2010, global production of farmed food fish was 59.9 million mt, an increase of 7.5% from 55.7 million mt in 2009 (FAO 2012). Farmed food fish include finfishes, crustaceans, molluscs, amphibians (frogs), aquatic reptiles and other aquatic animals such as sea cucumbers, sea urchins, sea squirts and jellyfishes. The total farmgate value of food fish production from aquaculture was estimated at US$119.4 billion in 2010 (FAO 2012).

Most aquaculture production of fish, crustaceans and molluscs is derived from freshwater with over 240 different aquatic animal and plant species reported as being farmed in 2004 with countries in the Asia-Pacific region accounting for over 91.0 % of global production and 80.5% by value (FAO 2006).

With increasing emphasis on aquaculture many countries have turned to exotic culture species (Hewitt *et al*. 2006) including marine shrimps, tilapia and freshwater prawns. According to Naylor *et al.* (2001), the most important aquatic species that are cultured in the USA are exotic species and this is also true in many other countries. In fact, growth of aquaculture has greatly accelerated interest in importing exotic species and has allowed research and development costs to be reduced via application of technological advances. Use of exotic species for farming however, rarely provides a zero risk of accidental release and can be problematic from biodiversity protection and transboundary perspectives. For example some exotic aquatic species have become serious feral pests (e.g. the Mozambique tilapia) in the Asia/Pacific region (De Silva *et al*. 2004; 2006).

While many see aquaculture as having huge potential to increase and stabilize the world's supply of fish available for food, as well as to generate employment, and to relieve pressure on wild stocks, the degree to which this potential can be realised under existing aquaculture practices remains unclear. This is because aquaculture production is vulnerable to adverse impacts of disease and environmental conditions. In recent years disease outbreaks have affected farmed Atlantic salmon in Chile, oysters in Europe, and marine shrimp farming in several countries in Asia, resulting in partial or sometimes total loss of production. For example, in 2010 aquaculture in China suffered production losses of 1.7 million mt caused by natural disasters, disease and pollution (FAO 2012). In addition, under present conditions and methods, some forms of aquaculture (specifically use of exotic species and intensive production systems) appear unsustainable when viewed from an ecological and economic standpoint. This is because intensive aquaculture largely depends on fisheries to harvest seafood for feed production that is given to cultured species in the form of pellets in intensive culture systems. This practice can also generate significant environmental impacts that affect spawning grounds of commercially valuable species and shellfish, as well as recruitment of larvae to aquaculture itself (Naylor *et al*. 2000). Some of the impacts include diminished size of native populations, local extinctions, and altered community structures and ecosystem functions (OTA 1993) including altered or impoverished natural biodiversity in receiving ecosystems via interbreeding, predation, competition for food and space and/or habitat destruction (Folke & Kautsky 1989). Potential for introducing infectious diseases to wild stocks, for example, Infectious Salmon Anaemia (ISA) is also an issue (www.arts.usask.ca). In addition to disease epidemics, cultured fish often escape from pens impacting on biodiversity of natural fish stocks.

Most marine shrimp available on international markets prior to 1970 were supplied from capture fisheries, however increased demand for shrimp after 1970 resulted in development of mass culture techniques for commercially important marine shrimp species and giant freshwater prawn, *M. rosenbergii* (Sandifer & Smith 1975; Webster & Tidwell 1995; Boyd & Clay 1998). At this time marine shrimp were more desirable to

consumers and profitable to farm than freshwater prawns (Webster & Tidwell 1995; Jory 1996) and this cleared the way for development of intensive shrimp aquaculture production systems (Boyd & Clay 1998; Gujja & Finger-Stich 1996). While initially it appeared that the shrimp culture industry would continue to expand rapidly, the spread of shrimp farms to mangrove swamp areas and estuaries caused damage to coastal ecosystems (Gujja & Finger-Stich 1996) and also impacted land-based activities. Intensive shrimp culture operations also produced conditions, i.e. self-pollution due to inappropriate pond management and use of water already polluted by neighbouring shrimp farms, leading to outbreaks of diseases, for example, Infectious Hypodermal and Hematopoietic Necrosis (IHHN) and Taura Syndrome Virus (TSV). Once farmers detect disease in their stocks, they often harvest and freeze the shrimp to prevent losses. Contaminated shrimp were then sold internationally, spreading viral disease problems (Boyd & Clay 1998; Nunan *et al*. 1998); causing production to crash in other farms and many farmers to abandon or convert their farms to culture of other species (Gujja & Finger-Stich 1996; Boyd & Clay 1998). Collectively, these impacts on shrimp culture have decreased the availability of shrimps in markets and increased prices. Had shrimp culture diversified, many of the problems described above may have been reduced or may even have been avoided (Patil & Krishnan 1997; Nunan *et al*. 1998; Islam & Wahab 2005).

Following from above, the issues of damage to coastal ecosystems and diseases that currently faces shrimp farming can be addressed partly by developing freshwater prawn farming in suitable locations. In addition, freshwater prawn farming is recognised as an efficient way of producing prawn products with minimal environmental impact (Valenti & New 2000). Saline water is not required for the grow-out phase excluding the need for coastal sites, thus reducing potential salinization of soil and water. Freshwater prawns are normally reared in inland areas that neither competes for coastal resources nor harms coastal ecosystems. There is also little risk of industry collapse due to diseases and there are simple ways to produce freshwater prawns with low environmental impact (New *et al*. 2000a). This is because low-stocking density is commonly practiced by most

freshwater prawn farmers and prawns can adapt to a range of feeding habits since most are omnivorous and grow well in polyculture with tilapia and carps (Zimmerman & New 2000). They also show good potential for integration with agriculture and animal husbandry (Chatopadhay *et al*. 1995) and can be more profitable than rice production (Fegan & Sriram 2001). While freshwater prawn farming may be more sustainable than marine shrimp farming (e.g., New *et al*. 2000a), there are still issues with respect to the farming systems employed to increase production in sustainable ways, and to develop approaches and technologies that will increase the contribution of prawn farming to food supply with low impact on natural systems particularly in Southeast Asia and Pacific Island Countries and Territories (PICTs).

1.1.2 Status and potential of aquaculture in PICTs

The capture fisheries and aquaculture sectors are of fundamental importance to the Asia-Pacific region in terms of food security, revenue generation and employment. Since the yield from capture fisheries is not expected to increase greatly, there is an emphasis being placed on the aquaculture sector's ability to provide increasing quantities of fish to satisfy demand. In PICTs, many indigenous people have a tradition of eating fish and have practiced simple fish farming in fresh and brackish water from ancient times. These traditions offer a useful basis for development of aquatic food production programs. Thus, there is optimism that animal protein supply in PICTs can be enhanced by developing aquaculture. However, several conditions must be satisfied in order that aquaculture can aaddress this expectation. Most importantly a substantial expansion of aquaculture regionally will be required. According to Sugiyama *et al*. (2004) obtaining the suitable land and water may be possible provided that the value of fishery products increases so that aquaculture can challenge other production systems for the use of feeds, land and water to effect this production. Alternatively, increased water use efficiency and intensified production can reduce land requirements. While current intensity of production, for example, tilapia farming in Vanuatu and Fiji, is such, that there is considerable scope for increased production per unit area, increased feed usage and probable increased water requirements are likely to be constraints. The current reliance

on fish meal as a protein source for aquaculture feeds, and more specifically, reliance on imported aquaculture feed in Vanuatu, for example, is a potential constraint. Also aquaculture currently competes with the livestock sector for fish meal and other feed ingredients for feeds and if the fish value continues to increase the "purchasing power" of aquaculture may draw this resource away from the livestock sector (Sugiyama *et al*. 2004). Other options include enforcement of conservation regulations to halt or reduce declines in fishery resources and to promote development of simple aquaculture systems.

1.1.3 Freshwater prawn culture in PICTs

Culture of freshwater prawns and brackish water prawns in the genus *Macrobrachium* has been practiced using simple culture systems and in polyculture with other crustacean and fish species for hundreds of years. The first experiments reported in the literature on *M. rosenbergii* culture would appear to be those of Ling (1962, 1969a, 1969b) and Ling and Merican (1961) after a significant breakthrough was achieved in Penang, Malaysia, when it was discovered that *M. rosenbergii* larvae require brackish water during their larval phase to complete their metamorphosis to post larvae. *M. rosenbergii*, according to Ling (1969a) "has since time immemorial been highly esteemed as food by people of the tropical countries of Asia and the Far East." *M. rosenbergii* is one of the largest species in the genus, with old males potentially attaining a body weight in excess of 300g. Ling's studies investigated larval rearing as well as the raising of juveniles to adult size in freshwater ponds and in irrigated paddy fields. At the time he concluded that well-managed ponds could produce two crops of commercial sized prawns each year in Malaysia. Later in 1965, Takuji Fujimura of the Hawaii Division of Fish and Game imported a small number of broodstock of *M. rosenbergii* from Malaysia and successfully developed commercial production of post larvae (PL) and pond culture of juveniles to produce a harvestable cohort (Ling & Costello 1979). The technological breakthrough of closing the life cycle of *M. rosenbergii* in captivity rapidly produced widespread interest in commercial culture of this species across the world. Following this development, broodstock of *M. rosenbergii* were translocated from S.E. Asia and Hawaii

to many countries and many commercial farms were established in Hawaii and elsewhere during the 1970s including the Fiji islands by the late 1970s.

The development described above produced a major stimulus to development of freshwater prawn farming in many tropical and subtropical regions around the world (New 2000; Fast & Leung 2003). By the 1980s, freshwater prawn culture contributed approximately 6% to global production of shrimps and prawns (New 1988, 1990), but this has expanded by up to 1300% since this time mainly due to improvements in culture technologies and wider recognition of the greater environmental sustainability of freshwater prawn culture compared with marine shrimp farming (Valenti & Tidwell 2006). Freshwater prawn culture has become one of the fastest growing aquaculture sectors and farmed freshwater prawns now contribute to global markets and have also become very significant in some domestic markets. This development has stimulated renewed interest in *Macrobrachium* culture in many regions of the world including in several PICTs where fish farming is a relatively new production sector.

Several species of indigenous *Macrobrachium* of commercial value occur in the western Pacific with *M. lar* native to most islands that possess suitable freshwater streams while *M. rosenbergii* is an exotic species in most of the western Pacific. The natural distribution of *M. lar* is very extensive (see Chapter 2), while *M. rosenbergii*, commonly known as giant freshwater prawn (GFP) has a more restricted distribution and is limited to north-west India across southeast Asia to Vietnam and to the Philippines, Papua New Guinea (PNG), northern Australia and Palau (Holthuis 1980). No natural populations of this species occur to the east of PNG.

Broodstocks of *M. rosenbergii* were imported to Hawaii to develop mass rearing (hatchery) techniques, beginning with 36 individuals sourced from Malaysia in 1965 (Fujimura & Okamoto 1972). Some individuals were also released into wild streams on all of the major Hawaiian Islands (Maciolek 1972); but, Davidson *et al* (1992) reported that the species did not establish viable feral populations. While *M. rosenbergii* were also

taken to several PICTs including Fiji in 1975 for simple grow out trials, there is no evidence that they have established in the wild there even today (Nandlal 2010).

Like *M. rosenbergii*, *M. lar* is also not native to Hawaii, but this species was introduced to Honolulu in Hawaii from Guam in 1956. Ninety-four individuals were released on Molokai and a year later 27 on Oahu (Brock 1960). Reports indicate that at present, *M. lar* has established feral populations in streams on all of the main Hawaiian Islands. Maciolek (1972) acknowledged problems with introducing new species into insular freshwater ecosystems and observed that *M. lar* should probably only be cultured where it occurs naturally. Thus, *M. lar*, a species native to most western Pacific island chains, potentially offers a large indigenous freshwater prawn species on which local farming systems in the western Pacific could be developed.

A brief summary of *M. lar* nomenclature, taxonomy and morphology are given below and, specific biological characteristics and attributes are compiled from the literature (e.g., New & Singholka 1985) and refined from personal observations during field studies in Vanuatu.

M. lar is referred to colloquially as the 'monkey river' prawn or 'monkey river' shrimp, (taxonomic serial No. 96308-FAO database: www.itis.gov/servelet). Current classification (taxonomic hierarchy) and synonyms can be found at: http://www.itis.gov/servlet/ and Holthuis (1980). Common local names used in Vanuatu include: '*naura*', *'ura'* and *'rauravai'*. Some details on *M. lar* life history traits are given below or are assumed *a priori* from that of *M. rosenbergii*.

Nomenclature, taxonomy and morphology

A schematic drawing of a generalized *Macrobrachium* prawn showing main morphological features is illustrated in Figure 1.1.

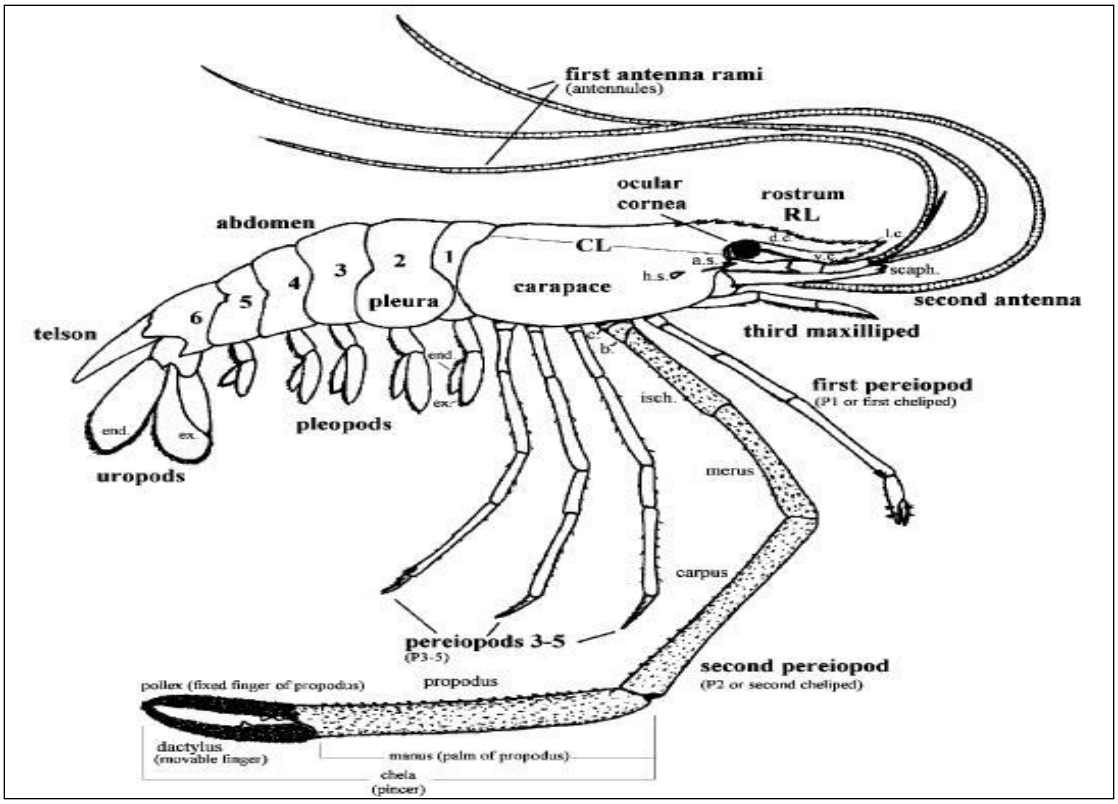


Figure 1.1: Schematic drawing of a *Macrobrachium* showing main morphological features. Abbreviations: a.s., antennal spine; b., basis; h.s., hepatic spine; c., coax; CL, carapace length; d.c., dorsal carina (of rostrum); end., endopod; ex., exopod; isch., ischium; l.c., lateral carina (of rostrum). Modified from Short (2004).

Morphological features that have been used to identify *Macrobrachium* species include the rostrum and the second pereiopods. *M. lar* has a long rostrum with 7-9 dorsal teeth compared with 8-13 in *M. rosenbergii*. Fully developed males are highly distinctive and are easily distinguished by long, robust second pereiopods with widely gaping fingers, each bearing a very large incisor tooth on the cutting edge. Specifically in the case of *M. lar*, the P2 merus in the second pereiopod in adult specimens is longer than the carpus and this character is diagnostic and distinguishes this species from other *Macrobrachium* taxa, e.g., *M. rosenbergii* that has the carpus distinctly longer than the merus. Other important phenotypic traits used for identification include that females are in general, smaller than males, have shorter second thoracic legs and are more slender with a smaller head and carry fertilized eggs for incubation until they hatch.

M. lar are sexually dimorphic (as is the case for *M. rosenbergii*) with mature males being larger and having longer second pereiopods and a larger cephalothorax than females. In a mature *M. lar* population, three male morphotypes can be distinguished by claw colour and their relative size ranges within the population (Nandlal 2010), similar to *M. rosenbergii* as reported by Ismael & New (2000) and Cohen *et al* (1981). Black claw (BC) males are relatively large while orange or brownish claw (OC) males possess medium-sized claws and small males (SM) possess short delicate claws, often with little pigment that are translucent.

Biology

Habitat

M. lar is diadromous as an adult with individuals occupying freshwater environments that are connected to the sea due to the fact that larval development must be completed in full seawater (35 ppt) before individuals must return to freshwater habitats as juveniles to complete their life cycle. Adults have been observed up to 5 km up-stream from estuaries (e.g. Teouma River) on Efate Island, Vanuatu and over 50km inland in Fiji (Nandlal 2010). Juveniles are found in estuaries, lowland fresh waters and inshore marine areas (Short & Meek 2000). Gravid females are usually found in under brush in streams where eggs hatch as free-swimming larvae. After eggs hatch and until metamorphosis to the PL stage the larvae pass through up to 13 zoeal stages (Atkinson 1977; Nandlal 2010; Lal 2012). After metamorphosis postlarvae assume a more benthic lifestyle and begin to migrate upstream where they complete their life cycle in freshwater.

M. lar are usually found in streams and creeks, and in pools below waterfalls. They can also climb steep waterfalls up to 90º angle and move to new pools with water. In instances when pools essentially dry out due to drought, individuals can tolerate very high water temperatures and tend to crawl on moist surfaces into remaining stagnant pools as flowing water ceases. This includes a limited capacity to ‘walk’ short distances over land where vegetation is moist (Nandlal 2010). At night individuals hunt for food as is typical of most *Macrobrachium* spp. During the day, individuals usually hide under

shrubs, brush, logs, rocks and in crevices and burrows in streams. They are also found extensively in wetland taro swamps and in other aquatic vegetation including vegetable crops, for example watercress (*Nasturtium officinale*, *N. microphyllum*) (Nandlal 2010). Natural abundance observed in Neslep stream on Epao, an area situated on the eastern side of Efate Island, in Vanuatu can exceed 10-20 individuals per square meter in streams covered with watercress.

Life cycle

The life cycle involves four distinct phases; egg, larva, postlarvae and adult. Gravid females are usually present in the upper reaches of streams, where mating and egg-laying takes place followed by egg-hatching which produces free-swimming larvae (zoea) that must reach saline water within 24-36 hours to survive (Nandlal 2010). After completing their larval development, the zoea metamorphoses into postlarvae and migrate to freshwater streams and creeks. Individual larvae become benthic feeders as juveniles and later as adults where they live on the stream bottom. They normally grow at temperatures ranging from 18-32^{o}C.

Food preference

M. lar is an omnivore and is most active at night. Common food includes: fruits, seeds, grains, aquatic insect larvae, nuts and detrital material. Individuals held in tanks will also readily consume pelleted feeds, animal flesh including molluscs, bivalves, fish, crustaceans and coconut flesh during the day (Nandlal 2010). They have also been observed in tanks to become cannibalistic when not fed for a couple of days. Food is located mainly by touch using filaments found on the antennae and antennules where it is picked up and brought to the mouth by the first and second pairs of thoracic legs. Visual observations indicate that in some larger streams in Vanuatu, males can reach 100g in weight, a relatively large size for any freshwater prawn species.

Moulting and growth

Growth in prawns or crustaceans can be expressed as the increase of length with time, volume, wet weight, or dry weight (Hartnoll 1982), i.e., growth increases with a succession of moults separated by the inter-moult period. During moulting, prawns shed their exoskeleton resulting in somatic tissue growth. Frequency of moulting depends on age with young individuals moulting more frequently than older individuals. The frequency of moulting depends on the amount and quality of food consumed, and also other factors like ambient water temperature.

Growth rates so far reported in the literature for *M. lar* indicate that juveniles can reach 20-40g/120 days or 0.25g/day (Nandlal 2010). Males generally grow to larger size than do females, and females tend to grow at a more uniform rate. This differential growth rate contributes to wide variation in size within populations, a pattern that also occurs in *M. rosenbergii* (Ra'anan & Cohen 1984b).

Types of culture systems

Freshwater prawns are normally cultured in earthen ponds supplied with freshwater under several types of monoculture, polyculture and in integrated systems. Monoculture systems can be extensive, semi-intensive or intensive and systems differ according to the level of intervention in the production process that may range from a production of less than 500 kg/ha/yr to in excess of 5,000 kg/ha/yr (Valenti & New 2000).

In extensive systems, various types of structures and systems are used and stocking density is usually quite low (1-4/m^2), with PLs or juveniles stocked that are generally wild-caught (Valenti & New 2000). Little or no control of water quality, growth or mortality is practiced and nearly all nutritional requirements are derived from natural sources, i.e., prawns in culture feed on natural food although sometimes natural productivity can be increased with application of organic fertilizers, animal and vegetable by-products or feed supplements (supplementary feeding is not normally provided). Harvesting of prawns is often difficult and inefficient, since most culture water bodies cannot easily be drained or cleaned before seine harvests. Construction and operating

costs are generally low and productivity is usually less than 500 kg/ha/yr (Valenti & New 2000). This simple type of farming or culture generally has a low environmental impact and requires only simple technology and minimal financial and physical infrastructure investment. Hence it is suitable for many rural farms in developing countries (Lin & Lee 1992). Extensive culture of freshwater prawns however, has become less common nowadays due to declines in availability of wild-caught prawn juveniles in many regions where they occur naturally.

Semi- intensive culture of freshwater prawns is carried out in ponds ranging in size from a few hundred square meters up to 2-3ha and is the most common culture system practiced in southeast Asia (Lee & Wickens 1992). Stocking is carried out after pond preparation mainly with hatchery produced PLs or juveniles. Stocking densities range from 5 to 20/m^2 (Lee & Wickens 1992), and produce yields from 500 to 5,000 kg/ha/yr (Valenti & New 2000). Formulated or farm-made feeds are usually supplied daily and ponds are fertilized at specific times. Predators and competitors are controlled where possible. Water quality, prawn health and growth rate are usually monitored across the production cycle. This method of farming has been practiced under a wide range of conditions and management strategies worldwide and the practice is still very popular especially in small rural farms where labour and energy costs are low. Feed costs can however, constitute from 40 to 60% of the total operational cost in grow out farms (Shang & Fujimura 1977) but in large farms, labour and energy costs are significantly higher, driving the component of feed cost lower (Correia *et al*. 2003).

Intensive prawn culture systems include culture in small earth ponds up to 0.20 ha (Valenti & New 2000) provided with a high water exchange rate, continuous aeration and supply of an artificial formulated feed (i.e. all nutritional requirements are met from external sources). Predators and competitors are eliminated and all water parameters are strictly controlled. Juveniles and PL's are stocked in densities above 20/m^2 and according to D'Abramo *et al*. (1989) stocking densities > 39.5/m^2 appear to be the most economical because a greater mean harvest weight of prawn can be realized. While construction and

operating costs are generally high, productivity in intensive culture can exceed 5,000 kg/ha/yr. Some studies have also suggested that productivity can be greatly improved by stocking juveniles rather than PLs (Willis & Berrigan 1977; Brody *et al*. 1980; Smith *et al*. 1981) and by separation of juveniles into weight classes prior to stocking, a practice that can further enhance total pond production (Ra'anan & Cohen 1983; Karplus *et al*. 1986a). This approach has been practiced, however, only at an experimental scale since the system is not easily compatible with many of the biological characteristics of *M. rosenbergii* particularly adult male aggressive territorial social behaviour combined with the fact that prawns vary widely in size at harvest.

In polyculture, prawns can be raised with one or more compatible species (e.g. tilapia, common carp, Chinese carps and catfish) in order to optimise pond production biomass using organisms with different feeding habits or that feed at a different trophic level or that have different spatial requirements. This system has been practiced for centuries and has the ability to increase total pond productivity via more efficient use of the natural food availability in ponds (Herpher & Pruginin 1981). The system utilizes available food resources e.g. tilapia and prawns feed on benthic detritus thus reducing biochemical oxygen demand in the complete water column (i.e., surface, pelagic and benthic) of the pond ecosystem efficiently, with the consequent effects of reducing costs (tilapia and prawns consume the faeces of common carp and grass carp) and this can increase productivity. Reports on the relative success of polyculture of *M. rosenbergii* with fish and other crustaceans however, vary. According to reviews by New (1990; 1995) polyculture can complicate the management of prawns in grow-out systems so outcomes can be variable. For example, reports show that presence of tilapia did not depress growth of prawns while others have suggested that tilapia reproduction can have an adverse effect on total prawn yield. Prawn-tilapia polyculture did however, provide a greater net return than did prawn monoculture. Stocking of *M. rosenbergii* juveniles weighing 0.25-0.50g at a density of $2/m^2$ with carps and tilapias has been recommended (Karplus *et al*. 1987; Hulata *et al*. 1988), while juveniles stocked at a density of $5\text{-}7.5/m^2$ in polyculture with Nile tilapia fingerlings stocked at 6000-7000/ha, yielded 1045 kg/ha

of prawns and 2700 kg/ha tilapia after 180 days with a net income of US$5,309/ha and US$4,095/ha, respectively (Mires 1987).

Integrated systems combine aquaculture with the farming of terrestrial animals and plants in various ways with the added advantage of reducing effluent, diversifying products and increasing productivity. For example, some systems utilize wastewater from fish-prawn polyculture for irrigation of trees and other crops raised on the bunds of ponds. In addition, rice-prawn and vegetable-prawn culture are now also commonly practiced in Vietnam (Zimmermann & New 2000). Wild-caught 2-5 g prawns are stocked in rice fields in peripheral trenches 2-3 m wide and 1 m deep at 1-2/m^2 during the planting of the rice crop. Prawns are fed with rice bran and harvests of 200-400 kg/ha/yr can be achieved, with a value of US$600-200 per cycle. This approach provides significantly higher returns to the farmers than just the rice crop (Zimmermann & New 2000) and this method has been practiced in nursery and grow-out systems in S.E. Asia for many years because of high returns, simplicity of culture and contribution to rural nutrition (Guerrero *et al*. 1982). Rice-prawn culture also generates better income returns than traditional rice-fish culture due to the higher price of prawns. Several species of prawns cultured with a variety of methods and combination of crops have been reported. Some studies indicate rice-prawn integrated farming can be made more efficient by employing polyculture, i.e., increased stocking density with supplemental feeding may enhance production and generate higher income; the nutrients derived from feed and metabolic wastes may also serve to fertilize the rice crop.

1.1.4 Why farm indigenous freshwater prawn species in Vanuatu

In Vanuatu a number of indigenous *Macrobrachium* species are known to occur naturally in rivers systems and inland waters and constitute freshwater prawn fishery resources. Among these species, *M. lar* is the most common and widely occurring species. Demand for *M. lar* exceeds supply and wild stocks are consequently often over harvested. In many remote rural areas, *M. lar* provides an important source of cash income and has important

economic and cultural significance but as catches from the wild have declined, local people cannot access the resource. The economic value of this species to Vanuatu and in general to PICTs has led to increasing pressure to conserve wild stocks and to develop stock enhancement and to trial culture practices (SPC 2002).

Recent successful trials on *M. lar* culture have indicated that this species is a viable candidate for aquaculture (Vanuatu Aquaculture Plan 2008-2013). In order to meet increased demand for fisheries products aquaculture across the region will need to grow and this needs to be carried out in a sustainable manner with an emphasis on simple systems, and where possible, farming indigenous species. This is because indigenous species in general pose little threat to natural ecosystems (New *et al*. 2000a), as opposed to an introduced species that can escape causing negative ecological impacts by dissemination of pathogens and establishment of exotic (feral) populations that may negatively impact on populations of native species (Valenti & New 2000; New *et al*. 2000). For example, there are reports of feral populations of *M. rosenbergii* becoming established following escapes from farms in South America (New *et al*. 2000a & b). While no adverse environmental impacts have been reported so far there, since natural populations of *M. rosenbergii* have been established in some rivers, establishing farming in new locations has been opposed due to possible environmental risks to the biodiversity of native freshwater prawn species should it (*M. rosenbergii*) escape (Graziani *et al*. 2003). In countries where *M. rosenbergii* is not indigenous however, development of the culture of native *Macrobrachium* spp. has been encouraged. Some examples include farming of *M. nipponense* in China, grow-out trials on painted river prawn, *M. carcinus*; Amazon River prawn, *M. amazonicum* and cinnamon river prawn and *M. acanthurus* in South America (Holthuis 2000; Kutty *et al*. 2000), and recent pond grow-out trials of *M. lar* integrated with wetland taro in Vanuatu.

Where indigenous species are available, they are also generally the preferred option for culture over exotic species because they constitute natural components of ecosystems and, as such, are not a potential threat to native wildlife. People in Vanuatu also generally

prefer *M. lar* and thus demand is high for local species. In addition, people have depended for long periods of time on local environments for the provision of a variety of resources including *M. lar* so they have developed a stake in conserving, and in some cases, enhancing biodiversity. They are also increasingly aware that biological diversity is a crucial factor in generating the ecological services and natural resources on which they depend. In general, people with a historical continuity of resource-use practices (e.g., collection of *M. lar* from the wild) often possess a broad knowledge base about complex ecological systems in their own localities. Thus species that enjoy a high local demand and where some local knowledge also exists may also have good potential to contribute to expansion of production. Therefore, efforts should be made where possible to develop production technologies for indigenous species that have recognized local demand and associated local knowledge. The development of production techniques for local species and their successful culture may also help to protect natural prawn populations that are already threatened in many places by unsustainable fishing practices. Studies of indigenous species in culture are needed therefore, to assess their commercial viability and to develop appropriate farming technologies and contribute to sustainability of wild stocks of farmed species.

M. lar has desirable ecological traits for culture in Vanuatu because of its relative large size compared with other *Macrobrachium* species present there. Investigations into potential for farming *M. lar* began in Vanuatu in mid 2005. The species was already known to have been reared on a subsistence basis in combination with wetland taro on the northern islands of the country, including Maevo, Pentecost and Santo. *M. lar* fetches US$10-15 /kg and is sold mainly to restaurants in Efate and Santo. Meat is also virtually indistinguishable from that of the high value exotic *M. rosenbergii* when cooked in a similar way. In Vanuatu, this species also has important cultural and economic significance. As the largest species in the genus that occurs naturally there, *M. lar* is present in almost all freshwater systems provided they are connected to the sea. The

species is usually harvested extensively from the wild except in some localities where it is grown in simple culture ponds in wetland taro farms with other aquatic vegetables.

Simple aquaculture of *M. lar* originated a long time ago along with wetland taro farming (Nandlal 2010) and this technology has been improved over the years. Juveniles and prawns of all sizes enter ponds or wetland taro farms with the natural flow of water or are brought in by stream flow or flood waters. This method is simple and appears to take advantage of the peculiar aspects of the biology of prawns in the family- Palaemonidae. With returns from marine fisheries also declining significantly in Vanuatu and in PICTs, many island nations are looking to develop sustainable aquaculture industries to supplement their fish protein requirements (Bell *et al*. 2009). Marine shrimp culture in the Pacific is limited to New Caledonia, Fiji, Vanuatu and Tahiti, and exotic freshwater prawn (*M. rosenbergii*) culture has been developed in Fiji (Nandlal 2010). There is, however, a large market for fresh prawns for the tourist trade, for export and for local sales and consumption in Vanuatu, so *M. lar* could have potential in culture there. But before a freshwater prawn aquaculture program is developed in Vanuatu that encompasses over 80 islands, it is important to address a number of important issues namely: at what scale should sustainable production of *M. lar* species be based and is *M. lar* a productive culture species? This can be encapsulated in the question ‘what is the best choice of freshwater prawn species for Vanuatu’?

While *M. lar* is still abundant in the wild and is important to both commercial and recreational fisheries across Vanuatu, preliminary work has suggested that it may not be an easy species to grow under pond conditions (Nandlal, 2010). For decades *M. lar* was thought to be impervious or not affected by overfishing due to a high natural recruitment rate, but recent reports of overfishing have raised concerns about health of some natural populations (Nandlal 2005). Like many species, *M. lar* has unique life history traits that can lead to predictions about natural population structure. While information on the extent of genetic differentiation in *M. lar* is available for the region, i.e., at a broad geographical scale, there is a lack of information however, at more local scales. *M. lar*

spawns inland and the larvae flow with the stream currents to the ocean where they go through many pelagic stages over a 3-month period (Atkinson 1977) before re-entering estuaries, following which they return to inland waters. Field observations and experiments have shown that as adults *M. lar* do not undertake extensive migrations in streams (Nandlal 2010). From these observations one might expect broad genetic homogeneity across the species' natural range. There are, however, some factors that could lead to low effective population sizes, local inbreeding or genetic drift impacts at either local or broad scales. For example, use of poisons, destructive fishing methods and drought can kill large numbers of the broodstock or the entire population present in a stream, resulting in a drastic decrease in prawn catches (Nandlal 2010). Recovery from population crashes relies on the high fecundity of *M. lar* (mean fecundity of 11,416) where large females can produce over 20,000 eggs (Nandlal 2010). In addition, our current knowledge of the scale at which broodstock should be sourced from the wild so as to capture sufficient genetic diversity to produce a strong genetic base for a culture industry while at the same time not mixing genetically discrete stocks, has yet to be examined. Thus, for *M. lar* or any new species being developed for culture, we need to understand at what scale natural populations are divergent to limit any impacts that hatchery and grow-out operations may have on wild populations.

While optimization of early life history environments forms the basis for development of economically viable production systems for most aquatic species, for a small country like Vanuatu, if the target species is indigenous then hatchery produced larvae could also be used for replenishment and enhancement of wild stocks while providing the foundation for a future freshwater prawn culture industry. Supplementation of 'seedlings' for stocking ponds and wild stocks has already been attempted in the Indo-Pacific region. Numerous reports have indicated that aquaculture and stock enhancement needs to be continued and expanded as human impacts on inland environments increase (Nandlal 2012a & b; SPC 2002, 2007). Therefore, before any serious attempt is made to develop *M. lar* or *M. rosenbergii* culture in Vanuatu, it is important to find out if *M. lar* and *M.*

rosenbergii can be grown productively under similar pond conditions to allow development of appropriate technologies for grow-out in ponds.

Results of the current study can contribute to development of pond rearing methodologies for both *M. lar* and *M. rosenbergii* and may also be relevant to development of pond grow-out techniques for other indigenous freshwater and brackish water species (in mixed culture or even polyculture) for which rearing conditions are yet to be trialled (e.g. there are a number of other *Macrobrachium* and *Palaemon* species in the Pacific considered by some people to have culture potential). Data obtained from the study on conditions for survival, development and growth in ponds of *M. rosenbergii* and *M. lar* and wild stock structure of *M. lar* can provide the basis on which to select the best candidate species for culture in Vanuatu in the future.

1.2 Aims and objectives

The specific research questions that will be addressed in the study include:

i) At what scale do wild *M. lar* disperse and re-colonize freshwater habitat (to be inferred from genetic (mtDNA data) and hence should be managed for wild stock enhancement and potential future aquaculture development?

ii) What are the basic environmental parameters required for growing juvenile wild *M. lar* and hatchery-reared *M. rosenbergii* to market size in small scale ponds?

iii) Can reasonable growth rates for *M. lar* and *M. rosenbergii* be achieved in simple pond culture systems in Vanuatu?

These questions will be addressed by:

(i) Characterizing the levels and patterns of genetic diversity in wild *M. lar* stocks across Vanuatu to identify discrete gene pools that may need to be managed independently in restocking programs or as hatchery stocks for aquaculture in the future there.

(ii) Undertaking grow-out trials of wild-caught *M. lar* juveniles and hatchery-reared *M. rosenbergii* juveniles to assess relative growth rates in simple pond culture systems.

Characterization of population genetic structure of wild *M. lar* stocks was addressed by screening mtDNA haplotype diversity across the country that also included out-group samples collected from Fiji and the Cook Islands. As pond grow-out of *M. lar* and *M. rosenbergii* species have been documented sufficiently in earlier studies, the current study focussed on identifying parameters that affect growth rate of wild caught juveniles of *M. lar* and hatchery reared juveniles of *M. rosenbergii* in Vanuatu. Grow-out trials of juveniles in ponds were undertaken in Vanuatu as part of the author's major study while genetic studies were undertaken as part of Vanuatu Fisheries Department (VFD), Australian Centre for International Agricultural Research (ACIAR) and Secretariat of the Pacific Community (SPC) collaborative research where the author here was a contributing scientist.

1.3 Thesis overview

This thesis is organized into 4 chapters. The first chapter presents a general argument about the need for the research project and a justification for trialling aquaculture of *M. lar* and *M. rosenbergii*. Chapter 2 describes an analysis of patterns of genetic diversity in wild *M. lar* populations collected across Vanuatu Islands (and Fiji Islands and Cook Islands) and how this has implications for development of *M. lar* aquaculture and stock enhancement, while Chapter 3 details the methods used and results of grow out experiments on wild caught *M. lar* juveniles and hatchery-reared *M. rosenbergii* juveniles in simple pond systems in Vanuatu. The final chapter summarises the findings and makes an assessment about the potential choice of either *M. lar* or *M. rosenbergii* as an aquaculture species in Vanuatu while suggesting directions for future research.

2.0 Evaluation of the scale at which wild populations of *Macrobrachium lar* in Vanuatu are differentiated

2.1 Introduction

The 'monkey river' prawn (*Macrobrachium lar*) has one of the most extensive natural distributions across tropical and subtropical waters of any primary freshwater prawn species. *M. lar* occurs on islands and on continents from French Polynesia in the central-western south Pacific in most island chains across the western Pacific to New Guinea and Australia, north to the Ruyuku Islands in southern Japan, across SE Asia and South Asia to Madagascar and across to the east coast of Africa (Holthuis 1980; Maciolek 1972; Short & Meek 2000). This species has also been introduced to the Hawaiian Islands where it has become an exotic pest. This is one of the most remarkable natural distributions reported for any primary freshwater invertebrate species that likely results from a unique life cycle.

M. lar like many *Macrobrachium* species is amphidromous where individuals migrate across biome boundaries from freshwater to saltwater at specific stages of their lifecycle (McDowell 2007). Unlike many amphidromous *Macrobrachium* species however, *M. lar* adults and juvenile prawns are confined to freshwater water bodies while their pelagic larvae spend relatively long periods of time drifting in the marine environment. The life cycle requires mating in freshwater following which females release their larvae into freshwater streams and the larvae then drift passively to the sea. Larvae can then spend up to an estimated three months passing through numerous (>11) larval stages before they must find and colonise freshwater streams so that they can complete their metamorphosis to the juvenile stage and return to freshwater stream habitats.

Thus the lifecycle includes a freshwater phase, a brief brackishwater phase and then a marine phase before individuals return to freshwater. Given the very extensive marine larval phase (longer than any other known *Macrobrachium* species), it would appear that the primary dispersal phase is the time planktonic larvae spend drifting passively in the ocean and it is this extended period of larval drift that potentially can explain why *M. lar*

has achieved such a remarkably wide natural distribution and is capable of colonising remote freshwater environments. Thus, unique life history traits would predict high connectivity between populations in widely dispersed freshwater habitats across two oceans (Pacific and Indian Oceans).

An extended marine larval phase in theory, offers potential for extensive geographical dispersal but potential to colonise new freshwater habitats must also be influenced by factors external to the species and include; the direction of prevailing winds and water currents in the oceans, mean water temperatures (*M. lar* is a tropical/subtropical species) and the probability of contacting land where appropriate freshwater streams are available to colonise at the right developmental stage within the larval cycle. Given that both the Pacific and Indian oceans have very large expanses of open water dissected by only very small landmasses (island chains) widely dispersed in very large areas of open ocean, most larvae will fail to find sites to colonise during the critical developmental period before they must metamorphose into juveniles in freshwater. Since however, natural populations of *M. lar* are still large in many localities and individual females can produce up to 20,000 eggs routinely per spawning event and can spawn multiple times, a large larval resource is therefore available at any one time to colonise freshwater habitats. These factors would suggest high dispersal connectivity between even very disjunct freshwater sites combined with low probability that individual larvae will be successful colonists.

In the southern hemisphere of the Pacific Ocean, prevailing major water currents and major winds (south-east trade winds) flow essentially from east to west, suggesting that passive larval gene flow in the marine environment is also likely to be primarily from east to west. Mean water temperature is also a critical factor for ectotherms like *M. lar* because larval development will slow and then stop as temperature declines, so where larvae are dispersed to areas of relatively low marine water temperature, even if appropriate freshwater habitats are available there, they are unlikely to survive and colonise these habitats.

As stated above, *M. lar* occurs naturally in freshwater streams with connections to the sea in most western Pacific island chains including the focus of the present study, the islands of Vanuatu. Traditionally *M. lar* is an important natural food resource there, among islands where freshwater faunas are depauperate and there are very few alternative indigenous protein resources in streams available to local people. *M. lar* is an important high value, 'bush food' on many islands in the western Pacific and as a consequence has cultural significance to many native people. Some (particularly native women) also use harvesting of wild freshwater prawns to supplement their incomes, so *M. lar* contributes to both lifestyles and the indigenous economy in many places. While another exotic species of freshwater prawn, *M. rosenbergii* (giant freshwater prawn or GFP), is the most-favoured freshwater prawn species used in aquaculture widely around the world, because *M. lar* is indigenous to the Pacific region and has cultural significance in many places it has been considered for development in farming, either via collection of wild juveniles for pond grow out in simple culture systems (e.g. taro/prawn simple polyculture) or potentially, if domesticated stocks can be developed and an efficient hatchery process optimised, it could offer a natural, indigenous alternative to farmers and reduce the need to import exotic culture stocks. Before this could be considered however, it is first important to determine at what spatial scale such an industry (ies) should be based. Specifically, it is important to assess the geographical scale at which wild populations have been exchanging genes via effective dispersal naturally, so as to avoid potentially contaminating local gene pools if 'foreign alleles' escape into wild stocks from hatcheries that have produced larvae from non-indigenous strains. This is referred to as 'genetic contamination' and could result from either escape of farmed stock from captivity, translocations or restocking of natural environments from a non-native hatchery stock. This has become an increasing problem as aquaculture has developed in many places and the result can be the loss or homogenisation of wild genetic resources often before they are well documented or harvested for breeding and stock improvement programs. Patterns of diversity in marker genes can be used to assess degree of panmixus/differentiation among sample sites where out-group sites are available as a reference and the information used to infer the scale at which populations should be

considered to be a single as opposed to multiple management units. Essentially this constitutes a phylogeographic approach to resolving the scale at which populations should be managed and farmed to conserve patterns of wild diversity.

Phylogeography emerged as a new science in the 1980s because it can act as a bridge between historical processes that produce diversity and contemporary patterns of genealogical lineages based on geographical distributions. Avise (2000) defined phylogeography as; “the field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species". Essentially phylogeography is a sub-discipline of biogeography that studies variable patterns of genealogical lineages and correlates this with natural geographical distributions at the intraspecific level in order to test hypotheses about the history of populations over evolutionary time (Avise 2000; Avise *et al*. 1987; Arbogast & Kenagy 2001; Bermingham & Moritz 1998). Basically, phylogeography deals with two fundamental axes: space and time, to jointly map targeted genealogical genes (Avise 2000; Bermingham & Moritz 1998; Freeland 2005). Such analyses require comprehensive input from a variety of disciplines including; population genetics, molecular biology, demography, evolutionary geography and geology. From a molecular perspective, DNA sequences provide an important resource for inferring relationships between biotic and earth history. DNA sequences can be used to infer the current patterns of diversity in species or populations as well as their geographical distributions that are affected indirectly by evolutionary processes in deep time (e.g. speciation, habitat adaptation and biogeographical history) and in shallow time (e.g. range shifts).

Phylogeographic analysis has been used for many applications including to reveal the extent of relatedness among cryptic species by identifying genetic boundaries (Palumbi 1996, 1997; Arbogast & Kenagy 2001; Bermingham & Moritz 1998). The approach has also been useful for identifying the origin of exotic species (e.g. invasive or introduced species) and as a way of tracing their paths of colonization and has also proven valuable

for distinguishing between indigenous and introduced (by humans) populations (Freeland 2005; Beebee & Rowe 2008; Palumbi 1996). In addition, phylogeography is also a powerful tool for assessing the timing of population divergence. This crucial role permits us to detect correlations between external or internal factors that have influenced species formation. Phylogeographic studies are also useful for providing a better understanding of the environmental factors that impact gene flow among populations that can influence species formation (Palumbi 1997; Encalada *et al*. 1996; Arbogast & Kenagy 2001; Avise 2009).

Dispersal and vicariance are considered to be significant opposing mechanisms that affect phylogeographic patterns that will ultimately determine population structure (Encalada *et al*. 1996; Arbogast & Kenagy 2001). Molecular data assessed in a phylogeographic context can be used to reveal if dispersal has homogenized a population by gene flow (i.e. producing low genetic differentiation), or if vicariance has isolated previously connected populations (i.e. resulting in high genetic differentiation) (Avise *et al*. 1987; Avise 2000). Phylogeographic inference depends therefore, on evaluating genetic variation patterns that either enhance population connectivity or restrict natural gene flow (Balloux & Lugon-Moulin 2002; Avise 2009). Dispersal has the capacity to introduce new alleles and/or to change the frequencies of existing alleles in populations (Bohonak 1999; Johnson & Gaines 1990). Therefore, gene flow can promote genetic diversity in wild populations (Freeland 2005) and will limit divergence among them.

Genetic drift in contrast, is considered to be an opposing force to gene flow. It is often the most powerful force that can erode genetic variation, particularly in small populations. Therefore, its impact is usually a function of effective population size (*Ne*) overtime. Genetic drift can be defined as random changes in allele frequencies from one generation to the next that results from sampling error (Freeland 2005). Such outcomes occur, from random processes, when more surviving offspring are produced by chance from a non-random subset of individuals in the population. This can result in loss of some alleles in the next generation or even random fixation of particular alleles, processes that can

reduce genetic diversity levels in small populations over time (Slatkin 1987; Bohonak 1999; Cook 1976; Allendorf 1983; Freeland 2005; Allendorf & Luikart 2007). Other forces that need to be considered in phylogeographic studies include; mutation (a mechanism for generating new alleles) and various kinds of natural selection where specific phenotypes are either favoured or are not favoured under certain environmental conditions.

It is apparent therefore, that differences in the amount of genetic diversity present within and among populations is a function potentially of many forces generated from the combined effects of gene flow, genetic drift, mutation and various forms of natural selection. Genetic drift, mutation and natural selection generally enhance divergence among sub-populations while gene flow has the opposing effect by homogenizing diversity across populations and inhibiting subpopulation development (Slatkin 1987; Bohonak 1999; Allendorf 1983; Balloux & Lugon-Moulin 2002).

Gene flow can be constrained when there is a barrier (e.g. physical or biological) to dispersal that in turn disrupts population connectivity. This potentially will result in populations becoming isolated and subsequently evolving independently. Consequently, such changes in allele frequencies will enhance divergence among populations over time (Bohonak 1999; Palumbi 1996; Balloux & Lugon-Moulin 2002; Freeland 2005).

The extent of gene flow differs remarkably among various taxa depending on both external (extrinsic) and internal (intrinsic) behavioural factors (i.e. life history traits) (Slatkin 1985; Johnson & Gaines 1990; Planes, Parroni & Chauvet 1998). The level and patterns of intraspecific population structure is influenced largely by a species' life-history traits (Bay *et al*. 2004). For example, populations of many freshwater species are often structured at the level of among streams and/or drainages. This results, from their stenohaline nature that limits dispersal and consequently obligates them at fine spatial scales (Bohonak and Jenkins 2003). In contrast to most freshwater species, many terrestrial and marine organisms often show shallow population structure due to extensive

dispersal by their zygotes and/or gametes via various dispersal vectors (e.g. wind and water currents etc.) (Jenkins *et al*. 2007).

A general perception widely held about population structure in marine species is that they often exhibit less spatial variation among populations than that present in equivalent freshwater or terrestrial species (Bernardi 2000). This is due to greater potential to disperse large distances and also that in the marine environment, barriers hindering gene flow are often less common or even absent (Graves 1998). This can produce genetic homogeneity among populations of many marine species as has been reported for Atlantic cod (Pogson *et al*. 2001), some sessile taxa (Dias, Duarte & Solferini 2006), reef fishes (Lessios and Robertson 2006) and gastropods (Kano & Kase 2004).

A larval dispersal phase is a common characteristic of many marine species that can strongly influence population connectivity and natural distribution patterns. For example, many aquatic species that possess relatively long-lived pelagic larval phases tend to exhibit extensive natural distributions, low genetic differentiation among populations and high rates of gene flow due to extensive dispersal. Therefore, the length of the pelagic larval stage is a critical factor that can affect population structure and is often correlated positively with the extent of individual dispersal and negatively with recognition of discrete populations (Bohonak 1999; Bay *et al*. 2004). For instance, some mollusk species have high potential to disperse (including many oyster, sea urchin and starfish taxa) because they have long-lived planktonic larval phases (e.g. pelagic eggs or larvae) that is manifested by essentially passive movement (dispersal) in water currents for time intervals from several days to weeks or even longer time frames. Potentially this can result in dispersal over large open oceanic distances (even between oceans) and consequently this can homogenize populations when compared with some sedentary species that lack a pelagic larval phase (Bohonak 1999; Freeland 2005; Jenkins *et al*. 2007). Some studies have shown however, that trans-oceanic dispersal patterns can often be much more complex and non-intuitive than had previously been anticipated, as a result of the existence of unseen barriers to dispersal and/or behavioural traits that affect gene

flow across many habitats and regions of open ocean (Freeland 2005; Bohonak 1999; Bay, Crozier & Caley 2006; Doherty, Planes & Mather 1995; Planes, Parroni & Chauvet 1998). A diverse array of marine taxa while possessing long lived larval phases have shown limited connectivity among populations including, some marine invertebrates (Sotka 2005), and starfish (Williams & Benzie 1998). Tracing dispersal patterns in some taxonomic groups across vast areas of open ocean was often a difficult task before the development of molecular markers as a method for estimating indirectly, levels and patterns of genetic diversity (Freeland 2005).

Vanuatu Fisheries Department (VFD) is currently trialling development of freshwater prawn aquaculture in the country and this could involve either farming the exotic *M. rosenbergii* and/or the indigenous *M. lar.* If farming *M. lar* proves to be a viable option then initially it will require collection of juveniles from natural streams for grow out in ponds until the life cycle is closed for this species in captivity and appropriate hatchery practices can be developed. To avoid potential for genetic contamination of wild populations by either escape of wild-caught juveniles from ponds or hatchery-reared individuals of different genotype to local wild stocks, it is first important to determine if wild populations of *M. lar* in the target area are differentiated and are genetically structured geographically in the wild. So the aim of the present study was to assess population structure of wild populations collected from five sites on the two main islands of Vanuatu, and to compare the patterns of variation in a mtDNA gene marker with that of reference populations collected from sites at distance in the western Pacific region (Fiji and the Cook Islands). Previous unpublished work at QUT (Wyatt, Hons. thesis 2001 unpubl.; Stephan Hons. thesis 2004 unpubl.; Hunter MSc, 2011 unpubl.) had shown that *M. lar* populations in both the Fiji Islands and Cook Islands were essentially panmictic respectively, but belonged to two distinct genetic clades indicating that they had not exchanged genes for a considerable period of evolutionary time (based on molecular clock calibration), and so constituted independent management units. Currently the status of populations in Vanuatu is unknown.

The genetic analysis of wild *M. lar* populations in Vanuatu was conducted as part of a SPC funded project directed at evaluating population structure and levels of divergence to assess the scale at which a local culture industry should be established to avoid potentially contaminating discrete gene pools. The author (Sompert Gereva) was a member of the research group engaged in this project and contributed to sampling design, collection of samples and analysis and interpretation of the genetic data. The molecular analysis was conducted with technical support from Queensland University of Technology (QUT).

2.2 Methodology

2.2.1 Study area and sampling sites

M. lar wild populations were sampled from seven sites in the western Pacific namely: five sites on two islands in Vanuatu (Rentapao, Sarete and Warore-East Santo I, Warore-East Santo II, Epao-North East Efate) by the author, SPC and VFD officers and a single reference site from both Fiji, and the Cook Islands that were available from previous studies of *M. lar* diversity across the western Pacific region (collected by Dr Satya Nandlal). Sampling was carried out from natural water bodies to assess *M. lar* wild populations. A brief description of site including country and site name are given in Table 2.1.

Table 2.1: Details of sampling sites.

Country	Site name	Code	Description
Vanuatu	Rentapao	RP	(S17.78511 E 168.45117 and S 17.78071 E 168.44803)
	Sarete	SR	(S 15.57396 E 166.99896)
	Warore, East Santo I	WR	(S 15.29546 E 167.16090)
	Warore, East Santo II	WO	(S15 18'47.21", E 167 09'45.53"
	Epao N.E Efate	EF	(S17 38'55.31", E168 13'.98"
Fiji Islands		BUA	(S16.59, E179.1)
Cook Islands		ATIU	(S19.59, W158.6)

2.2.2 Sampling

Samples were collected from three sites on Santo Island and from two sites on Efate Island in Vanuatu. In addition reference samples from the Fiji Islands and Cook Islands were available at QUT from previous studies (Figure 2.1a & b). Pleiopod or muscle tissue was taken from approximately 50 individuals at each site, and tissue preserved in 70% ethanol for genetic analysis. Initially, 24 individuals chosen randomly from each site were screened for their individual mtDNA CO1 haplotype, because only 23 individuals were available from the Warore site. This strategy was employed to determine the minimum haplotype diversity level per site based on the site with the lowest number of individuals with sample sizes able to be increased later, if required.

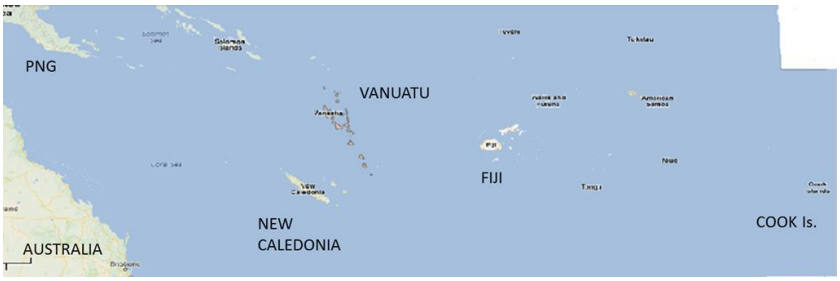


Figure 2.1a: Regional sample sites in the Pacific (Vanuatu, Fiji, and Cook Islands).

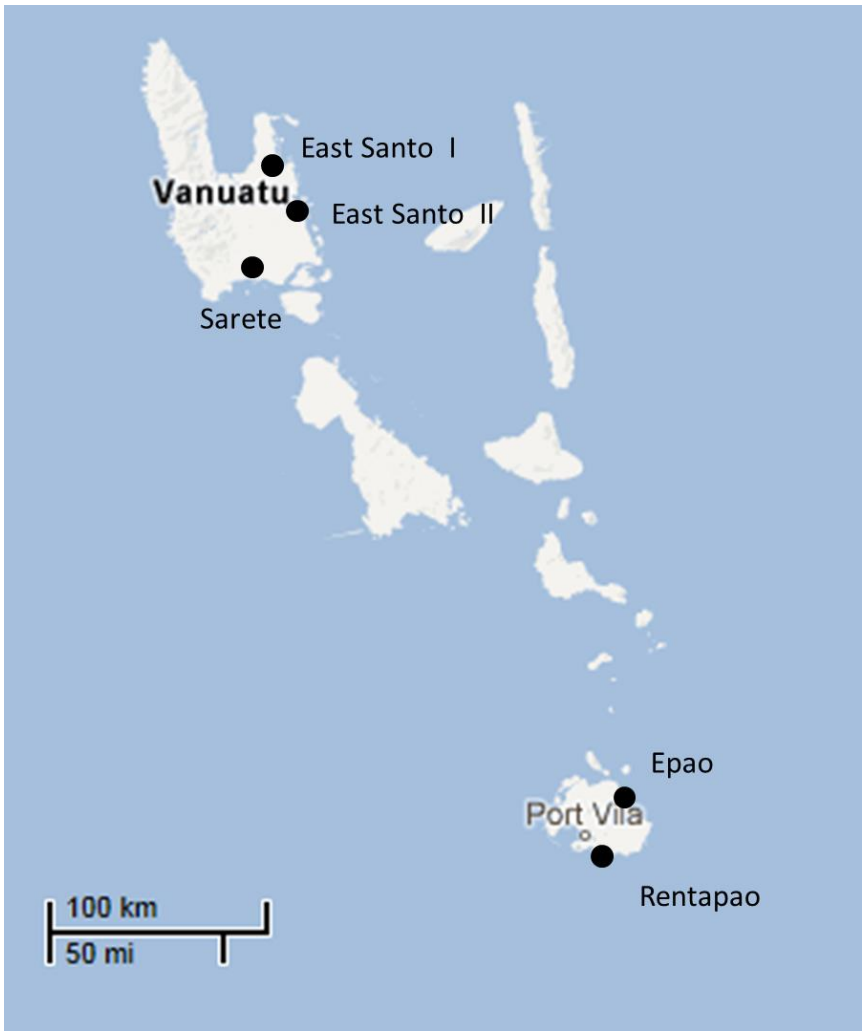


Figure 2.1b: Sampling sites in Vanuatu Islands

2.2.3 Genotype screening

Total genomic DNA was extracted from pleiopod or white muscle using a modified salt extraction method (Miller *et al*., 1988). Tissues were treated with proteinase *K* to enhance tissue digestion. Following DNA extraction, PCR amplification was carried out for a mtDNA Cytochrome oxidase (COI) fragment for each individual using internal primers MLF (5' – TCAACATACGATCCCCAGGT – 3') and MLR (5' – CTATTCTGATTCTTCGGCCACCC -3') (Duffy pers. comm.) modified from Palumbi (2002) primers. Mitochondrial PCR reactions consisted of 2.5µl Roche 10X buffer, 2.0µl 25mM Fisher $MgCl_2$, 1.0µl Roche deoxy nucleotide tri phosphate (dNTP), 0.5µl 10 mM primer, 0.1µl Roche Taq DNA polymerase, 1µl DNA template to a final volume of 25µl

with ddH_2O. PCR conditions were, 5 minutes at 94^0C for initial denaturation, then 30 cycles of 30 seconds at 94^0C, 30 seconds at 50^0C annealing temperature, 30 seconds at 72^0C, with the final extension step 8 minutes at 72^0C.

Amplified PCR products were checked on 1% agarose gels stained with 'gel red'. PCR products were then purified for direct sequencing using QIAGEN PCR Purification kits. Concentration of purified PCR products were measured by running 3µl in a 1% agarose gel with a concentration standard. Sequence PCR mixture consisted of ~600ng of purified PCR products, 3.5µl sequencing buffer, 1µl of 3.2 pmol/µl MLF forward primer, 1µl Big Dye terminator and ddH_2O to a total volume of 20µl. A specific PCR program was used for sequencing; initial denaturation at 94^0C for five minutes, followed by 29 cycles of 96^0C denaturation for 10 seconds, 50^0C annealing for 5 seconds and 60^0C extension for four minutes. Sequencing was carried out using an ABI 3500 DNA sequencer in the Molecular Genetics Research Facility (MGRF), at QUT. Sequences were checked in CHROMAS (version 2.1.3, http//www.technology. com.au/chromas.html) and then edited and aligned using the BIO-EDIT (version 5.0.6) sequence alignment editor (Hall 1999) software.

2.2.4 Data analysis

mtDNA analysis

All unique COI sequences detected in the sampled individuals from the seven sites were aligned and mutation positions identified and used to define the total number of unique haplotypes present and their respective sequences.

2.2.5 Phylogenetic analysis

A neighbour-joining (NJ) phylogenetic tree (Saitou & Nei 1987) was constructed in MEGA version 4.0.2 (Tamura *et al*. 2007) to characterise relationships among all unique haplotypes detected in the sequence analysis.

2.2.6 Genetic diversity

Relative haplotype diversity was assessed initially as relative mtDNA haplotype frequencies at each site.

2.2.7 Genetic differentiation and gene flow

Pairwise Φ_{ST} analysis was carried out between all site pairs to assess the levels of genetic differentiation between sites in ARLEQUIN version 3.11 (Excoffier *et al*. 2005). Φ_{ST} applying the Tamura and Nei distance method is an analogue of *F*st (sequence data) that estimates degree of genetic differentiation between population pairs that incorporates both haplotype frequency heterogeneity and divergence when partitioning genetic variation within and among populations. Φ_{ST} estimates range from 0 (complete sharing of haplotypes at identical frequencies) to 1 (no sharing of haplotypes).

2.2.8 Haplotype network & evolutionary history

A mtDNA parsimony cladogram of haplotypes (haplotype network) was constructed (95% level of connectivity) using TCS version 1.18 (Clement *et al.* 2000). Haplotype networks reconstruct the genealogical history of haplotypic variation and illustrate the evolutionary relationships among unique haplotypes, and include the amount of divergence between individual haplotypes (number of base pair mutational steps). So a mtDNA parsimony cladogram can provide information on the demographic and geographical history of a population and includes information about population expansions, bottlenecks etc. Haplotype frequency and sample site information were incorporated into the network to illustrate the distribution of haplotypes among sites.

2.3 Results

2.3.1 mtDNA data and genetic diversity

After poor sequences were excluded, final genetic analyses were conducted on a total of 37 individuals from 7 sites (namely; Rentapao, Sarete, Warore, East Santo, Warore, East Santo, Epao, N.E Efate, Fiji Islands, and the Cook Islands). MtDNA COI haplotype sequence data produced an alignment of a 589 bp fragment. Four *M. lar* individuals sampled from the Cook Islands and three individuals from the Fiji Islands were included

in the analysis as reference out groups. A number of individuals from sites in Vanuatu did not amplify successfully or produced highly divergent haplotype sequences that could not be connected to the *M. lar* network. They were inferred to belong to cryptic *Macrobrachium* species that had not been identified correctly as *M. lar* based on external morphology at the time individuals had been sampled in the wild and so these sequences were excluded from the analysis.

All *M. lar* sequences grouped into 26 unique haplotypes. Of the 26 unique haplotypes, 23 haplotypes were singletons (only occurred in a single individual). The distribution of each unique haplotype at each site is shown in Table 2.2 Figure 2.2 contains pie charts of haplotype frequency distribution at each site.

2.3.2 Phylogenetic data

All haplotypes from Vanuatu sites and Fiji grouped into a single shallow clade indicating that they were all closely related (Fig 2.3). In contrast, the Cook Islands samples were highly genetically divergent from the remaining samples and formed a separate clade as identified earlier for Fiji vs. Cook Island samples by Stephan (2004). The minimum number of mutational steps (bps) that separated the most closely related Vanuatu/Fiji haplotype to a Cook Island haplotype was 35bp (~6%) while no Vanuatu or Fijian haplotype differed by more than 12bps (2%).

Table 2.2: Haplotype distribution among sites (Vanuatu, Fiji, and Cook Island)

Haplo #	RP	EF	SR	WO	WR	COOK	FIJI	n
1		1	4	2	2			9
2	1	1		1				3
3	1	1						2
4		1						1
5					1			1
6							1	1
7							1	1
8							1	1
9		1						1
10	1							1
11	1							1
12	1							1
13	1							1
14	1							1
15			1					1
16	1							1
17	1							1
18					1			1
19			1					1
20		1						1
21		1						1
22						1		1
23						1		1
24						1		1
25						1		1
26					1			1
	9	7	6	3	5	4	3	37

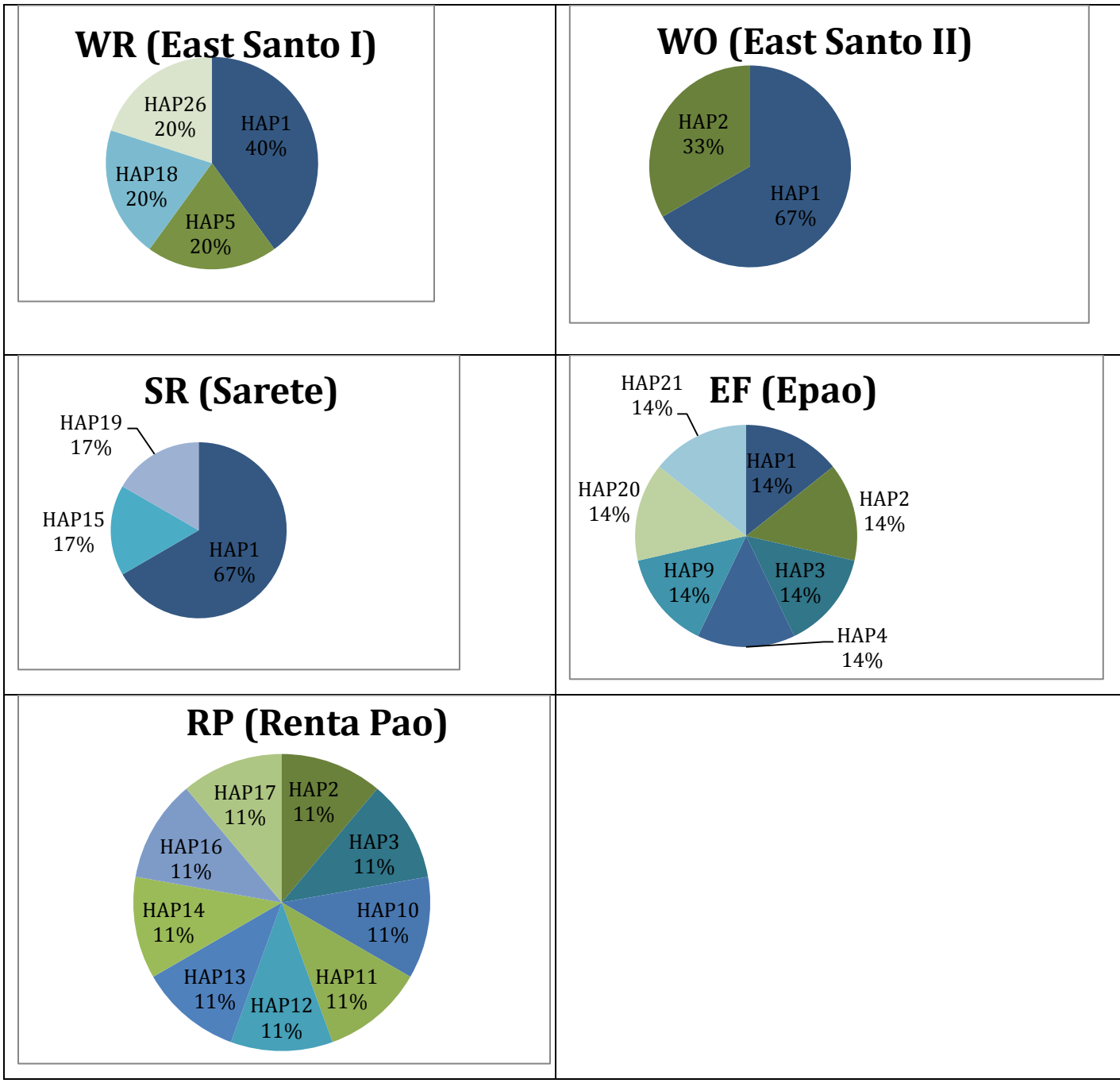


Figure 2.2: mtDNA haplotype frequency distribution at each sampling site in Vanuatu.

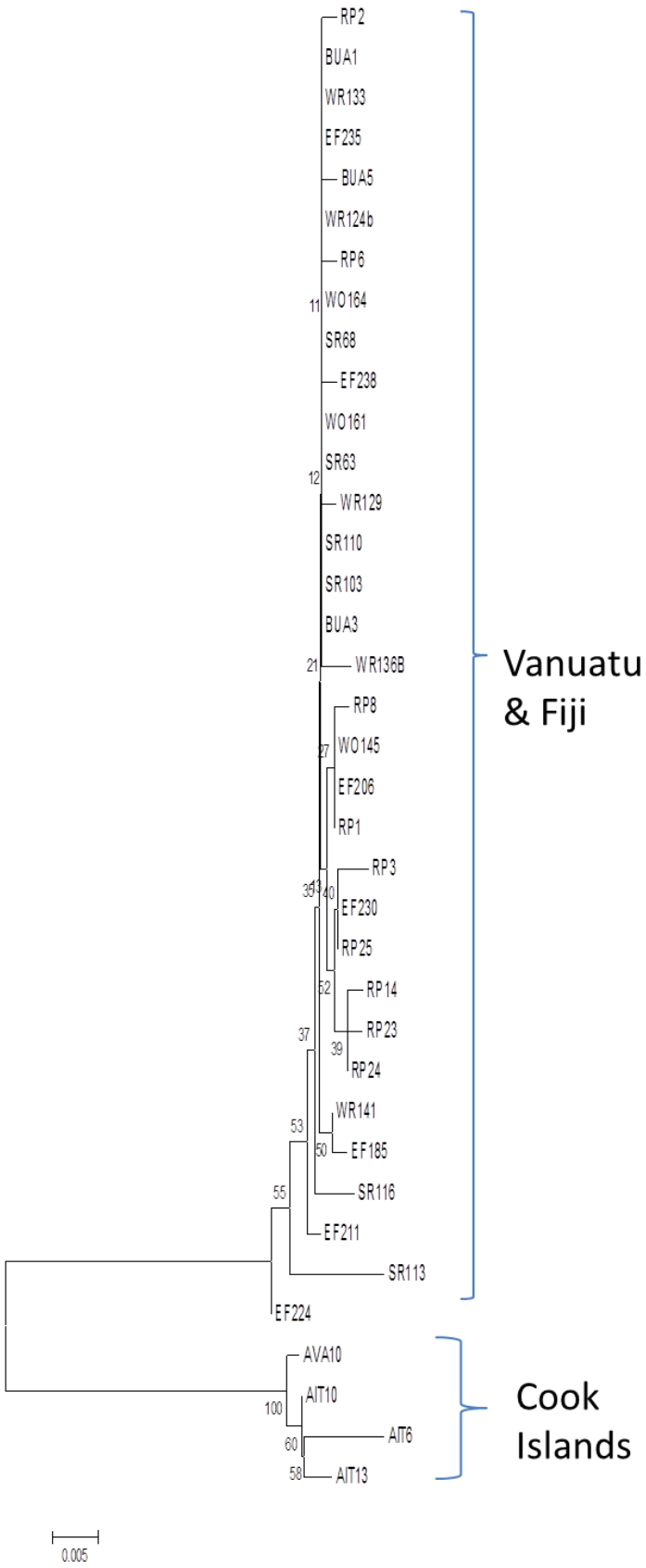

RP2
BUA1
WR133
EF235
BUA5
WR124b
RP6
11
WO164
SR68
EF238
WO161
SR63
12
WR129
SR110
SR103
BUA3
21
WR136B
RP8
27
WO145
EF206
RP1
RP3
35
40
EF230
RP25
52
RP14
RP23
37
39
RP24
WR141
53
50
EF185
55
SR116
EF211
SR113
EF224
AVA10
AIT10
100
AIT6
60
58
AIT13
Vanuatu & Fiji
Cook Islands
0.005

Figure 2.3: Unrooted neighbour joining tree of *M. lar* haplotypes based on Tamura *et al*. (2007) genetic distances.

2.3.3 Genetic differentiation and gene flow

Spatial genetic variation among *M. lar* sampling sites was assessed statistically using pair wise Φ_{ST} analysis. Pair-wise Φ_{ST} analysis results for the entire collection are shown in Table 2.3. While highly significant genetic variation was evident between virtually all pairs of sites, after Bonferroni correction, only 1 pair of 10 comparisons were significantly different. Overall pair-wise Φ_{ST} analysis suggested that gene flow among sites was relatively low.

Table 2.3: mtDNA pair-wise Φ_{ST} among *M. lar* sampling sites of after Bonferroni correction for entire collection (initial $\alpha = 0.05/21 = 0.002$). Distance method: Tamura *et al.* (2007)

	RP	WR	EF	SR	WO
RP	*	*0.009*	*0.072*	***0.000***	*0.631*
WR	0.17686	*	*0.396*	*0.604*	*0.838*
EF	0.06189	0.00719	*	*0.640*	*0.973*
SR	0.17022	-0.02158	-0.01589	*	*0.784*
WO	-0.02767	-0.07133	-0.16719	-0.09801	*

Above the diagonal are *P* values. Bold font indicates significant *P* values after Bonferroni correction for multiple tests ($\alpha = 0.050/21 = 0.002$). Below the diagonal are Φ_{ST} values. Negative values are not significantly different from zero.

2.3.4 Haplotype network

The network showing evolutionary relationships among unique haplotypes identified in all the samples screened here is presented in Figure 2.4.

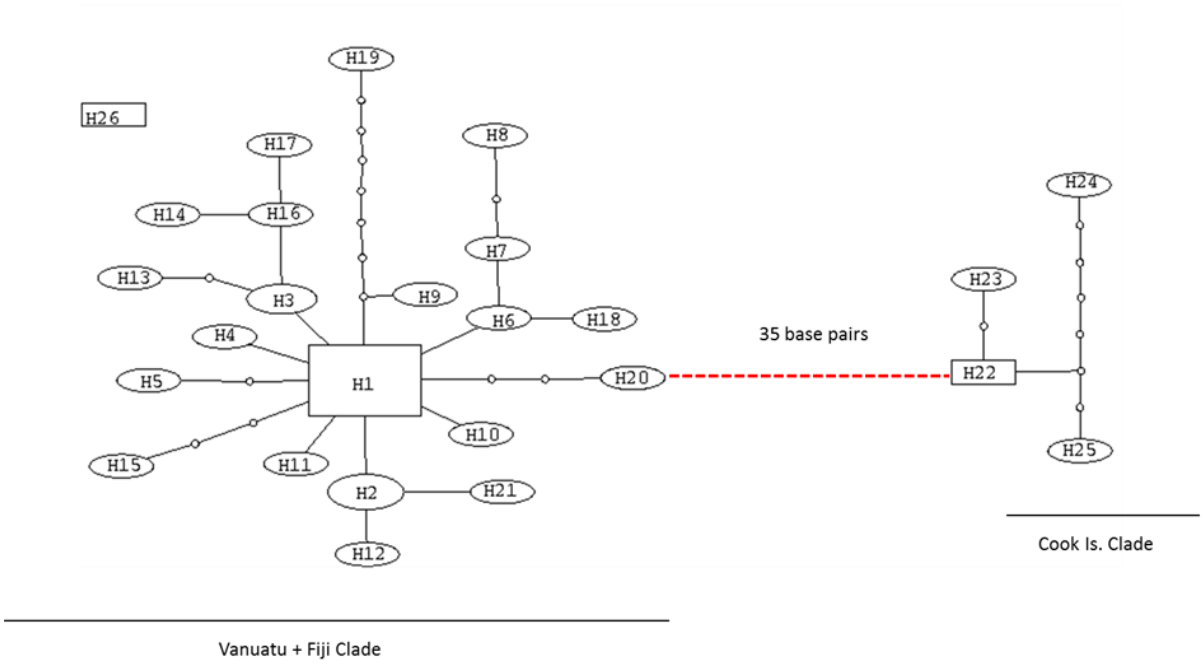


Figure 2.4: Parsimony network of *M. lar* COI haplotypes showing the evolutionary relationships among all unique haplotypes. Each circle represents a unique haplotype in the sample, and the size of each circle represents the relative frequency of each haplotype among the samples. Dots between circles represent the number of base pair difference between individual haplotypes. Small circles (and the dotted line) between individual haplotypes represent missing haplotypes not detected in the analysis.

The haplotype network shows two distinct clades (sets of distinct haplotype groups), with the Cook Island haplotypes forming a single discrete group and the Vanuatu/Fiji haplotypes forming a second divergent genetic clade.

2.4 Discussion

The outcome of the molecular analysis of genetic diversity in the sampled populations are clear, two distinct sets (clades) of haplotypes were detected among the sampled populations with all sites in Vanuatu and Fiji forming a single clade and the Cook Islands forming a second clade. In simple terms, this means that *M. lar* apparently are capable of dispersing over very large geographical distances and that populations from Fiji and Vanuatu are linked to the exclusion of the Cook Islands populations. The distinction of the Cook Island clade from all other sites in the western Pacific was recognised in earlier

unpublished studies of *M. lar* diversity (included sites in Fiji, Samoa, New Caledonia, American Samoa and New Britain (PNG)). Data presented here confirm that *M. lar* in Vanuatu belong to the western Pacific clade (Fiji etc.) that is highly divergent from the clade present in the Cook Islands. We can infer from this result that while they obviously had shared a common ancestor, gene flow is now absent between the Cook Islands and other western Pacific island chains and has been absent for a considerable period of time. In contrast, recognition of the shallow nature of the evolutionary tree of haplotypes identified from Vanuatu and Fiji indicates that gene flow has occurred much more recently (and may be ongoing) between these island chains. While no haplotypes were shared between Vanuatu and Fiji, Fijian haplotypes were in some cases more closely-related to some haplotypes found in Vanuatu than some Vanuatu haplotypes were to each other (1 to 2bp difference only). Comparisons also show however, some significant differences both between Fiji and sites in Vanuatu and among some Vanuatu sites themselves (e.g. sites RP and SR). Given that they belong to the same genetic clade, this most likely reflects the impacts of a number of factors (a) large natural populations with high haplotype diversity all possessing large numbers of rare alleles (b) sample effects - sampling for the current project was probably not sufficiently intensive to allow capture of all rare haplotypes at individual sites and (c) low probability of successful colonisation of sites following larval drift leading to local genetic drift (sampling error) effects biasing the frequency of genetically related haplotypes at individual sites. Considering that the western Pacific Ocean covers a very large geographical area and islands are small in physical area and are widely dispersed, it is not surprising that the probability of an individual pelagic larvae finding a freshwater stream away from their natal stream within three months of reaching the marine environment would be extremely low and this could lead to strong genetic drift affects influencing the frequencies of individual haplotypes at different (even adjacent sites) on islands. Thus turnover rate of individual haplotypes in streams is likely to be quite high producing stochastic effects on haplotype frequencies over time. If stream isolation in the western Pacific region however, had inhibited dispersal between Fiji and Vanuatu for relatively long periods of evolutionary time we

should expect to see equivalent levels of divergence between clades as is evident between all other western Pacific sites and the Cook Islands that was apparent here.

The results of this study are therefore clear and indicate that *M. lar* wild populations in Vanuatu and Fiji to the exclusion of Cook Island populations constitute a single genetic population for management and farming purposes. From this perspective, there are no apparent genetic reasons that would preclude *M. lar* translocations among sites for farming, restocking of wild sites or establishment of hatcheries that service multiple regions within Vanuatu, should the life cycle of the species be closed in captivity in the future. Recognition of the essential panmixus of wild *M. lar* stocks across Vanuatu reduces the complexity and cost of establishing a simple local farming program for this species across the country because a single hatchery could in theory, produce sufficient postlarvae to service the industry without compromising wild diversity if escapes from culture ponds occur. Whether this would encourage further attempts to close the life cycle of *M. lar* in captivity to allow a hatchery to be developed to produce postlarvae rather than depending on wild-caught juveniles therefore will depend (in part) on the relative cost-effectiveness of farming *M. lar* vs. *M. rosenbergii* for which hatchery technologies are already well established.

3.0 Comparative growth and survival of *M. rosenbergii* and *M. lar* in simple pond culture environments in Vanuatu.

3.1 Introduction

In the Pacific Islands taro is an important staple food, in particular farming of wetland taro with other crops (e.g. plantain etc.) including native prawn species, is particularly important because it is a traditional practice. This practice is also of important socio-cultural significance to the people. Cultivation of taro, plantain, yams, sweet potatoes and kava are important components of the subsistence lifestyle, particularly in rural areas where the custom of feasting is an important part of local rituals. Plantations have normally been tended for generations and there is a regard for local land tenure systems. Taro is the most important staple food in Vanuatu and plays a critical role in household, community and national food security. Similarly, freshwater prawn in particular *M. lar* is a highly valued commodity and in the past was used in exchange for goods and gifts in traditional life, but in recent times it has become a very important fishery in Vanuatu because individuals can be collected easily and sell for very high prices in restaurants and hotels following recent increases in the local tourist trade. The species also provides a very important source of protein for inland and coastal communities. Traditionally on some islands, *M. lar,* had been farmed in terraces of wetland taro. Prawns are collected by diverting water from streams with large sized individuals shared between villages, while small individuals are returned to the taro terrace. High demand for native fresh water prawns especially in urban centres (e.g. Port Vila) in recent decades has in some instances encouraged fishers to use prohibited fishing practices including use of chemicals to collect wild prawns from river systems. While use of poison or dynamite in open river systems and streams is illegal (Vanuatu Fisheries Act 2005), some fishermen continue to use these techniques and this can harm local wild populations.

Currently the VFD is promoting development of fish farming to supply much needed animal protein to the market and to reduce over exploitation of important natural aquatic resources such as giant clams, trochus etc. and wild freshwater prawns. Realizing that *M.*

lar is an important natural resource for local people, VFD began trial farming of this species to investigate growth rates and the economic viability of culture. This project was developed with funding assistance from ACIAR and was conducted on Santo Island with collaboration from SPC Aquaculture Section in 2005. The trial was successful with a follow up strongly recommended to confirm the findings before local farmers were encouraged to farm this species. In 2008, the VFD launched a five year Plan (Vanuatu Aquaculture Development Plan 2008 to 2013) and this plan classified freshwater prawn culture as a high priority for development. The plan also highlighted the VFD's strong commitment to successful development of a fresh water prawn industry to supply markets as well as to help reduce impacts on wild aquatic resources. While trials of *M. lar* culture have been successful, production levels are low, simply because the species has yet to be fully domesticated and culture depends entirely on collection of wild juveniles for stocking into culture ponds. Collection of juveniles however, can be a tedious and time-consuming task. It can take several days to collect sufficient juveniles to stock a single pond with culture performance depending on the quality of the stocked wild juveniles. This approach may suit the traditional taro patch integrated farming method but it will not meet commercial farming requirements.

The VFD recognises that for Vanuatu to develop a commercial freshwater prawn culture industry, the country will require a species of *Macrobrachium* that can perform well in Vanuatu's local environment. The exotic *M. rosenbergii* that has been domesticated for over 30 years and is now farmed widely around the world potentially offers such a resource. While *M. rosenbergii* was introduced to Vanuatu in the 1970s it did not survive, and since both *M. lar* and *M. rosenbergii* appear to have many attributes in common with the exception that *M. lar* has a much longer larval development phase compared with *M. rosenbergii,* and requires full marine conditions to complete its life cycle, both species potentially could be used for farming there. *M. rosenbergii* also requires a shorter hatchery cycle than *M. lar* allowing it to be successfully spawned and reared to PL stage in 25-35 days after which it can be used for pond stocking. The hatchery technology for *M. rosenbergii* is also well established and is quite simple making this species potentially

suitable for culture in countries like Vanuatu. Local fisheries officers have recently been trained in producing *M. rosenbergii* PLs in simple hatcheries, so if it can be demonstrated that the species is productive under basic culture systems in Vanuatu, then *M. rosenbergii* may offer a more simple and more productive option than developing *M. lar* farming over the long term.

A number of farmers have shown interest in developing simple prawn farming systems in Vanuatu and believe that the species that is indigenous to Vanuatu, *M. lar,* does have culture potential. This is because simple grow-out ponds integrated with wetland taro, plantain, water cress or other vegetables were stocked with wild caught juveniles collected from natural sources in Vanuatu in 2004 (Nandlal 2010). The practice at the time stimulated great interest in farming mainly due to the high price achieved for large size (farmed) prawns compared with prawns of variable sizes collected from the wild. Development of *M. lar* farming in Vanuatu (if successful) would parallel that of *M. rosenbergii* juveniles collected from the wild and grown to market size in simple culture systems in Thailand.

While commercial production has been developed for the exotic *M. rosenbergii* and other *Macrobrachium* species in various types of culture systems, industry development in some places has not been as rapid as expected for several reasons. It is often difficult to obtain sufficient wild juveniles at stocking time. While utilizing prawn juveniles collected from natural waters for stocking in ponds has a disadvantage due to their great variation in size, additionally, farmers in Vanuatu have been raising prawns in simple culture systems with little basic knowledge about efficient types of culture systems and so harvests can be highly variable and even uneconomic. Resolving which species therefore; either the native *M. lar* or the exotic *M. rosenbergii* may be the best option for assisting local industry development will be crucial to promoting aquaculture development in Vanuatu and more broadly across the southwest Pacific region.

In its most basic form, juveniles of *M. rosenbergii* can be produced easily in hatcheries and can be stocked directly into ponds using well-established farming techniques and practices. Since wild *M. lar* occur naturally in the creeks, juveniles can be collected from the wild and these prawns should derive most of their dietary needs from the organic micro-fauna that is present naturally in the pond. Farming prawns in ponds can also make efficient use of low value marginal land and water as it can in theory, produce greater overall yields than is available from some other crops. Existing farms in Fiji show that within a pond ecosystem, the plants and animals present compliment and interact with each other, making ponds very productive in terms of both quantity and variety of natural food organisms for prawns with nutrients recycled efficiently by culturing prawns. Vegetables and fruit trees can also be planted on bunds and provide natural shelter and with weeds on the sides of the banks, can increase feeding surface area for prawns in such systems. This basic system can also be upgraded to fit more productive and commercially oriented production systems whereby ponds could be used for prawn monoculture with feed inputs to supplement dietary needs and substrates provided. Adopting such semi-intensive farming systems is likely to be relatively easy to establish and this will enable production to grow in response to market demand.

With returns from capture fisheries declining significantly in many parts of Vanuatu, VFD is looking to develop aquaculture to supplement local fish protein and address food sustainability requirements. While the exotic species, *M. rosenbergii* has been present in Fiji since 1978 (Nandlal 2010), and has been released deliberately into the wild, (where it has yet to establish feral populations) the two species (*M. rosenbergii* and *M. lar*) seem to represent low threats to natural ecosystems. While both species have been suggested as potential candidates for culture in PICTs with *M. rosenbergii* now farmed commercially in Fiji, some preliminary work undertaken on *M. lar* has suggested that this species may not necessarily be an easy species to culture under pond conditions. Therefore, before any serious attempt is made to develop a local prawn culture industry based on either the indigenous species *M. lar*, or the exotic *M. rosenbergii* in Vanuatu, it will be crucial to

trial grow-out of juveniles in culture environments, and to determine which species (*M. lar* vs. *M. rosenbergii*) offers the best local option for farmers.

Currently, aquaculture of *M. rosenbergii* in Fiji is expanding rapidly and VFD has expressed an interest in developing a similar freshwater prawn culture industry. *M. rosenbergii* however, is exotic in the Pacific and broodstock will need to be sourced from Fiji for culture. If growth performance of the indigenous prawn (*M. lar*) is similar or approaches that of an exotic species like *M. rosenbergii*, then industries based on either species could provide significant opportunities without potential for negative effects. Although *M. lar* does not grow as large and yields less meat than *M. rosenbergii* per individual, it is still abundant in many streams, and so juveniles could be collected and stocked and thereby may provide advantages not available with *M. rosenbergii*. Currently, little if anything is known however, about relative survival and growth rates of the indigenous (*M. lar*) compared with the exotic *M. rosenbergii* in simple culture systems.

The development of these technologies can provide important outcomes including:

- Provide farmers with the basic technology necessary to culture either species in combination or individually in simple pond systems.
- Permit trials to be undertaken to evaluate *M. lar* and *M. rosenbergii* as a new culture species for Vanuatu and assist in improving local human nutrition and employment while offering a sustainable simple new farming option that is compatible with the culture of local people.

The current study therefore assessed growth rates of wild caught juvenile *M. lar* with those of hatchery reared *M. rosenbergii* juveniles in simple pond culture systems in Vanuatu. The objective was to undertake basic trials to determine the specific

environmental conditions that need to be satisfied to allow juveniles of both species to grow to adult stage.

The aim was also to compare the performance of the two species under identical simple culture conditions to determine which species was most suitable for local farmers.
Therefore the main goal of this study was to investigate growth and survival rates of *M. lar* and *M. rosenbergii* in simple pond monoculture in Vanuatu. Results could then be compared with survival and growth rates achieved for similar species under similar production environments elsewhere. The data will allow the VFD to make an informed choice about which species may be best to target in the future for local industry development.

3.2 Materials and methods

3.2.1 General methods

The study was initiated in March 2011 following development of a basic freshwater prawn hatchery by VFD with a total of about 40m^2 floor space. The room was designed for culture of larvae with space allocated for feed preparation and holding of freshwater and larval rearing water (12‰) in a 2000L Fibre Reinforced Plastic (FRP) tank.

Acquisition of *M. rosenbergii* larvae to serve as an initial broodstock stock began in March 2011 with importation of 3000 PLs from Fiji. Following completion of quarantine procedures, PLs were transferred in mid June 2011 to ponds at Tagabe (17.42'14S 168.19'.11E) on Efate, Vanuatu, approximately 5km from Port Vila (see Figure 3.1 and Figure 3.2), to be raised to broodstock age.

The first hatchery operation of *M. rosenbergii* was conducted at the end of October 2011 following the procedures outlined by Nandlal and Pickering (2005). Ovigerous females were collected on 27th October 2011 and two females hatched the following day resulting in a first brood of larvae of approximately 50,000. Larvae were reared in 3 x 200L FRP

clear flat bottom tanks. Towards the end of the rearing trial, high mortality occurred with only 50 PLs surviving. Larval rearing techniques were then refined, in particular more attention was paid to tank hygiene and thereafter all subsequent larval rearing cycles to produce *M. rosenbergii* PLs for the trials was accomplished successfully.

For Trial 1, a hatchery run to produce *M. rosenbergii* PLs was carried out in March/April 2012, resulting in a total production of 10,500 PLs. PLs were nursed in cement tanks for a period of 75 days before stocking in ponds on 30th June 2012, while in Trial 2, the hatchery run was carried out in December 2012 with a production of 12,000 PLs that were nursed to 19th of February (15 days) and stocked on 20th February 2013.

Preparation for pond trials and associated facilities began with building of 4 earth ponds and water holding tanks at Tagabe, Port Vila (see Figure 3.1). Ponds were excavated and were rectangular in shape with surface area ranging from 43- 47m^2 (refer to details given in Table 3.1). The pond bottom was levelled and adjusted to a suitable height and lined with a 1.5mm low-density polyethylene sheet (plastic liner) on the pond bottom and the internal sides of the dikes. A layer of top soil was spread evenly (≤ 10cm thick) across the plastic surface area to mimic an earthen pond, i.e., a natural pond environment when filled with water. Pond trials were carried out in these 4 ponds. The ponds were supplied with well water that was stored in 2 x 10,000L tanks.

Table 3.1: Details of pond length, width, surface area and water depth at Tagabe.

Pond No.	**Length (m)**	**Width (m)**	**Surface area (m^2)**	**Depth (m)**	
				Inlet	**Outlet**
1	9.0	4.8	43.2	0.37	0.53
2	10.2	4.6	46.9	0.43	0.55
3	9.0	4.8	43.2	0.47	0.61
4	10.2	4.6	46.9	0.45	0.69

3.2.2 Pond preparation

A week prior to the anticipated stocking date, a 2mm plastic mesh of 1m height was positioned in the middle portion (see bottom right of Figure 3.1) to partition each pond into two equal compartments (from the inlet side to the outlet side into two sections) to allow co-stocking of both species of prawns in the same pond. The mesh was fixed about 3cm into the pond bottom and soil was also placed on either side at the bottom of the mesh to add strength to the base so to hold the mesh firmly in place and to prevent the two species mixing together while still allowing water to be shared. The mesh was supported by aluminium poles staked into the pond bottom and plastic straps were used to attach the mesh onto the pipes.

Water was supplied via 25 mm polyethylene pipes and this also served as inlet pipes. For the outlet, 100mm PVC pipes were installed at opposite ends (of the inlet pipe) at the centre of the pond dyke. The pipes were screened with fine mesh (2mm) to stop prawns escaping from ponds. Two days prior to stocking the ponds were filled with water via inlet pipes that were connected to 2x10,000l tanks that stored the water pumped from a tube well (bore hole), (Figure 3.1). All ponds were supplied with a continuous flow of water and water depth maintained by adjusting the position of the outlet pipe at 70cm across the trial period. Water depth ranged from 0.37-0.45m at the inlet side to 0.53-0.69m at the outlet end. Approximately 10% of pond water volume was exchanged every 2-4 days over the trial period.

Ponds were provided with 5 ‘green’ coconut palm fronds each about 60-100cm wide and 2-3m long that were suspended horizontally across each pond to provide refuge and substrate for prawns. These fronds were removed at the time of monthly prawn sampling and replaced with new ones. The inlet pipe-screen was cleaned regularly to avoid clogging. Water flow rate (at the inlet pipe) was 0.15l\L/s in all ponds to maintain water levels in the ponds and to ensure that there was adequate water to continuously supply the 4 ponds. On average, water flow rate was 0.1L/s, i.e., waste water flowed out of each pond through the outlet pipes into a drain.

Prior to stocking in Trial 1, cow manure (1000kg/ha) was broadcast over the floor of the ponds and then ponds were filled with water to a depth of 20cm. After one day, the level of the water was raised to 30cm and juvenile prawns were stocked in each pond.

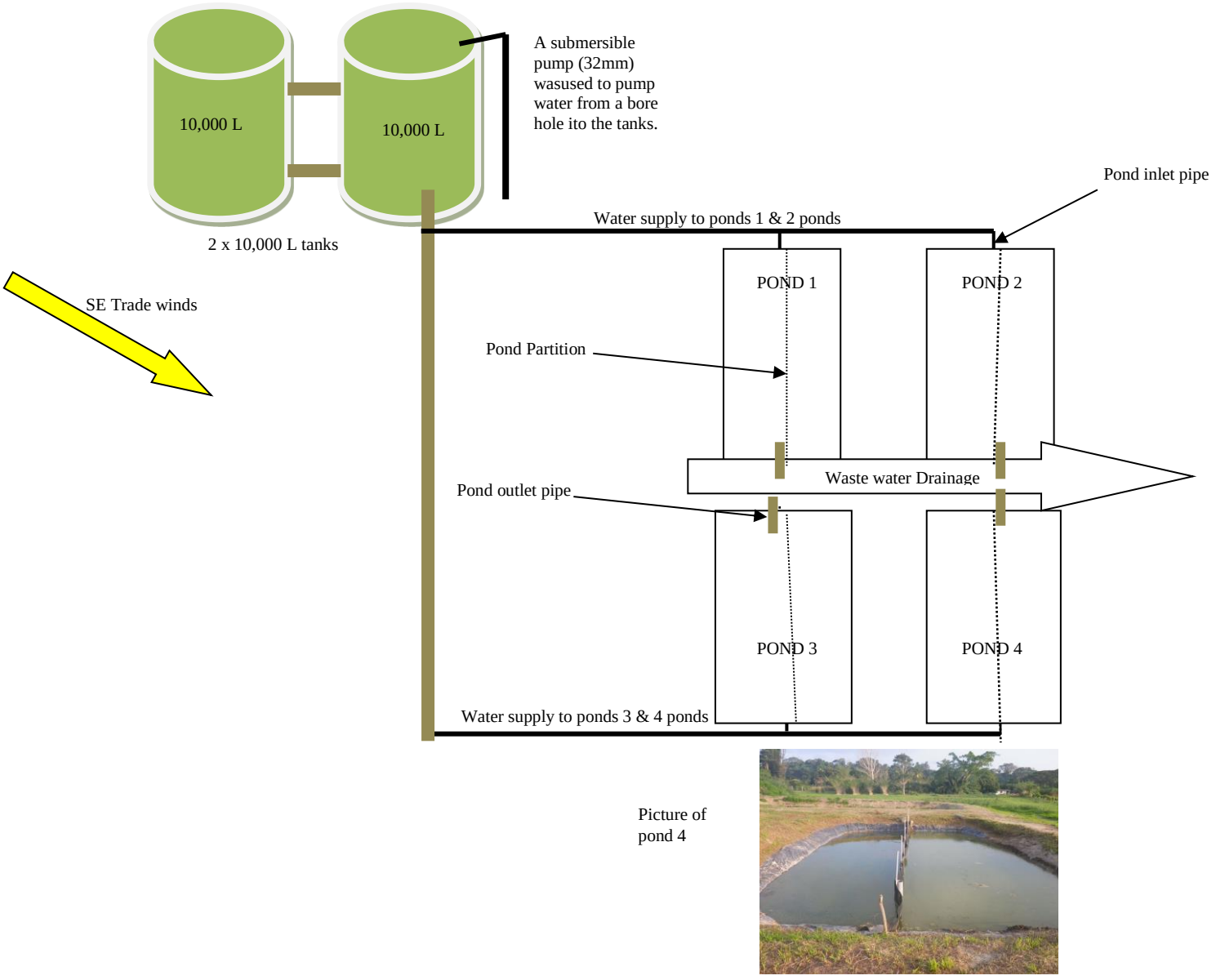


Figure 3.1: Layout of ponds and water supply system at Tagabe Freshwater Aquaculture Centre (TFAC).

3.2.3 Water quality management

Water quality parameters including temperature, pH and flow rate were monitored regularly. Water temperature was recorded in all ponds in both trials using a Temperature Logger (HOBO brand) while pH was measured prior to and a day after stocking in both trials using a standard pH meter.

3.2.4 *M. lar* juvenile collection

Three days prior to the anticipated stocking date, *M. lar* juveniles were collected from Neslep and Mangaliliu Creeks on the island of Efate (see Figure 3.2). Neslap Creek is a small creek flowing eastward before entering the sea. *M. lar* are present in the Creek, approximately 100m inland from the highway where they are abundant throughout the year. Mangaliliu is a small stream that runs from the coast through Mangaliliu village to the hills-about 1km in length.

Juveniles were collected manually from 26-29 June 2012 using a push net (1.5m in length and 3mm mesh size) applied across the bottom of the stream after disturbing the debris and partially submerged vegetation along the stream banks. Captured juveniles were placed in 20L containers filled with creek water and aeration provided using a portable battery operated aerator. Precaution was taken with juveniles that were soft (moulted) with the bucket placed in a larger bin with a handful of crushed ice to maintain temperature at ~21^{o}C inside the bucket (temperature of creek water).

In addition, aquatic plants from the collecting site were added to the buckets and covered with a lid to avoid escapes. Juveniles were then transported to VFD hatchery site for the prawns to be kept overnight.

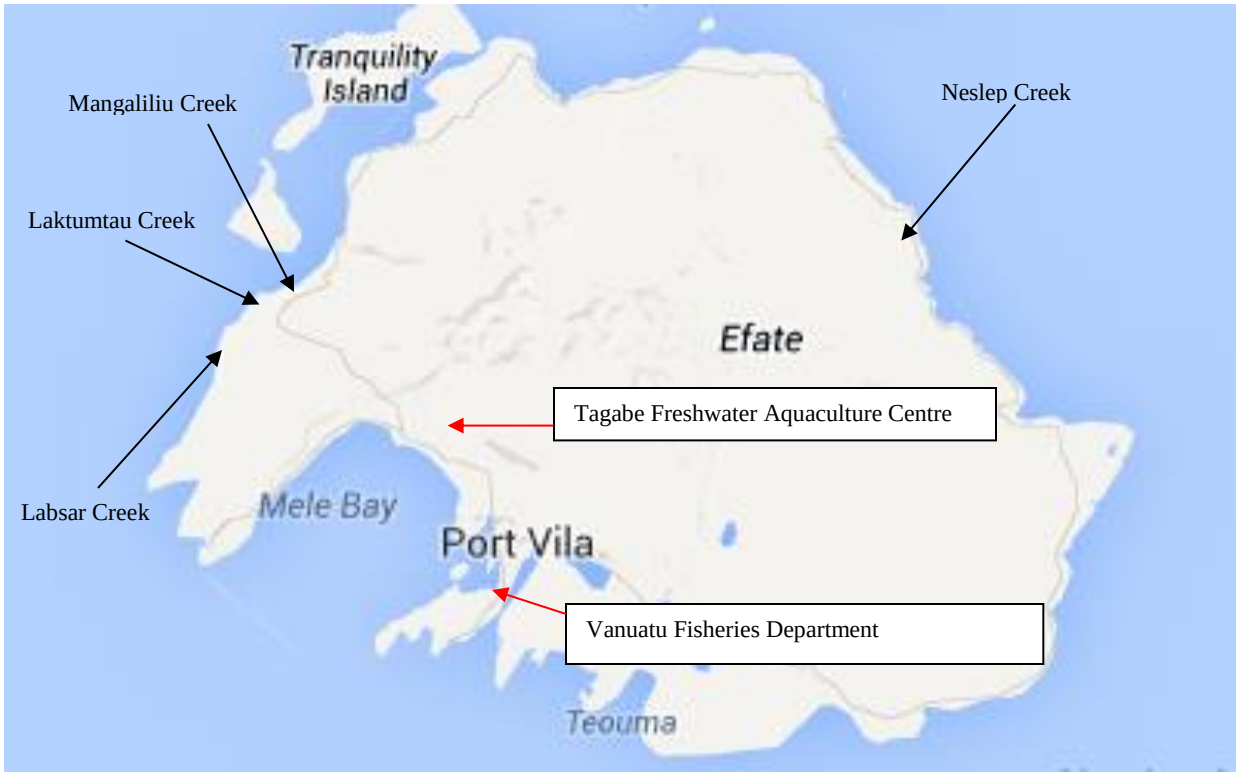


Figure 3.2 Map of Efate Island showing location of Tagabe Freshwater Aquaculture Centre (TFAC) and collection sites for *M. lar* juveniles

3.2.5 Conditioning of juveniles

On arrival at VFD hatchery, buckets were floated (with prawns inside) for 15 minutes in the holding tank (6000L fibre-glass tank filled to 80% capacity) until water temperature in the buckets equilibrated to tank water temperature (26-28^{o}C). Once prawns had recovered (from transport stress) they were examined and confirmed as *M. lar* using the number of spines on the rostrum as a marker, i.e., upper margin of the rostrum having 7 or 8 teeth (Short 2000) and the number recorded before release into a holding tank where they were maintained at ambient temperature (26-28^{o}C) until sufficient numbers of juveniles were available for the trial. The tank was provided with aeration and 10% of the water changed every day. Ten air stones were placed inside the tank and aeration adjusted to produce a

steady stream of bubbles at all times. To provide refuges for juveniles and to help prevent cannibalism, several plastic mesh screens (1-cm mesh) folded into a cylindrical shape and secured with binding wires were placed inside the tank. In addition, several 30-cm segments of 100-mm diameter PVC pipe were also placed in the tank as shelters. Animals were fed a commercial tilapia pellet containing approximately 30% crude protein (obtained from Goodman Fielder Fiji Limited) two times a day. Special care and attention was taken (aeration in the tank was stopped) to siphon out all uneaten feed before providing new feed. Care was also taken at all times to remove excess feed and to avoid over-feeding so that decay of uneaten food did not foul the water.

Trial 1 (commenced on 30th June 2012; cold season going into warm season)

On the day of stocking, juveniles of similar size in appearance were separated and body weights of a sample of 50 individuals of both species were measured using an electronic balance. The total number required for stocking in each pond was determined for both species at 50,000/ha (at a stocking density of 5 prawns per m^2).

The required number of juveniles of each species for each pond were placed in separate 30L containers and aerated for transport to the TFAC site. At the pond site, water temperature and pH of all ponds were measured and compared with that in the containers holding the juveniles. Juveniles were acclimated to pond water conditions for 15-20 minutes by placing the container (with juveniles) in the pond and draining approximately half of the water from the container and replacing with pond water. Juveniles were then allowed to swim freely into the pond. Remaining juveniles were emptied gently into the pond. Any dead prawns settled on the pond bottom and these were counted and replaced with live individuals to balance numbers of both species.

While age and size classes obviously varied among stocked juvenile *M. lar* individuals, selection of juveniles for stocking of each pond was random, so each pond received a

random assortment of available juveniles of both species for both size and age. This resulted in 108, 120, 108, and 120 juveniles of each species stocked into their respective compartments in the four ponds. Juveniles of *M. rosenbergii* (mean weight 0.79g) were stocked into the four ponds on the same day following a similar procedure to *M. lar* stocking, i.e., mean stocking weight for each of the ponds was determined from a sample weight of 50 juveniles (Appendix 1). For trial 1 all the ponds were stocked on 30/6/2012. Details of pond stocking are given in Table 3.2 and Appendix 2. The culture period was 144 days. Apart from sampling at the initial stocking time and at final harvest, prawns were also sampled at day 38, 67 and 97th followed by a complete harvest on day 144th (see appendix 1 and 2)

Trial 2 commenced on 20th February 2013-warm season into cold season. Details of pond stocking are given in Table 3.3 and Appendix 2. The culture period was 120 days. Apart from sampling at the initial stocking time, prawns were also sampled at day 48, 71 and 99th followed by a complete harvest on day 120th (see appendix 1 and 2)

3.2.6 Feed and feeding

A commercial tilapia pellet containing approximately 30% crude protein (imported from Fiji) was fed twice daily. This feed is currently used routinely by prawn farmers in Fiji.

The amount of feed provided per day was based on estimated prawn biomass per pond determined on levels employed for *M. rosenbergii* culture in semi-intensive ponds (currently practiced by farmers in Fiji) with the rate deceasing from 10% to 4% of body weight as prawns increased in size over the trial period. Initial feeding rate was 10% on a live wet weight basis for the first month and daily feed rations were adjusted after sampling based on a percentage of body weight reducing to 4% (of the body weight) by the last month of the grow-out period. An estimated 5% juvenile mortality rate per month was applied when calculating feed rations. Daily feed ration was derived from the following calculation: $N_i \times S_t \times W_t \times FR_t$ = daily ration (in g)

Where

N_i = the initial number of prawns stocked in the pond;

S_t = estimated survival at time t-a rough mortality estimate of 5% per month;

w_t = mean prawn weight (in g) at time t.

FRt = the recommended feed rate for period t.

Feed was distributed over the entire surface of each pond twice daily with 30% of the ration provided in the morning (between 0800 to 1000 hours) and 70% in the evening (between 1700 and 2000 hour). Feeding was reduced when water supply was disrupted temporarily between 12th and 15th of October 2012.

Table 3.2 Trial 1- Pond stocking data from 30/6/2012 to 21/11/2012

	Pond Number							
	1		2		3		4	
Species	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*
Pond size (m^2)	21.6	21.6	24	24	21.6	21.6	24	24
SD (PL/m^2)	5	5	5	5	5	5	5	5
Total stocked	108	108	120	120	108	108	120	120
Mean weight (g)	0.79	1.06	0.71	0.73	0.72	1.3	0.65	1.14
Total weight (g)	85.32	114.48	85.2	87.6	77.76	140.4	78.0	136.8
Standard Error	±0.06	±0.17	±0.04	±0.11	±0.06	±0.17	±0.04	±0.11

M.r = Macrobrachium rosenbergii; *M.l = Macrobachium lar*; SD = stocking density

3.2.7 Sampling

Prawns were sampled initially (before stocking) for mean weight (Appendix 1) and then monthly to estimate daily feed ration. Mean weights were determined by seining a sample of approximately 30 prawns from each compartment of the ponds which were then group weighed and returned after weighing to their respective compartments in the ponds.

Feeding rates used were 10, 8, 6 and 4% of prawn biomass for the 1st, 2nd, 3rd and 4th month, respectively. Prawns were sampled three times over the trial period followed by complete harvest at the end of the trial (see Appendix 2-monthly sampling data).

3.2.8 Final sampling

On the day of final sampling the water level in each pond was lowered to approximately 20cm and all substrate was removed. Prawns were collected using push nets and following this all pond water was drained completely and remaining prawns were captured by hand. All harvested prawns were purged in clean water and transported to the VFD prawn hatchery. Measurements of length, weight and sex of all individuals were recorded and individuals were then graded and packed into bags and stored in the fridge for later sale.

Trial 2 commenced on 20 February 2013- warm season going into cold season

Protocols for pond preparation, collection of juveniles, stocking, feeding, sampling and final harvesting were identical to those used in Trial 1 except for additional procedures and equipment specific to Trial 2 that are given below.

Additional soil was placed at the base of the partition to compensate for lost soil and also to stop prawns from moving from one pond compartment to the other i.e., to avoid mixing of the two species. No cow manure was broadcast into the ponds in Trial 2. Screens at outlet pipes were cleaned regularly to avoid water overflowing from the ponds during heavy rainfall.

M. lar juveniles were captured from Laktumtau and Labsar creeks from 17-19th February 2012. These creeks are located on the north western side of Efate Island (see Fig 3.2).

3.3 Data analysis

Means and standard deviations of body weight, survival rate and water quality parameters were estimated. Pond productivity was also assessed by standardising the total pond biomass over the number of juveniles stocked initially. Parameter differences among species were assessed using a 2 tailed paired t-test (α=0.05).

Food conversion ratio (FCR) was calculated as FCR = total diet fed (kg)/total wet weight gain (kg). Daily cost of operating the four grow-out ponds was estimated based on feed, utility and labour use from the records maintained. Electricity consumption was estimated from the number of kilowatt hours needed per day to run a tube-well pump (to pump the water into storage tanks for water exchange). Juvenile price to stock the ponds was set at US$ 0.10/individual for both species, diet price (imported from Fiji, retail price in Fiji is US$ 0.76/kg) plus freight cost was estimated at US$1.00/kg, and electricity price was US$ 0.50/Kwh (average retail commercial price for year 2012 and 2013 in Port Vila (Nettie Naviti pers. comm.).

Diet quantity data (per trial) were available from the maintained records. Labour requirement was assumed to be limited to 2 hours/day @ US$ 1.60/hour for feeding, water quality monitoring etc.

The tube well pump is rated at 1.1kw (1.2 HP -230 volts) and operated approximately 40% of 24 hours/day, i.e., 9.6 hours /day. The remaining cost items were considered to be time-independent (e.g., management, maintenance, truck fuel, security costs, etc. and non-cash costs such as equipment depreciation). These costs did not change over length of the grow-out trials and were not included in the production cost estimates.

Table 3.3: Trial 2 - Pond stocking data from 20/02/2013 to 20/06/2013.

	Pond Number							
	1		2		3		4	
Species	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*
Pond size (m^2)	22	22	24	24	21.6	21.6	24	24
SD (PL/m^2)	5	5	5	5	5	5	5	5
Total stocked	108	108	120	120	108	108	120	120
Mean weight (g)	0.16	0.58	0.19	0.34	0.10	0.18	0.12	1.06
Total weight (g)	17.28	62.64	22.8	40.8	10.8	19.44	14.4	127.2
Standard Error	±0.06	±0.17	±0.04	±0.11	±0.06	±0.17	±0.04	±0.11

M.r = Macrobrachium rosenbergii; *M.l = Macrobrachium lar*; SD = Stocking Density

3.4 Results

3.4.1 Pond water quality

The water supply was consistent over both trial periods and hence no significant differences in water quality parameters were found among ponds in either Trial 1 or Trial 2. The water quality parameters are given in Table 3.3 and Figure 3.4, respectively.

Mean water temperature ranges differed in Trial 1 and Trial 2 reflecting seasonal differences. The water temperature in Trial 1 ranged from 20.90 ± 0.04 to 34.05 ± 0.04ºC. Maximum recorded pH was 8.18. Complete data on pH and DO were not recorded on a regular basis due to repeated equipment malfunction.

Table 3.4: Water quality parameters (mean ± s.e) and flow rate of water in the ponds in Trial 1

Parameter	**Pond numbers**			
	Pond 1	**Pond 2**	**Pond 3**	**Pond 4**
Water Temperature (ºC)	26.36 ±0.04	26.37±0.04	26.45±0.04	26.41±0.04
pH	8.13	8.02	8.18	7.6
Water flow rate (L/s)	0.13	0.14	0.10	0.24

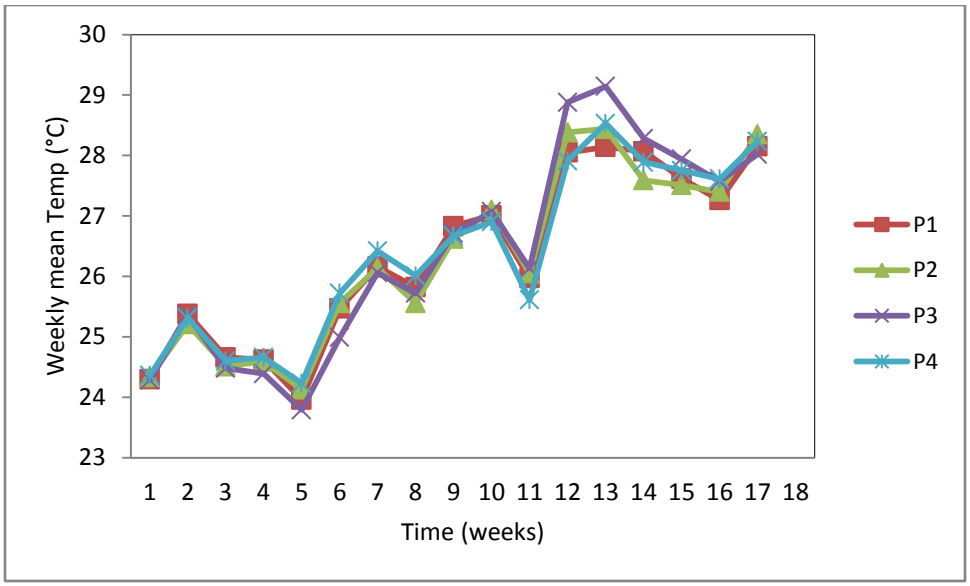


Figure 3.3: Record of weekly mean water temperature in Trial 1.

In Trial 2, the water temperature ranged from 22.24 ±0.04 to 34.69±0.04ºC. The mean water temperature is given in Table 3.5. While ambient temperatures fluctuated over the study period, variation among ponds within trials was minimal (Figure 3.4).

Table 3.5: Water quality parameters (mean ± s.e) and flow rate of water in the ponds in Trial 2

Parameter	**Pond numbers**			
	Pond 1	**Pond 2**	**Pond 3**	**Pond 4**
Water Temperature (ºC)	27.56 ±0.04	27.62±0.04	27.44±0.04	27.20±0.04
pH	8.68	7.86	8.75	7.67
Water flow rate (L/s)	0.13	0.14	0.10	0.24

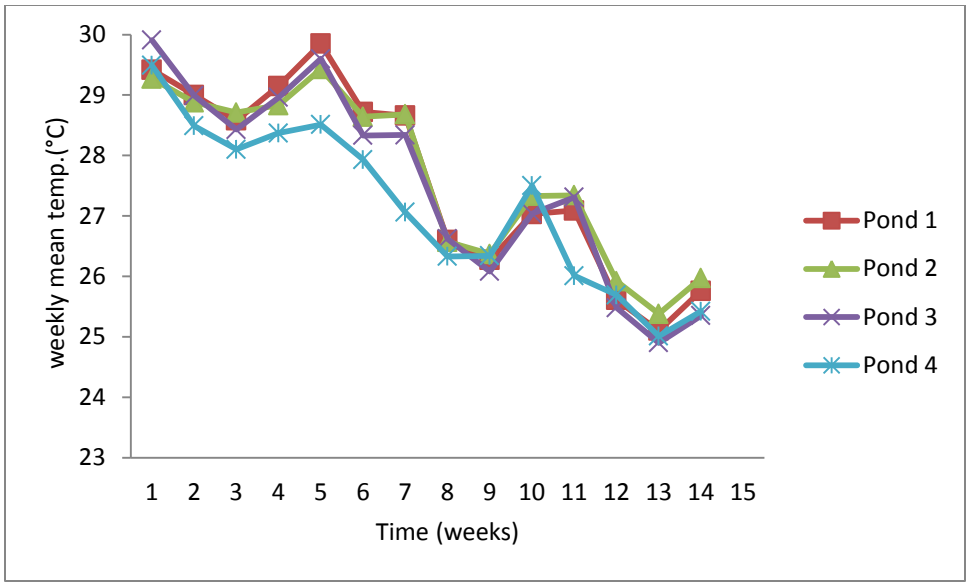


Figure 3.4: Record of weekly mean water temperature in Trial 2

3.4.2 Stocking data

Mean weight of *M. lar* juveniles at stocking in Trial 1 was 1.38 ± 0.17g, ranging from 0.12 to 4.90g, while mean weight of juveniles stocked in Trial 2 was 0.35±0.06g, ranging from 0.04 to 2.29g

Mean weight of *M. rosenbergii* juveniles stocked in Trial 1 was 0.89 ± 0.06g, ranging from 0.31 to 1.76g, while mean weight of juveniles stocked in Trial 2 was 0.13±0.006g, ranging from 0.07 to 0.25g.

3.4.3 Growth, survival rate and yield

Trial 1

Table 3.6 presents a summary of results for growth rate, survival rate, productivity and FCR per pond. At the end of the 144 day grow out period, a total of 370 *M. rosenbergii* and 255 *M. lar* individuals were harvested from the 4 ponds. All ponds showed very consistent prawn growth patterns in both species, expressed in mean weight over the trial period as shown in Figure 3.5. *M. rosenbergii* achieved a significant mean growth advantage over *M. lar* in all ponds.

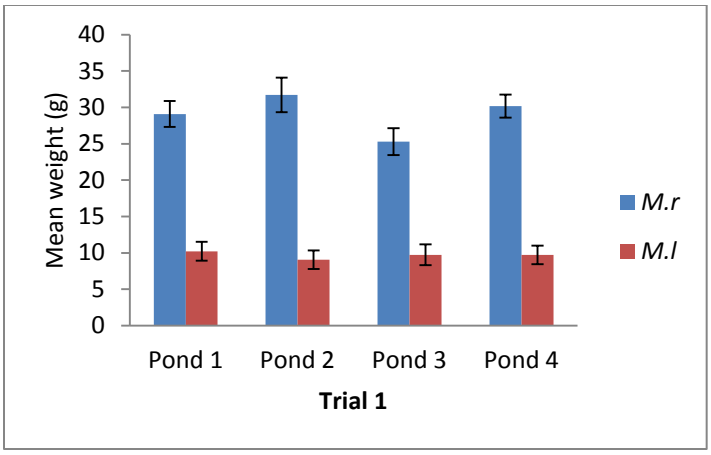
Table 3.6: Summary of survival, growth, productivity and FCR for the two species in Trial 1

Pond No.	Pond size (m^2)	Species	No. stocked	Survival No. (%)	Mean wt(g)	s.e	Productivity (kg/m^2)	FCR
1	21.6	*M.r*	108	91 (84.26)	29.08	±1.78	0.12	2.38
2	24	*M.r*	120	81 (67.5)	31.72	±2.38	0.11	3.46
3	26.1	*M.r*	108	91 (84.26)	25.29	±1.87	0.11	2.94
4	24	*M.r*	120	107 (89.17)	30.19	±1.58	0.13	2.37
	Mean				29.07	±1.9	0.12	2.79
1	21.6	*M.l*	108	74 (68.52)	10.08	±1.3	0.03	7.83
2	24	*M.l*	120	53 (44.17)	9.05	±1.28	0.02	7.33
3	21.6	*M.l*	108	48 (44.44)	9.23	±1.43	0.02	4.06
4	24	*M.l*	120	80 (66.67)	9.72	±1.29	0.03	5.98
	Mean		.	.	9.52	±1.33	0.03	6.3

M.r =Macrobrachium rosenbergii; *M.l = Macrobrachium lar*

M. rosenbergii out-performed *M. lar* in all ponds. Significant differences were apparent for mean growth between the two species, with *M. rosenbergii* attaining a significantly higher mean weight than *M. lar* ($t_{3,0.05} = 14.102$, $P < 0.001$).

Figure 3.5 displays the final mean weight for the two species in each pond. While the stocking density was the same for the two species, it is clear from Figure 3.6 that *M. rosenbergii* outperformed *M. lar*.



M. r = Macrobrachium rosenbergii; *M.l = Macrobrachium lar*

Figure 3.5: Comparison of final mean weights ±s.e for *M. rosenbergii* and *M. lar* in 4 ponds in Trial 1

With respect to species productivity per pond the results also show that *M. rosenbergii* out-performed *M. lar* in both trials (Table 3.7). In Trial 1 the productivity results for *M. rosenbergii* ranged between 21.15 – 26.91g while for *M. lar* in Trial 1 this varied between 4.07 – 6.48g. Overall, the mean weight of *M. rosenbergii* was 23.47g compared with 5.26g for *M. lar*, ($t_{3,0.05}$ = 12.125, P < 0.00). Essentially the mean weight per *M. rosenbergii* individual was approximately 5 times that of *M. lar* individuals.

Table 3.7: Number of individuals stocked, survival, final weight and productivity per species for four ponds in Trial 1.

Trial 1	Pond 1		Pond 2		Pond 3		Pond 4	
Species	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*
Number stocked	108	108	120	120	108	108	120	120
Survival	91	74	81	53	91	48	107	80
Final total weight (g)	2646.4	664	2537.5	488.5	2301.4	467	3230.5	777.5
Productivity	24.50	6.15	21.15	4.07	21.31	4.32	26.92	6.48

M.r = Macrobrachium rosenbergii; *M.l = Macrobrachium lar*

FCR for *M. lar* in Trial 1 ranged from 4.06 to 7.83, compared with 2.37 to 3.46 for *M. rosenbergii*.

In Trial 1, *M. rosenbergii* also showed a higher survival rate than *M. lar* ($t_{3,0.05} =$ 4.956, P = 0.016), (Table 3.6) (details in Appendix 3). Differences in mean weight between species was also investigated for sexes separately and social condition within sex (data not shown).

Trial 2

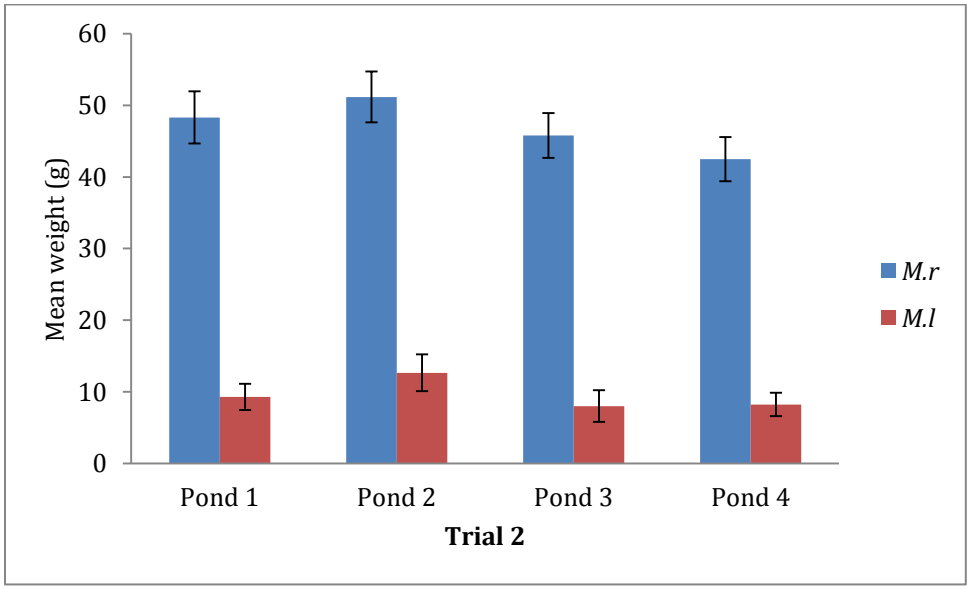
At the end of the 120 days grow out period a total of 452 *M. rosenbergii* and 242 *M. lar* individuals were harvested from the 4 ponds. Table 3.8 presents a summary of results for survival rate, productivity and FCR per pond. Mean weight (±s.e) of juveniles stocked in Trial 2 was 0.13 ± 0.006g, range 0.07 to 0.25g, respectively (details in Appendix 2).

As with Trial 1, survival rate of *M. rosenbergii* was significantly higher than for *M. lar* ($t_{3,\ 0.05}$ = 9.076, P = 0.003). The mean weight for *M. rosenbergii* was 46.93±1.85g compared with 9.56±1.07g for *M. lar* ($t_{3,\ 0.05}$ = 34.488, P = 0.002). Figure 3.6 displays the final mean weight for the two species and it is clear in Trial 2 that *M. rosenbergii* showed an approximate 5 fold difference in mean weight at harvest over *M. lar*.

Table 3.8: Summary of growth, survival, productivity and FCR for the two species in Trial 2

Pond No.	Pond size (m^2)	Species	No. stocked	Survival No. (%)	Mean wt. (g) ± s.e		Productivity (kg/m^2)	FCR
1	21.6	*M.r*	108	108 (100)	48.3	±1.82	0.24	1.45
2	24.0	*M.r*	120	120 (100)	51.16	±1.77	0.25	1.62
3	26.1	*M.r*	108	104 (96.29)	45.79	±1.57	0.22	1.72
4	24.0	*M.r*	120	120 (100)	42.46	±1.44	0.21	1.58
	Mean				46.93	±1.65	0.23	1.59
1	21.6	*M.l*	108	49 (45.37)	9.29	±0.91	0.02	7.06
2	24.0	*M.l*	120	77 (64.17)	12.67	±1.28	0.04	3.62
3	21.6	*M.l*	108	62 (57.40)	8.02	±1.11	0.02	3.81
4	24.0	*M.l*	120	54 (45.00)	8.25	±0.81	0.02	10.43
	Mean				9.56	±1.03	0.03	6.23

M.r = Macrobrachium rosenbergii; *M.l = Macrobrachium lar*



M.r = Macrobrachium rosenbergii; *M.l = Macrobrachium lar*

Figure 3.6: Comparison of final mean final weights (±se) for *M. rosenbergii* and *M. lar* in Trial 2

As with results of Trial 1, *M. rosenbergii* out-performed *M. lar* in Trial 2 with mean weight ranging between 42.46 to 51.16g while for *M. lar* the range was 3.71 – 8.23g .

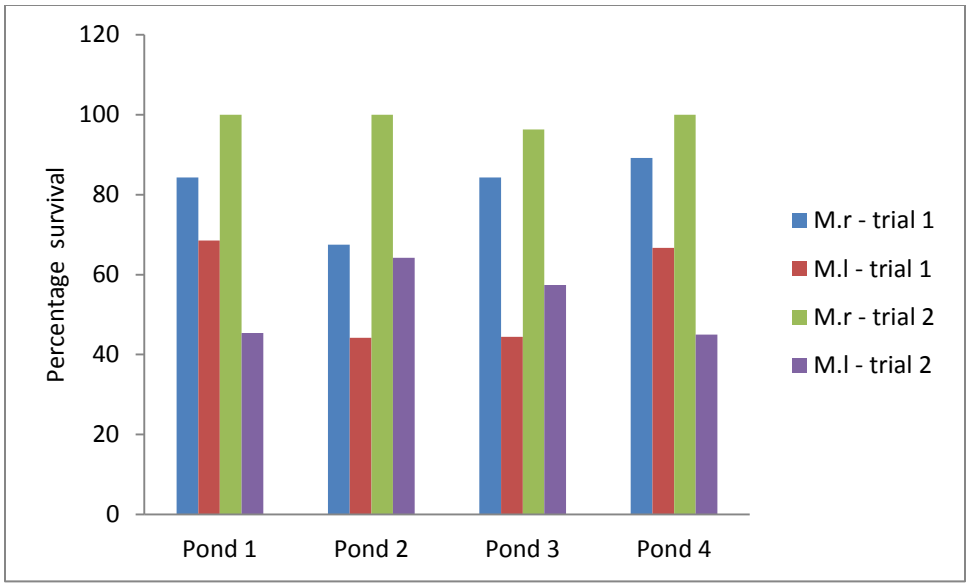
Table 3.9: Productivity of *M. rosenbergii* and *M. lar* for the four ponds in Trial 2

M.r = Macrobrachium rosenbergii; *M.l= Macrobrachium lar*

Trial 2	Pond 1		Pond 2		Pond 3		Pond 4	
Species	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*	*M.r*	*M.l*
Total initial stocked	108	108	120	120	108	108	120	120
Survival	108	49	120	77	104	62	120	54
Total weight (g)	5216.41	455.12	6139.72	988.06	4807.5	497.09	5095.73	445.43
Productivity (g)	48.30	4.21	51.16	8.23	44.51	4.60	42.46	3.71

Overall mean productivity of *M. rosenbergii* in Trial 2 was 46.61±1.94g compared with a mean productivity of 5.19±2.06g for *M. lar* ($t_{3,\ 0.05}$ = 33.076, P = 0.00).

In Trial 2 survival of *M. lar* followed a similar pattern as for Trial 1, with 45.7 to 64.17% while *M. rosenbergii* survival approached 100% in all ponds except for pond 3 with 96.3% (Figure 3.7). Proportion of males and females surviving were not different between trials.



M.r = Macrobrachium rosenbergii; *M.l = Macrobrachium lar*

Fig: 3.7: Survival rates for *M. rosenbergii* and *M. lar* for the 4 ponds in Trials 1 and 2

In Trial 2, the FCR for *M. lar* ranged from 3.62 to 10.43, while for *M. rosenbergii* was 1.45 to 1.72 (Table 3.10). The data for *M. lar* indicate that the rate of incremental growth decreased over the course of each trial indicating that it would probably not be profitable (in terms of time and space usage) to continue growing *M. lar* to a larger size in such culture systems beyond three months.

Table 3.10: Survival, mean weights, productivity and FCR for *M. rosenbergii* in Trials 1 and 2

Trial 1 & 2	**Pond Number**							
	1		2		3		4	
Species	*M.r 1*	*M.r 2*	*M.r 1*	*M.r 2*	*M.r 1*	*M.r 2*	*M.r 1*	*M.r 2*
Pond size (m^2)	21.6	21.6	24	24	21.6	21.6	24	24
SD (PL/m^2)	5	5	5	5	5	5	5	5
Total initial stocked	108	108	120	120	108	108	120	120
survival	91	108	81	120	91	104	107	120
Survival (%)	84.26	100.00	67.50	100.00	84.26	96.30	89.17	100.00
Harvest weight (g)	2646.4	5216.4	2537.5	6139.7	2301.4	4807.5	3230.	5095.7
Mean weight (g)	29.08	48.3	31.72	51.16	25.29	45.79	30.19	42.46
Standard Error (±)	±1.78	±1.82	±2.38	±1.77	±1.87	±1.57	±1.58	±1.44
Productivity(kg/m^2)	0.12	0.24	0.11	0.25	0.11	0.22	0.13	0.21
FCR	2.38	1.45	3.46	1.62	2.94	1.72	2.35	1.58

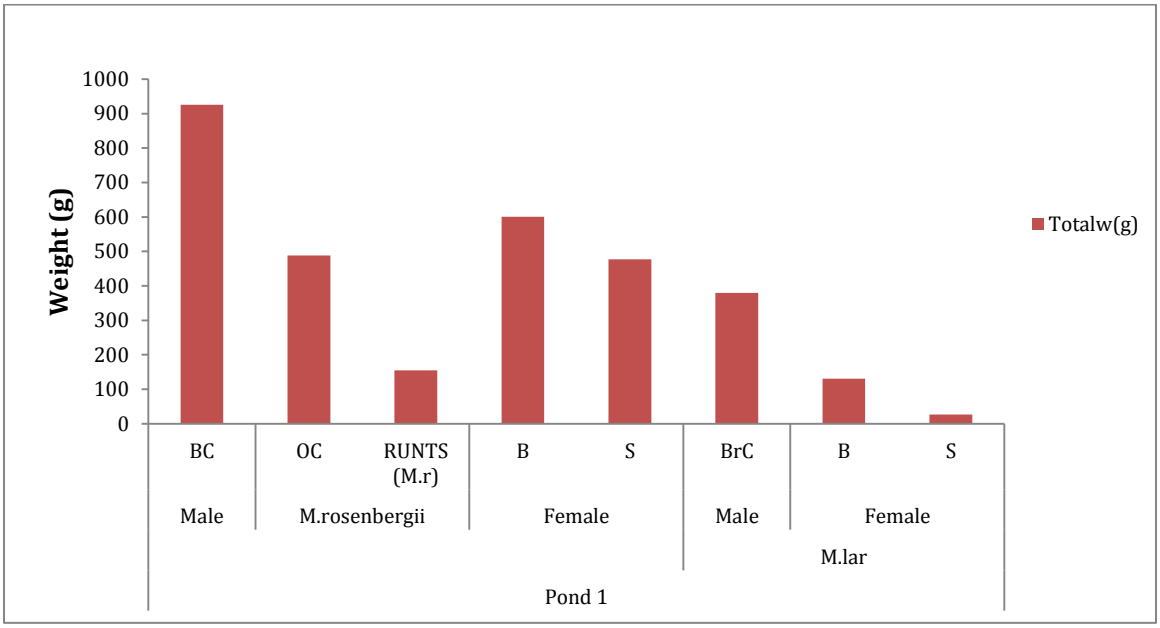
M.r =Macrobrachium rosenbergii; *M.l = Macrobrachium lar*; SD = stocking density.

The mean weight of each sex and total yield showed significant differences between species and between trials. Large differences were observed for each sex in Trial 1. The average weight of males at harvest was more than twice that of females and the average weight of all prawns reared in Trial 2 was slightly higher than that of those reared in Trial 1. Weight distribution for males, females and combined sexes showed a tendency towards positive skewness. Skewness in females was almost 3-fold higher than that for males, while weight distribution of prawns in Trial 1 showed a higher skewness than that of prawns reared in Trial 2.

3.4.4 Population structure

The distribution of male and female morphotypes for *M. rosenbergii* at final harvest in Trial 1 is shown in Figure 3.8 for Pond 1. A similar distribution was evident in all ponds in both trials. Male morphotypes include blue claw males (BC) that are large sized and

socially dominant with prominent chelae, smaller orange claws (OC) are behaviourally submissive to the BC males while runts (SM) occupy the most submissive (to both BC and OC males) position in the dominance hierarchy and make up the smallest value mode in the size distribution. Female morphotypes are based on individual reproductive status, not body size and social status as in males, and are described as gravid females carrying eggs-berried (B) and females that have passed the 'B' stage and have shed their eggs, as shed, (S).



BC= Blue claw male; OC= Orange claw; B = berried female; S = shed female; BrC = Black claw male

Figure: 3.8: Final crop of *M. rosenbergii* and *M. lar* gender classes according to morphotypes from pond 1 in Trial 1.

3.4.5 Production cost estimate

The mean productivity in Trial 1 of *M. lar* was 30g/m^2 (300kg/ha/cycle) compared with 230g/m^2 (2,300kg/ha/cycle) for *M. rosenbergii* (see Table 3.8). The feed conversion ratio (FCR) ranged from 4.06 to 7.83 for *M. lar* and 2.37 to 3.46 for *M. rosenbergii*.

In trial 2, productivity of *M. rosenbergii* ranged from 0.21 to 0.25kg/m^2 that translates to an estimated total production of 2,100-2,500 kg/ha/cycle. The FCR ranged from 1.45 to 1.72. The cost of production under this management was estimated at US$4.00-5.00/kg.

3.5 Discussion

Water quality data collected in both trials indicate that conditions were within reported ranges for good growth of *M. rosenbergii* (e.g., New & Singholka 1982; Sandifer & Smith 1985). Water temperature within the 18-34ºC range is considered to be in the preferred range by New & Singholka (1982), but lower than the range of 29-31ºC identified for *M. rosenbergii* as reported by Tidwell and D'Abramo (2000). The mean pH was 8.24 (all ponds combined), and this is within the acceptable range of 6.5–9.0 as reported for *M. rosenbergii* by New and Singholka (1982). While the record of dissolved oxygen in the ponds has not been included here because of periodical malfunction of the test equipment, since water flow was continuous through all ponds during both trials and no pond water quality problems were experienced in either trial, the water quality was considered suitable for grow out of both species. Additionally, high survival rates (almost 100%) were achieved in 3 ponds in Trial 2 for *M. rosenbergii* indicating that the grow-out conditions were extremely favourable for the experiment. It can often be difficult however, to control water quality in ponds during grow out because it can change with season and rainfall levels. Visual observations indicated however, that the water was clear and in general, of high quality over both trials.

Following from above, the climate of Vanuatu varies from hot, very humid with little seasonality in the North, to a warm, less humid and more seasonal climate in the South. Ambient temperature range from 18°C to 31°C and the cold drier season is from June to September, while the hot rainy season is from October to April. Changes in temperature on a daily basis from summer to winter however, are relatively small. The average temperatures differ by only 2-4^{O}C between the coolest months (July and August) and the warmest months (December-February). Based on information from Vanuatu

Meteorological Service (VMS), the average night temperatures can be as low as 18°C and the average day-time temperatures can be as high as 32°C. There are also no pronounced long periods of rainfall that could lower water temperature in the ponds by reducing the intensity of light and causing turbidity in the water supply. Importantly, the culture site has adequate sunlight, and has overall conditions that are considered ideal for prawn grow out all year round.

Water flow rate and water supply are the key limiting factors in calculating the potential for pond water exchange, and can directly influence prawn productivity (New & Singholka 1985). Mean water flow rate in all ponds was 0.1l/sec. New and Singholka (1985) suggested flow rates of 0.14 to 0.28m^3/min/ha (2-4%/day) to replace evaporation and seepage losses, and 0.56m^3/min/ha (8%/day) to keep continuous flow in the ponds while others have opted for lower flow rates. The flow rate in Trial 1 was 0.1m^3/min/ha and in Trial 2 was 0.24 m^3/min/ha, both well within the recommended rates suggested above. Thus, results obtained suggest that conditions in the culture ponds were most likely close to optimal for normal growth of both species.

Results obtained here confirmed that in 144 days (Trial 1) of culture, *M. rosenbergii* grew three fold faster in terms of mean weight compared with *M. lar*, while in trial 2 (120 days), growth rate was even higher (five fold), and higher than has been reported in the literature elsewhere for *M. rosenbergii* under similar climatic conditions, e.g. see Hurwood *et al.*, (2012). *M. lar* grew about 60% more slowly, i.e., combined mean weight of prawn harvested was 9.52g in Trial 1 and 9.56g in Trial 2 compared with 29.07g (Trial 1) and 46.93g (Trial 2) for *M. rosenbergii*. The growth performance outcomes in this study showed substantial differences between species. The differences in productivity (Table 3.8) were also large with mean values of 230g/m^2 (2,300kg/ha/cycle) for *M. rosenbergii* compared with 30g/m^2 (300kg/ha/cycle) for *M. lar*.

Variation in prawn weight during sampling for each species was relatively small and was similar in both trials. For crustaceans that have a rigid exoskeleton, growth is essentially a

discontinuous process achieved via a succession of moults separated by intermoult periods. Almost all growth occurs incrementally, immediately after a moult and before hardening of the new tegument. After this growth spurt, only limited growth is possible between moults due to limited flexibility of the membranes and plates forming the largely rigid exoskeleton (Hartnoll 1982) and this occurs at a much slower rate (Sampaio & Valenti 1996).

Though both species were of similar size and weight when stocked, at harvest no *M. lar* males had attained a weight over 50g in Trial 1. A similar trend was observed for growth of females that varied over a range of approximately three fold. The average weight of males at harvest (25.7 ± 0.6g) was 2.7 times that of females (9.5 ± 0.2g). In Trial 2, a similar trend was found, whereby mean weight of males at harvest (19.1 ± 0.8g) was 2.8 times greater than for females (6.8 ± 0.6g). The distinct variation in growth rate of male and female prawns recorded here is widely recognised for *M. rosenbergii* (Wohlfarth *et al*. 1985; Karplus *et al*. 1986a & b), in *M. malcolmsonii* (Kanaujia *et al*.1997), and in *M. acanthurus* and *M. carcinus* (Dobkin *et al.* 1974). Variations in weights of male *M. lar* individuals were much higher compared with females because they grow considerably more slowly after achieving sexual maturity, a pattern that results from territorial and/or aggressive behaviour among males in this genus. Differences in growth rate of adult prawns in same-age populations are associated with three kinds of morphologically distinguishable males that are termed ‘blue claw’ male (BC), ‘orange claw’ male (OC) and ‘small males’ (SM). The relative frequencies of the three morphs in a culture cohort at harvest can affect overall pond productivity outcomes significantly, with OC males having higher growth rates than the other two morphs, so changes in their relative frequency can affect mean growth rate of a pond population.

Based on the pattern described above, the two *Macrobrachium* species compared here exhibit a bimodal growth pattern, in which males potentially exhibit superior growth to females constituting a possible disadvantage in the management of these species. Management of sexually dimorphic growth has been improved by a variety of methods to

increase yields for *M. rosenbergii*, including improving environmental and nutritional conditions and manipulation of the population structure by selective stocking and harvesting (Ra'anan & Sagi 1985; Sagi *et al*. 1986; Hulata *et al*. 1988).

Based on the growth, survival rates and production data of prawns in each pond, significant differences ($p<0.05$) were found in growth rate for males and females between the two trials except for small *M. lar* individuals in Trial 1. The proportion of females to males however, was not affected.

A higher proportion of females at harvest are a common outcome in both mono-and polyculture of *M. rosenbergii* (Willis & Berrigan 1977; Smith *et al*. 1981, 1982; Sandifer *et al*. 1982; Karplus *et al*.1986a). The processes responsible for higher survival rates of females in freshwater prawn populations has been described by Smith *et al*. (1978) and Karplus *et al*. (1986b) in studies carried out under different culture conditions on *M. rosenbergii*. The higher proportion of females to males recorded in Trial 1 is likely to reflect selective male mortality (reported in *M. rosenbergii* by Segal and Roe 1975).

Tables 3.6 and 3.8 show that there were significant differences in the mean percentage survival of *M. lar* prawns between Trial 1 (55.95%) and Trial 2 (52.98%). A problem in the supply of water arose after one month of culture in Trial 1 leading to reduced daily water exchange. In addition, a problem with screening of incoming water arose towards the middle of the growout period in Trial 1. This does not explain however, the relatively poor survival rate in Trial 2, suggesting that overall survival in culture for *M. lar* could be a limiting factor for the development of this species in culture,

Growth can sometimes be inversely related to survival, so higher survival could have impacted negatively on growth rate in this study, however, no significant differences for *M. rosenbergii* males was evident between the two trials. Differential growth of *M. rosenbergii* has often been attributed to territorial behaviour as reported by Daniels *et al*. (1995) and Menasveta and Piyatiratitivokul (1982), competition for food and loss of

exuvia (Segal & Roe 1975), a social dominance hierarchy and/or early sexual maturation (Cohen *et al*. 1981; Cohen & Ra'anan 1983). Similar reasons may perhaps apply to *M. lar* here, where females may have matured at a much smaller size and then spent much of their energy in gonadal maturation and repeated breeding cycles. Production as well as survival was better in Trial 2 compared with Trial 1. Based on these results, a relatively low stocking density of 50,000 juvenile/ha is therefore recommended to optimise pond productivity.

Investigations of other *Macrobrachium* species have been made under pond conditions by several workers. In monoculture, Rao *et al*. (1986) obtained 534.2 to 690.4 kg/ha with a survival of 44.2 to 57.2% over 13 months. Rama Rao *et al*. (1992) recorded higher production of 774 kg/ha/year with a survival of 64.77%. In the present study, a production in the range of 0.21 to 0.25kg/m^2 was achieved in 120 days in Trial 2 that translates to an estimated total production of 2,100-2,500 kg/ha/cycle. The FCR ranged between 1.45 (Pond 1) and 1.72 (pond 2) and this is within the range reported in semi intensive farming systems in Bangaldesh (Ahmed *et al*, 2008). The FCR can be improved by improving feed formulation, i.e., using a diet specific for freshwater prawns since a commercial 'tilapia' pellet (imported from Fiji) was used and also by reducing waste. Waste can be reduced by monitoring and feeding the right amount of feed and where possible providing more feed during the night. Overfeeding results in higher cost of feed per unit prawn produced and in water pollution. According to Shang *et al*., (1998), the unit cost of feed can also be lowered using locally available materials for feed instead of commercial feed. The costs of production relate to the level of inputs, the price of inputs, the culture systems, and institutional factors such as costs of credit and marketing (Shang & Tisdell 1997). In the present study, more specifically, in Trial 2, cost of production based on yield, costs and returns were collected for *M. rosenbergii* and was estimated at US$4.00-5.00/kg. This is higher than previously reported production levels and low cost of production achieved under similar conditions elsewhere in *Macrobrachium* species. In integrated systems, a much lower production level has been reported compared with the findings here. These results suggest that production rates of *M. rosenbergii* have the

potential to be improved further via developing a better understanding of optimum management practices for this species and by improving culture techniques and practices and removing bottlenecks in support services, or a combination of these alternatives (Shang & Tisdell 1997). Prospects for total pond production would be greatly enhanced by management procedures that employ separation of juveniles into weight classes prior to stocking as shown for *M. rosenbergii* by Ra'anan and Cohen (1983) and Karplus *et al*. (1986) at the start of summer as with Trial 2 reported here. A partial or selective harvest designed to remove the largest animals in a pond population after a specified period may also be advantageous if the growing season exceeds four months duration. This procedure will permit compensatory growth of smaller males that have been growth inhibited by social factors from larger males present in the population.

Results obtained here also suggest that there is no critical need to provide special feed supplements to *M. rosenbergii*. This species appears to be able to adjust to differences in diet quality by increasing consumption of benthic detrital fauna. This is consistent with studies by Coyle *et al*. (1996) that revealed juvenile prawns (≥ 2g) fed a variety of live foods grew as fast or faster than prawns fed a nutritionally-complete pelleted diet. The amount of feed supplied to the prawns was abundant and sufficient, however, there are strong indications that *M. lar* depends fundamentally on natural food being available in ponds and this may have impacted the results obtained here. Accordingly, increased competition for food may occur at higher densities, regardless of the amount of additional feed provided. The influence of limited food supplies on growth depends on reaching a critical biomass in the pond rather than simply total prawn numbers per unit area. Thus while *M. lar* can apparently tolerate high densities of conspecifics, survival and growth may depend on adequate natural feed being available that is not always available in ponds with high stocking rates.

Results presented here suggest that while *M. rosenbergii* has significant potential in low input aquaculture in Vanuatu, there is also an opportunity to refine existing culture techniques and practices in terms of optimal pond location, layout, and construction to

optimise growth performance. The factors including; feed (locally available), pond management and various other innovations (shade system) could be experimented with to further optimise productivity. On average, *M. rosenbergii* mean weight in all ponds combined exceeded 29g after 144 days in Trial 1 while in Trial 2 it was 46g after 120 days of culture. Compared with performance of *M. rosenbergii* in Fiji, this is a very good growth rate (Hurwood *et al*. 2012) and can be attributed to use of a high quality feed (tilapia feed) and availability of optimal pond water conditions.

The high survival rate of *M. rosenbergii* observed here compared with previous reports suggest that it may be possible to increase stocking densities in ponds above that employed here. More recently, biologists have become interested in systems less dependent on open natural environments in order to better control conditions affecting animal growth and mortality in both research and commercial settings. For example, Danaher *et al*. (2007) have indicated that high-density polyculture of tilapia and prawns (*M. rosenbergii*) increased pond profit over low density polyculture management practices and prawn monoculture by 165% and 561%, respectively. Thus, it may be possible to increase initial stocking density in the ponds from $5/m^2$ to near $10/m^2$ if good water exchange, appropriate feed, sufficient substrate and shelter are provided.

In combination, the results suggest that *M. rosenbergii* has significant potential for development in culture in Vanuatu and can create a positive impact on small scale subsistence rural farming communities there, provided the technology used is simple, low cost and allows most locals access to the system and supply of juveniles. The use of local knowledge, particularly with regard to feed and pond design should be incorporated into future culture systems especially in remote areas where access to transport and facilities are often difficult, e.g. supplementing high quality prawn feed with kitchen wastes, using coconut leaves instead of black plastic liners on pond bottoms to reduce seepage etc.

Importation of commercial feeds to Vanuatu would present problems due to quarantine and customs department requirements, not to mention the large costs involved. For the

long term, the VFD and other line agencies in Vanuatu should consider trialling locally available alternative feeds such as stock feeds and marine shrimp feeds from local companies after a cost-benefit analysis.

M. rosenbergii clearly has a role to play in successful commercial aquaculture in Vanuatu, similar to what is already being practiced notably in Vietnam, India, Brazil, China and Fiji and that has been the subject of trial experiments in many other countries. The trials described here constitute an exploration intended to be simple in technical design and operation. Overall the results obtained were encouraging, but, more effort needs to be put towards optimizing *M. rosenbergii (*and perhaps even to *M. lar*) culture methods to increase overall profitability to determine optimal stocking densities based on feeding rates and to better understand and limit territorial and aggressive behaviour, inherent in both species.

4.0 General discussion

4.1 Implications of study findings

The main objective of the current study was to assess the scale at which the indigenous freshwater prawn *M. lar* disperse and re-colonise freshwater, and the basic environmental parameters required for developing simple aquaculture systems for *M. lar* and the exotic *M. rosenbergii* in Vanuatu. The study resulted from wide recognition that freshwater prawn fisheries now play important roles in food supply, food security and income generation, however due to over-exploitation catches have declined in recent years and therefore to ensure long-term sustainability of this industry there is a need to establish economically viable small-scale aquaculture. In addition, in Vanuatu there are some serious local economic and social problems coupled with relatively few opportunities to develop new industries because economic conditions are generally unfavourable and thus scale of industries must, by compulsion of land ownership rights, be quite small. If these issues are considered with growing levels of poverty, declining natural resources and increasing impacts of climate change, it is essential that new opportunities like as small-scale freshwater prawn farming can be developed to allow locals to participate in these activities and to live in their rural communities.

While developing freshwater prawn aquaculture industries in Vanuatu can potentially help to address some of these problems, it must be done in a way that does not require huge changes to local life styles, and allows all levels of society to benefit from such developments. While there are few indigenous freshwater aquatic species in Vanuatu that have potential for development as farmed species, *M. lar* is one species that has been identified as having potential for culture (Nandlal 2010). Although *M. lar* culture in simple ponds has proven to be practical in Vanuatu, at least on Santo Island (Nandlal 2010), exotic species like *M. rosenbergii* has been very successful elsewhere and offers a direct comparison. Potential advantages of using native species over exotics include, wild juvenile availability, better tolerance of local environmental conditions, satisfactory growth rates under lower temperatures, and better acceptance in local markets. Furthermore, most of the inland fishery in Vanuatu involves harvesting native

Macrobrachium species from streams and creeks and so people have developed a stake in conserving and in some cases, enhancing biodiversity so they are regarded highly. Freshwater prawn wild fisheries however, have been decreasing over the last 10 years mostly due to over-fishing and use of destructive fishing methods. Identifying and demonstrating that freshwater prawn culture is practical and efficient in Vanuatu while also identifying the best species option can assist development goals there. Thus, the objective here was to attempt to address a number of important issues recognised by the Vanuatu government and the regional organization- SPC, affecting the development of freshwater prawn culture in a simple culture system for rural farmers.

4.2 Geographical scale of wild *M. lar* population structure

Results of the study confirm that *M. lar* wild populations in Vanuatu and Fiji constitute a single genetic population and that gene flow between and within these two countries has been extensive. Since *M. lar* adults are essentially confined to freshwater, gene flow is most probably mediated by dispersal of larvae via the marine environment. A review of the literature on larval development and duration in *M. lar*, for example, Atkinson (1977) and Nandlal (2010) add support to this view i.e., early larval stages show tolerance of full marine conditions and development of larvae requires at least 90 days in the marine environment for newly hatched larvae to reach PL stage. Other studies of *M. lar* provide additional support that larvae are capable of wide natural marine dispersal. For example, 94 specimens of *M. lar* were released into a freshwater stream on Molokai in 1956, and 27 specimens into a stream in Oahu in 1957 in the Hawaiian island chain (Maciolek 1972; Hanson & Goodwin 1977). Subsequently, in the absence of further introductions, larvae have apparently dispersed naturally and colonized every island in the Hawaiian chain that has suitable freshwater habitat available as reported by Hanson and Goodwin (1977). This would only be possible for an animal able to survive long periods in seawater. Natural dispersal in Hawaii has been so successful, that this exotic species is now considered to be a pest and it has displaced some indigenous palaemonid species in certain streams (Kubota unpublished).

This implies that larvae are naturally adapted to long periods of exposure to marine conditions, a trait that allows potential for wide geographical dispersal in ocean currents. Dispersal in the wild is most probably passive in currents both in freshwater streams and in the sea. These data have implications for both, long term persistence of wild stocks and for how a sustainable culture industry could be developed based on this species. For wild populations in Vanuatu, it is clear that where over-fishing or destructive fishing methods cause population declines in individual streams or islands, there is high likelihood of stream and creek populations being recolonised naturally or self-recruiting from adjacent streams or even distant neighbouring countries, for example, Fiji, to which they are apparently connected by natural larval drift. Thus there is a limited potential for local population structure to develop due to isolation and populations are likely to be replenished naturally by recruitment from adjacent islands over extended periods of time. Local gene frequencies at individual sites, particularly among islands, may fluctuate over time however; as probability of colonization by a large PL cohort is likely to be low and random colonization events will tend to skew gene frequencies due to local genetic drift effects. Changes in gene frequencies at a local scale in Vanuatu or Fiji are unlikely however, to lead to the evolution of significant genetic differentiation over time, simply because the randomness of larval colonization are likely to average out relative genotype frequencies at any single site, over time. Genetic diversity is also likely to be greater in Vanuatu populations as they will tend to accumulate mutations that have drifted from east to westward (for example, from Fiji) as components of westerly larval drift in major ocean currents (via the Pacific South Equatorial Current and South Pacific Current) and winds (South East Trade Winds).

In terms of any future development of a local *M. lar* culture industry in Vanuatu, the genetic data are also very clear and indicate that wild populations in Vanuatu are similar in ‘structure’ but divergent from Cook Islands that should be considered to be discrete stocks. The two stocks (Vanuatu/Fiji and Cook islands) have apparently been diverging for significant evolutionary time. Hatchery production of PLs and juveniles for use in Vanuatu and Fiji could be established, for example, at TFAC in Vanuatu and broodstock

for hatchery operations could easily be obtained from the wild across the islands. In addition, for any future stock improvement program broodstock could also be developed with very high levels of genetic diversity if strategic sampling of compatible wild stocks was practiced. Thus, based on available data, the basis for developing a sustainable culture industry for *M. lar* in the future is now clear but for this potential to be realised in practical terms there is a need for improvement in pond grow out practices, and mass hatchery production of PLs to be developed.

The status of the Cook Islands *M. lar* populations remains unresolved since stocks there could be divergent due to their remote position and the great distances between the Cook Islands and, for example, Fiji (minimum distance of approximately 2,356 km). This great distance could be perhaps beyond natural larval drift potential and thus successful colonization of Fiji and Vanuatu may be limited by geographical distance. In theory, *M. lar* larvae should have moved or dispersed via currents from east to west, allowing passive dispersal of Cook Island genotypes to move more westerly to Fiji. In the past however, when sea levels were significantly lower during the Pleistocene era, dispersal may have been possible as distances between land masses were less and land masses were probably larger and more islands were present within the Pacific Ocean than remain today. It remains to be seen if *M. lar* populations in more easterly Pacific sites including French Polynesia are related to Cook Islands stocks or also may constitute additional 'evolutionary significant' units for this species.

4.3 Comparative analysis of simple farm production of *M. lar* and *M. rosenbergii* in Vanuatu

Results of grow-out trials show that performance of *M. rosenbergii* in simple small pond systems in Vanuatu has matched or have even exceeded that of this species in farms in Fiji and S.E. Asia. Thus, *M. rosenbergii* can apparently be farmed successfully and economically in Vanuatu. There is considerable potential for aquaculture development there, particularly in areas where agriculture development is limited. In this regard areas of swamps, wetland taro farms and other low-lying areas with water in Vanuatu provide

an opportunity for farmers to participate in aquaculture and hence to profit from available (sometimes marginal) land with a little extra investment required for pond construction. On average, *M. rosenbergii* weight exceeded 29g after four months of culture in Trial 1 (mean productivity 0.12kg/m^2) while in Trial 2 it reached more than 46g (0.23kg/m^2). Projections based on minimum production rates, indicate that between 2400 kg/ha/year and 4,600kg/ha/year could be achieved in Vanuatu. Comparatively, this is an excellent production level and can be attributed to favourable water temperature, use of high quality feed (tilapia feed) provided in the trials combined with favourable water quality. These results parallel that of *M. rosenbergii* juveniles grown to market size in simple culture systems in S.E. Asian countries. Since hatchery production of *M. rosenbergii* has been successful in Vanuatu and prawns derive part of their dietary needs from the organic micro-fauna already present in the ponds, in theory, farming prawns makes better use of land and water and can produce greater overall yields and returns than is available from some similar farming systems, for example, wetland taro farming. This basic system can be modified to fit more productive and commercially oriented systems whereby fallow ponds could be used for mono-culture prawn farming with feed inputs to supplement dietary needs. Semi-intensive farming systems are also likely to be relatively easy to establish and this will enable production to grow in response to local market demand.

4.4 Future work

While we did observe some movement of *M. rosenbergii* individuals into compartments occupied by *M. lar* and vice versa after initial stocking, an outcome that compromised in part, attempts to assess growth rates rigorously, the fact that mean survival rate across the ponds exceeded 90% suggests that it may be possible to increase stocking densities in ponds significantly above that employed in the trials here. For example, perhaps from 5 prawns/m^2 to approximately 10prawns/m^2, a density not usually practiced in *M. rosenbergii* culture in Fiji. Either pond conditions at TFAC are more favourable for *M. rosenbergii* culture or food requirements in ponds were able to sustain higher survival than has been reported elsewhere. Either way these data are interesting and should be

followed up to determine the maximum stocking density that can be employed without impacting relative productivity and survival rates of stocked prawns.

Outcomes of the grow-out trials here clearly indicate that a low-input approach to *M. rosenbergii* culture can be successful and is an acceptable model for sustainable aquaculture in Vanuatu because it exemplifies judicious use of resources with minimal environmental impacts while providing sound economic benefits to small rural communities. The simple farming systems trialled here, especially *M. rosenbergii* culture have recently been extended and implemented in the Mangaliliu village, about 40km from TFAC and this has created a new small enterprise for some farmers. Prawns are sold soon after harvest at US$15.00/kg and this is a very good return on the investment made by the farmers compared to returns, for example, from their taro farms. According to VFD many farmers have requested technical assistance to establish prawn farms, thus there is significant potential for many more prawns farms to be established across the islands. A number of aquaculture systems have been trialled in Vanuatu and other PICTs in the past but few have been successful over the long term. While the reasons for failures are diverse, perhaps establishing simple, small-scale pond culture of *M. rosenbergii* that compliments rural life-styles well may be a better long term option and allow local people to become familiar with simple prawn farming systems before more high technological approaches are considered.

In comparison with the faltering production and future production uncertainty of the wild *M. lar* fishery in many localities across Vanuatu, simple small-scale *M. rosenbergii* culture appears to have a bright future as a source of income and human food. Based on the results of this study, culture of *M. rosenbergii* can be semi-intensive using water from tube well, streams, creeks or rivers in monoculture systems. Since the time that the trials were conducted for this project, several local farms have been stocked with *M. rosenbergii* juveniles in earthen ponds using the production techniques developed in the current study: crops of 5 to 6 prawns/m^2 in 40-50 m^2 ponds. Farms are small, ranging from 1 or 2 ponds and harvest results are comparable with results obtained in the trials

here (Lency Dick, VFD pers. comm.). Increased production can be achieved by expanding production areas, improving existing culture techniques, removing bottlenecks in government (and donors) service, improving extension support services, or some combination of these factors.

Following from above, the relative costs of inputs, especially formulated feeds will be high and hence the environmental impacts of different culture intensities and systems should be an important consideration in selecting the type of prawn farming practices that are suited to the local people in Vanuatu. Polyculture and integrated farming are usually ecologically more sound than monoculture due to more efficient use of waste and energy on the farm. Farm wastes can be recycled to produce multiple products, and water pollution can be reduced. These farming systems tend to be more favourable than monoculture from an environmental view point, especially in rural areas and outer islands with high prices of imported feeds and energy, and low family labour costs. In addition, the integrated farming systems are often more profitable as observed in prawn farms in Fiji and also reported in the literature, e.g., Bardach 1986.

4.5 Some issues that remain to be tackled in the future

Key issues for current and future research and development for any *M. rosenbergii* culture industry in Vanuatu will include optimising hatchery production of PLs followed by nursery rearing and transport of PLs and juveniles to remote farms and to farms on outer islands. This is because PL and juvenile prawn production relies on sustainable hatchery production of quality PLs. High cost of larval feeds, especially *Artemia* (imported from Taiwan) often affect hatchery operations. If prawn culture is to be introduced successfully, some degree of certainty over PL and juvenile supply will need to be developed to maintain farmer participation. Several other specific areas that require further research are mentioned in each of the chapter discussions. The following summarises areas that should be considered for future work.

1. Even though *M. lar* is widely distributed in Vanuatu, the high value and demand for this species has resulted in some local populations being over-harvested in some

localities, and in some localities non-sustainable capture methods are employed to collect them from the wild. In addition, construction of coastal roads has affected them negatively, not only by preventing migration but also by reducing connectivity of their habitats, i.e., in some cases, small streams and creeks are completely destroyed. These practices have depleted local populations significantly in some locations and therefore may have significant implications for biodiversity of stream communities and for the long-term viability of the *M. lar* wild fishery. In addition, in recent times, there has been increased pressure on the part of VFD to develop aquaculture to supplement fish supplies from aquaculture due to declines from capture fisheries before stocks decline to a critical level. In the current study some variation was also observed in individual body weight, growth and differences in other economically important traits that justify a need for establishing a good genetic foundation for any future hatchery production based on *M. rosenbergii*. This will provide the basis for improving culture attributes as has been achieved successfully for other cultured species including Nile tilapia, common carp and the shrimp, *Litopenaeus vannamei*.

2. Current *M. rosenbergii* broodstock were imported from Fiji and came from a very small population without consideration of genetic issues that can lead to deterioration in the genetic quality of stocks over time. Before any serious attempt is made to develop a standard broodstock management plan and hatchery and pond grow-out systems for *M. rosenbergii*, it would be beneficial to develop a genetically diverse and outbred broodstock/culture line for Vanuatu.

3. Crustacean pond culture operations require determination of optimum production environments, stocking densities and foods for effective production. In the current study, 100% survival (in 3 ponds in Trial 2) at harvest most likely resulted from a failure to stop encroachment of *M. rosenbergii* into compartments occupied by *M. lar* and vice- versa. It seems likely that *M. rosenbergii* individuals may have accessed additional resources intended for *M. lar* and this could have influenced survival and growth rates. This will require further experimentation.

4. Another source of variability in the results of the grow-out trials here may be due to the condition of *M. lar* juveniles. Juveniles were collected from creeks and transported to VFD (see sections 3.2.4 and 3.2.5). Some juveniles were stocked soon after arrival at the pond site while others were conditioned for 1 to 3 days and stocked after careful handling. In general practice, juveniles must be held for conditioning to overcome transport stress and provided with proper nutrition before stocking. Under these circumstances, juvenile health and subsequent development are highly dependent upon good nutrition and handling prior to stocking. Since the *M. lar* juveniles were collected from the wild, all their nutrition came from food that was available in the streams and creeks and may not have been the proper nutrition for optimal growth in comparison to that of *M. rosenbergii* that were nursed in hatchery using quality feeds. The nursery diets must contain essential nutrients (Cohen *et al.* 1981; Ra'anan & Cohen 1982) for good growth performance in ponds and this clearly suggests the possibility of enhancing the condition of juveniles via improvement of diets and handling, thus more research is needed to establish dietary requirements for effective *M. lar* nursery rearing in tanks.

5. Locally available feed ingredients (agricultural by-products) and kitchen wastes have been used as an alternative culture feed for *M. lar* trials in the past (Nandlal 2010). Unfortunately, it was not possible to compare the relative growth and survival performance of prawn cohorts fed high quality tilapia feed versus local feeds, to quantify the impact that low-cost feeds have on production efficiency. This work will contribute greatly to developing freshwater prawn culture in Vanuatu since the cost of feed is probably the most important cost item for a relatively small island nation of Vanuatu. Feeds costs often constitute 40 to 60% to production costs (D'Abramo & Sheen 1991), and since feeds are imported, this may also present problems due to quarantine requirements. Cost of feed per unit of prawn produced depends mainly on the conversion ratio of feed to prawn and the unit price of feed. Therefore, the cost of feed can be reduced by improving the conversion ratio or by lowering the unit price of feed, or by a combination of these two factors. The conversion ratio in turn can be improved by reducing waste, pond fertilization and improving the feed formula, for example,

developing a feed specifically made for freshwater prawns instead of using tilapia pellets. According to Corbin *et al*. (1983) the major portion of macronutrients for cultivated freshwater prawns need to be provided in prepared diets and the required levels of micronutrients (i.e., vitamins and minerals) can be obtained from natural productivity. In addition, Tidwell *et al*. (1993a and 1993b) demonstrated that fish meal in freshwater prawn diets could be replaced with less expensive plant proteins with no adverse effects on prawn growth. Following from above, the waste can be reduced by feeding the right amount of feed, while the unit cost of feed may be lowered by utilizing locally available feed ingredients instead of including imported ones (e.g., tilapia pellets from Fiji). Other practices to reduce the costs of feed and fertilizer are to use integrated aquaculture-agriculture and polyculture systems. These farming practices diversify and minimize risks and uncertainty, and also safeguard the environment. Thus, a better understanding of the role of natural productivity in *M. rosenbergii* and *M. lar* nutrition could lead to management strategies that selectively enhance desirable food organisms and lower feed costs by utilizing low-cost agricultural by-products as feed. And since *Macrobrachium* prawns are benthic omnivores (Ling & Merican 1961), and can adjust to differences in diet quality by increasing their consumption of benthic fauna (Hill *et al*. 1997), identifying a low cost, local alternative feed would enhance industry development.

6. A sustainable aquacultural development project depends not only on increased production and income, but also on the existence of a potential market, an efficient marketing system, and other supporting systems including hatcheries, feed mills, credit, research and extension support services. While all supporting services need to be well coordinated for sustainable development, currently there is also a need to improve local hatchery and pond grow-out techniques and practices, i.e., to determine how best to improve PL production, to nurse PLs, feed zoea larvae (partial replacement of *Artemia*), optimise pond location, layout, and construction, and pond management and various other innovations (tube well water system). In addition, more attention should be directed toward stocking at the commencement of summer (to take advantage of relatively high pond water temperatures) in order to maximize final harvest weight and to minimize the

degree of variation in individual sizes. There is also a need to control or effectively minimize the contribution of economically undesirable morphotypes by sorting juveniles just prior to stocking. This procedure would be designed to select individuals that were within a certain percentage of the upper end of the weight frequency distribution to capitalize upon an already established social structure and lead to a greater mean harvest weight with significant reduction in the degree of variation as has been suggested in *M. rosenbergii* studies elsewhere.

4.6 Contribution of the study

The major outcome of this project has been a step towards meeting the requirements to grow *M. rosenbergii* and *M. lar* communally in captivity and to identify the scale at which a local freshwater prawn culture industry could be based in Vanuatu.

Planning a specific aquaculture project is a complex and interdisciplinary task. Successful aquaculture at any location involves both technological and nontechnological components that must be assessed, planned for, decided on, and managed using a holistic approach. Other site-dependent factors include economics, markets, community sociology and culture, land tenure systems, government policy, regulation and politics. Recent experience in Vanuatu shows that failure in, or a major conflict with, any of these components can slow or stop project development or cause a successful operation to fail and not be sustainable. Thus closer collaboration among government and donor agencies in Vanuatu and PICTs with interest in developing local aquaculture industries will enable a concerted effort to be directed towards use of exotic and as well as native aquatic species in a developing culture framework. Other regional countries through regional collaborations, can also share experience and expertise on existing methods to build towards more cost-effective methods of farming *M. rosenbergii,* and where applicable, *M. lar.* Aquaculture development in Vanuatu in general, has not had a favourable history in part, perhaps due to a lack of knowledge and cultural understanding of the exotic species introduced for farming purposes. Results here demonstrate that at least one species of freshwater prawn (*M. rosenbergii*) offers excellent potential in simple pond

culture systems and can potentially provide nutrition, employment and improved livelihoods for local people in Vanuatu in the future.

5.0 References:

Ahmed, M. & Delgado, C. 2000. Introduction to the issues and context of rapid changes in world demands for fish. *Fish to 2020: implications of global fish outlook for developing countries*. Penang Malaysia: World Fish Centre.

Ahmed, N., Ahammed, F & Brakel, M.V. 2008. An economic analysis of freshwater prawn, *Macrobrachium rosenbergii*, farming in Mymensingh, Bangladesh. *Journal of the World Aquaculture Society*, 39(1): 37-50.

Akpaniteaku, R.C., Weimin, M & Xinhua, Y. 2005. Evaluation of the contribution of fisheries and aquaculture to food security in developing countries. NAGA, WorldFish Centre Newsletter 28, No.1 & 2.

Allendorf, F.W. 1983. Isolation, gene flow and genetic differentiation among populations. 51–65. *In:* Schonewald-Cox, C., S. Chambers, B. MacBryde, and L. Thomas, editors. *Genetics and Conservation*. Benjamin-Cummings. Menlo Park.

Allendorf, F.W. & Luikart, G. 2007. *Conservation and the Genetics of Populations*. Blackwell Publishing, USA.

Arbogast, B.S. & Kenagy, G.J. 2001. Comparative phylogeography as an integrative approach to historical biogeography. *Journal of Biogeography*, 28: 819 – 825.

Atkinson, J.M. 1977. Larval development of a freshwater prawn, *Macrobrachium lar* (Decapoda, Palaemonidae), reared in a laboratory. *Crustaceana*, 33(2): 119 – 132.

Avise, J.C. 2000. Cladists in wonderland. *Evolution*, 54: 1828 – 1832.

Avise, J.C. 2009. Phylogeography: retrospect and prospect. *Journal of Biogeography*, 36: 3 – 15.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., & Neigel, J.E. 1987. Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. *Annual Review of Ecology and Systematics*, 18: 489 – 522.

Balloux, F. & Lugon-Moulin, N. 2002. The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, 11(2): 155 – 165.

Bardach, J.E. 1986. Constraints to poluyculture. *Aquacult. Eng*. 5: 287-300

Bay, L.K., Choat, J.H., Herwerden, L. & Robertson, D.R. 2004. High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorusus sordidus*); evidence of an unstable evolutionary past. *Marine Biology*, 144(4): 757 – 767.

Bay, L.K., Crozier, R.H. & Caley, M.J. 2006. The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great barrier reef. *Marine Biology*, 149(5): 1247 – 1260.

Beebee, T. & Rowe, G. 2008. *An Introduction to Molecular Ecology*. New York, USA: Oxford University Press Inc.

Bell, J. D., Kronen, M., Vunisea, A., Nash, J. W., Keeble, G. & Demke, A. 2009. Planning the use of fish for food security in the Pacific. *Marine Policy* 33: 64-76.

Benardi, G. 2000. Barriers to gene flow in *Embiotica jacksoni*, a marine fish lacking a pelagic larval stage. *Evolution*, 5491: 226 – 237.

Bermingham, E. & Moritz, C. 1998. Comparative phylogeography: concepts and applications. *Molecular Ecology*, 7: 367 – 369.

Bohanak, A.J. 1999. Dispersal, gene flow and population structure. *Quarterly Reviews of Biology*, 74(1): 21 – 45.

Bohanak, A. J. & Jenkins, D. G. 2003. Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters* 6(8): 783 – 796.

Boyd, C. E. & Clay, J.W. 1998. Shrimp aquaculture and the environment. *Scientific American*, 278(6): 42-49.

Brock, V. E. 1960. The introduction of aquatic animals into Hawaiian waters. *Internat. Rev. Ges. Hydrobiol*. 45: 463-480.

Brody, T., Cohen D., Barnes, A. & Spector, A. 1980.Yield characteristics of the prawn *Macrobrachium rosenbergii* in temperate zone aquaculture. *Aquaculture*, 21: 375-385.

CGIAR (Consultative Group on International Agricultural Research). 1995. From hunting to farming fish. Consultative Group on International Agricultural Research. Press release, May 1995. Rome, Italy.

Chatopadhay, G.N., Biswas, C.R., Chakraborti, P.K., Bandopadhyay, A.K. & Ghosh,A. 1995. Possibilities of rice-fish culture in high rain-fed coastal soils. In: *Proceedings of National Symposium on Agrosystems Management*, Vishwa Bharati, Santiniketan, West Bengal, February 18-21 1989. pp. 129-133. Central Inland Capture Fisheries Institute, Barrackpore, West Bengal, India.

Clement, M. and Posada, D. 2000. TCS. Department of Zoology, Brigham Young University.

Cohen, D. & Ra'anan, Z. 1983. The production of freshwater prawn *Macrobrachium rosenbergii* in Israel. III. Density effect of all-male *Tilapia* hybrids on prawn yield characteristics in polyculture. *Aquaculture*, 35: 57-71.

Cohen, D., Raanan, Z. & Brody, T. 1981. Population profile development and morphotypic differentiation in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *Journal of World Aquaculture Society:* 12: 231-243.

Cohen, D., Ra'anan, Z. & Barnes, A. 1983. Production of freshwater prawn *Macrobrachium rosenbergii* in Israel. I. Integration into fish polyculture systems. *Aquaculture*, 31: 67-76.

Cook, L.M., Thomason, E.W. & Young A.E. 1976. Population structure, dynamics and dispersal of the tropical butterfly, *Heliconius charitonius*. *Journal of Animal Ecology*, 45(3): 851 – 863.

Corbin, J.S; Fujimoto, M. M. & Iwai, T.V. 1983. Feeding practices and nutritional considerations for *Macrobrachium rosenbergii* culture in Hawaii, pp.391-412. *In*: CRC Handbook of Mariculture, Volume 1, Crustacean Aquaculture, McVey, J.P (eds). CRC Press, Boca Raton, FL.

Corriera. E.S., Pereira, J.A. & Silva, A.P. 2003. Growout of freshwater prawn *Macrobrachium rosenbergii* in fertilized ponds with reduced levels of formulated feed. *Journal of the World aquaculture Society*. 34(2): 184-191.

Coyle, S., Najeeullah, T. & Tidwell, J. 1996. A preliminary evaluation of naturally occurring organisms, distillery by-products, and prepared diets as food for juvenile freshwater prawn *Macrobrachium rosenbergii*. *Journal of Applied Aquaculture*, 6(2): 57-66.

D'Abramo, L.R., Heinin, J.M., Robinette, H.R. & Collins, J.S. 1989. Production of freshwater prawn *Macrobrachium rosenbergii* stocked as juveniles at different densities in temperate zones ponds. *Journal of World Aquaculture Society*, 20(2): 81-89

D'Abramo, L.R. & Sheen, S.S. 1991. Nutritional requirements, feed formulation and feeding practices for intensive culture of the freshwater prawn *Macrobrachium rosenbergii*. *Review of Fisheries Sciences*, 2: 1-21.

Danaher, J.J., Tidwell, J.H., Coyle, S.D., Dasgupta, S. 2007. Effects of two densities of caged monosex Nile tilapia, *Oreochromis niloticus*, on water quality, phytoplankton populations, and production when polycultured with *Macrobrachium rosenbergii* in temperate ponds. *Journal of World Aquaculture Society*, 38(3): 376-382.

Daniels, W.H., D'Abramo., Fondren, M.W. & Durant, M.D. 1995. Effects of stocking density and feed on pond production characteristics and revenue of harvested freshwater prawns *Macrobrachium rosenbergii* stocked as size-graded juveniles. *Journal of World Aquaculture Society*, 26(1): 38-47.

Davidson, J.R., Brock, J.A. & Young, L.G.L. 1992. Introduction of exotic species for aquaculture purposes. Pp. 83-101. *In* A. Rosenfield and R. Mann (eds). *Dispersal of Living Organisms Into Aquatic Ecosystems*. Maryland Sea Grant Publications.

De Silva, S. S., Subasinghe, R. O., Bartley, D. M. & Lowther, A. 2004. Tilapias as alien aquatics in Asia and the Pacific: a review. FAO Fisheries Technical paper 453. FAO, Rome.

De Silva, S., Nguyen, T.T.T., Abery, N.W. & Amarasinghe, U.S. 2006. An evaluation of the role and impact of alien finfish in Asian inland aquaculture. *Aquaculture Research*, 37: 1-17.

Delgado, C. L., Rosegrant, M.W., Meijer. S. Wada, N. & Ahmed, M. 2002. Fish as food: projections to 2020. In *Fish to 2020: implications of global fish outlook for developing countries*. Penang, Malaysia: Worldfish Center.

Delgado, C. L., Wada. N., Rosegrant, M.W., Meijer. S. & Ahmed, M. 2003. Fish to 2020: Supply and demand in changing global markets, Washington: IFPRA/Worldfish Center. 226 pp. Available at: http://www.ifpri.org/pubs/books/fish2020book.htm

Dias, G., Duarte, L. & Solferini, V. 2006. Low genetic differentiation between isolated populations of the colonial ascidian, *Symplegna rubra*, Monniot, C. 1972. *Marine Biology*, 148(4): 807 – 815.

Dobkin, S., Azzinaro, W.P. & Montfrans, J. 1974. Culture of *Macrobrachium acanthurus* and *M. carcinus* with notes on the selective breeding and hybridization of these shrimps. Proc. 5th Ann. Meeting, World Mariculture Soc., 5: 51-62.

Doherty, P.J., Planes, S. & Mather, P. 1995. Gene flow and larval duration in 7 species of fish from the Great Barrier Reef. *Ecology*, 76: 2373-2391.

Encalada, S.E., Lahanas, P.N., Bjorndal, K.A., Bolten, A.B., Miyamoto, M.M. & Bowen, B.W. 1996. Phylogeography and population structure of Atlantic and Mediterranean green turtle, *Chelonia mydas*: a mitochondrial DNA control region sequence assessment. *Molecular Ecology*, 5: 473 – 483.

Excoffier, L., Laval, G. & Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**: 47-50.

FAO 1995. Precautionary approach to capture fisheries and species introductions. *FAO Technical Guidelines for Responsible Fisheries* 2, FAO, Rome.

FAO. 2006. *The state of world fisheries and aquaculture* 2006. Food and Agriculture Organization of the United Nations, Rome 2007.

FAO. 1997. Review of the state of world aquaculture, FAO Fisheries Circular 886 (Rev 1)./ FAO, Rome.

FAO. 1999. *The State of World Fisheries and Aquaculture* 1998, FAO, Rome.

FAO. 2010. *The state of world fisheries and aquaculture* 2008. Food and Agriculture Organization of the United Nations, Rome 2011.

FAO. 2012. *The state of world fisheries and aquaculture* 2012. Food and Agriculture Organization of the United Nations, Rome.

FAO 2005-2013. Aquaculture topics and activities. State of world aquaculture. Text by Rohana Subasinghe. In: FAO Fisheries and Aquaculture Department [online]. Rome. http://www.fao.org/fishery/topic/13540/en

Fast, A.W. & Leung, P.S. 2003. Rise and fall of freshwater prawn (*Macrobrachium rosenbergii*) culture in Hawaii: 1965-1999. *Reviews in Fisheries Science*, 11(3): 243-290.

Fegan, D. & Sriram, R. 2001. *Macrobrachium* boom: freshwater prawn takes off in India. *Global Aquaculture Advocate:* 4(3), 65.

Folke, C. & Kautsky, N. 1989. The role of ecosystems for a sustainable development of aquaculture. *Ambio*, 18: 234-243.

Freeland, J. R. 2005. *Molecular Ecology*, John Wiley & sons, England.

Fujimura, T. & Okamoto, H. 1972. Notes on progress made in developing a mass culturing technique for *Macrobrachium rosenbergii* in Hawaii. *In*: T.V.R Pillay (editor). *Coastal Aquaculture in the Indo-Pacific Region*. Fishing News (Books) Ltd., London. pp. 313-327.

Graves, J.E. 1998. Molecular insights into the population structures of cosmopolitan marine fishes. *Journal of Heredity*, 89(5): 427 – 437.

Graziani, C., Moreno, C., Villarroel, E., Orta, T., Lodeiros, C. & De Donato, M. 2003. Hybridization between the freshwater prawns *Macrobrachium rosenbergii* (De Man) and *M. carcinus* (L.). *Aquaculture*, 217: 81-91.

Guerrero, L.A., Circa, A.V. & Guerrero 111, R.D. 1982. A preliminary study on the culture of *Macrobrachium lanchesteri* (De Man) in paddy fields and without rice. *In*: New, M.B (Ed). *Giant prawn farming: developments in aquaculture and fisheries science*, Vol, 10. Elsevier Scientific Publishing Company, New York. Pp 203-206.

Gujja, B. & Finger-Stich, A. 1996. What price prawn? Shrimp aquaculture's impact in Asia. *Environment*: 38(7): 12-15

Hall, T.A. (1999). BioEdit: A user friendly biological sequence alignment editor and analysis programme for Windows 95/98/NT. *Nucleic Acids Symposium*. **41**: 95-98.

Hanson, J. & Goodwin, H. (eds.), 1977. *Shrimp and prawn farming in the western hemisphere. State-of-the-art reviews and status assessments*. Dowden, Hutchinson & Ross, Inc. Stroudsburg, PA. 1977. pp. 439.

Hartnoll, R.G. 1982. Growth. In *The Biology of Crustacea, Volume 2, Embryology, Morphology, and Genetics*. Abele, L.G (Eds.). Academic Press, London. pp. 111-196.

Hempel, E. 1993. Constraints and possibilities for developing aquaculture. *Aquaculture International* 1: 2-19.

Herpher, B. & Pruginin, Y. 1981. Commercial fish farming-with special reference to fish culture in Israel. John Wiley & Sons, New York.

Hewitt, C.L., Campbell, M.L. & Gollasch, S. 2006. *Alien species in aquaculture. Considerations for responsible use*. IUCN, Gland, Switzerland and Cambridge, UK. 32 pp.

Hill, S.J., Sedlacek, J.D., Weston, P.A., Tidwell, J.H., Davis, K.D. & Knight, W.L. 1997. Effects of diet and organic fertilization on water quality and benthic macroinvertebrate populations in ponds used to culture freshwater prawns, *Macrobrachium rosenbergii*. *Journal of Applied Aquaculture*, 7(3): 19-32.

Holthuis,1980. FAO Species Catalogue. Vol. 1. Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries. FAO Fisheries Synopsis 1: 1-126.

Holthuis, 2000. Nomenclature and taxonomy. In: New, M.B. and Valenti, W.C. (eds). Freshwater prawn culture- the farming of *Macrobrachium rosenbergii*. Blackwell Science, Oxford, U.K., 12-17.

Hulata, G., Karplus, I., Wohlfarth, G.W., Halevy, A., Cohen, D., Sagi, A. & Ra'anan, Z. 1988. The production of *Macrobrachium rosenbergii* in monosex populations. 11. Yield characteristics in polyculture ponds. *Israeli Journal of Aquaculture- Bamidgeh*, 40: 9-16.

Hunter, B.E. (2011). Population genetic structure and patterns of dispersal in the Giant lon-armed prawn, *Macrobrachium lar* (Fabricus, 1798) (Decapoda: Palaemonidae). Unpublished MSc Thesis, Queensland University of Technology

Hurwood, D.A., Singh, S., Dammannogoda, T., Nandlal, S. & Mather, P.B. 2012. Experimental evaluation of the culture performance of three exotic and a 'local' Giant Freshwater Prawn (*Macrobrachium rosenbergii*) culture strain in Fiji. *Aquaculture Research* 1-12.

http://www.feral.org.au/wp-content/uploads/2012.)

http://www.itis.gov/sevlet/SingleRpt/

Imamura, K. 1999. The organization and development of sea farming in Japan. In: *Stock Enhancement and Sea Ranching*, pp. 91–101 (B. R.Howell, E. Moksness, and T. Sv°asand, Eds.). Oxford: Fishing News Books, Blackwell Publishing.

Islam, S. & Wahab, A. 2005. A review on the present staus and management of mangrove wetland habitat resources in Bangladesh with emphasis in mangrove fisheries and aquaculture. *Hydrobiologia*, 542:165-190

Ismael, D. & New, M. B. 2000. Biology. In: New, M.B & Valenti, W.C. (eds). *Freshwater prawn culture- the farming of Macrobrachium rosenbergii*. Blackwell Science. Oxford, England, 18-40.

Jenkins, D.G., Brescacin, C.R., Duxbury, C.V., Elliot, J.A., Evans, J.A. & Grablow, K.R. 2007. Does size matter for dispersal distance? *Global Ecology*, 16(4): 415 – 425.

Johnson, M.L. & Gaines, M.S. 1990. Evolution of dispersal: Theoretical models and empirical tests using birds and mammals. *Annual Review of Ecology and Systematics*, 21: 449 – 480.

Jory, D.E. 1996. Marine shrimp farming development and current status, perspectives and the challenge of sustainability. *Aquaculture Magazine Annual Buyers Guide*, 25: 33-43.

Kanaujia, D.R., Mohanty, A.N. & Tripathi, S.D. 1997. Growth and production of Indian river prawn *Macrobrachium malcolmsonii* (H.Milne Edwards) under pond conditions. *Aquaculture* 154: 79-85.

Kano, Y & Kase, T. 2004. Genetic exchange between anchialine cave populations by means of larval dispersal: the case of a new gastropod species, *Neritilia cavernicola*. *Zoologica Scripta*, 33(5): 423 – 437.

Karplus, I., Hulata, G., Wohlfarth, G.W. & Halevy, A. 1986a. The effect of size-grading juvenile *Macrobrachium rosenbergii* prior to stocking on their population structure and production in polyculture 1. Dividing the population into two factions. *Aquaculture,* 56: 257-270.

Karplus, I., Hulata, G., Wohlfarth, G.W. & Halevy, A. 1986b. The effect of density on *Macrobrachium rosenbergii* raised in earthen ponds on their population structure and weight distribution. *Aquaculture*, 52: 307-320.

Karplus, I., Hulata, G., Wohlfarth, G.W. & Halevy, A. 1987. The effect of size-grading juvenile *Macrobrachium rosenbergii* prior to stocking on their population structure and production in polyculture 11. Dividing the population into three fractions. *Aquaculture,* 62: 85-95.

Kutty, M.N., Herman, F. & Le Menn, H. 2000. Culture of freshwater prawn species. In M. B. New & W.C Valenti, eds. *Freshwater prawn culture: the farming of Macrobrachium rosenbergii*, pp 393-410. Oxford, England. Blackwell Science.

Lal, M.M., Seeto, J., Pickering, T.D. & Hodge, S. 2012. Salinity and temperature requirements of laviculture of the Monkey river prawn *Macrobrachium lar* (Fabricius, 1798) (Decapoda: Caridea: Palaemonidae). *Aquaculture* 366-367: 1-8.

Lee, D.O'C. & Wickins, J.K. (1992). *Crustacean Farming*. Blackwell Scientific Publications, London. 392 pp.

Lessios, H.A. & Robertson, D.R. 2006. Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society B*. doi:10.1098/rspb.2006.3543

Lin. C.K. & Lee, C. 1992. Integration of crustacean aquaculture with coastal rice farming in Vietnam. *Naga, The ICLARM Quarterly*, 15(2): 24-26.

Ling, S. W. 1962. Studies on rearing of larvae and juveniles and culturing of adults of *Macrobrachium rosenbergii* (de Man). Indo-Fisheries Council, Current Affairs Bulletin 35: 1-11.

Ling, S.W. 1969a. The general biology and development of *Macrobrachium rosenbergii* (de Man). FAO Fisheries reports (57) 3: 607-619.

Ling, S.W. 1969b. Methods of rearing and culturing *Macrobrachium rosenbergii* (De Man)). FAO World Science. Conf. Biol. Shrimps and Prawns, Mexico City, 12-24 June, 1967.

Ling, S.W. & Merican, A.B.O. 1961. Notes on the life cycle and habits of the adults and larval stages on *Macrobrachium rosenbergii*. *Indo-Pacific Fish. Council. Current Affairs Bulletin*, 9(2): 55-60.

Ling, S.W. & Costello, T. J. (1979). The culture of freshwater prawns: a review. In Advances in Aquaculture. Papers presented at the FAO Technical Coonference on aquaculture, 26 may- June 1976, Kyoto, (Ed. By T.V. Pillay and W.A. Dill. Pp.299-304. Fishing News Books, Blackwell Science, Oxford.

McDowell, R.M. (2007). Onamphidromy, a distinct form diadromy in aquatic organisms. *Fish and Fisheries*, 8(1): 1 – 13.

Maciolek, J.A. 1972. *Macrobrachium lar* as a culture prawn in the tropical insular Pacific. In: *Proceedings of the 52nd Annual Conference of the Western Association of State Game Fish Commissioners* 52: 550-558.

Menasveta, P. & Priyatiratitivokul, S.1982. Effects of different culture systems on growth, survival, and production of the giant freshwater prawn (*Macrobrachium rosenbergii* De Man). pp. 175-189. *In* : *Giant Freshwater Farming, Developments in Aquaculture and Fisheries Science*, 10. New, M.B. (eds). Elsevier Science publishing, Amsterdam.

Miller, S.A., Dykes, D.D. & Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from nucleated cells. *Nucleic Acids Research*. **16**: 1215.

Mires, D. 1987. An improved polyculture management for freshwater prawn *Macrobrachium rosenbergii* and sex inverted *Oreochromis niloticus*. Bamidgeh, 39: 109-119.

Nandlal, S. 2005. Monoculture of the native freshwater prawn *Macrobrachium lar* in Vanuatu, and integrated with taro in Wallis and Futuna, *SPC Fisheries Newsletter*, 112-January /March 2005.

Nandlal, S. 2010. A new species for culture in the Pacific: evaluaton of the potential of the indigenous *Macrobrachium lar* (Fabricius 1798). Unpublished PhD Thesis, The University of the South Pacific, Suva, Fiji.

Nandlal, S. 2012a. *Investment opportunities in aquaculture in Pacific Island Countries*, Proceedings of World Aquaculture Society- the Australian Aquaculture Conference- 'Indigenous and Pacific session' 1- 4 May, 2012 Melbourne, Australia

Nandlal, S. 2012b. *Freshwater aquaculture production in the South Pacific region: increasing regional food security and sustainability*. Proceedings of 56th Conference of- The Australian Agricultural and Resource Economics Society (AARES)-mini symposia 'Food security in the South Pacific, PNG and East Timor', Fremantle, Australia

Nandlal, S. & Pickering, T. 2005. Freshwater prawn *Macrobrachium rosenbergii* farming in Pacific Island countries. Volume one. Hatchery operation. Noumea, New Caledonia: Secretariat of the Pacific community.

Naylor, R.S., Goldberg, R.J., Primevera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folkes, C., Lubchenco, J., Mooney, H. & Troel, M. 2000. Effect of aquaculture on world fish supplies. *Nature*, 405: 1017-1024.

Naylor, R. L., Williams, S.L & Strong, D.R. 2001. Aquaculture- a gateway for exotic species. *Science* 294: 1655-1656.

New, M.B. 1988. Freshwater prawn: Status of Global Aquaculture 1987. NACA Technical Manual 6, Bangkok, Thailand, 58 pp.

New, M. B. 1990. Freshwater prawn culture: a review. *Aquaculture* 88: 99-143.

New, M. B. 1991. Turn to the millennium aquaculture: Navigating troubled waters or riding crest of the wave? *World Aquaculture* 22(3): 28-49.

New, M.B. 1995. Status of freshwater prawn farming: a review. *Aquaculture Research* 26: 1-54.

New, M.B. 1997. Aquaculture and the capture fisheries; balancing the scales. *World Aquaculture* 28 (2): 11-30.

New, M. B. 1999. Global aquaculture: current trends and challenges for the 21st century. *World Aquaculture* 30(1): 8-13, 63-79.

New, M. B. 2000. Commercial freshwater prawn farming around the world. In: New, M.B. and Valenti, W.C. (eds). *Freshwater prawn culture- the farming of Macrobrachium rosenbergii*. Blackwell Science, Oxford, U.K., 290-325.

New, M.B. & Singholka, S. 1982. Freshwater prawn farming. A manual for culture of *Marcbrachium rosenbergii*. 1985 Rev.1. *FAO Fisheries Technical Paper 225*. FAO, Rome.

New, M.B. & Singholka, S. 1985. Freshwater prawn farming. A manual for culture of *Marcbrachium rosenbergii*. 1985 Rev.1. *FAO Fisheries Technical Paper 225 (Rev 1)*. FAO, Rome.

New, M. B., D'Abramo, L.R., Valenti, W.C. & Singholka, S. 2000a. Sustainability of freshwater prawn culture. In M. B. New & W.C Valenti, eds. *Freshwater prawn culture: the farming of Macrobrachium rosenbergii*, pp 429-434. Oxford, England. Blackwell Science.

New, M. B., Singholka, S. & Kutty, M.N. 2000b. Prawn capture fisheries and enhancement. In M. B. New & W.C Valenti, eds. *Freshwater prawn culture: the farming of Macrobrachium rosenbergii*, pp 411428. Oxford, England. Blackwell Science.

Nunan, L.M., Poulos, B.T. & Lighter, D.V. 1998. The detection of white Spot Syndrome Virus (WSSV) and Yellow head Virus (YHV) in imported commodity shrimp. *Aquaculture*, 160: 19-30.

OTA (Office of Technology Assessment) (U.S Congress). 1993. Harmful non-indigenous species in the United States. OTA Publication OTA-DF-565, U.S Government Printing Office, Washington, D.C.

Palumbi, S.R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. *Journal of Experimental Marine Biology and Ecology*, 203: 75 – 92.

Palumbi, S.R. 1997. Molecular biogeography of the Pacific. *Coral Reefs*, 16(5): 847 – 852.

Palumbi S., Martin A., Romano S., McMillan W., Stice L., & Grabowski G. (2002). The simple fool's guide to PCR (version 2.0). Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu.

Patil, P.G. & Krishnan, M.1997. The social impacts of shrimp farming in Nellore district, India. *Aquaculture Asia*, 2: 3-5.

Planes, S., Parroni, M. & Chauvet, C. 1998. Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. *Marine Biology*, 3(130): 361 – 368.

Pogson, G.H., Taggart, C.T, Mesa, K.A. & Boutilier, R.G. 2001. Isolation by distance in the Atlantic cod, *Gadua morhua* at large and small geographic scales. *Evolution* 55:131-146.

Ra'anan, Z. & Cohen, D. 1982. Production of the freshwater prawn, *Macrobrachium rosenbergii*, in Israel. Winter activities 1980/81. *Israeli Journal of Aquaculture-Bemidgeh* 34: 47-58.

Ra'anan, Z. & Cohen, D. 1983. Production of the freshwater prawn, *Macrobrachium rosenbergii*, in Israel. II. Selective stocking of size subpopulations. *Aquaculture*, 31: 369-379.

Ra'anan, Z. & Cohen, D. 1984a. Characterization of size distribution development in the freshwater prawn *Macrobrachium rosenbergii* (De Man) juvenile populations. *Crustaceana*, 46(3): 271-281.

Ra'anan, Z. & Cohen, D. 1984b. The effect of group interactions on the development of size distributions in *Macrobrachium rosenbergii* (De Man) juvenile populations. *Biological Bulletin*, 166(1): 22-31.

Ra'anan, S. & Sagi, A. 1985. Alternative mating strategies in male morphotypes of the freshwater prawn *Macrobrachium rosenbergii* (De Mann). *Biol. Bull*. 169: 592-601.

Rama Rao, P. V. A.R., Prasad Rao, K.V., Ramadrishna, R. & Haribabu, P. 1992. Studies on growth of two *Macrobrachium* spp. in monoculture. Proceedings of National Symposium on Freshwater Prawns (*Macrobrachium* spp), Kerala Agricultural University, Kochi, pp. 183-186.

Rao, K.G., Reddy, R., Rao, P.V.A.N.R. & Ramakrishna, R. 1986. Monoculture of Indian freshwater prawn, *Macrobrachium malcolmsonii* (Milne Edwards). *Aquaculture*, 53: 67-73.

Sagi, A., Ra'anan, Z., Cohen, D., Wax, Y., 1986. Production of *Macrobrachium rosenbergii* in monosex populations: yield characteristics under intensive monoculture conditions in cages. *Aquaculture.* 51: 265-275.

Saitou, N. and Nei, M. (1987). The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution.* 4: 406-425.

Sampaio, C. M. S & Valenti, W.C.1996. Growth curves for *Macrobrachium rosenbergii* in semi-intensive culture in Brazil. *Journal of World Aquaculture Society*, 27(3): 353-358.

Sandifer, P.A. & Smith, T.I.J. 1975. Effects of population density on growth and survival of *Macrobrachium rosenbergii* reared in recirculation water management systems. *Proceedings of the World Mariculture Society*, 6: 43-53.

Sandifer, P.A., Smith, T.I.J., Stokes, A.D & Jenkins, W.E. 1982. Semi-intensive growth of prawns (*Macrobrachium rosenbergii*). Preliminary results and prospects. In: M.B. New (Editor), *Giant Prawn farming*. Elsevier, Amsterdam, pp.161-172.

Segal, E. & Roe, A. 1975. Growth and behavior of post juvenile *Macrobrachium rosenbergii* (de Man) in close confinement. *Proceedings of World Mariculture Society*, 6: 67-88.

Shang, Y.C & Fujimura, T. 1977. The production economics of freshwater prawn (*Macrobrachium rosenbergii*) farming in Hawaii. *Aquaculture*, 11: 99-110.

Shang, Y.C. & Tisdell, C.A. 1997. Economic decision making in sustainable aquaculture development. Pages 127-148. *In* J.E. Bardach, editors. Sustanable aquaculture. John Wiley and Sons, Inc., Honolulu, Hawaii, USA.

Shang, Y.C., Leung, P. & Ling, B.H. 1998. Comparative economics of shrimp farming in Asia. *Aquaculture*, 164: 183-200.

Short, J. W. 2000. Systematics and biogeography of Australian *Macorbrachium* (Crustacea: Decapoda: Palaemonidae) –with description of other new freshwater Decapoda. Pp. 401. Zoology. University of Queensland, Brisbane.

Short, J. W. 2004. A revision of Australian river prawns, *Macrobrachium* (Crustacea: Decapoda: Palaemonidae). *Hydrobiologia*: 525: 1-100.

Short, J. W. & Meek, P. 2000. New records of *Macrobrachium* (Crustacea: Decapoda: Palaemonidae) from Christmas Island, Indian Ocean. *Records of the Western Australian Museum*, 20: 81-86.

Slatkin, M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics*, 16: 393 – 430.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. *Science*, 236 (4803): 787 – 792.

Smith, T.I.J. & Sandifer, P.A. 1982. Influence of three stocking strategies on the production of prawns, *Macrobrachium rosenbergii*, from ponds in South Carolina, U.S.A., In Proc. Symp. Coastal Aquaculture, Cochin, India, 76-

Smith, T.I.J., Sandifer, P.A., Jenkins, W.E. & Stokes, A.D. 1981. Effects of population structure at stocking and density on production and economic potential of prawn (*Macrobrachium rosenbergii*) farming in temperate climates. *World Mariculture Society*, 12(1): 233-250.

Smith, T.I.J., Sandifer, P.A., Jenkins, W.E., Stokes, A.D. & Murry, G. 1982. Pond rearing trials with Malaysian prawns *Macrobrachium rosenbergii* by private growers in South Carolina. *Journal of world Aquaculture Mariculture Society*. 13, 41-55.

Sotka, E.E. 2005. Local adaptation in host use among marine invertebrates. Ecology *Letter*, 8(4): 448 – 459.

SPC -Secretariat of the Pacific Community, Aquaculture Action Plan. 2002. Secretariat of the Pacific Community, Noumea, New Caledonia.

SPC (Secretariat of the Pacific Community). 2007. The future of Pacific fisheries-planning and managing for economic growth, food security and sustainable livelihoods. 5th meeting of the Conference of the Pacific Community, Apai, Samoa, November 2007. Secretariat of the Pacific Community, Noumea, New Caledonia.

Stephan, R. (2004). Pattern of mtDNA variation in the freshwater prawn (*Macrobrachium lar*) between American Samoa and Madagascar: genetic consequences of an unusual life history strategy. Unpublished Honours Thesis, Queensland University of Technology.

Sugiyama, S., Staple, D & Funge- Smith, S. 2004. Status and potential of fisheries and aquaculture in Asia and Pacific. FAO Regional office for Asia and the Pacific. RAP Publications 2004. p25- 53.

Tamura, K., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.

Tidwell, J.H., Webster, C.D., Clark, J.A. & D'Abramo, L.R. 1993a. Evaluation of distillers dried grains with solubles as an ingredient in diets for pond culture of the freshwater prawn, *Macrobrachium rosenbergii*. *Journal of the World Aquaculture Society* 24(1): 66-70.

Tidwell, J.H., Webster, C.D., Yancey, D.H. & D'Abramo, L.R. 1993b. Partial and total replacement of fish meal with soyabean meal distillers' by-products in diets for pond culture of the freshwater prawn (*Macrobrachium rosenbergii*). *Aquaculture*, 118: 119-130.

Tidwell, J.H. & D'Abramo, L.R. 2000. Grow-out systems- culture in temperate zones . In: *Freshwater Prawn Culture: The Farming of Macrobrachium rosenbergii* (Ed. By M.B. New & W.C. Valenti), pp.177-202. Blackwell Science, Oxford, UK.

UN. 2010. World Population Prospects: The 2010 Revision. Department of Economic and Social Affairs. Population Division, New York.

Valenti, W. C. & New, M. B. 2000. Grow-out systems-monoculture. In *Freshwater Prawn culture-The farming of Macrobrachium rosenbergii*. (Eds. M.B. New and W.C. Valenti.) Blackwell Science Limited. Pp. 157-176.

Valenti, W.C. & Tidwell, J.H. 2006. Economics and management of freshwater prawn culture in Western hemisphere. In *Shrimp culture: economics, market & trade* . (Eds. P. Leung and C. Engle), pp.261-276. Blackwell Publishing.

Vanuatu Aquaculture Development Plan, 2008-2013. Vanuatu Department of Fisheries, Vanuatu.

Webster, C.D. & Tidwell, J.H. 1995. Diets, feeding and production of freshwater prawn in Kenturky. *Aquaculture Magazine*, 22 (6): 47-60.

Williams, S. T. & Benzie, J. A. H. 1998. Evidence of a biogeographic break between populations of a high dispersal starfish; congruent regions within the Indo-West Pacific defined by color morphs, mtDNA and allozyme data. *Evolution*, 85(1): 87 – 99

Willis, S. A. & Berrigan, M. E. 1977. Effects of stocking size and density on growth and survival of *Macrobrachium rosenbergii* (de Man) in ponds. *Proceedings of the 8th annual Meeting of the World Mariculture Society*, 8: 251-264.

Wohlfarth, G.W., Hulata, G., Karplus, I. & Halevy, A. 1985. Polyculture of freshwater prawn *Macrobrachium rosenbergii* in intensively manured ponds, and the effects of stocking rate of prawns and fish on their production characteristics. *Aquaculture*, 46: 143-156.

Wyatt, M. L. (2001). Patterns of mtDNA variation in the freshwater prawn (*Macrobrachium lar*) in Fiji: Genetic consequences of an unusual life history. Unpublished Honours Thesis, Queensland University of Technology.

Wijkstrom, U.N. 2003. Short and long-term prospects for consumption of fish. *Veterinary Research Communications*, 27 Suppl.1: 461-468

Zimmerman, S. & New, M. B. 2000. Grow-out systems- polyculture and integrated culture. In: *Freshwater Prawn Culture: The Farming of Macrobrachium rosenbergii* (Ed. By M.B. New & W.C. Valenti), pp.187-2002. Blackwell Science, Oxford, UK.

6.0 Appendix

Appendix 1: Weights of individuals stocked in Trials.

30th June 2012

	M.lar	*M.rosenbergii*
Prawn count	**Weight (g)**	**Weight (g)**
1	1.81	1.12
2	1.09	0.98
3	0.32	1.20
4	2.20	0.85
5	1.28	0.56
6	2.13	1.07
7	0.85	1.24
8	0.16	0.78
9	0.87	0.65
10	1.37	0.35
11	2.31	0.69
12	0.84	0.68
13	2.49	0.65
14	1.44	1.50
15	1.88	0.65
16	4.41	1.36
17	0.82	1.77
18	3.33	1.05
19	3.59	1.66
20	1.67	1.73
21	0.34	1.18
22	0.53	1.22
23	1.33	1.22
24	1.07	0.34
25	0.31	1.43
26	0.38	0.39
27	0.43	0.78
28	0.15	0.78
29	2.70	0.35
30	0.19	0.79
31	0.24	0.79
32	3.69	0.33
33	3.22	0.81
34	2.58	1.76
35	0.50	0.75
36	0.35	1.07
37	0.15	1.58
38	0.17	0.56
39	0.55	0.53
40	3.15	0.31
41	1.44	0.50
42	3.03	1.43
43	3.20	0.53
44	1.21	0.87
45	0.24	0.42
46	0.16	0.54
47	0.12	0.35
48	0.21	0.37
49	1.68	0.53
50	1.02	1.50

20th March 2013

	M.lar	*M.rosenbergii*
Prawn count	**Weight (g)**	**Weight (g)**
1	0.1	0.2
2	0.09	0.13
3	0.38	0.12
4	0.38	0.18
5	0.07	0.12
6	0.1	0.12
7	0.25	0.11
8	2.29	0.12
9	0.47	0.14
10	0.56	0.11
11	0.08	0.17
12	0.1	0.17
13	0.07	0.13
14	0.88	0.12
15	0.24	0.13
16	0.43	0.1
17	0.16	0.1
18	0.8	0.16
19	0.17	0.07
20	0.06	0.15
21	0.16	0.25
22	0.09	0.12
23	0.12	0.09
24	0.29	0.12
25	0.26	0.12
26	0.15	0.25
27	0.19	0.14
28	0.08	0.07
29	0.07	0.1
30	0.74	0.11
31	0.04	0.11
32	0.14	0.17
33	0.07	0.12
34	0.05	0.11
35	0.12	0.1
36	0.4	0.16
37	0.08	0.19
38	0.08	0.19
39	0.98	0.14
40	0.24	0.21
41	0.85	0.17
42	1.17	0.17
43	0.1	0.18
44	1.47	0.11
45	0.19	0.1
46	0.55	0.09
47	0.33	0.11
48	0.4	0.14
49	0.08	0.11
50	0.65	0.18

Appendix 2: Monthly sampling data of Trial 1 and Trial 2.

Pond	Stocking Date	No. of Prawns	Sampling Date	Species	Sampling Number	ABW (grams)	Feed %	Feed/day (g)	
								Morning	Afternoon
1	30/06/12	108	30/06/12	M.lar	0	1.06	10%	3.5	9.5
				M.rosbgii	0	0.79		3	6
		103	7/8/2012	M.lar	1	6.25	8%	15.5	36
				M.rosbgii	1	4.5		11	26
		98	5/9/2012	M.lar	2	7.79	6%	14	31
				M.rosbgii	2	10.21		18	42
		94	5/10/2012	M.lar	3	12.79	4%	14	34
				M.rosbgii	3	21.08		24	54
		74	20/11/2012	M.lar	4	10.08			
		91	20/11/12	M.rosbgii	4	29.08			
2	30/06/12	120	30/06/12	M.lar	0	0.73	10%	3	6.5
				M.rosbgii	0	0.71		3	6
		114	7/8/2012	M.lar	1	1.33	8%	4	8
				M.rosbgii	1	2.5		7	15
		109	5/9/2012	M.lar	2	5.67	6%	11	26
				M.rosbgii	2	15.71		31	71.5
		104	5/10/2012	M.lar	3	13.17	4%	16.5	38
				M.rosbgii	3	28.54		35.5	83
		53	20/11/12	M.lar	4	9.05			
		81	20/11/12	M.rosbgii	4	31.72			
3	30/06/12	108	30/06/12	M.lar	0	1.3	10%	4.5	10
				M.rosbgii	0	0.72		2.5	5.5
		103	7/8/2012	M.lar	1	2.45	8%	6	14
				M.rosbgii	1	4.92		12	28.5
		98	6/9/2012	M.lar	2	8.29	6%	14.5	34
				M.rosbgii	2	9.13		16	37.5
		94	5/10/2012	M.lar	3	12.71	4%	14.5	33.5
				M.rosbgii	3	24.83		28	64.5
		48	20/11/12	M.lar	4	9.23			
		91	20/11/12	M.rosbgii	4	25.29			
4	30/06/12	120	30/06/12	M.lar	0	1.14	10%	4.5	10
				M.rosbgii	0	0.65		2.5	5.5
		114	7/7/2012	M.lar	1	3.21	8%	9	20
				M.rosbgii	1	2.83		8	18
		109	6/9/2012	M.lar	2	9.17	6%	18	42
				M.rosbgii	2	12.92		25.5	59
		104	5/10/2012	M.lar	3	18.63	4%	23.5	54
				M.rosbgii	3	24.5		30.5	71.5
		80	20/11/2012	M.lar	4	9.72			
		107	20/11/12	M.rosbgii	4	30.19			

Pond	Stocking Date	No. of Prawns	Sampling Date	Species	Sampling Number	ABW (grams)	Feed %	Feed/day (g)	
								Morning	Afternoon
				M.lar	0	0.58		2	4.5
		108	20/02/13	M.ros	0	0.16	10%	0.5	1.5
				M.lar	1	4.92		12	28.5
		103	10/4/2013	M.ros	1	9.58	8%	24	55
1	20/02/13			M.lar	2	10.46		18.5	43
		98	3/5/2013	M.ros	2	17.92	6%	31.5	74
				M.lar	3	8.88		10	23.5
		94	31/05/13	M.ros	3	37	4%	41.5	97.5
			20/06/2013	M.lar	4	9.29			
			20/06/2013	M.ros	4	48.3			
				M.lar	0	0.34		1	3
		120	20/02/13	M.ros	0	0.19	10%	0.5	2
				M.lar	1	5		13.5	32.5
		114	10/4/2013	M.ros	1	8.17	8%	33	52
2	20/02/13			M.lar	2	6.38		12.5	29.5
		109	3/5/2013	M.ros	2	20.5	6%	40	94
				M.lar	3	13.75		17.5	40
		104	31/05/13	M.ros	3	47.13	4%	59	137
			20/06/2013	M.lar	4	12.67			
			20/06/2013	M.ros	4	51.16			
				M.lar	0	0.18		0.5	1.5
		108	20/02/13	M.ros	0	0.1	10%	0.5	1
				M.lar	1	2.63		7	15
		103	10/4/2013	M.ros	1	10.58	8%	33	76
3	20/02/13			M.lar	2	6.17		11	25.5
		98	3/5/2013	M.ros	2	20.29	6%	36	83.5
				M.lar	3	6.29		7.5	16.5
		94	31/05/13	M.ros	3	34.41	4%	39	90.5
			20/06/2013	M.lar	4	8.02			
			20/06/2013	M.ros	4	45.79			
				M.lar	0	1.07		4	9
		120	20/02/13	M.ros	0	0.12	10%	0.5	1
				M.lar	1	7.54		21	48
		114	10/4/2013	M.ros	1	5.29	8%	14.5	34
4	20/02/13			M.lar	2	8.25		16.5	37.5
		109	3/5/2013	M.ros	2	21.54	6%	42.5	98.5
				M.lar	3	8.29		10.5	24
		104	31/05/2013	M.ros	3	37.58	4%	47	109
			20/06/2013	M.lar	4	8.25			
			20/06/2013	M.ros	4	42.46			

Appendix 3: Survival number, mean and total weights, and percentage survival for *M. rosenbergii* in Trial 1 and Trial 2

Trial no	Total	No	Av Wt(g)	SE	Total wt(g)	% survival
1	Males	151	40.35	±1.61	6052.4	40.81
1	Females	128	30.09	±0.53	3850.9	34.59
1	Runts	91	8.89	±0.56	800	24.59
1	BC	59	57.85	±2.01	2412.9	15.95
1	OC	92	29.01	±1.34	2639.5	24.86
1	B	71	31.23	±0.65	2217	19.19
1	O	57	28.66	±0.84	16339	15.41
2	Males	287	52.78	±1.16	15146.58	63.50
2	Females	154	36.31	±0.48	5592.24	34.07
2	Runts	11	43.38	±5.97	520.54	2.43
2	BC	171	54.87	±1.51	9387.13	37.83
2	OC	116	49.69	±1.77	5764.5	25.66
2	B	34	35.06	±0.61	1192.05	7.52
2	O	120	36.67	±0.59	4400.19	26.55

.

.