COMPONENT 2A - Project 2A1

Knowledge, monitoring, management and beneficial use of coral reef ecosystems

November 2008

TECHNICAL REPORT

POSTLARVAL FISH CAPTURE, CULTURE AND RELEASE IN FIJI: EFFECTS OF CULTURE CONDITIONS ON SURVIVAL IN THE WILD

By Shirleen BALA
Master student

THE UNIVERSITY OF THE SOUTH PACIFIC

CRISP
Coral Reef Initiatives for the Pacific
Initiatives Corail pour le Pacifique

CNRS
CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE
The CRISP programme is implemented as part of the policy developed by the Secretariat of the Pacific Regional Environment Programme for a contribution to conservation and sustainable development of coral reefs in the Pacific.

The Initiative for the Protection and Management of Coral Reefs in the Pacific (CRISP), sponsored by France and prepared by the French Development Agency (AFD) as part of an inter-ministerial project from 2002 onwards, aims to develop a vision for the future of these unique ecosystems and the communities that depend on them and to introduce strategies and projects to conserve their biodiversity, while developing the economic and environmental services that they provide both locally and globally. Also, it is designed as a factor for integration between developed countries (Australia, New Zealand, Japan, USA), French Overseas Territories and Pacific Island developing countries.

The CRISP Programme is divided into three major components, which are:

**Component 1A:** Integrated Coastal Management and Watershed Management
- 1A1: Marine biodiversity conservation planning
- 1A2: Marine Protected Areas (MPAs)
- 1A3: Institutional strengthening and networking
- 1A4: Integrated coastal reef zone and watershed management

**Component 2:** Development of Coral Ecosystems
- 2A: Knowledge, monitoring and management of coral reef ecosystems
- 2B: Reef rehabilitation
- 2C: Development of active marine substances
- 2D: Development of regional database (ReefBase Pacific)

**Component 3:** Programme Coordination and Development
- 3A: Capitalization, value-adding and extension of CRISP Programme activities
- 3B: Coordination, promotion and development of CRISP Programme

---

**COMPONENT 2A**

**Knowledge, monitoring and management of coral reef ecosystems**

- **PROJECT 2A-1:** Postlarvae (fish and crustacean) capture and culture for aquarium trade and restocking
- **PROJECT 2A-2:** Improvement of knowledge and capacity for a better management of reef ecosystems
- **PROJECT 2A-3:** Synopsis and extension work on indicators for monitoring the health of coral ecosystems and developing a remote sensing tool
- **PROJECT 2A-4:** Testing of novel information feedback methods for local communities and users of reef and lagoon resources
- **PROJECT 2A-5:** Specific studies on i) the effects on the increase in atmospheric CO2 on the health of coral formation and ii) the development of ecotourism

---

This CRISP component is funded by the following agency:
DECLARATION

I declare that this thesis is my original research. Any form of assistance or source of information by other people have been properly acknowledged and cited in the thesis.

.................................................. ..................................................

Shirleen Ajeshni Bala Date

This research thesis was performed under my supervision and to my knowledge is the sole work of Ms Shirleen Ajeshni Bala.

.................................................. ..................................................

Dr Joeli Veitayaki Date
University of the South Pacific
Laucala, Fiji

.................................................. ..................................................

Dr Timothy Pickering Date
Secretariat of the Pacific Communities
New Caledonia

.................................................. ..................................................

Prof. René Galzin Date
University of Perpignan
France
DEDICATION

This thesis is dedicated to my loving husband for his support and guidance
ACKNOWLEDGMENT

This thesis would not have been completed without the help of several people and organizations.

I would like to thank Coral Reef Initiative in the South Pacific (CRISP), and Prof. René Galzin for fully sponsoring my Masters Degree. I am grateful to the French Embassy in Fiji for providing me with additional funding which enabled me to travel to New Caledonia for a training attachment in the Institute of Research and Development (IRD). I thank IRD for their great hospitality and in providing me will all essentials during my stay in New Caledonia.

Sincere thanks to my supervisor Dr Timothy Pickering, for identifying me as a potential candidate for the Master Scholarship and for helping me throughout my study period. I am grateful for your continuous support, guidance and your assistance in my field work and report writing.

Sincere thanks also to Dr Dominique Ponton of IRD. Thank you your support, guidance and patience during and after my stay in IRD. I thank you for assisting me with my lab work, data analysis and report writing.

I am also grateful to Julien Grignon for all his support, guidance and enormous help in the field, to Laisasa Cava for assisting in field and laboratory work, and to Mr. Ed Lovell for identification of corals. I acknowledge the assistance of Shital Swarup, Prema Chand and Cherie Morris for proof reading and providing me with relevant articles. The assistance of the Marine Science Programme (MSP) staff, especially Jone Lima, Shiv Sharama, Deepak Kissun, Alfred Tavo, Rusiate Vadiga, Josephine Singh and Hitesh Patel is also appreciated.

Lastly I would like to acknowledge the assistance given to me by all my family and friends in Fiji and New Caledonia.
ABSTRACT

Capture and culture of pre-settling (prior to settling on reefs) reef fishes for the aquarium trade, live reef food fish trade and reef fishery restocking is believed to provide an alternate to capture of wild stock. The possibly of restocking using post-larval reef fish is still in its infancy. This study was part of a wider preliminary study to investigate the viability of such restocking in Fiji. Pre-settling Chromis viridis were collected using light traps outside Makaluva reef and Muaivuso reefs, eastern Fiji. After successful weaning of fish onto artificial diets, experiments were done to investigate the growth and post-release survival of fish fed on three different diets (imported Otohime feed, local tilapia feed, and fresh egg custard) and culture duration (60 day, 90 days and 158 days). Fishes were released in enclosures containing one predatory fish and six microhabitats; live and dead Montipora altasepta, live and dead Porites cylindrica, artificial “coral” (made from galvanized chicken wire) and coral rubble. The relative growth rates of fishes were calculated using standard lengths for 158 days. Similar growth rates (0.54, 0.45 and 0.50 % per day) were obtained for all three feeds (Otohime, tilapia and egg custard respectively). A significant difference was only detected after 60 days of culture, by which time the mean standard length of fish fed on Otohime feed was significantly larger than the others. After release, post-release survival ranged between 50-100 %. Microhabitat chosen by the fishes was mainly live corals. There was no apparent trend seen for different feeds or culture duration, however fish in 90 days culture exhibited a more structured behavior. Additional studies on wild C. viridis were done by collecting 15 different schools of juveniles from three different positions of the Suva reef (far from reef crest (F), middle reef (M) and near reef crest (N)), observing their surrounding habitat and studying their size and age structure. Otolith analysis was used to back-calculate size-at-age to examine differences of fish in the 15 schools, to compare between position on the reefs, different habitats, and between wild and cultured fish. All 15 samples were found to have variable sizes and ages of fish, and there was no difference in size at age within samples and between locations. Size-at-age was significantly higher for fish in intermediate density (50-100 indiv.m\(^{-2}\)) from 10 days after settlement and significantly lower when water depth was <1.0m, at settlement and after settlement. Size-at-age of cultured fish was slightly
lower than wild fish, during later larval stages up till 10 days after settlement. This was followed by slightly larger size of cultured fish 20 days after settlement and the difference became significant between wild and cultured fish at 60 days after settlement.

This study has demonstrated that post-larval reef fish can be successfully grown in captivity using local feeds. Growth rates obtained in culture were found to be similar to those of wild fish, indicating normal growth in captivity. Restocking by using post-larval reef fish has potential, and species specific knowledge is important in understanding their preferences in terms of social interactions and habitat. In the case of Fiji, this method is possible using lower-cost local feeds and materials; however further research is recommended to implement community level projects for either restocking or fish trade.
# TABLE OF CONTENTS

DECLARATION ................................................................................................................................. ii
DEDICATION ......................................................................................................................................... iii
ACKNOWLEDGMENT ........................................................................................................................ iv
ABSTRACT ............................................................................................................................................... v
TABLE OF CONTENTS ........................................................................................................................ vii
LIST OF FIGURES AND TABLES .......................................................................................................... xi
LIST OF APPENDICES ........................................................................................................................... xiii

1.1 Global marine resources status ................................................................................................. 1
1.2 Role of aquaculture ..................................................................................................................... 2
  1.2.1 Definition ................................................................................................................................. 2
  1.2.2 Aquaculture and conservation .............................................................................................. 2
1.3 Stock enhancement and restocking ........................................................................................... 3
  1.3.1 Definition ................................................................................................................................. 3
  1.3.2 History ...................................................................................................................................... 3
  1.3.3 Effectiveness of stock enhancement and restocking ............................................................. 4
1.4 Aquaculture and restocking programs in Pacific Island Countries .......................................... 5
1.5 Coral reefs and reef fishes ........................................................................................................ 7
  1.5.1 Uses of corals and coral reef fishes ....................................................................................... 8
    1.5.1.1 Subsistence fishery ........................................................................................................ 8
    1.5.1.2 Live food fish and aquarium industry ........................................................................... 8
  1.5.2 Threats to corals and coral reef fish ..................................................................................... 9
1.6 Capture and culture of post-larval reef fishes .......................................................................... 10
  1.6.1 What are post larval reef fishes? .......................................................................................... 10
  1.6.2 Using post larval fish for aquaculture or stock enhancement ............................................. 11
  1.6.3 Methods of capture ............................................................................................................ 12
  1.6.4 Methods of culture ............................................................................................................... 13
  1.6.5 Effects of culture conditions on post release survival ....................................................... 14
1.7 General objective ....................................................................................................................... 15
1.8 Study species ............................................................................................................................ 16
1.8.1 Taxa ........................................................................................................................ 16
1.8.2 General Description ............................................................................................... 16
1.8.3 Importance of *C. viridis* ..................................................................................... 16

2.1 Introduction ................................................................................................................... 18
2.1.1 Use of wild post larvae for restocking reefs .......................................................... 19
2.1.2 Rearing conditions ................................................................................................. 20
2.1.3 Feeding requirements ............................................................................................. 20
2.1.4 Behavioral competency of juveniles before release ............................................... 22
2.1.5 Effects of size at release on post release survival .................................................. 25
2.1.6 Objectives and implication .................................................................................... 26

2.2 Methodology ................................................................................................................. 27
2.2.1 Experimental setup ................................................................................................. 27
2.2.1.1 Capture ............................................................................................................ 27
2.2.1.2 Culture ............................................................................................................. 28
2.2.1.3 Calculation of specific growth rate using mean standard length ..... 29
2.2.2 Releasing *C. viridis* into the wild ........................................................................ 29
2.2.2.1 Building of enclosures .................................................................................... 29
2.2.2.2 Releasing of fish ............................................................................................. 30
2.2.2.3 Monitoring post-release survival .................................................................... 30
2.2.3 Otolith analysis ...................................................................................................... 31

2.3 Results ........................................................................................................................... 31
2.3.1 Survival and growth of *C viridis* in aquaria ........................................................ 31
2.3.2 Post release survival and habitat choice of cultured fish for different culture period (size-at-release) .................................................................................................................. 35
2.3.2.1 Culture period (size at release) and post release survival ......................... 35
2.3.2.2 Culture period (size at release) and microhabitat choice ...................... 37

2.4 Discussion ..................................................................................................................... 41
2.4.1 Growth and survival in aquaria .............................................................................. 41
2.4.2 Post-release survival .............................................................................................. 44
2.4.3 Post-release microhabitat choice ............................................................................ 45

3.1 Introduction .................................................................................................................. 48
3.1.1 Settlement of coral reef fish ................................................................. 48
  3.1.1.1 Definition of settlement ................................................................. 48
  3.1.1.2 Competency to settle ................................................................. 48
  3.1.1.3 Selection of habitat for settlement .............................................. 49
3.1.2 Survival and growth after settlement .................................................. 51
  3.1.2.1 Post-settlement mortality ............................................................. 51
  3.1.2.2 Post-settlement growth ............................................................. 52
3.1.3 Otolithometry ......................................................................................... 53
  3.1.3.1 What are otoliths? ........................................................................ 53
  3.1.3.2 Use of otoliths in ecology ............................................................. 54
  3.1.3.3 Determining fish age and growth rate using otolith microstructures... 55
3.1.4 Objectives and implications ................................................................. 56
3.2 Methodology ............................................................................................ 56
  3.2.1 Study site .......................................................................................... 56
  3.2.2 Field work ......................................................................................... 56
    3.2.2.1 Capture of juveniles ................................................................. 56
    3.2.2.2 Description of habitat and surrounding environment ............... 57
3.2.3 Laboratory work- otolith analysis ......................................................... 57
  3.2.3.1 Sub sampling of colonies per size and per location ....................... 57
  3.2.3.2 Extraction of otoliths ................................................................. 58
  3.2.3.3 Polishing of otoliths ................................................................. 60
  3.2.3.4 Interpreting microstructures (age and growth) ............................ 61
    3.2.3.5 Relationship between otolith size and fish size ......................... 61
    3.2.3.6 Calculating post-larval duration (PLD) ...................................... 62
3.3 Results ..................................................................................................... 62
  3.3.1 General description of surrounding habitat ...................................... 62
  3.3.2 Size distribution .............................................................................. 64
  3.3.3 Age distribution .............................................................................. 66
  3.3.4 Hatch and settlement dates and PLD .............................................. 67
  3.3.5 Relationship between otolith size and fish size .................................. 69
    3.3.5.1 Isometric test ........................................................................... 69
3.3.5.2 Allometric test.............................................................. 69
3.3.6 Growth rates of wild C. viridis .............................................. 71
3.3.7 Influence of habitat on growth................................................. 73
3.3.8 Growth of wild and cultured fish ............................................ 75
3.4 Discussion .............................................................................. 76
  3.4.1 Size and age distribution...................................................... 76
  3.4.2 Growth rate of wild fish.................................................... 77
  3.4.3 Influence of habitat on growth of wild fish ......................... 78
  3.4.4 Growth comparison of wild and cultured fish ................. 79
4.1 Review of objectives............................................................ 81
4.2 Constraints and limitations of this study............................... 83
4.3 Discussion and conclusion........................................................ 83
4.4 Recommendation for further research ................................. 86
REFERENCES .............................................................................. 88
APPENDICES .................................................................................. 99
LIST OF FIGURES AND TABLES

Figure 1: Map of Suva barrier reef and Muaivuso fringing and barrier reef, showing the capture (light traps) and release sites................................................................. 27
Figure 2: Cross-sectional diagram showing the layout of the enclosures, where LC is live coral, DC is dead coral, RB is Rubble and AC is artificial coral.......................... 30
Figure 3: Mean standard lengths with time for the three feeds. With the first point (day 30) being the estimated number of days in captivity and the start of the feed trials thereafter. ... 32
Figure 4: Standard lengths of *C. viridis* for different feeds at different days of culture. With start of feeding trial corresponding to day = 0. (** Anova test p-value = 0.0001)........... 33
Figure 5: Mean standard lengths of the three feeds after 60, 90 and 158 days of culture.... 34
Figure 6: Post release survival trends of *C. viridis* fed with different feeds, for culture periods (a) 60 days, (b) 90 days and (c) 158 days. O = Otohime feed, T = Tilapia Feed and E = Egg Custard................................................................................................................................... 36
Figure 7: Post-release microhabitat choice of *C. viridis* for the three feeds after 60 days of culture. ...................................................................................................................... 38
Figure 8: Post-release microhabitat choice of *C. viridis* for the three feeds after 90 days of culture. ...................................................................................................................... 39
Figure 10: Comparison of mean standard lengths between the current study and Durville *et al.* (2003).................................................................................................................. 43
Figure 11: Position of otoliths within the inner ear (a) Dorsal view in a typical Teleost species, (b) Otoliths position within the inner ear of Teleost and Cyprinoid. Ast = asteriscus, Lag = lagena (vestibule), Lap = lapillus, Sac = sacculus (vestibule), Sag = sagitta, and Utr = utriculus (vestibule). (Source: Panfili *et al.*, 2002) ........................................ 54
Figure 12: (a) *Chromis viridis* colony on coral head just before capture, (b) coral head covered with plastic sheets prior to clove oil injection................................................. 57
Figure 13: Representative sub samples used for analysis from the three positions. With F = far from reef crest, M = middle and N = near reef crest ................................................. 58
Figure 14: Example of the three pairs of otoliths found in most teleosts. L – lapillus, S-sagitta and A – asteriscus (Source: Panfili *et al.* 2002). ................................................. 59
Figure 15: (a) Internal and external face of a typical sagitta illustrating the component parts, (b) the three planes of orientation of a typical sagitta (Source: Panfili *et al.* 2002). .... 59
Figure 16: a, b, and c shows the different positions of otolith placements while polishing and (d) otolith after the process of polishing under X60 magnification. ................................. 60
Figure 17: (a) microstructures in otolith marked with dots while counting, and (b) Graph of relative growth between microstructures vs microstructure number, giving PLD. .................. 62
Figure 18: Picture of surroundings of a branching coral harboring C. viridis at position M. 63
Figure 19: Picture of the general surrounding of position N. ....................................................... 63
Figure 20: Size distribution of C. viridis in the 15 analyzed samples. ..................................... 65
Figure 21: Size distribution of C. viridis when grouped by positions. With F = far from reef, M = middle and N = near reef crest. ................................................................. 66
Figure 22: Age distribution of C. viridis in the 15 analyzed samples........................................ 67
Figure 23: Age distribution of C. viridis when grouped by position. With F = far from reef crest, M = middle, and N = near reef crest. ................................................................. 68
Figure 24: a, b and c: graphs representing residual points along the zero line for isometric test and d, e, f: graphs for allometric tests................................................................. 70
Figure 26: Back-calculated size-at-age of fish in different water depths. Where H = hatching, and H+05d equals to 5 days after hatching etc. and S = settlement and S + 05d equals 5 days after settlement etc. ................................................................. 74
Figure 27: Back-calculated size-at-age of fish under different densities................................. 75
Figure 28: Back-calculated mean size at age of wild and cultured fish with time of settlement corresponding to age = 0................................................................. 76

Table 1: Summary of habitat and microhabitat description for the 15 samples. .................. 73
LIST OF APPENDICES

Appendix 1: Map of Suva barrier reef (site of juvenile capture for otolith analysis) .......... 99
Appendix 2: ANOVA analysis of variance for culture periods (Chapter 2) ......................... 99
Appendix 3: ANOVA analysis for back-calculated size-at-age at different positions, F, M and N of wild fish. ................................................................................................................................. 100
Appendix 4: GLM analysis output of size-at-age of C. viridis at the three positions F, M and N. .................................................................................................................................................. 101
Appendix 5: ANOVA analysis for back-calculated size-at-age (at stages) of wild fish by positions. .......................................................................................................................... 102
Appendix 6: GLM analysis output for C. viridis size-at-age versus percentage of sand cover. C1 = <50% and C2 > 50% sand substrate. .................................................................................. 103
Appendix 7: GLM analysis output for C. viridis size-at-age at water depths. Where C1 =<1.0m, C2 = 1.1-1.2m and C3 = 1.3-1.8m. ........................................................................................................ 104
Appendix 8: GLM analysis output of size at age of C. viridis grouped in different densities. Where C1 =<50 indiv.m⁻², C2 =50-100 indiv.m⁻², C3 = >100 indiv.m⁻² ......................... 105
Appendix 9: ANOVA analysis for back-calculated size-at-age of wild and cultured fish .... 106
Appendix 10: Picture of different traps designed to collect post larval reef fish. ............ 107
Chapter 1

LITERATURE REVIEW

1.1 Global marine resources status

Food fish and marine invertebrates are among the world’s leading sources of protein and, due to their rich omega-3 polyunsaturated acid composition, fat and water soluble vitamins, minerals and trace elements, they are superior to terrestrial protein sources (Food and Agriculture Organization 2000). The global protein demand is increasing rapidly along with increase in population and supply from capture fisheries is not sufficient to meet this demand. Constant pressure such as over fishing, increased fishing effort and pollution, have resulted in a decline of marine finfish and invertebrate populations in the wild, forcing the governments to set control measures such as reduced quotas, closed seasons, marine protected areas and increase in fishing license fees. However, these conservation methods alone are not enough to revive and sustain the natural marine ecosystems. Many coastal fisheries are no longer capable of fulfilling the demand for two reasons: 1) the number of spawning fishes has been reduced to below optimum levels, and 2) fish habitat alteration/destruction (FAO 2004). The Convention for International Trade of Endangered Species (CITES) has identified nine fish species as critically endangered and 68 species as endangered worldwide (www.cites.org).

Marine resources are further subjected to overexploitation by the recently developed Aquarium Trade and Live Reef Food Fish Trade (LRFFT). The market demand for both aquarium and food fish has rapidly increased in the past decade (Hair et al. 2000). Tropical and subtropical countries are amongst the world’s largest exporters of live fish for the private aquarium trade (Olivotto et al. 2003). Trade includes live corals, fish and other reef associated organisms (Pomeroy et al. 2004).

High demand for fishery resources has resulted in introduction of high-tech fishing gear and increased fishing effort, which has led to global decreases in fish stocks. Nevertheless,
subsistence and commercial fishing cannot be stopped. The livelihood of thousands of fishers is dependent on fisheries (food fish and aquarium fish) in source countries such as Indonesia, Philippines and even Pacific Island Countries (Hair et al. 2000; Pomeroy et al. 2004). Conservation organizations, governments and scientists are now exploring the possibilities of aquaculture, stock enhancement/restocking and more recently post larval fish capture and culture as alternatives to wild capture.

1.2 Role of aquaculture

1.2.1 Definition

“Aquaculture” is defined by the Food and Agriculture Organization (FAO) as ‘the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants’. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, and protection from predation (FAO 1999). It also includes catching juveniles from wild and rearing them to a larger size in captivity. Aquaculture can be done for protein supplement, stock enhancement, baitfish industry and live fish trade. A closed system of aquaculture does not depend on the wild for juvenile or broodstock. Juveniles are produced using broodstock that are maintained within a closed facility (for example: tilapia, freshwater prawns, carps). An open system is where the hatchery is dependent on the wild at some point in time, either for broodstock or juveniles (for example: milkfish - food fish, turtles- stock enhancement, marine invertebrates- aquarium trade/stock enhancement).

1.2.2 Aquaculture and conservation

With the serious decline of natural marine fisheries all around the world, aquaculture is expected to play an increasingly crucial role in meeting the demand for marine fish and invertebrates globally. It provides substantial income, and helps meet demand for fish by providing high value alternative to fish caught in the wild (Job 2005). During the 1990s, global production of farmed fish and invertebrates (shrimps, clams, oysters) doubled in weight and value whereas wild-caught production remained the same (Naylor et al. 2001). Production of food fish has increased by a gradual 1.2 % annually since the early 1970s while annual production from aquaculture has increased by more than 6 % (excluding China).
(FAO 2004); if China’s aquaculture production is included, the percentage increase becomes 9.1%.

1.3 Stock enhancement and restocking

1.3.1 Definition

Fish are introduced into the wild for different purposes, either to increase the number of juveniles to the full carrying capacity of their habitat or to increase the number of spawning stock. According to Bell et al. (2005), stock enhancement and restocking are two different terms. Stock enhancement is the process of releasing hatchery reared juveniles to overcome recruitment limitations. This allows yield levels to increase beyond the level supported by natural recruitment. Restocking is the process where the spawning biomass of a severely depleted stock is restored until the normal level of yield is reached.

Terms such as “sea ranching”, sea farming, marine farming, and reseeding are also used as synonyms for stock enhancement (Munro and Bell 1997; Anna et al. 2004).

1.3.2 History

Stock enhancement and restocking projects in many countries did not materialize until the mid 19th century (Anna et al. 2004). With the increase in fish demand and the decrease in wild fishery stock, many countries started to develop management strategies to protect fisheries. Stock enhancement using cultured fish was one of these management strategies.

The popularity of stock enhancement decreased amongst most scientists after half a century of hatchery releases showed no indications of increased yield (Blankenship and Leber 1995). In the United States, marine stocking programs were abandoned after discovering that there was no clear impact on fisheries landings (Blankenship and Leber 1995; Leber et al. 1996). Species such as Atlantic cod Gadus morhua, haddock Melanogrammus aeglefinus, pollock Pollachius virens, winter flounder Pleuronectes americanus, and Atlantic mackerel Scomber scombrus were released without any apparent success (Blankenship and Leber 1995).
According to Munro and Bell (1997), most early enhancement programs failed due to poor detection of the high mortality rates that were suffered by the released juveniles.

Current failures are specifically attributed to inappropriate time of release, unsuitable habitat, insufficient knowledge on species biology and ecology, fish quality, and behavioral deficits (Anna et al. 2004).

1.3.3 Effectiveness of stock enhancement and restocking

Even though early stock enhancement attempts in most parts of the world failed, there were also some significant successes in a few countries. For example, Japanese stock enhancement projects have been successful for quite some time. In 1994, the Japanese used 34 species of finfish, 12 species of crustaceans, 25 species of shellfish and 8 other species in such projects. For many of these species, juveniles were artificially reared in hatcheries, while a few species were collected from the wild, reared in a hatchery and re-released in the wild (Masuda and Tsukamoto 1998). Although a high number of species are used for stock enhancement in Japan, the salmonids have been the most popular (Masuda and Tsukamoto 1998; Kaeriyama 1999). Species popularity later diversified to red sea bream (*Pagarus auratus*), Japanese flounder (*Paralichthys olivaceus*) (Munro and Bell 1997), black sea bream (*Acanthopagrus schlegeli*), herring (*Clupea pallasi*), sand fish (*Arctoscopus japonicus*), mud dabs (*Limanda yokohamae*), jacopever (*Sebastes schlegeli*), ocellate puffers (*Takifugu rubripes*) and wrasses (*Halichoeres poecilepterus*) (Masuda and Tsukamoto 1998). The highest releases amongst fishes were of red sea bream and Japanese flounder (Munro and Bell 1997; Masuda and Tsukamoto 1998). Several million of prawns, shrimps, crabs and sea urchin were also released for stock enhancement purposes.

Similar industrial-level projects have also succeeded in other countries, for example, the shrimp fishery (*Penaeus orientalis*) in China, the scallop fishery (*Pecten novaezelandiae*) in New Zealand, and the recreational fishery of red sea drum (*Sciaenops ocellatus*) in Texas (Munro and Bell 1997). Salmonids have been used for stock enhancement for over a century, and salmon hatcheries provide a substantial portion of juveniles to most of the North Pacific and North Atlantic fisheries (Munro and Bell 1997). In 1998, Welcommee and Bartly
(in Brown and Day 2002), estimated that more than 300 aquatic species internationally were reared in hatcheries and later released in the wild, and every country contributed to some aspect. Since approximately 290 out of the 300 were freshwater species, this clearly proves that marine stocking was relatively uncommon, even in the late 1990s. This may be mainly due to difficulty in reproducing marine species in captivity and controlling their survival in the ocean.

1.4 Aquaculture and restocking programs in Pacific Island Countries
The importance of aquaculture has been recognized by most Pacific Island governments and investments have been made in freshwater and marine aquaculture (Adams et al. 2001), however aquaculture is still in its developmental stages in most of these countries. Only a few large-scale commercial enterprises have emerged over the years, such as pearl oyster farming in French Polynesia and Cook Islands, shrimp farming in New Caledonia and micro-algae in Hawaii (Bell 1999).

The slow development of aquaculture in the Pacific has been mostly due to lack of expertise and infrastructure (Adams et al. 2001), but this is changing now. The local governments are now realizing the importance of aquaculture as an alternative to fishing for food security, and as a means of income generation.

Most Pacific Islands are involved in small-scale subsistence level aquaculture such as Tilapia in American Samoa, Fiji, French Polynesia, Samoa, Vanuatu and Tuvalu; Milkfish in Kiribati, Nauru and Tuvalu; Freshwater prawns in Vanuatu, Fiji and Solomon; Barramundi in French Polynesia; cage culture of jackfish and rabbit fish in French Polynesia, Pearl oyster in Papua New Guinea (www.spc.org).

A few Pacific Island countries are also producing aquaculture products at semi-commercial to near commercial level such as Tilapia in Fiji, Vanuatu and Papua New Guinea, Pearl oyster in Cook Islands, Micronesia, French Polynesia and Fiji; Shrimp in French Polynesia, New Caledonia and Fiji; Seaweed in Kiribati and Solomon Islands; Freshwater prawns in Fiji and Vanuatu (www.spc.org).
Most Pacific Island countries are also culturing giant clams and *Trochus* for aquarium trade and for stock enhancement (Bell 1999). Attempts at stock enhancement of giant clams are being carried out in Solomon Islands, Fiji, Cook Islands, Western Samoa (Adams et al. 2001), Micronesia and Vanuatu (www.spc.org). *Trochus* enhancement projects were carried out in Tonga and Vanuatu with the assistance of Japan International Cooperation Agency (JICA) and Australian Center for International Agriculture Research (Adams et al. 2001). However these restocking and stock enhancement programs are still at experimental stage. This may be due to (1) long timeframes for breeding and grow out to juvenile stage, (2) strategies not in place for releasing, (3) time-consuming follow-up of successes and failures of pilot-scale releases. (Bell 1999)

In Fiji, marine aquaculture includes pearl oyster, giant clams, and *Trochus*. Black lip pearl oyster (*Pinctada margaritifera*) farming started in 1998, whereby spat were collected from the wild and then reared in long-line farms (Fisheries Annual Report 2001). In 2004 there were 4 farms operational in the Northern Division (Fisheries Annual Report 2004). A Giant clam and *Trochus* culture facility has been setup on the island of Makogai where captive breeding of the two commodities has been done over the years. While pearl oyster and *Trochus* farming is more on a commercial basis for export, the production and breeding of giant clams appears to have two purposes; aquarium trade, and restocking in areas with depleted stocks (Fisheries Annual Reports 2001-2004). According to fisheries annual reports, 300 giant clams were restocked in 2001, followed by 15,500 clams in 2002 and 730 clams in 2004. Data from the year 1997 suggest restocking of 21,800 clams in ocean nursery, but the intention was not clear as to whether they were for restocking or for grow-out to harvest for the aquarium trade.

The freshwater fish Tilapia was stocked in major rivers all around Fiji as food fish from 1950s onwards (Nandlal and Pickering 2004), however data is unavailable about releases since then. The practice has been criticized for impacts on native fish populations.
Aquaculture and restocking can assist island nations in income generation, food security, employment generation and conservation (Bell 1999). Future development in aquaculture and restocking of marine aquarium animals is being increasingly advocated, since the demand for aquarium animals is increasing. These include coral reef organisms such as spiny lobsters, hard corals, soft corals, angel fish, clown fish and other colorful damselfish (Bell 1999). Aquaculture and restocking of coral reef organisms will assist in meeting the demand while conserving wild stock.

1.5 Coral reefs and reef fishes
Coral reefs have been around for approximately 450 million years, and throughout their existence they have been an essential habitat for many marine organisms (Bellwood and Wainwright 2002). Coral reefs are known to harbor a high diversity of marine fish, invertebrates and plants (Sebens 1994), making them one of the most productive and biologically diverse ecosystems on earth.

Several estimates on global coral cover ranges from 0.1 - 0.5 % of the ocean floor, nevertheless almost one third of the world’s marine fish species are associated with coral reefs (Moberg and Folke 1999). The reef ecosystem has proven to be biologically and economically valuable to humans. It supplies a vast array of commodities and services such as seafood, medicinal extracts, raw materials, recreational possibilities, and coastal protection, as well as cultural benefits (Moberg and Folke 1999).

Many fish are intimately associated with coral reefs, and it is here that numerous fish families attain their utmost species diversity and abundance. Over 1200 fish species (food and non food) is supported by the Great Barrier Reef alone (Randall et al. 1990; Sale 2002). Subsistence and commercial fisheries target multiple species, depending on their availability. According to Sale (2002), 200 – 300 fish species are harvested in the Indo-Pacific, more than 100 species in the Caribbean, and more than twice these numbers are consumed in Pacific and Atlantic oceans.
1.5.1 Uses of corals and coral reef fishes

1.5.1.1 Subsistence fishery

The livelihood of thousands of fishers is dependent on coral reef fisheries in countries such as Indonesia, Philippines and the Pacific Island Countries (Hair 2000; Pomeroy et al. 2004). Some people catch fish only for their own consumption, while most catch fish and sell it in local markets at artisanal level, or exploit fishing grounds for commercial purposes such as food fish for restaurants, resorts and export.

1.5.1.2 Live food fish and aquarium industry.

Coral reef resources are further subjected to exploitation by the recently developed Aquarium Trade and Live Reef Food Fish Trade (LRFFT). The aquarium trade is focused on coral reef fish which are otherwise not considered as key food sources in many countries (Sadovy and Vincent 2002). The market demand for both aquarium and food fish has rapidly increased in the past decade (Hair 2000), a consequence of economic and technological changes (Sadovy and Vincent 2002).

A commercial trade of colorful reef fish for aquaria has developed rapidly in the past decade. A wide diversity of non-food coral reef fish are being caught for the trade, which also includes live corals and other reef associated organisms (Pomeroy et al. 2004). Over 1000 fish species from more than 50 families are being currently traded in the global market (Sadovy and Vincent 2002), to satisfy marine aquarists. Tropical and subtropical countries are amongst the world’s largest exporters of live fish for the private aquarium trade (Olivotto et al. 2003). The aquarium trade is estimated to earn US$200-330 million annually, with destination markets in USA, EU and to a lesser extent Japan (Wabnitz et al. 2003). There is no doubt that the demand for aquarium fish will further increase in the future.

In addition to commercial and subsistence fishing for protein, many fisheries are used for demand-driven commodity markets. This industry includes trade of high value live reef food fish for consumption in luxury restaurants, largely in Asia. Production modes for this luxury industry are either wild capture of market size, grow-out of wild captured juvenile, or mariculture, however the wild caught fish were estimated to make up half to two thirds of the
total trade (Wabnitz et al. 2003). Species such as *Cromileptes altivelis* (Family Serranidae) and *Cheilinus undatus* (Family Labridae) are in greatest demand; however other varieties of grouper, snapper and rock cods also supplement the market demand (Samoilys 1997; Hair 2000). Hong Kong has been identified as one of the major importers of live food fish, which received approximately 32,000 metric tons in 1997 alone (Graham 2001) and which has approximately 800 seafood restaurants selling live reef fish (McGilvary and Chan 2002).

### 1.5.2 Threats to corals and coral reef fish

Throughout the world, reefs are being over exploited, polluted, and destroyed. Any threats to corals are also threats to the diversity of coral reef fishes. A long term study (1996-2003) in PNG indicated that fish biodiversity abundance is directly proportional to coral cover. Survey results of eight separate reefs showed coral cover decline from 66% in 1996 to as low as 7% in 2002. Over 75% of reef fish declined in abundance due to habitat degradation, however the effects is even greater in species which has a greater dependence on living coral as juvenile recruitment sites (Jones *et al.* 2004).

It is obvious that subsistence and commercial use of reefs has increased in recent years. Intense over fishing and use of destructive fishing methods are the most obvious and widespread threats to reef ecosystems. Usage of reef for recreational purpose also poses a threat to reefs if the activities are not conducted sustainably (Sebens 1994).

For example, the live reef food fish trade often targets spawning aggregations of selected high value species, leaving a population with relatively less spawning stock, thus resulting in recruitment limitation (Pomeroy *et al.* 2004). A study conducted in Borneo showed a significant exponential decline in the population of reef fishes targeted by LRFT (Scales *et al.* 2007). Furthermore a review done by Ziemann (2001) indicates that much stock of marine ornamental fish has been severely depleted. For example, abundance of eight marine ornamental fish species have declined in Hawaii, along with declines in butterfly fish in Sri-Lanka and anemone fish in Kenya (Ziemann 2001).
In addition to overfishing, destructive fishing methods such as use of cyanide are also a concern. Fishermen in some countries use cyanide to stun fish which eases collection, however, cyanide not only stuns fish but also destroys the fish habitat. These luxury wildlife trades (marine aquarium trade and food fish trade) are a major concern. High prices, together with strong demand, ensure incentives for constant heavy exploitation. Fishing efforts are being increased to cater for the high market demand, which leads to fish stocks being severely depleted.

Along with human activities, natural events such as hurricanes, predator outbreaks (*Acanthaster planci*) and periods of high temperature are also causes of reef destruction. Reef and reef organisms are further threatened by climate change phenomena such as global warming. These are predicted to include more frequent storms, increased sea level, and increased temperature stress. The recent coral bleaching of the Caribbean and eastern Pacific is believed to be caused by increased periods of high water temperature (Sebens 1994).

1.6 Capture and culture of post-larval reef fishes

1.6.1 What are post larval reef fishes?

Most coral reef fishes are known to have a biphasic life cycle (Jones 1987, Leis *et al.* 1991, Irisson *et al.* 2004). In the first phase, adult and juvenile stages live and feed primarily in coral reef areas. In the second phase eggs and pelagic larval stages float around in the open ocean as plankton. Larval fishes are generally minute and transparent. This phase can last for as little as a week in some damsel fish (Pomacentridae) (Sale 2001) and as high as 64 weeks in some porcupine fishes (Diodontidae) (Sale 2002). The duration of the larval phase is species specific but it can also be influenced by environmental cues (Jones 1987). The pelagic larval phase ends when larvae metamorphose and migrate from the planktonic environment to substrate-associated (coral reef) adult populations (Armsworth 2000; Durville *et al.* 2003). After metamorphosis the larvae reaches the post larval stage.

During settlement, larvae are exposed to the complex coral reef environment for the first time since hatching. Along with potential substrate, the environment also contains competitors, and predators (Ohman *et al.* 1998), which may have an influence on survival during and after
settlement. The growth and survivorship of settling fish could be further determined by factors such as reef location, coral type, habitat structure, and presence of conspecifics and heterospecifics (Ohman et al. 1998). Furthermore, selective mortality studies during larval-juvenile transition suggest that juvenile survival may also be related to early life history events that affect their physiological condition (Searcy and Sponaugle 2001).

1.6.2 Using post larval fish for aquaculture or stock enhancement

While most aquaculture activities and stock enhancement programs use hatchery bred juveniles, some use wild captured juveniles. This technique of using juveniles captured from the wild for aquaculture is not new. Italians in the Mediterranean used to traditionally harvest juvenile mullet, sea bream and European sea bass for rearing purposes. This practice was also being adopted for milkfish *Chanos chanos* and Japanese amberjack, *Seriola quinqueradiata* for over a century (Durville et al. 2003).

Capture based fish culture is still practiced and the technology has recently been applied to coral reef fishes, where colorful juveniles are collected for the lucrative aquarium trade. On the other hand, the scientific basis for collecting juveniles from the wild is being debated, as to whether harvesting juveniles from the wild affects natural replenishment of reefs and what may be the long term effects on the reef ecosystem (Bell et al. 2005). However, considerable research has been carried out on reef fish life cycles, breeding, recruitment, factors affecting recruitment, spatial and temporal variations of recruitment, factors affecting survival after settlement, and so on. These studies help us to further understand the ecology of reef fishes.

It is generally known that most coral reef fish spend their early days as larvae floating around in the plankton, before metamorphosis and settlement on reefs (Irisson et al. 2004; Planes and Lecaillon 2001; Hair et al. 2004). Studies investigating settlement patterns and rates of recruitment have shown that larvae suffer very high levels of mortality when they come to settle on reefs (Searcy and Sponaugle 2000; Steele and Forrester 2002). Leis (1991) pointed out two crucial periods when larvae are subjected to high levels of mortality, firstly during the planktonic larval development stage and secondly during settlement on coral reefs. According to Leis (1991) less than 1 % of hatched planktonic larvae survive to become
successful colonists of coral reefs. The mortality rates are highest during and after settlement. It is generally assumed that mortality is mainly due to predation by other bigger reef fishes (Searcy and Sponaugle 2000; Steele and Forrester 2002).

Post-larval capture and culture techniques enable us to capture and use pre-settling reef fish, which will otherwise be subjected to exceptionally high rates of mortality. Captured fish may include both food fishes (for example, high-value LRFT species) and aquarium fishes. These can be reared in land-based hatcheries for certain period before being used either for capture-based aquaculture or for capture-based stock enhancement. The current study will focus on the latter, where captured fish will be reared and released in the wild, and will help assess the viability of such releases. Stock enhancement can focus on either or both of food fishes and aquarium fishes. Release of aquarium fish can be practiced to replenish areas of low species diversity, and tourist snorkeling areas.

1.6.3 Methods of capture
Post larval fishes can be captured using various methods, which have been developed after studying larval recruitment and behavioral patterns. According to Anderson et al. (2002), the two most common methods of collection/capture are using light traps and stationary nets such as channel and crest nets.

Light traps (Appendix 10) are used mainly to attract settlement-stage larvae, which are coming to settle on reefs at night. This nocturnal phase, as highlighted by Lecaillon (2004), is the most critical phase for recruitment of coral reef fish. It is at this stage that larvae experience high levels of mortality. Light traps are designed to efficiently capture various species of fish post-larvae. Many settling larvae will swim actively towards light, and into a transparent or semi-transparent trap with a light source (Anderson et al. 2002). The stationary traps (crest nets {Appendix 10} and channel nets) capture larval fish passively. Larvae are carried into the nets by wave action (crest) and currents (channel).

Light traps are known be selective. They capture older and stronger larvae, which are able to swim actively towards the light. Traps using lights are non destructive and can be used as
sustainable alternatives of collection of fish for aquarium trade and fishery enhancement programs (Watson et al. 2002).

The original design of light trap, designed by Doherty in 1987, was expensive and thus unsuitable for most small projects, especially at community level (Watson et al. 2002). Keeping this in mind, various reef fish researchers have designed cheaper versions of light traps. One such example is the CARE system developed by ECOCEAN (Appendix 10), a private company. The C.A.R.E (Collect by Artificial Reef Eco-friendly) device comprises a water proof light, and a trap, that is termed a “lighted artificial reef”. The C.A.R.E is attached to a mooring (anchor) near reefs. Post larval fishes which come to settle on reefs get attracted to the light and thus enter the trap. The trap design has been improved after conducting several tests in the Indian Ocean. The design has also been used to collect post larval fish in Florida, New Caledonia, French Polynesia and China Sea (Lecaillon 2004) and is currently being used in Fiji for experimental purposes.

Post larval collection using stationary crest nets are being used experimentally by a Masters student in Fiji. Similar crest nets are also in use in the Solomon Islands where community level aquarium fish projects are being carried out using capture and culture of post larval reef fish. The project, which is run by Australian Center for International Agricultural Research (ACIAR) has proven to be successful, both in collection of competent and healthy post larvae and in short term culture methods (Hair et al. 2004)

1.6.4 Methods of culture

Few studies have documented the culture methods for raising post-larval reef fishes. Durville et al. (2003) adopted an intensive hatchery rearing system in glass aquaria, with the fish density ranging from 2.5 – 1125g /m³ depending on species. Larger fishes were reared in 2m³ polyester tanks. Open circuit filtered water exchange system was adopted, and tanks were siphoned daily to remove waste and uneaten feed. Results of this culture method were favorable on the survival of ten of the twelve species studied. The importance of using nutritionally complete feed and suitable feed particle size was emphasized.
Experiments on grow-out of post-larval reef fish in Solomon Islands were carried out by Australian Center for International Agricultural Research (ACIAR). Rearing of post-larvae fish and crustaceans were initially done in land-based concrete tanks; however later rearing was done in floating sea-cages. This improved the growth and survival of fish; and was recommended as more appropriate for village situations. Survival of crustaceans, on the other hand, improved when in stationary benthic cages. The position of floating cages was found to be important for fish survival. Experimental results showed high mortality of small fish in cages, and this was due to strong currents and rough weather. Thus fixed floating cages in shallow, near-shore sheltered waters are recommended (Hair et al. 2004). Parameters such as good quality water, food, photoperiod, noise and daily feeding cycle has been found to be important in rearing of post-larval reef fishes (Dufour 2002).

Culturing post-larval reef fish with substantial survival is possible provided an adequate environment is provided. Culturing can be done in intensive systems or more cost effective extensive system (sea cages), however proper husbandry techniques are necessary. Disease outbreak has been identified as one of the major setbacks in any culture system, and the chances of this may be increased by poor water quality (Dufour 2002).

1.6.5 Effects of culture conditions on post release survival
The success of any stock enhancement and restocking program is judged by the survival of released fish and their interaction with, and contribution to, the natural population. In most enhancement programs, the survival of newly released juveniles is drastically low, which results in the enhancement being ineffective. This is a key issue which requires increased research effort to make stock enhancement more effective.

Many factors are assumed to contribute to the high levels of mortality after release. Changes in temperature, salinity, habitat and transporting/handling stress are only some of the factors that cause mortality. In addition, more complex factors have also been identified by Fairchild and Howell (2004). After release of hatchery reared juveniles, the high density of fishes at the site results in an increase in intra-specific competition, followed by high mortality. Hatchery reared juveniles generally possess behavioral anomalies or deficits, and
this may also be a cause of high mortality. Most importantly, the inability of released fish to switch from formulated diets to live prey, and inability to effectively capture prey, can reduce chances of survival (Fairchild and Howell 2004).

The behavior of predator and prey generally determines the result of their interaction, and the outcome of such interactions has a major influence on fish populations (Fuiman et al. 2006). It has been shown that cultured fish experience sensory deprivation, which in turn leads to inability to recognize, react to and avoid predators in the wild. Predatory fish exhibit a sequence of behaviors from searching, recognizing prey, stalking, and attacking, to defeating and consuming. In turn the prey may exhibit its own set of behaviors in avoiding predators. However, prey fishes are believed to have fewer behavioral options, which mainly are avoidance and evasion (Fuiman et al. 2006)

1.7 General objective
The aim of the present study was to assess the potential of PCC (post-larval reef fish capture and culture) techniques for stock enhancement and address some issues that are known to cause low survival in fish released for stock enhancement.

The four major objectives of this study were:

1) investigate the effects of three different diets on growth and post-release survival of a coral reef fish,
2) investigate the effects of size-at-release on post-release survival and microhabitat choice,
3) compare growth of cultured fish with that of fish in the wild, and
4) determine the colonization pattern, growth, and age of fish within and between different schools (colonies) of fish in the wild.
1.8 Study species

1.8.1 Taxa

Kingdom : Animalia
Phylum : Chordata
Class : Actinopterygii
Order : Perciformes
Family : Pomacentridae
Genus : Chromis
Species : viridis

Chromis viridis
(Source : www.aquariumsetc.com.au)

1.8.2 General Description

*Chromis viridis* (Cuvier 1830), formerly known as *Chromis cyanea*, is a common attractive damselfish which is found throughout the Indo-Pacific, from Eastern Africa and the Red Sea towards the Pacific Ocean west of North America.

This species is known to be planktivorous and associated with branching *Acropora* corals. These fishes are found in sheltered areas such as sub-tidal reef flats and lagoons (www.reefbase.com) at depths down to approximately 12 feet. The blue-green *Chromis* is easily identified by its shining green – light-blue coloration. They are non territorial, schooling fishes. While the juveniles are closely associated with coral heads (www.reefbase.org), adults are free-swimming and forage in the water column. The male is the dominant parent when it comes to breeding. Breeding is done on sand or rubble substrate, where the nest is prepared by a male, who later breeds in the nest with several females. The male further guards the nest and ventilates the fertilized eggs with their caudal fins. Almost all eggs will hatch 2-3days after spawning (www.reefbase.org).

1.8.3 Importance of *C. viridis*

*C. viridis* serves an important ecological role as a patchily distributed and highly aggregated planktivore, i.e. they compete for resources with other planktivore organisms. (Lecchini *et al*, 2007). It is also one of the most exploited groups of reef fish for the global aquarium market.
For these reasons, and because it can be readily found in good numbers at the Suva Reef, both on the reef (post-settlement) and in light traps outside the reef (pre-settlement), *C. viridis* was the species chosen for this study.
Chapter 2

Post Larval Fish Capture, Culture and Release for Restocking of Coral Reef Fisheries in Fiji: Effects of diet on growth and post release survival.

2.1 Introduction
Coral reefs have been subjected to over exploitation in the past few decades due to the high demand in food fish and invertebrates for food and aquarium trade (Olivotto et al. 2003; Job 2005). In efforts to reduce pressure on marine resources, aquaculture protocols for some organisms (fish and invertebrates) have been successfully implemented (Olivotto et al. 2003; Job 2005), with some farms producing fish commercially. One example is production of flame angel Centropyge loriculus in Hawaii (Job 2005)

While aquaculture is now being adopted to reduce pressure on natural stocks of some species, the possibility of captive rearing of wild caught larvae for restocking depleted reef areas is also now being investigated (Bell et al. 2005; ). Post-larval Capture and Culture (PCC) has been proposed for restocking (Dufour 2002; Bell et al. 2005) and for the marine aquarium trade (Dufour 2002; Hair et al. 2004; Bell et al. 2005). The use of wild caught juveniles is not a new concept for aquaculture (Pomeray et al. 2004). Various aquaculture industries are using wild caught larvae or juveniles. Hair et al. (2002) has identified a substantial number of such industries which use numerous methods of capture and culture, depending on species. Groups identified by Hair et al. (2002) includes penaeid shrimps (in South America, Indo-china, Asia and India), spiny lobster (in Australia, New Zealand, Asia and Indochina), scallops (in Australia, New Zealand, Europe, North and South America, United Kingdom and some parts of Asia), oyster (in Australia, New Zealand, Europe, North and South America, United Kingdom, China and Japan), mussels (in New Zealand, Europe, North and South America, Australia, Asia, Africa, India and Pacific Islands), blacklip pearl oyster (in Pacific Islands), milkfish (in Philippines, Sri Lanka, Pacific Islands and Indonesia), eels (in Japan, Asia, Europe, Australia, United Kingdom, China, North Africa and North...
America), Serranidae reef fish (in Asia), small and colourful coral reef fishes (in Pacific Islands), yellowtail (in Japan and Indo-china) and Southern Bluefin tuna (in Australia).

Post larval reef fish and invertebrates have also been identified by the Secretariat for Pacific Communities (SPC) as high interest commodities in the Pacific Islands for the aquarium and live reef food fish trades. The two major places involved in this are French Polynesia and Solomon Islands (www.spc.org).

Keeping in mind the increasing demand for corals, fish and invertebrates by the marine aquarium trade, PCC techniques were transferred to community level in Solomon Islands for household-level businesses (Hair et al. 2004) with the aim of catching and growing wild caught larvae sustainably and at a low cost for the aquarium and live reef fish trade (Bell et al. 2005).

2.1.1 Use of wild post larvae for restocking reefs
Following the recognition of PCC techniques for the marine aquarium trade, efforts are now being made to study ways of using this method for restocking reefs with depleted fish stocks or to achieve stock enhancement. While the technique of catching pre settling coral reef fish for small-scale (community level) marine aquarium trade has been successfully developed (Meekan et al. 2001; Watson et al. 2002; Hair et al. 2004), research on restocking reefs with the above technique is still in its infancy.

The first study to assess the viability of this technique was done in French Polynesia, where pre settling coral reef fish were caught using reef crest nets and “hoa” net (channel net) on the islands of Moorea and Bora Bora respectively (Grignon 2005). All captured fish were reared in captivity for 3-14 weeks depending on the time of capture, and released simultaneously on the reef. Out of the 1500 ornamental fish (30 species) and 1000 food fish (20 species) released, the overall survival rate was calculated to be 2-3% for ornamental and 4-6 % for food fish. It was, however, suggested that the survival rate may have been underestimated due to the inability to detect some species which may have been hiding in corals or had probably left the survey area. These fish exhibit cryptic behavior and are often
unaccounted for in survival studies. Improving assessment methods of released fish on reefs and training fish for habitat and predator recognition before release has been strongly recommended (Grignon 2005).

2.1.2 Rearing conditions

After capture of post-larval fishes, the next critical stage is to rear them in proper culture conditions. Some of the most important factors that need addressing in any culture system are: proper infrastructure (tanks, ponds), good water quality (depending on species) and, most importantly, good quality feed (Pillay 1990). These factors may differ between species and between culture systems. Fish can be cultured in: 1) intensive systems which involves high-tech rearing at high densities, 2) semi-intensive systems involves low-tech rearing with medium densities and the use of both natural food and artificial feeds and 3) extensive rearing involves rearing at low densities using naturally occurring food.

2.1.3 Feeding requirements

Proper feeding in culture conditions ensures production of good quality fish, which will have better growth rate and will result in better survival after release. Several studies have proven that faster growing reef fish have better quality and have higher chances of survival compared to slow growing fish (Vigliola and Meekan 2002; Hawn et al. 2005).

Feeding of reef fish larvae in hatcheries is usually done by providing live prey like Brachionus plicatilis (rotifer) or Artemia salina (brine shrimp), however recent research shows that live prey may be replaced by a formulated diet (Yufera et al. 1999; Cahu and Infante 2001). Fishes need different levels of proteins at different stages of their life, with a decrease/change in the requirement level as the fish grows (Cahu and Infante 2001). Marine fish larvae require complete formulated diets containing required amounts of proteins, vitamins and minerals (Craig 2002), essential fatty acids such as long chain polyunsaturated fatty acid (Day and Howell 1997), and high energy level, supplied by neutral lipid and phospholipids (Cahu and Infante 2001). Larvae may exhibit deficiencies in morphology if nutrition is inadequate (Cahu and Infante 2001). Masuda and Tsukamoto (1997) also reviewed effects of dietary ascorbic acid and docosahexaenoic acid on schooling behavior of
ayu, *Plecoglossus altivelis* and yellowtail, *Seriola quinqueradiata*. Both species showed enhanced schooling behavior when fed with higher levels of dietary ascorbic acid, while juvenile yellowtail fed with feeds containing docosahexaenoic acid had normal schooling behavior compared to fish fed without docosahexaenoic acid which did not school.

The challenge of providing essential nutrients in diets for early stage larvae at an economic cost can be avoided by using pre-settling post larval reef fish from the wild. These fish are already past the larval stage and are ready to adapt to the benthic juvenile lifestyle. While feeding of larvae is more challenging than feeding juveniles, there is always a need to utilize suitable feed (50% crude protein) to maximize growth, but at the same time be cost effective (Day and Howell 1997) since feed may represent 40-50% of the production costs (Craig 2002).

Grow-out of wild caught juvenile fish in land based facilities using artificial diets has been done previously. In a comprehensive study done by Durville *et al.* (2003), experimental rearing of twelve coral reef species was done in captivity. Species studied were: *Monodactylus argenteus, Gerres acinaces, Stegastes nigricans, Chromis viridis, Dascyllus aruanus, Chrysiptera glauca, Stethojulis albovittata, Scarus sordidus, Valamugil cunnesius, Zebrasoma desjardinii, Naso unicornis* and *Rhinecanthus aculeatus*. It was shown that fish at post larval stages were sufficiently developed to accept artificial feed, but a 7-10 days weaning/acclimatization period seemed to be necessary, where fish were fed initially with live feed. Ten out of the twelve species studied exhibited reasonable survival rates, out of which three species; *Monodactylus argenteus, Valamugil cunnesius* and *Scarus sordidus*, showed high specific growth rates. These results clearly suggest that captive rearing of wild caught post-larval or juvenile reef fish is possible.

Most extensive and semi-intensive aquaculture systems use natural live feeds like phytoplankton, *Artemia* and rotifer, but supplementary artificial feed is also important to promote adequate growth and increase production (Pillay 1990).
Feed cost is known to be one of the major expenses in culturing fish, thus it is important to identify a feed which contains essential nutrients and promotes adequate growth at the lowest cost. Since marine finfish culture is almost insignificant in Fiji, obtaining feeds manufactured locally that are specifically formulated for marine fish is difficult. But freshwater food-fish feed is locally produced in Fiji, while pet shops do sell imported feeds for freshwater aquarium fishes (though at high cost). Using feeds made from local ingredients may offer advantages in terms of availability, easy access and lower cost. But these feeds are not nutritionally formulated for marine fish, thus research on growth rate of marine fish using local feeds needs to be done. Favorable results will definitely be welcome, in terms of availability, easy access and most importantly, lower cost.

2.1.4 Behavioral competency of juveniles before release

While these fishes do perform well in captivity, releasing them back into the wild may not be so promising (Griffin et al. 2000; Brown and Day 2002). Most restocking programs of hatchery bred and reared juveniles which failed over the years attribute their failure to inappropriate time of release, unsuitable habitat (due to insufficient knowledge on species biology and ecology) and to fish quality and behavioral deficits (Masuda and Tsukamoto 1997, Brown and Laland 2001; Anna et al. 2004; Fairchild and Howell 2004). In addition to the above three sources of failure which are unique to fish release scenarios, Fairchild and Howell (2004) also identified routine culture-protocol factors such as changes in temperature and salinity, stress by excessive handling, stress induced impairment, and competition for resources due to high artificial densities, which may also cause high mortality after release. Such factors have also been highlighted by Masuda and Tsukamoto (1997), as inducing behavioral deficits in hatchery reared fish.

Captive breeding can induce behavioral deficits in fish, especially regarding feeding and predator avoidance (Griffin et al. 2000; Brown and Laland 2001; Fairchild and Howell 2004,). The inability of hatchery reared juveniles to recognize predators and react appropriately has caused severe mortality within a few days (Anna et al. 2004) or even a few hours of release (Grignon 2005). Consequently most restocking studies are now focusing on addressing these problems of low post release survival. Behavioral and ecological
characteristics of the species to be enhanced should be considered (Masuda and Tsukamoto
1997) as top priority.

Different species-specific behaviors play critical roles in post-release survival (Tsukamoto et
al. 1999) and the levels of behavior may differ between wild and hatchery reared stocks
(Masuda 2004). For example wild Plecoglossus altivelis exhibited stronger jumping behavior
than hatchery reared juveniles and wild Paralichthys olivaceus had different feeding
behavior than reared fish of the same species (Masuda 2004). The presence of these
behaviors may serve as an indicator of fitness of fish.

In an experiment on the release of red sea bream, Pagrus major, a tilting behavior of the
species was identified as an index relating to predator avoidance. This behavior was
observed in both wild and hatchery reared fish while handling. Fish aggregated on the
bottom, tilted their body, extended the dorsal fin and stayed still with frequent eyeball
movement. The duration of tilting was quantified as cautiousness of individual, when
subjected to external stimuli. Further experiments showed that non tilting fish were more
vulnerable to predators compared to tilting fish, thus tilting behavior was suggested to be a
predictive index for effective enhancement of P. major (Masuda and Tsukamoto 1997).

Masuda and Tsukamoto (1997) also reviewed some other behaviors that were proven to be
essential for survival in the wild. For example, the jumping behavior in amphidromous
Salmonoid fish (Plecoglossus altivelis) was identified as an important index for upstream
migration. This behavior was influenced by water temperature, light intensity, fish density,
feed availability and water depth. Furthermore, the schooling behavior in masu salmon
(Oncorhynchus masou) was suggested to play an important role in seaward migration.
Experiments revealed that wild juveniles had stronger schooling behavior compared to reared
ones. This suggests that hatchery rearing weakens schooling behavior, an aspect which
needs to be addressed during stock enhancement of this species. Japanese flounder
Paralichthys olivaceus exhibited deficits in feeding behavior, whereby hatchery reared
juveniles were seen to spend more time in off-bottom feeding than wild stock. This makes
them more vulnerable to predators. Experiments show that the species also exhibited deficits
in burying behavior at night, which may cause higher mortality (Masuda and Tsukamoto 1997).

The possibility of teaching hatchery reared fish some survival skills has been suggested. According to Olla et al. (1998) behavioral capabilities of these fish can be improved by training fish in identifying and avoiding predators, feeding with feeds from the wild, reducing stress of handling and transportation and control of social environment. In an experiment conducted by Brennan et al. (2006), tagged hatchery reared common snook *Centropomus undecimalis* were released in the wild in two sets. One set was acclimatized in cages for 3 days, while the other set was released directly. The recapture rates suggested that *in-situ* acclimatization can improve post release survival, whereby the stock was allowed to get used to the environment in the wild while being protected from potential threats. Similarly in-situ acclimatization was also suggested for striped jack, *Pseudocaranx dentex*, where acclimatization allowed the released fish to slow down stress induced spiral diving which cause them to drift from release sites (Masuda and Tsukamoto, 1997).

In another behavioral study, the ability of winter flounder (*Pseudopleuronectes americanus*) to camouflage according to sediment color and bury in sand (predator avoidance) was tested in both hatchery reared and wild caught fish. Results showed that even though wild caught fish exhibited better behavioral capability, cultured fish were also capable of exhibiting these qualities provided they were given enough training (Fairchild and Howell 2004).

Other studies indicate better survival of hatchery reared fish after skill training, for example striped jack exhibited better feeding behavior after training (Kuwada et al. 2000). In a review by Brown and Laland (2001) it has been pointed out that exposure to predators, schooling with wild conspecifics, and learning foraging skills in captivity, does allow aquacultured fishes to become more behaviorally competent in the wild.

Thus restocking using wild juveniles (PCC) may also exhibit some behavioral deficits (Grignon 2005). These can be reduced by training fish in predator avoidance, habitat identification, catching live prey and reduction of stress during handling and transportation.
2.1.5 Effects of size at release on post release survival

The size of released fish has been found to affect post-release survival. In a review by Yamashita and Yamada (1999), on the release strategy of Japanese Flounder (*Paralichthys olivaceus*), results show that survival rate was higher for a particular size. Juveniles ranging between 40mm – 140mm total length were tagged and released. Recapture rates showed that fish of 100mm total length had the highest survival. This size-at-release effect was also seen after experimental release of red sea bream, *Pagrus major* in Japan during a 3 year period (1987-1989). Three size ranges of 10mm, 20mm and 40mm (total length) were released, and results showed that mass mortality occurred in the 10mm and 20mm size groups (Masuda and Tsukamoto 1997; Tsukamoto *et al.* 1999).

A large-scale experiment conducted in Norway also identified the importance of size at release on survival. Atlantic Cod, *Gadus morhua*, were released in different areas, i.e. closed area with low productivity and open coastal areas with higher productivity. The effect of size at release on survival were found to be different in areas. While one size group exhibited high survival in closed areas, the same size group exhibited low survival in open coastal areas. This difference was found to be related to the size of predators in areas, where the open coastal and high productive areas might have had larger and greater number of predators (Svasand 2004).

A similar trend has also been noted by Leber *et al.* (1998), but seasonal variation was also thought to have an effect on survival of size groups. Recapture results of released Pacific threadfin, *Polydactylus sexfilis*, in Hawaii showed that larger fish had higher survival after winter while smaller fish had higher survival after summer and fall (Leber *et al.* 1998). This effect of season was also noted by Kristiansen (1999), where Cod, *Gadus morhua*, had a difference in survival during summer and winter. Large cod (20-30cm) had higher survival during summer while few smaller cod (19-20mm) were observed to survive in winter.

Size group of released juveniles may have implications on restocking and stock enhancement programs. Greater body size of released fish may help in avoiding predators; however predator size may also be influential (Yamashita and Yamada 1999). Survival rates may
vary according to season, productivity of area, food composition and predator size and presence (Svasand 2004). It has been recommended by Blankenship and Leber (1995) that experiments to determine appropriate size at release, release season, release habitat and magnitude of species in question, should be conducted before the actual large scale release. These will help determine release strategies.

2.1.6 Objectives and implication

This restocking study is among the first to be done in Fiji, in collaboration with the French-funded Coral Reef Initiative in the South Pacific (CRISP). Some components of a wider Post Larval Reef Fish Capture, Culture and Release strategy will hopefully be determined, and these will complement other research being done by PhD candidate Julien Grignon in Fiji. The overall project aims to find out the viability of PCC and release in Fiji, and put in place possible release strategies.

In this study, the possibility of restocking coral reefs using wild caught fish was investigated. The first restocking trial done by Grignon (2005) in French Polynesia showed that restocking using this technique is possible. However several aspects still need to be addressed, such as culture conditions, size-at-release, and release strategies.

In this study, the effects of three different diets on fish growth and survival, both in captivity and post release, were investigated. This will firstly enable us to find out which is a suitable feed that promotes good growth rate at reasonable cost, and enables adequate survival in the wild after release in enclosures where predators are present. Secondly, the effect of size-at-release on post release survival will be investigated.

Specific aspects to be addressed by this study were:

- Identification of a fish feed that promotes good growth of fish in culture,
- Identification of a feed that enables adequate post-release survival, and
- Determination of the size-at-release (culture duration) that is linked to best post-release survival.
2.2 Methodology

This study consist of two major parts, the first being the culture/rearing of settlement stage, wild caught *Chromis viridis* and the second being the releasing of the cultured fish into enclosures in the wild.

The culture and rearing experiments were done in the Seawater Laboratory at the School of Marine Studies of the University of the South Pacific, while the releasing experiments were done on a fringing reef area at Muaivuso near Suva (Figure 1).

![Figure 1: Map of Suva barrier reef and Muaivuso fringing and barrier reef, showing the capture (light traps) and release sites.](image)

**2.2.1 Experimental setup**

**2.2.1.1 Capture**

Post larvae were captured using light traps on reefs at Nukubuco (Laucala Bay) and Muaivuso (Figure 1). The fishing was done by a hired fisherman, who received prior training in the handling of the light traps and post larval fishes. Collected fishes were brought to the laboratory, sorted by species where possible and placed in glass aquaria where they were weaned on live prey *Artemia* and artificial pelleted diets for approximately three
weeks before the experiment started. The decision about which species of fish to use for this study was determined by the numbers and species of fish which could be caught in the light traps. In particular, the choice of species was determined by whether sufficient numbers of a single cohort could be obtained for a statistically valid experiment.

2.2.1.2 Culture

Once the fishes were able to consume artificial feed, they were moved to the experimental tanks. A total of 90 fish were anaesthetised using clove oil (0.05ml/l) and measured for standard length (± 0.01) using a vernier caliper. Fish were then separated at random into three treatments of 30 fish each. The three treatments were: (1) fed on imported Otohime Marine Fish Pellet, (2) fed on local Tilapia Pellet and (3) Egg Custard (fresh feed consisting of eggs, squid and cod liver oil respectively). Each treatment had three replicates of 10 fish each (5 extra fish per replicate was also put in to cover for mortality and for otolith analysis). Fish were fed three times per day for a total of 158 days, with Day 0 being the day of separation and first feeding using the three experimental diets. Ten fish from each feed application respectively were randomly selected, anaesthetised, measured for standard length and put aside for release at days 60, 90 and 158, while 3 fish each per diet (1 fish per replicate) were preserved for otolith analysis for the three respective days at release.

Feeds that were used in this experiment were chosen on the basis of their protein content and availability. The imported Otohime feeds contains 58% crude protein and ingredients used (as documented in advertisement of the feed) are 86% animal by-product (squid, fish, and krill meal), 2% grains (wheat flour) and the remaining consist of essential oils, vitamins, minerals, and binders. The local tilapia feed is also produced using animal and plant by-products. Tilapia pellet contains 29% crude protein and consists of 25% animal by product (fish meal) and 85% plant by-products (copra, mill-mix, rice pollard etc) and vitamins, minerals and binders. Raw materials of the local tilapia pellets are also occasionally replaced by other similar raw material, especially when one is out of stock. The egg custard consist egg, squid and cod liver oil. Each preparation of one liter consists of 350g fresh squid, 6 eggs and 2 ml cod liver oil.
2.2.1.3 Calculation of specific growth rate using mean standard length.
Mean standard lengths of fish from all feed groups were used to calculate the specific growth rate after 158 days of culture. The specific growth rate (SGR) can be defined as the percentage of daily growth of fish at time = t (Durville et al. 2003). SGR can be determined by a number of parameters such as physiology, morphology and biomass (James and Drenovsky 2007), thus factors such as weight, length, and height can be used to express specific growth rate. In the current study the following formula was used:

$$\text{SGR} = \frac{100 \times (\ln \text{final length} - \ln \text{initial length})}{\text{time}}$$  

(Ezhil et al. 2008)

where time = 158 days and lengths are mean standard lengths in millimeters(mm).

2.2.2 Releasing *C. viridis* into the wild
2.2.2.1 Building of enclosures
Enclosures were built at the back reef area of Muaivuso fringing reef flat (Fig. 1). The site was accessible by foot at low tide (0.8m) and was also accessible by boat at high tide (2.5m). The site had a sandy bottom with patchily distributed branching and massive corals.

Octagonal enclosures were built using a 2mm polyester mesh material. Star pickets (steel stakes) and binding wire were used to support the material (Fig. 2). Each cage had a door which was closed using bolts. The mesh material was held in place by using cable ties to attach the mesh material to the existing skeleton, while sand bags were used to place the mesh material on the bottom and to cover any possible holes in between. It was ensured that the enclosures were closed properly, and had no holes for fish to escape, however the very top of the mesh enclosures were submerged under 30cm of water at high tide, giving fish an option to leave if they were unhappy with the microhabitat provided. Six different microhabitats for fish colonization were placed within the enclosures (Fig. 2). These were live and dead *Montipora altasepta*, live and dead *Porites cylindrica*, artificial coral (made from galvanized chicken wire) and coral rubble.
2.2.2.2 Releasing of fish

Fishes to be released were packed in oxygenated plastic bags and transported to the release site by boat. Fish were acclimated for 24 hours in a separate acclimation box (inside enclosures), before being released into predator-free enclosures. Releasing occurred on the falling tide, by opening the acclimation box and letting the fish swim out. Predatory fish, *Parapercis hexophthalma* (sand perch) were put in enclosures one hour after release, and monitoring of released fish by observation commenced from that time onwards.

2.2.2.3 Monitoring post-release survival

Monitoring of fish commenced at 10am and finished at 4pm for 3 consecutive days. Fish were observed in terms of what they were doing, which microhabitat they were associated with, and in what numbers they were present, every two hours for three days. Relevant data like water depth, tide level, weather, and cage condition were noted during the survey. Two people were involved in observing the cages. Snorkeling gear was used at low tide, while scuba gear was used at high tide. Data recording per cage was done after consultation between both observers.
2.2.3 Otolith analysis
3 fish from each culture treatment were preserved in 97% ethanol for otolith analysis to
determine growth patterns. The otolith extraction and analysis was done at the Institute of
Research and Development (IRD) in New Caledonia. See Chapter 3 for detailed
methodology.

2.3 Results
2.3.1 Survival and growth of C. viridis in aquaria
Survival of C. viridis in the culture system was exceptionally high. Only one fish died during
the entire feed trial.

Specific growth rate calculated using mean standard lengths for Otohime pellet, tilapia pellet
and egg custard was 0.54, 0.45 and 0.50 % per day respectively.

Growth trajectory from the start (day 0) till the end of the experiment (day 158) did not differ
significantly between the three feeds (ANOVA test; p-value = 0.5) (Fig. 3, 4).
**Figure 3:** Mean standard lengths with time for the three feeds. With the first point (day 30) being the estimated number of days in captivity and the start of the feed trials thereafter.
Figure 4: Standard lengths of *C. viridis* for different feeds at different days of culture. With start of feeding trial corresponding to day = 0. (**) Anova test p-value = 0.0001

When mean standard length for days 60, 90 and 158 were compared between feeds, differences were only detected at day 60. The mean standard length of fish fed with Tilapia feed was significantly lower than fish fed with Otohime pellet (ANOVA test; p-value = 0.0001), while difference was also detected between fish fed with Egg custard and Otohime feed which had a p-value of 0.003 (ANOVA - Appendix 2). No difference in mean standard length was detected between Tilapia feed and Egg custard. Similarly no statistical difference was detected between feeds at 90 (ANOVA test; p-value = 0.33) and 158 days of culture (ANOVA test; p-value = 0.06) (Fig. 5). Fish size was more consistent between feeds at 90 days of culture, compared to that of 158 days of culture (Fig. 4)
Figure 5: Mean standard lengths of the three feeds after 60, 90 and 158 days of culture.

** Anova test p-value = 0.003
*** Anova test p-value = 0.0001
2.3.2 Post release survival and habitat choice of cultured fish for different culture period (size-at-release).

2.3.2.1 Culture period (size at release) and post release survival.

2.3.2.1.1 Culture period of 60 days.
A post-release survival rate of 100% was observed for fish fed with tilapia feed while survival rate of fish fed with Otohime feed decreased to 90% by the end of first day, followed by a steady survival (70%) on the second and third day and the lowest survival was observed in fish fed with egg custard, where the survival decreased to 70% in the first day and ended with 50% on the third day. (Survival trend: T > O > E) (Fig. 6a)

2.3.2.1.2 Culture period of 90 days.
Fish fed with Tilapia feed had 100% survival on the first, which decreased to 80% on the second day and ending up with 70% on the third day. Survival rate of fish fed with Otohime and Egg custard decreased by 90% and 80% respectively by the end of the first day and ended up with 60% and 70% survival respectively at the end of third day. (Survival trend: T = E > O) (Fig. 6b)

2.3.2.1.3 Culture period of 158 days.
A 100% survival rate was observed in fish fed with Otohime feed, while fish fed with Tilapia feed also had 100% survival for the first and second day, and decreased to 70% by the end of the third day. Fish fed with Egg custard had 100% survival on the first day, but this decreased to 70% on the second day. 70% survival was also noted on the start of the third day, however no fish were seen afterwards. (Survival trend: O > T > E) (Fig. 6c)

Generally the numbers of C. viridis were seen to decrease over the 3 day observation period, except for two groups (tilapia feed at 60 days and Otohime at 158 days of culture) which had 100% survival (Fig. 6a and 6c). Generally survival rate was between the range of 50-100 % for all culture durations, however all fish went missing from one cage (fed with egg custard for 158 days) on the last day of observation (Fig. 6c). The survival trend between size-at-release (culture duration) and respective feeds were similar after 60 days and 158 days of
culture, where there was larger difference in survival rate between feeds (Fig. 6a and 6c), however this was not the case in fish after 90 days of culture. In this group there was much less difference in survival rate between feeds (Fig. 6b).

**Figure 6:** Post release survival trends of *C. viridis* fed with different feeds, for culture periods (a) 60 days, (b) 90 days and (c) 158 days. O = Otohime feed, T = Tilapia Feed and E = Egg Custard.
2.3.2.2 Culture period (size at release) and microhabitat choice.

2.3.2.2.1 Culture period of 60 days.
Fish fed with Otohime feed were mostly swimming in the water column and was seen on the live *Porites cylindrica* only on one occasion throughout the 3 days monitoring period. Fishes fed with tilapia feed were observed to be more active, swimming around the cage and also occasionally associated with both live corals and rubble, while fish fed on egg custard was very closely associated with live *Montipora altasepta* all throughout the monitoring period. (Fig. 7)

2.3.2.2.2 Culture period of 90 days.
Fish in this group were all observed to be swimming around the cage for the first day (1-4 observations) and were later closely associated with live corals. Fish fed with Otohime and Tilapia feed settled on live *Porites cylindrica* while fish fed with egg custard were associated with live *Montipora altasepta*. (Fig. 8)

2.3.2.2.3 Culture period of 158 days.
Fish in this group were also active. Fish fed with Otohime pellet were mostly swimming around the cage and occasionally closely associated with both live corals. Fish fed with Tilapia feed were observed to be either swimming or on live *Montipora altasepta*. Fish fed with egg custard were mostly on live *Montipora altasepta* and swimming, but were also seen on live *Porites cylindrica* on one occasion. (Fig. 9)

In general, the microhabitat selected by fish differed at times. Fishes were observed to mostly associate with live coral microhabitats. Some were also observed to be actively swimming when water level was high but were close to microhabitat when water level was low in the cages. The monitoring observations showed that most groups inhabit only one or two habitats throughout the monitoring period, but fish were also observed to be occasionally swimming in the water column away from the microhabitats (Fig. 7, 8 and 9).
Figure 7: Post-release microhabitat choice of *C. viridis* for the three feeds after 60 days of culture.
Figure 8: Post-release microhabitat choice of *C. viridis* for the three feeds after 90 days of culture.
Figure 9: Post-release microhabitat choice of *C. viridis* for the three feeds after 158 days of culture.
2.4 Discussion

2.4.1 Growth and survival in aquaria

Survival rate in culture showed exceptionally good survival of *C. viridis*. This proves that wild caught post larval fish of this species are able to adapt to culture environments and survive on artificial feeds. Their survival may have been favored by captive conditions where natural influential factors, like heterospecific and conspecific competition for resources and predatory fishes, were absent.

The three feeds that were used in this experiment were chosen according to their protein content, availability in Fiji, and price. Another aim was to find out if locally available feeds can be used to promote reasonable growth of fish, compared with growth using an imported formulated feed. While the imported feed (Otohime pellet) had 58% crude protein and is formulated specifically for post larval marine fish to promote vitality, the locally produced tilapia feed contains only 29% crude protein and mostly uses low cost local ingredients. The third feed included locally available fresh ingredients (egg, squid and cod liver oil), which can also be easily prepared. Even though the three feeds contained different ingredients and qualities of protein, they all produced fish with similar growth rates.

This indicates that local feeds can indeed be used for culture of this species of fish, resulting in lower feed cost. The imported feed costs $24.50 per kg and the local tilapia feed cost $0.98 per kg. The price of fresh egg custard was $7.70 per liter, but price may vary depending on the price of squid and or other materials used like minced fish. These results show that post larvae of *C. viridis* can be captured and successfully reared in captivity using lower-cost, locally available feed, rather than expensive imported feeds.

Despite the growth difference being insignificant between feeds, fish cultured for 90 days showed more consistency in standard lengths between feeds. This consistency was not visible for 60 and 158 days after culture (Fig. 4). This may indicate that culture duration of 90 days gives a more consistence size fish, which may be of advantage when releasing these fish.
Several studies have suggested that fish within the same cohort have different rates of growth, and this variability in traits may be subjected to selectivity (McCromick 1998; Searcy and Spongule 2001; Raventos and Macpherson 2005). Post settlement processes such as predation and food availability can further influence these growth trajectories, but such was not the case here. This could be because the culture environment was controlled, meaning a predator free environment, constant stock density, and readily available feed three times a day.

The specific growth rates calculated were 0.54, 0.45 and 0.50 % per day, with otohime feed having the highest and tilapia feed having the lowest, and since there was no significant difference detected, specific growth rate value was averaged giving a relative growth of 0.496 % per day for C. viridis.

Specific growth rate figures for juveniles of C. viridis have been provided in only one other scientific study, but this study (Durville et al. 2003) used weights to calculate SGR while in the current study mean standard lengths were used. Determination of lengths rather than weights were preferred for the current study because the fish were destined for a release experiment upon completion of the feeding experiment, so the stress on fish and the potential for mortality caused by handling during weight determinations had to be avoided.

Mean size (SL) in the current study was compared with data published by Durville et al. 2003, who also calculated specific growth rate of C. viridis. The specific growth rate of C. viridis calculated by Durville et al. (2003) was 0.9 % per day, however these calculations were done using fish weight (W), not standard lengths (SL). For comparison purposes, the weights reported by Durville et al. (2003) (at a particular day, as extrapolated from the plot published in their paper) of C. viridis were back-calculated to standard length, according to the formula \( W = a \cdot SL^b \) and the constants \( a = 0.074, b = 2.3 \) that they used for the fish that were weighed in their study. The specific growth rate (based on length) of the fish in their experiment was thus 0.43 % per day, which is
slightly lower than that determined in the current study. The graphical comparison (Fig. 10) of data from this current study and that of Durville et al. (2003), shows similarity in mean lengths except for 90 days after captivity, where mean standard lengths of Durville et al. (2003) are lower.

![Graph showing comparison of mean lengths](image)

**Figure 10:** Comparison of mean standard lengths between the current study and Durville et al. (2003).

The suggestion made by Durville et al. (2003) that *C. viridis* had difficulties in adapting to culture environments and underwent an abnormal development process in their culture conditions, especially in terms of growth rate and length-weight relationship, may be an overstatement. Their observed trend may simply be the natural growth trajectory of this species (see chapter 3 for comparison of cultured fish with wild fish). While the fish of both studies were of the same species, they were not of the same cohort and country. Fish studied have been subject to totally different environmental conditions and have different larval history. Despite this both study demonstrated a similar growth trajectory. However future research on fish of the same cohort will reveal more quality data on growth trajectory of the species.

Most aquaculture industries use live feeds in rearing early stage fish and prawn larvae. The two most common live feeds used are *Artemia* and *Rotifer*. Crude protein of *Artemia* ranges from 40 – 60 % (Sorgeloos 1978), and *Rotifer* has a range of 30-70%
(Tucker 1998), depending on the stage of lifecycle. These live feeds can further be enriched with algae and other diets (Sorgeloos 1978). Live feeds do have an advantage over most supplementary feeds, especially in nutritive value and because it remains in the aquaria (as live prey) for a longer period of time. Supplementary feeds become essential for aquaculture after early larval rearing, since fish start to eat more when they grow bigger. This study has indicated that supplementary diets are capable of producing normal growing fish, however a totally different (better) growth rate can be achieved by either increasing the feeding time per day (5 times a day instead of 3 times a day) or by adopting a feeding regime consisting of both live and supplementary feeds.

Since this technology (PCC) may be transferred to communities (village level) for small-scale culture and restocking at a later stage, it is necessary to find the easiest and cheapest way of raising good quality fish. The results of this study have shown that low cost local feeds do promote normal growth, thus using live feeds can be avoided because: 1) live feeds are expensive and have to be imported, 2) they need special separate tanks for rearing, and 3) they need to be enriched with live algae or other feeds which is also expensive. An ACIAR funded study will soon be carried out in Vanuatu, where clown fish will be reared with naturally caught plankton. The plankton is proposed to be caught using lights and scoop nets at night (Antoine Teitelbaum, pers. comm., 2008). Results from this study will be very interesting, as it may provide information on fish growth using natural plankton and also indicate if the practice is feasible and cost effective.

2.4.2 Post-release survival
Post-release survival of captive-reared juveniles was between 50-100 %. Survival trends show a slightly enhanced survival for fish fed with local tilapia feed in all three culture durations, whereas no apparent trend could be seen between the other two feeds. On the other hand, when compared between different culture durations, survival rate between feeds was more consistent after 90 days of culture (Fig. 6). This may indicate better quality fish at 90 days of culture, a trend similar to that of growth (size) at 90 days (Fig. 4).
It is difficult to say whether the disappearance of fish from the enclosures was due to predation, or due to the fact that fish were unhappy with all of the microhabitat choices and simply left the cage. The cages were properly sealed from the bottom; however the top of the cage left a 30cm gap between the upper limit of the mesh and the sea surface at high tide. Personal observation of fish behavior at high tide showed that fish were actively swimming on the surface of the water. The fish disappearance in some occasions (during the monitoring period) was observed to occur at the time of high tides (Fig. 7 and 9), for example a drop in fish numbers in observation no. 5 (90 days- Fig 6b) and no.9 (158 days- Fig 6c). Thus it cannot be said for sure that the post release survival has a lower limit of 50%. This may well have been higher had the top of the cage not been submerged at high tide. The survival of *C. viridis* may therefore have been underestimated in this current study.

On the other hand, predators are known to be the common cause of mortality on juvenile coral reef fish, and the survival rate of *C. viridis* in this study may be argued by critics as an overstatement, since they were released in cages with only one predator, while in more natural scenarios there is greater diversity and sizes of predators on the reefs. But since it is difficult to monitor fish when they are directly released in the wild (Grignon 2005), releasing was done in enclosures.

While releasing fish in enclosures does not portray the real scenario of post-release survival, Brennan *et al.* (2006), has suggested that the post-release survival of common snooks (*Centropomus undecimalis*) were significantly improved by initially releasing them in predator free enclosures for three days. This indicates that releasing in enclosures is essential in improving post-release survival of released fishes.

### 2.4.3 Post-release microhabitat choice

Microhabitat choices by fish from among the types provided showed that fish overwhelmingly preferred to settle on live corals and not dead or artificial corals, but they were also observed to move around the cage throughout the day. This choice of live
coral over artificial corals for microhabitats by *C. viridis* has previously been demonstrated by Lecchini *et al.* (2007) and Feary *et al.* (2007). Out of all the nine groups of fish released (3 feed by 3 size-at-release), more fish chose live *Montipora altasepta* as their microhabitat than *Porites cylindrica*. The choice of one microhabitat over the other is most probably due to the structural complexity of the corals (Lecchini *et al.* 2007). The importance of this is acknowledged by several scientists (summarized in Clua *et al.* 2006). While live hard corals provide better structural complexity and shelter than artificial structures, one live coral may be preferred to another due to the extent of complexity i.e. size of branches and spacing between branches (Lecchini *et al.* 2007), which may explain why more groups chose *Montipora altasepta* over *Porites cylindrica* in this experiment. Branching *Montipora* and *Porites* are similar; however *Montipora altasepta* has characteristic thin branches (6mm thick) which are often irregularly fused (Veron 2000), giving a better shelter. *Porites cylindrica* corallites are thicker, shallow and smooth (Veron 2000).

There was no association detected between feed type and microhabitat choice, or microhabitat choice and time of day. However fish which were cultured for 90 days showed a more structured behavior in terms of their movement, while other two groups (60 days and 158 days of culture) seemed to be actively moving around the cage. The structured behavior of fish in 90 days of culture may be an indication of better precautionary behavior, which may assist in predator avoidance. While the movement of groups throughout the day is most likely explained in terms of foraging for food around the enclosures, this might not be the best behavior for predator avoidance in a real restocking scenario.

In summary, post larval reef fish (*C. viridis*) capture and culture for restocking may yet prove to be successful, however more research is needed to validate this. This study was a preliminary study for Fiji, and results suggest that restocking of reefs with this and other species may indeed be viable if proper procedures are followed.
It is encouraging that fish diets in culture need not be expensive, and that size-at-release (over the size ranges tested here) are not critical to success, however fish cultured for 90 days exhibited less variability in size, survival and microhabitat choice between feeds. Ninety days may therefore be optimum culture duration for *C. viridis*, but further scientific research is needed to verify this.

Fish can be raised with locally available feeds, which performed just as well as imported feed, while being much cheaper. Survival during culture was also high, showing that wild caught *C. viridis* are capable of adapting to the captive environment. However, as for any species in aquaculture, proper culture protocols need to be followed to minimize the risk of disease. This can be done by having regular water exchanges with good quality water and cleanliness.

Post-release results of this study show that survival can be as high as 100%, and the lowest figure of 50% is an underestimate. This is because some fish escaped from the enclosures. Acclimatization in small cages before releasing is recommended, since this gives the fish a chance to adapt to the wild environment. Provision of proper microhabitat was also found to be important, thus there is a necessity to assess the habitat quality prior to any release. Size-at-release and diet did not affect survival greatly however, this may be due in part to the relatively small range of the three sizes-at-release chosen for this experiment. Further research over a wider size range is recommended, in particular over a range of sizes smaller than the 60 day-old fish. It should also be noted that 90 days culture duration fish did show more consistency in sizes between feeds and microhabitat choice between feeds.

The use of PCC techniques for restocking of coral reef fish is possible in Fiji, provided that fish are released initially into a controlled environment to allow them to adapt to the wild conditions, and live branching corals are present at the release sites at the same time (keeping in mind that these factors will differ between species). It is essential to determine the best size-at-release, microhabitat preference, intra-specific and inter-specific interactions, etc., before restocking of any species.
Chapter 3

Growth patterns and age structure of Damselfish *Chromis viridis* within and between different colonies and locations on Suva Barrier Reef.

3.1 Introduction

Variability in size at age is common with fish populations of the same cohort, whether they are artificially cultured (Dou *et al.* 2004) or in the wild. These size hierarchies may also affect social interactions and future growth of juvenile fish. Dou *et al.* (2004) found that small size Japanese flounder *Paralichthys olivaceus* did show suppressed growth in the presence of larger juveniles, which is believed to be due to aggressive interactions of larger individuals rather than food acquisition. Studies on coral reef fish have shown that size and age structure may differ between species, for example territorial fish may not colonize with larger conspecifics (Webster and Hixon 2000), while some species may prefer to colonize with conspecifics (Ohman *et al.* 1998). *C. viridis* have been shown to prefer the latter (Lecchini *et al.* 2005); however, the effect on their growth is unknown.

3.1.1 Settlement of coral reef fish

3.1.1.1 Definition of settlement

After completing the pelagic larval phase, coral reef fish settle on reefs and adopt a benthic lifestyle. The process of occupying benthic habitat after metamorphosis is called settlement. While settling on reefs, fish larvae face the complex coral reef environments consisting of potential habitats along with competitors and predators (Bergenius *et al.* 2005).

3.1.1.2 Competency to settle

The competency of larvae to settle and adapt a benthic lifestyle is a much debated subject. Pelagic larval duration (PLD) of fish can vary within the same cohort (Searcy and Sponaugle 2000; Bergenius *et al.* 2002). Short PLD may indicate that larvae have attained competency to settle, while longer PLD may be due to larvae not getting proper
settlement cues, thus larvae will delay metamorphosis until a proper settlement cue is obtained (Searcy and Sponaugle 2000).

Recent studies have identified factors or processes other than settlement cues that may also be the cause of variable larval growth. In a study done by Searcy and Sponagule (2000) it was found that *Thalassoma bifasciatum* exhibited variation in larval growth and PLD, and the availability of food during the planktonic stage was proposed as the reason for this variation. The condition of larvae and the energy stores of the larvae are important factors in final settlement, since larvae with high energy stores are able to metamorphose while weaker larvae may be forced to remain in the planktonic stage until sufficient energy stores are obtained. Proper feeding can result in a doubling of the swimming capability of settling larvae, compared with that of unfed larvae (Leis and Clark 2005). The influence of environmental variables were examined by Bergenius *et al.* (2005) where it was suggested that solar radiation, rainfall and along-shore wind during the early larval stage can have significant effects on larval growth, PLD and, in turn, competency to settle.

### 3.1.1.3 Selection of habitat for settlement

Distribution of species among habitats on the same reefs depends upon suitability in terms of competition, predator distribution/abundance and habitat selection. Habitat characteristics (structure, location, size, shape) have been identified as important factors determining the fate of juveniles after settlement (Ohman *et al.* 1998; Holbrook *et al.* 2002; Lecchini *et al.* 2007).

The ability of larvae to detect coral reefs and potential habitats in the vast ocean is an interesting question. Previously it was believed that larvae settled wherever the currents took them (Wright *et al.* 2005), but this has been proven incorrect (Tolimieri *et al.* 2000). Firstly, pre-settling larvae are now known to have swimming capabilities stronger than the currents (Armsworth 2000; Fisher and Bellwood 2003). Secondly, studies have shown that the ability of pre-settling larvae to locate reefs and habitats within reefs may be due to their sensory abilities (Lecchini *et al.* 2005; Wright *et al.* 2005). In a study by
Wright et al. (2005), it was proven that *P. nagasakiensis* were capable of detecting auditory and olfactory cues that might indicate the proximity of reefs. The night noise of snapping shrimps and urchins (biological origin) are probable indicators of the presence of a reef (Tolimieri et al. 2000), and olfactory cues are thought to be important in the location of suitable habitat at larger distance (Lecchini et al. 2005).

After the process of finding a reef in the vast ocean, the task of identifying suitable habitats within the reef matrix follows. As mentioned earlier, habitat microstructure (size, shape) are important factors for the suitability of a particular habitat. The interaction between other individuals on the reef may also have implications on selection of habitat (Ohman et al. 1998). Past studies have also demonstrated that larval fish have some behavioral flexibility, such as a tendency to slow their growth and sustain active swimming periods until suitable settlement habitat is located (Victor 1986; Frederick 1997).

Damselfish *Chromis viridis* has demonstrated the ability to use visual, olfactory and auditory cues to detect and settle on suitable habitats, preferably on habitats containing con-specifics (Lecchini et al. 2005) and the same has also been noticed for *Pomacentrus moluccensis* (Ohman et al. 1998). The presence of conspecifics could indicate favorable settling conditions in terms of habitat quality and food availability (Ohman et al. 1998; Lecchini et al. 2007), however it could also have negative effects due to intra-specific competition for resources (Jones 1987; Webster and Hixon 2000). Ohman et al. (1998) demonstrated that pre-settling larvae have precise choices for habitats which are different between species. Spatial and temporal settlement and distribution of coral reef fish may be influenced by a combination of factors ranging from settlement environment (Wilson and Meekan 2001; Bergenius et al. 2005) and habitat availability to habitat suitability (Sale et al. 2005; Feary et al. 2007; Lecchini et al. 2007), presence and interaction between conspecifics and heterospecifics (Jones 1987; Ohman et al. 1998; Lecchini et al. 2005: 2007) and predators (Hoey and McCormick 2004; Almany and Webster 2006). Recruitment of coral reef fish varies amongst years and locations (Sale et al. 2005) depending on the above factors.
3.1.2 Survival and growth after settlement

Locating reefs and settling on suitable habitats is one part, but surviving and growing after the reef colonization process is a different aspect altogether. The settlement patterns of coral reef fish after the planktonic stage is critical for the survival and growth of these fish (Leis et al. 2002). Recent studies show that post-settlement survival and adult distribution patterns are strongly influenced by habitat selection. The difference in survival amongst different habitats within reef systems may be due to the variability in food abundance and predators, and/or location of settlement (Andrews and Anderson 2004) and presence of conspecifics and heterospecifics (summarized in Frederick 1997) collectively. Consequently, habitat and the surrounding environment can be one of the most important factors that contribute to the survival and growth of juvenile coral reef fishes.

3.1.2.1 Post-settlement mortality

Mortality is known to be very high (99%) during larval stages and the early settlement period (Hair et al. 2004; Hoey and McCromick 2004; Lecchini et al. 2007). According to Planes and Lecaillon (2001) between 30-70% of newly settled juveniles are lost within seven days after settlement, (also see Lecchini et al. 2006) however Holbrook and Schmitt (2003) suggest that such losses characteristically decline with the size and age of colonies.

Considerable research has been done on larval, pre-settling and post settlement mortality of coral reef fish and most researchers consider predation as the main cause (Hixon and Carr 1997; Steele 1999; Webster 2002; Almany and Webster 2006). Numerous predatory species, both benthic (snappers and groupers) and pelagic (trevally), inhabit the reefs. Numerous hypotheses have been tested to better understand the process of mortality, and the effects of mortality on growth and distribution of juveniles. It has been proposed that mortality is size selective (Vigliola and Meekan 2002; Hawn et al. 2005), a “growth – mortality hypothesis”. Other related factors have also been included in survival studies, such as density dependent/independent processes (Hawn et al. 2005; Lecchini et al. 2006) and fish behavior in predator avoidance and habitat selection (Steele
1999; Lecchini et al. 2007). Experiments conducted by Lecchini et al. (2007) into the effects of shelter type (shape and structure) on survival demonstrated that the physical structure of a particular habitat is very important in predator avoidance. At the same time, fish with better body condition (good feeding) have an advantage for survival after settlement (Booth and Hixon 1999). Thus survival of fishes after settlement may be influenced collectively by the larval growth history, fish behavior, the habitat selected and its location.

3.1.2.2 Post-settlement growth
Survival after settlement is thought to be determined partially by the growth of these fishes. Fish growth may vary between individuals, species and locations (food availability). Fast growing fish within the same cohort have been known to have survival advantages over other slower growing individuals (Booth and Hixon 1999; Wilson and Meekan 2002). This difference in growth has been attributed to firstly intrinsic factors (McCromick 1999; Wilson and Meekan 2002; McCromick and Hoey 2004) and settlement choice (McCromick and Hoey 2004; Lecchini et al. 2007).

For example, the size of larvae at hatching is not uniform, demonstrating genetic variation within cohorts (McCromick 1999). Several studies have demonstrated this variability in size at hatching and in the larval growth history of recruits (Searcy and Sponaugle 2000; Searcy and Sponaugle 2001; Raventos and Macpherson 2005) by using otoliths. However it has also been suggested that this variability in growth is less in juveniles (Searcy and Sponaugle 2000). As mentioned before, slower growing and smaller fish are subjected to higher rates of mortality than fast growing and bigger fish (Booth and Hixson 1999; Hoey and Mccromick 2004), however it has also been noticed that surviving slow growing fish have an ability to compensate with faster growth after settlement (Booth and Hixson 1999; Gagliano and McCromick 2007) when more favorable conditions are encountered. Few studies have been done on compensatory growth of damselfish; nonetheless some studies have demonstrated this fact by using otoliths. In a recent study, Gagliano and McCromick (2007) investigated the link between growth history and survival of damselfish *Pomacentrus amboinensis* by tagging
fish before settlement and recapturing after 30 days. Results showed that fish with larger size at settlement better survived the first month of benthic lifestyle. But when these results were compared to the growth of conspecifics collected at the same time and fed in experiments (producing fast and slow growing fish), the wild survivors showed rapid acceleration in growth following a period of slow growth just after settlement. This compensation in growth may be a result of fishes being able to settle properly in their new habitats and then find food.

The post-settlement survival and growth of damselfish are affected by a series of factors, as discussed above. Both survival and growth of these fishes seem to be related to early life history events. The study of early larval history traits and growth patterns, before and after settlement, has been made possible by the development of otolith analysis techniques over the past few decades.

3.1.3 Otolithometry

3.1.3.1 What are otoliths?
Otoliths, or fish ear bones, are small calcified structures found in the inner ear region of fish. These calcified structures assist in hearing and orientation (Begg et al. 2005; Popper et al. 2005).

The inner ear of a fish is a complicated paired structure consisting of canals, sacs and ducts filled with a viscous fluid known as endolymph. Teleosts have three semi-circular canals, which open into a series of interconnecting chambers or otic sacs containing a sensory tissue, the macula. The three otic sacs, saccus, utriculus and lagena, contain the otoliths, sagitta, lapillus, and asteriscus, respectively (Fig. 11). Each type of otolith has a different size and shape (Panfili et al. 2002). The movement of otoliths relative to the movement of fish or sound waves leads to the detection of mechanical signals, which assists fish in hearing and orientation. (Popper et al. 2005)
3.1.3.2 Use of otoliths in ecology

The study of otoliths and the scope of information that can be extracted from them has increased over the past decade (Begg et al. 2005). Interestingly, the otolith itself provides a record of fish age, growth rate, development and environmental history (Fey et al. 2005; Popper et al. 2005), which can be used in understanding fish systematics and evolution (Popper et al. 2005). Because of its chemical properties, such as being acellular and metabolically inert, the otoliths have a tendency to retain the elements or compounds accreted on their growing surface (Ghosh et al. 2007). Otoliths are formed at hatching and grow daily as calcium ions from the endolymph precipitate and form a new crystal layer around the core (Warner et al. 2005). These layers are known as microstructures, increments or growth rings.
3.1.3.3 Determining fish age and growth rate using otolith microstructures

According to Campana (2005) the study of microstructures has become the most applied approach in determining fish larval history stages, age and growth. Aging fish by counting the number of microstructures has been done for quite some time. Furthermore it is also possible to back-calculate the growth rate of some species of fish by sequentially measuring the width of each increment (Vigliola et al. 2000; Wilson and Meekan 2002; Campana 2005). The daily growth rings become narrower as the fish ages, making it difficult to determine the growth history of older fishes (Baumann et al. 2005).

Settlement marks or microstructures are species specific, whereby different species exhibit different types of microstructures after settlement. Wilson and McCromick (1999) studied microstructures of 44 tropical species and showed that there are three groups of settlement marks. Thirty-nine species out of the 44 had a rapid decrease in microstructure width over settlement (type 1), while another four species had wider microstructure width over settlement than pre settlement width (type 2), while one species showed gradual decrease in microstructure width over settlement (type 3).

While aging a fish is a fairly simple procedure of counting the number of microstructures from hatching until capture, extracting other information like growth rate requires further analysis of the microstructures. Some researchers in the past have debated the accuracy of growth trajectories shown in otoliths. While a coupled increase in somatic growth and otolith growth has been demonstrated in most species, this may not be the case in all species (Thorrold and Milicich 1990), thus indicating that back calculation for growth rate can only be done for species that exhibit strong correlation between somatic and otolith growth (Vigliola et al. 2000). But Vigilola et al. (2000) argue that the few studies that have demonstrated the lack of proportionality between otolith and fish growth may be a result of problems in regression analysis and sample selection. Various models have been developed to back calculate fish growth using otolith size, however some may and some may not accommodate the flexibility of changes in proportionality between otolith
and somatic growth over time, and variable growth among individuals (Vigliola et al. 2000).

Research relating to otoliths has increased in the 21st century, with the aim of better understanding the history and chemistry of processes occurring within a fish life history. In a review by Campana (2005), it is suggested that the focus of otolith research is shifting from aging of fish to microstructures and their chemistry.

3.1.4 Objectives and implications
This study aims to determine the effects of habitat choice and environment on the growth of colonies of Chromis viridis on Suva reef. Settlement of C. viridis in sites where conspecifics are present may demonstrate high habitat quality or enhanced survival. The study aims to investigate whether this interaction is still present after successful settlement. The age structure of selected colonies will be determined using otoliths, and the age groups living in each colony will be identified. It will also assist in determining any growth advantage in each colonization pattern. Growth comparison of wild fish with cultured fish will show how adaptable a species is towards a captive environment. The study will enable us to determine the age and growth of Chromis viridis at different locations of the reef, and gain insights that may help plan future restocking strategies.

3.2 Methodology
3.2.1 Study site
Damselﬁsh (Chromis viridis) were collected from the Suva Barrier Reef. This reef borders the lagoon of Laucala Bay which is situated in the southeastern part of Viti Levu and is bounded by the Suva Peninsula and the Rewa delta. (See appendix 1).

3.2.2 Field work
3.2.2.1 Capture of juveniles
Recently settled C. viridis juveniles were captured from three different locations, each different in terms of distance from the reef crest. The fish were collected from the Suva Barrier reef using clove oil, which acts as an anaesthetic. Clove oil was diluted with
freshwater and applied to the colony with syringes. To make the clove oil more effective, the coral in which the target fish colony sought refuge was covered with a sheet of transparent plastic to minimize the flow of currents prior to the injection of clove oil (Fig. 12b). The anaesthetic started affecting fish 1-2 minutes after injection. Fish were collected by slowly removing the plastic bag and picking up the immobilized fish by hand or with small scoop nets. All fish collected were placed in pre-labeled bottles and later sorted and preserved in 95% alcohol in the laboratory.

**Figure 12:** (a) *Chromis viridis* colony on coral head just before capture, (b) coral head covered with plastic sheets prior to clove oil injection.

### 3.2.2.2 Description of habitat and surrounding environment

Supporting data such as state of tide, time of collection, water depth, current speed and direction, GPS coordinates, coral species, and size and type of substrate was noted and recorded. Then the surrounding environment was described by surveying an area of 5m from each colony for presence of conspecifics, heterospecifics, predators and habitat composition.

### 3.2.3 Laboratory work- otolith analysis

#### 3.2.3.1 Sub sampling of colonies per size and per location

All collected fish were measured for standard length (±0.01mm) and sub sampled into groups of 1mm, which was later sub sampled according to the percentage of individuals required per size class over total number of fish and per location. Representative sub samples of available fish were used for analysis (Fig. 13). Each sub sample was coded, with each code having all necessary information on the colony and/or individual.
3.2.3.2 Extraction of otoliths

Otoliths of each coded fish were extracted using a binocular microscope and dissecting apparatus. Dissection was done from the frontal head section, by removing the upper part of the cranium, just above the eye. Once the upper cranium was removed, the exposed brain was also removed to have a view of the cavity containing the inner ear. Otoliths were teased out of the semi circular canals using forceps and placed in a clean petri dish for cleaning. Otoliths were cleaned of excess tissue, and glued to pre-labeled clean
microscope slides. Both sagittae and lapilli (Fig. 14) were extracted from each fish. However some otoliths were lost during transfer.

Figure 14: Example of the three pairs of otoliths found in most teleosts. L – lapillus, S- sagitta and A – asteriscus (Source: Panfili et al. 2002).

Figure 15: (a) Internal and external face of a typical sagitta illustrating the component parts, (b) the three planes of orientation of a typical sagitta (Source: Panfili et al. 2002).
3.2.3.3 Polishing of otoliths

Before polishing of extracted otoliths, a minor experiment was done to determine the best orientation of polishing. Horizontal and transverse positions were tried for both lapilli and sagittae (see Fig. 15 for illustration), and it was determined that the microstructures were clearer in lapilli when polished in the transversal plane (see illustration of transversal plane in Fig. 15b).

Lapillus of each fish was transferred with glue to a clean slide and positioned on the edge, with one side overlapping (Fig. 16a). Once set hard, the otolith was polished using lapping/polishing paper (Fig. 16b). The polished otolith was then positioned to be polished from the opposite side (Fig. 16c) to obtain a thin layer with visible core (center) and microstructures (Fig. 16d).

Figure 16: a, b, and c shows the different positions of otolith placements while polishing and (d) otolith after the process of polishing under X60 magnification.
3.2.3.4 Interpreting microstructures (age and growth)
A program called ImageJ, which was developed at the US National Institute of Health (NIH) and available at [http://rsb.info.nih.gov/ij](http://rsb.info.nih.gov/ij) was used to measure the width of each microstructure (Fig. 16d) to the core (Fig. 16d) along the longest axis of each otolith. Microstructures were counted by clicking the computer mouse on each microstructure starting from core (Fig. 17a). Microstructure width and counts were automatically saved in the software. The age of fish was determined by the number of microstructures present, however for further data analysis, SYSTAT statistical software was used, whereby all raw data was sorted by location, colonies and individual fish.

3.2.3.5 Relationship between otolith size and fish size
The size of otolith at age was back calculated into the form of fish size at age. The model used for back calculation was adopted from Vigliola et al. (2000). Since the fish size at age were to be determined by otolith size at age, it is essential to determine the relationship between otolith size and fish size. An isometric test was done to determine if there was a linear relationship between otolith size and fish size, while the allometric test was done to determine if there was a logarithmic relationship.

3.2.3.5.1 Isometric test
Firstly the isometric formula was used to test if there was a linear relationship (direct proportionality) between otolith size at capture (Rc) and fish size at capture (Lc):
\[ L_{cap} = L_{op} - b R_{op} + b R_{cap} \]
Where \( L_{op} \) and \( R_{op} \) are biological intercepts, fish size and otolith size at hatching respectively and \( L_{cap} \) and \( R_{cap} \) are fish size and otolith size at capture. \( L_{op} \) is 3mm and mean \( R_{op} \) was calculated to be 15.038mm.

3.2.3.5.2 Allometric Test
In order to test if there was negative or positive allometry between fish size and otolith size, a test was done using the formula:
\[ L_{cap} = L_{op} - b R_{op}^{c} + b R_{cap}^{c} \], where \( b \) and \( c \) is the slope and probability coefficient respectively.
3.2.3.6 Calculating post-larval duration (PLD)

Pelagic Larval Duration is known to vary amongst individuals within the same cohort (Bergenius et al. 2002; Searcy and Sponaugle 2000). PLD of C. viridis is assumed to be between the range of 18 -29 days after hatching (Lies and Carson-Ewart 2000), however there may be variations on temporal and spatial scale. PLD of samples from the Suva reef were calculated by plotting a graph of relative growth of otolith increment width against age. The time of settlement is marked by an abrupt decrease in the relative growth of increment, followed by a decrease in incremental width (Fig. 17b).

![Figure 17: (a) microstructures in otolith marked with dots while counting, and (b) Graph of relative growth between microstructures vs microstructure number, giving PLD.](image)

3.3 Results

3.3.1 General description of surrounding habitat

Habitat and environment at positions far from the reef crest (F), generally consisted of patchily distributed large (1-1.5m) and medium (0.5-1m) encrusting corals boulders, with isolated branching corals in between. This area generally has less fish diversity and mostly higher ratio of sand to rubble. Heterospecifics seen within 5 m radius of the colonies consist of genera Pomacentrus, Dassylus, Stegastes and Crypitera. Larger C. viridis (25-50 mm) were seen, but in small numbers (10-20 individuals). Few predatory fishes of families Lutjanidae (75-150 mm) and Pinguipedidae (sand perch) were seen.

The middle position (M) generally had a combination of branching and small encrusting corals (Fig. 18). Most corals were not in good condition, either damaged or bleached. The branching corals were either seen as massive beds of approximately 2-3m in length.
and width or smaller colonies isolated in between. Heterospecifics seen were similar to that of position (F) but in lesser numbers. Conspecifics were also less in number, only a few (10s) large 25-50 mm size seen. Predatory fishes seen were snapper (Lutjanidae) and sand perch (Pinguipedidae).

![Figure 18: Picture of surroundings of a branching coral harboring C. viridis at position M.](image)

The position nearest to the crest (N) consisted of massive beds of branching corals (95%) with a few patchily distributed and isolated branching coral colonies. Fish diversity is higher at this site. Several cohorts of *C. viridis* were present with mostly large (2-3 inches) and medium size (25 mm). Heterospecifics were the same as in other positions but in higher numbers and larger size. Predators were difficult to spot due to the complex coral structures; however a few large goatfish (Mullidae) and grouper (Serranidae) of the size 150-175 mm were seen.

![Figure 19: Picture of the general surrounding of position N.](image)
3.3.2 Size distribution

All analyzed samples consisted of fish of different standard lengths ranging from 9.0 to 20.9 mm. Sample B002 had the smallest and the largest fish, while the other two samples which also had high number of fish, B008 (25 fish) and B013 (16 fish) exhibited a narrower size range (9.1 – 16.9mm). Samples with less fish number (7 – 12 individuals) had a size range of (9.0 – 19.6mm), while samples which had fish number less than 5 also had a similar size range of 9.5 to 20.0mm. No apparent trend can be seen in size distribution within samples and in relation to total number per sample (Fig. 20)

Size distributions of fish did not differ between positions F, M and N, however two cohorts were visible for all three positions, the first consisting of individuals [9 – 15mm] and the second [15-21mm] (Fig. 21).
Figure 20: Size distribution of *C. viridis* in the 15 analyzed samples.
3.3.3 Age distribution

Fish age at capture ranged from 25 to 113 days. Each sample consisted of fish of different ages. Most fishes were between 31 and 46 days. Samples with less fish (B001, B005 and B018) exhibited the same number of ages as the total number of fish analyzed (see Fig. 22). No noticeable difference in age structure could be detected either between

Figure 21: Size distribution of *C. viridis* when grouped by positions. With F = far from reef, M = middle and N = near reef crest.
different colonies or between the three different positions from the reef (F, M, and N) (Fig. 23).

### 3.3.4 Hatch and settlement dates and PLD

Hatch dates ranged from 30\textsuperscript{th} May 2007 to 26\textsuperscript{th} August 2007 and settlement dates ranged from 28\textsuperscript{th} June 2007 to 19 Sept 2007. PLD of fishes ranged from 21 days to 36 days, giving a mean PLD of 28 days. Since relevant dates and PLD were calculated using the age at capture of fishes, no difference was detected between positions or within samples.

![Graphs showing age distribution of C. viridis in the 15 analyzed samples.](image)

**Figure 22:** Age distribution of *C. viridis* in the 15 analyzed samples.
Figure 23: Age distribution of *C. viridis* when grouped by position. With F = far from reef crest, M = middle, and N = near reef crest.
3.3.5 Relationship between otolith size and fish size.

3.3.5.1 Isometric test

Isometric test results showed unequal distribution of points above and below the best fit line (Fig. 24 a). This was further proven by unequal distribution of residuals around point zero in scatter plot and histogram (Fig. 24 b, c). The lilliefors P-value was also low (0.002), suggesting that the probability of being wrong when rejecting the isometric hypothesis is low, thus allometric formula (logarithmic) was used to back calculate size at age.

3.3.5.2 Allometric test

Results of this test showed negative allometry between fish size and otolith size, with almost even distribution of points along the best fit line and residuals around zero (Fig. 24 c, d, and e). The lilliefors probability was 0.76 suggesting higher chances of being wrong when rejecting this hypothesis (negative allometry).

Size of fish at age was back calculated using the Modified Fry formulae (Vigliola et al. 2000):

\[
L_i = a + \exp \left( \ln (L_{op} - a) + (\ln (L_{cap} - a) - \ln (L_{op} - a)) \frac{(\ln (R_i) - \ln (R_{op}))}{(\ln (R_{cap}) - \ln (R_{op}))} \right)
\]

Where \( a = \frac{(a_1 + a_2)}{2} \),

\( a_1 = L_{op} - b_1 R_{op}^{c_1} \) determined from \( L_{cap} = L_{op} - b_1 R_{op}^{c_1} + b_1 R_{cap}^{c_1} \)

\( a_2 = L_{op} - b_2 R_{op}^{c_2} \) determined from \( R_{cap} = ((L_{cap} - L_{op} + b_2 R_{op}^{c_2}) b_2^{-1}) \)
Figure 24: a, b and c: graphs representing residual points along the zero line for isometric test and d, e, f: graphs for allometric tests.
3.3.6 Growth rates of wild *C. viridis*

Back-calculated size at age was not significantly different in fish from different samples. Data from individual samples were pooled per position and compared within the three positions, but no statistically significant difference was detected (ANOVA test, p-value = 0.251). See appendix 3 for ANOVA output and appendix 4 for analysis output using General Linear Model (GLM) with repeated measurements. However, when size at age was calculated for particular time/stage (i.e. 10, 15, 25 days after hatching, at settlement and 10 - 70 days after settlement), a significant difference in size was detected 25 days after hatching and at settlement (Appendix 5). Figure 25 displays box plots of each stage for the three positions, using the mean back-calculated size-at-age.

At 25 days after hatching back calculation size differed between positions M and N (ANOVA test, p-value = 0.049), individuals at position M had a back-calculated mean size of 8.4 mm, while the mean size was only 8.05 mm at position N.

Size at settlement differed significantly between positions (ANOVA test, p-value = 0.005), individuals at position F settled at 8.6 mm while those at M settled at 9.2 mm.
Figure 25: Size-at age by position from hatching (H) to settlement (S) and after settlement (S+xd).
Where H + x = days after hatching and S + x = days after settlement. (* = p-value 0.049, ** =0.005)
3.3.7 Influence of habitat on growth

Descriptions of habitat and microhabitat were summarized (Table 1) and their influence on growth on *C. viridis* was investigated.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Water depth (m)</th>
<th>Micro-habitat species</th>
<th>Micro-habitat sq. area (m^2)</th>
<th>Substrate %sand : rubble</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B001</td>
<td>1.2</td>
<td><em>Acropora pulchra</em></td>
<td>0.093</td>
</tr>
<tr>
<td>2</td>
<td>B002</td>
<td>0.6</td>
<td><em>Acropora samoensis</em></td>
<td>0.090</td>
</tr>
<tr>
<td>3</td>
<td>B005</td>
<td>1.2</td>
<td><em>Acropora humilis</em></td>
<td>0.024</td>
</tr>
<tr>
<td>4</td>
<td>B006</td>
<td>1.1</td>
<td><em>Acropora elseyi</em></td>
<td>1.000</td>
</tr>
<tr>
<td>5</td>
<td>B007</td>
<td>1.0</td>
<td><em>Pocillopora dermiconus</em></td>
<td>0.200</td>
</tr>
<tr>
<td>6</td>
<td>B008</td>
<td>1.0</td>
<td><em>Acropora humilis</em></td>
<td>0.075</td>
</tr>
<tr>
<td>7</td>
<td>B010</td>
<td>1.3</td>
<td><em>Acropora humilis</em></td>
<td>0.176</td>
</tr>
<tr>
<td>8</td>
<td>B011</td>
<td>1.2</td>
<td><em>Acropora nasuta</em></td>
<td>0.302</td>
</tr>
<tr>
<td>9</td>
<td>B013</td>
<td>1.2</td>
<td><em>Acropora aspera</em></td>
<td>0.135</td>
</tr>
<tr>
<td>10</td>
<td>B014</td>
<td>1.2</td>
<td><em>Acropora aspera</em></td>
<td>0.120</td>
</tr>
<tr>
<td>11</td>
<td>B015</td>
<td>1.3</td>
<td><em>Acropora aspera</em></td>
<td>0.088</td>
</tr>
<tr>
<td>12</td>
<td>B016</td>
<td>1.8</td>
<td><em>Acropora nasuta</em></td>
<td>0.239</td>
</tr>
<tr>
<td>13</td>
<td>B017</td>
<td>1.8</td>
<td><em>Acropora nasuta</em></td>
<td>0.147</td>
</tr>
<tr>
<td>14</td>
<td>B018</td>
<td>1.8</td>
<td><em>Acropora humilis</em></td>
<td>0.312</td>
</tr>
<tr>
<td>15</td>
<td>B019</td>
<td>1.5</td>
<td><em>Acropora nasuta</em></td>
<td>0.216</td>
</tr>
</tbody>
</table>

Table 1: Summary of habitat and microhabitat description for the 15 samples.

Further analysis was done to detect if there was any influence of surrounding habitat and microhabitat on fish growth. Statistical analysis was done using the General Linear Model with repeated measurements (GLM) to compare size-at age vs. percentage sand, water depth and density.

Size-at-age of fish was not influenced by the percentage of sand cover near the microhabitat (see appendix 6) but significant differences were detected for water depth and density (Appendix 7 and 8 respectively).

Fish were grouped in three categories in terms of water depth (≤1.0m, 1.1-1.2m and 1.3-1.8m). Results indicated that at settlement (S) and after settlement (S + x), size-at-age are significantly lower when water depth was equal to or less than one meter (≤1.0m), whereas size-at-age were significantly same for the other two categories (Fig. 26).
Fish were grouped in three categories of density (≤ 50 indiv.m⁻², 50-100 indiv.m⁻² and >100 indiv.m⁻²). Results showed that mean growth differs with density of fish on a particular habitat. Size-at-age of fish was significantly higher 10 days after settlement in intermediate densities (Fig. 27).
3.3.8 Growth of wild and cultured fish

Growth of wild fish was compared to growth of wild caught post-larvae reared in captivity (referred to as cultured fish). Statistical analysis detected a significant difference (ANOVA test: p-value-0.001) in back-calculated size at age between wild and cultured fish. Consequently, growth-rate was calculated for a particular period (i.e. 10, 15, 25 days after hatching, at settlement and 10 - 70 days after settlement), and significant differences were seen on two occasions. Firstly in the late larval (day 15 and 25 with p-value of 0.027 and 0.001 respectively) to early juvenile stages (at settlement and day 10 with p-value of 0.001 and 0.026 respectively) and secondly, slight difference at 60 days (ANOVA test:p-value-0.04) and 70 days (ANOVA test: p-value-0.03) after settlement (Appendix 9). Figure 28 shows the growth trajectories before and after settlement, where the time of settlement is at x value = 0.
3.4 Discussion

3.4.1 Size and age distribution

There was no detectable trend in the size and age distribution between colonies of different positions, but it is clearly evident that all samples had several sizes and ages of fish within a particular colony. This reinforces the view that *Chromis viridis* prefer to settle on or colonize habitat with conspecifics. This effect of conspecifics in habitat selection by young recruits of *C. viridis* has previously been demonstrated in experimental cages. Results of experiments conducted by Lecchini *et al.* (2007) at Moorea Island showed that, when given a choice to settle on empty coral heads or corals already colonized with larger conspecifics, 100% of younger fish settled on corals already colonized with larger individuals of the same species. However, it is unknown whether this social interaction is present during colonization or whether it occurs after post-settlement emigration.
3.4.2 Growth rate of wild fish

Back-calculated size at age of fish does not seem to differ generally between the 15 samples or between the three reef positions, however slight difference in size at age was detected between M and N at 25 days after hatching (Fig. 25). This trend of slow growth can also be seen when comparing size at settlement of the three reef positions; however a statistically significant difference was detected between F and M (Fig. 25). While there may be some differences in size of fish at certain age, the general overview indicated is that there is no difference in growth rate of fish between positions, which suggests that parameters affecting growth of juvenile fish in the wild were somewhat similar throughout the three reef positions (near the reef crest (N), middle of crest (M) and far from crest (F)).

Larval growth history (Vigliola and Meekan 2002, Raventos and Macpherson 2005) and settlement environment (Lecchini et al. 2006, 2007) are known to collectively influence growth and survival of coral reef fish. Influential factors such as size at hatching, larval quality and planktonic duration, along with environmental conditions, induce variability in larval growth within the same cohorts of fish. In the study done by Vigliola and Meekan (2002), it was suggested that having larger size at hatching was an advantage which propagated throughout larval growth and also juvenile growth of damselfish Neopomacentrus filamentosus. Subsequently back calculated size at age showed that individuals which still survived after 1-2 months of settlement were fishes with larger size at hatching. Similar effects were also noticed in a study comparing pelagic larval growth and size at hatching of recently settled and two month old settlers of two temperate labrid fish (Raventos and Macpherson 2005). This may explain the similarities in growth of surviving C. viridis in this study. No difference was detected in size at hatching of fishes in the three reef positions F, M and N (3.003 ±0.037, 2.998 ±0.040 and 3.008 ±0.042 respectively) although hatching dates varied for cohorts. Fish that were collected and analyzed for this study were groups of successfully settled juveniles, which exhibited a close range of growth rate that probably helped them survive the period of size selective mortality, whereas the slower growing fish may have been selectively predated on.
Processes affecting growth and survival differ in a benthic lifestyle (after settlement). Pre-settling fish are exposed to different species of predators (Almany and Webster 2006; Hixson and Carr 1997) and prey, different types of habitat, different ages of con-specific and heterospecifics (Ohman et al. 1998; Lecchini et al. 2005). The ability of a juvenile to survive and grow better in the selected habitat is based on foraging skills and predator avoidance. Results of this study show that the growth trajectories (after settlement) of fish in the three reef positions were similar, indicating that any growth-limiting effects of food availability and sheltering space at the three sites were also similar. All 15 samples had different age groups of fish sharing the same habitat. Presence of adult con specifics at a potential settlement site may indicate to colonizers higher habitat quality and/or food availability at that site, which in turn may enhance their growth and survival (Ohman et al. 1998).

### 3.4.3 Influence of habitat on growth of wild fish

Abundance and distribution of recently settled reef fish is suggested to be habitat-dependent (Andrews and Anderson 2004). Furthermore specific habitat characteristics such as coral cover, presence and density of macroalgae or rock, and substratum have a strong relationship with coral reef fish recruitment and juvenile abundance (Andrews and Anderson, 2004) and may also have effects on juvenile growth.

Slight differences in size at age of *C. viridis* were detected with differences in water depth and density of fish. This difference may be due to increased food availability (zooplankton) due to increased water depth, and increased competition for resources such as food due to higher density. Fish with intermediate density (50-100 indiv.m\(^{-2}\)) had larger size at age compared to other densities (Fig. 27). While high densities of fish in a particular habitat indicates better conditions, too many fish in one particular microhabitat may also have unfavorable effects, such as increased competition for available food, and space in microhabitat, resulting in slower growth. This may explain the slightly smaller size at age of fish at high density (>100 indiv.m\(^{-2}\)). But it must be noted that the sample number of fish for each category of water depth and density were not similar, which may
have induced error and bias in results, thus more work should be done to verify the
effects of habitat on growth of *C. viridis*.

### 3.4.4 Growth comparison of wild and cultured fish

When comparing growth of wild and cultured fish, difference in size at age were detected
during late larval stages and the early juvenile stages. The size at age of cultured fish was
smaller just before settlement and this propagated until 10 days after settlement. However
the size at age increased and was similar to that of wild fish from 20 days after settlement
and onwards (Fig. 28).

The smaller size at age just before and after settlement may be due to larvae being caught
just before settlement by the light traps and being transferred to a totally different
environment (aquaria), where they were forced to switch from natural food to artificial
feeds. The increase in size after 20 days reflects the successful adoption by *C. viridis*
juveniles of artificial feed and a captive environment. The slightly larger size at age of
cultured fish after this point can be explained by the quality of the artificial feeds used,
and the predator free environment. Fish in the wild have to hunt for food by swimming
long distances and at the same time must avoid being eaten by predators. This is not the
case for cultured fish, which were not obliged to swim any long distance for food or to
avoid predators, allowing them to conserve energy and utilize this for growth. *(But see
recommendations)*

Having slow initial growth followed by a period of rapid growth is termed “growth
compensation” (McCromick 1998; Gagliano and McCromick 2007). This term may be
used to describe the growth trajectory of cultured fish in this study. *C. viridis* in captivity
had slow initial growth, but once favorable conditions were obtained, they had faster
growth that allowed them to catch up.

Evidence of growth compensation in wild fish also has been demonstrated by Gagliano
and McCromick (2007). Comparisons made between fast and slow growing captive
*Pomacentrus amboinensis* to that of wild stock revealed that wild fish had slow growth
during the first few days on the reef, but they were able to attain faster growth thereafter. Results of this current study shows that *C. viridis* also has the ability to compensate growth and even to reach a larger size at age than wild survivors, once adapted to their captive environment (Fig. 28). This feature may be an advantage when considering releasing of captive reared fish. The larger size at age of fish reared in captivity may assist in ensuring decreased effects of size-selective mortality after release.

In summary, *C. viridis* prefer to settle on live branching corals that already have conspecifics, resulting in colonies (samples) consisting of several sizes and ages of fish. Growth of fish in the wild was not influenced by the distance of colonies (samples) from the reef crest on the Suva Reef Flat; however this may change with locations and demography of reefs. Selected microhabitat and surrounding environment may be influential in the growth of *C. viridis*, but further research is needed to verify this. *C. viridis* fish which were captured by light traps and reared in captivity showed similar but slightly better growth than their wild counterparts, indicating positive implications for restocking of using captured and cultured fish.
Chapter 4

General Summary and Conclusion

4.1 Review of objectives

The major findings for each of the research objectives in this study are:

i) Investigate the effects of three different diets on growth and post-release survival of a coral reef fish (*C. viridis*).

*C. viridis* demonstrated good acclimatization capabilities and had reasonable survival and normal growth compared to wild conspecifics. There was no difference between the diets tested on growth and post release survival. Most importantly, this study demonstrated that local feeds can be used to grow these fishes, resulting in lower feed costs compared with imported feeds. The use of local feeds (tilapia feed, and egg custard) has been proven to contribute towards normal growth.

ii) Investigate the effects of size-at-release on post-release survival and microhabitat choice.

The results indicate that live branching corals are preferred over artificial or dead corals, as potential microhabitat. Survival rate after release ranged from 50% - 100%, with no apparent effects of feed type and culture duration (size-at-release) on post-release survival. However fish cultured for 90 days were more structured in terms of growth in captivity, post-release survival, microhabitat choice, and movement around the enclosures, which may be important in predator avoidance. Further
research is needed to verify this however, and other (particularly smaller) release sizes should also be tested.

iii) Compare growth of cultured fish with that of fish in the wild.

There were only slight differences detected between the growth trajectories of wild and cultured fish. Back-calculated size at age of cultured fish was significantly lower during late larval and early juvenile stages, and the same cultured fishes had a significantly larger size at age 60 days after settlement. Generally the growth of cultured fish was similar to that of wild fish with a few exceptions at certain stages.

iv) Determine the colonization pattern, growth and age of fish within and between different schools (colonies) of fish in the wild.

Wild *C. viridis* were associated with live branching corals of several species. Otolith analysis showed that they mostly form colonies with variable sizes and ages of fish, indicating settlement preference with conspecifics. Growth of the species was similar in all reef positions tested (far from reef crest (F), middle (M), and near reef crest (N)). Growth was also similar to that of the cultured fish reared in the first part of the study. No apparent effects of habitat on growth were detected; however, there was a slight size-at-age difference with water depth and density of fish per microhabitat. These factors (water depth and density) should be further investigated, as the sample size of fish used in these comparisons were not constant in all categories.
4.2 Constraints and limitations of this study

There were three major constraints that were encountered during the study, which also caused delays in the timetable for completion of this research.

Firstly, there was insufficient number of fish of any one species available from the light traps at any one time. Only one species (C. viridis) was caught in sufficient numbers to enable this study to be carried out and, even then, more highly replicated trials could not done due to limited fish numbers. Very few of the total days fished by the C.A.R.E system yielded numbers and species of fish sufficient for replicated experiments.

Secondly, the building of the enclosures took much more time than expected. Delays were mainly caused by late arrival of mesh material from Australia and bad weather conditions during construction. Enclosures were severely damaged on several occasions after storms, which resulted in more time being lost making repairs.

Thirdly, breakdown of outboard engines several times during the building of enclosures and while monitoring also caused unexpected delays. The C.A.R.E is operated in open sea, albeit not far from the reef crest. The logistics and equipment requirements (boat, engine, etc.) for operating at such sites in safety must not be underestimated.

Other minor problems of reliability of labour for field assistance, tide, and laboratory water quality during culture were also encountered. Rough weather after releasing caused damages to cages and on some occasions the weights that were used to seal the bottom were displaced.

4.3 Discussion and conclusion

The overall aim of this study was to find out whether PCC techniques can be applied to reef fish restocking in Fiji, and whether artificial feeds (local and imported) can be used to grow them at growth rates similar to those of wild fish. Furthermore, other influential factors that were believed to affect restocking success, such as habitat, site of release,
size-at-release and fish ecology, were also investigated to obtain the best releasing strategy.

Overall results of the study indicate that using PCC for restocking reefs (esp. small scale) in Fiji may indeed be possible. According to Durville et al. (2003), the best evidence that a species has adapted to captive environment is its ability to feed properly, grow normally and survive in artificial conditions. In the current study, pre-settling fish were able to adapt to the captive environment and to grow normally on artificial feed. Local feeds that were used in this study promoted growth similar to that of imported formulated feed; yet the cost of local tilapia feed was 25 times less than the imported one. This result is very important, because it will allow local communities to obtain low cost feeds locally and grow coral reef fish without compromising their quality for restocking.

Hatchery practices that were identified as important during fish rearing were; good quality water exchange, feeding 3 times daily, daily siphoning of aquaria, and general weekly cleaning. These practices were noticed to reduce the risk of disease outbreaks. Even though this sounds intensive, it can be done with minimum labor. In case land-based culture facilities are unavailable, other research has shown that post-larval coral reef fish can also be reared in floating cages in shallow, near shore sheltered waters (Hair et al 2004) and communities can easily feed and take care of fish, without worrying about water exchange, water quality and/or provision of aeration.

The growth and survival of fish in this study were not influenced by the type of feed or the duration of culture. Neither was size-at-release notably important for this species; however this result should be treated cautiously as it will need further testing over a wider range of release sizes. Previous studies on other species have proven that the best size-at-release needs to be established because at some sizes fish may have behavioral deficits (e.g. Masuda and Tsukamoto 1997; Yamashita and Yamada 1999; Svasand 2004). The *C. viridis* which were released 90 days after culture were more structured in terms of microhabitat choice and survival. Fish were noticed to be closely associated with the microhabitat, while fish in other groups (60 and 158 days) were observed to be actively moving around the enclosures. Thus fish cultured for 90 days may have better
chances of surviving after release due to their more cautious behavior, consistency in size, and survival trends.

Releasing practices such as steady transportation, acclimatization before release and releasing in enclosures are known to reduce stress-induced behavioral deficits and thereby help to further increase post-release survival. Releasing in enclosures has proven to be very helpful for monitoring purposes, minimizing predatory effects and giving the fish a chance to acclimatize to the wild environment. Ecological knowledge about the species to be released is very important to understand the best settlement strategy, habitat and microhabitat choice. This releasing experiment showed that *C. viridis* preferred to settle on live branching corals, and studies of wild fish had further revealed that the presence of conspecifics is also important for settlement and/or microhabitat choice. Thus it is important to provide proper habitat before release, if habitat is not available. The presence of conspecifics may also indicate favorable conditions for survival and growth. There was no difference in growth at different position on the Suva reef indicating suitable habitat and food availability throughout. However influential factors may be different in other places or for other species.

*Chromis viridis* can be successfully and economically cultured in captivity, using locally available feed. However site of release, microhabitat type and habitat are important to the success of their release.

From the results of the current study and after future research on other species, the methodology of rearing and releasing post-larval reef fish can be refined or modified to suit local communities. The PCC technique can be used to restock severely depleted reefs, or enhance fish populations in tourist snorkeling areas. The PCC technique can also be used to catch and grow out fish for the aquarium trade, while food fish can be used for cage culture aimed for live reef food fish trade or simply to supply local markets.
4.4 Recommendation for further research

Further research on this topic needs to be carried out, especially on the releasing aspect. While results were promising in using post-larval reef fish for restocking, it must be realized that these releases were done in enclosures in the wild, and supplementary field research should be done to investigate whether these fish further survive and become part of the breeding population.

Focus should also be shifted onto food fish species and the effects of local feeds on their growth. The potential for such fishes to be cultured to adult size in captivity (sea cages) should be investigated. This would be welcomed by communities and governments as a form of aquaculture which will assist in reducing pressure on natural stocks and at the same time be a form of community development project.

Culture of post-larvae for restocking after capture using light traps or crest nets is workable, subject to the underlying assumption being met that suitable species of fish are catchable in cohorts of sufficient numbers for mass culture. Further research on this aspect of PCC needs to be carried out in Fiji. It was not a focus of this study to investigate in detail the abundance, seasonality or lunar cycling of post-larval caught using C.A.R.E light traps in Fiji. However that the occasions when species interesting for re-stocking could be caught, in numbers sufficient even for experimental-scale culture, were unpredictable and were a very small portion of the total days fished. This will be a very rewarding area for more scientific research.

More scientific research is also needed to identify the best size-at-release of this species. While the current study did not show notable difference in fish behavior at different culture durations, a slightly better performance was seen with fish cultured for 90 days. More research and statistical analysis should be done on this aspect; at the same time keeping all other variables constant (e.g. feed type).

While the system of rearing pre-settling larvae in aquaria can be adopted by commercial aquarium traders or hotels, a more cost effective rearing method should be developed for
community level. Rearing of ornamental fishes in cages near village sites, later to be sold to aquarium traders, should be researched.

In order for this technique to work commercially, whatever the purpose, more research should be done on ways to increase fish numbers caught in traps (either using light traps or crest nets), at the same time making sure that the natural replenishment rate is not affected.

It is also recommended that similar studies be done with other ornamental and food species to further substantiate the usage of PCC technique for restocking, aquaculture or aquarium trade.
REFERENCES


Websites

  - [www.spc.org](http://www.spc.org)
  - [www.fishbase.org](http://www.fishbase.org)
  - [www.cities.org](http://www.cities.org)
  - [www.reefbase.org](http://www.reefbase.org)
APPENDICES

Appendix 1 : Map of Suva barrier reef (site of juvenile capture for otolith analysis)

Appendix 2: ANOVA analysis of variance for culture periods (Chapter 2)

Analysis of Variance (60 days of culture period)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEED$</td>
<td>45.197</td>
<td>2</td>
<td>22.599</td>
<td>12.457</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>48.982</td>
<td>27</td>
<td>1.814</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bonferroni Adjustment.

Matrix of pairwise comparison probabilities:

<table>
<thead>
<tr>
<th>Postn</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F)1</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(M)2</td>
<td>0.003</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>(N)3</td>
<td>0.934</td>
<td>0.0001</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Appendix 3: ANOVA analysis for back-calculated size-at-age at different positions, F, M and N of wild fish.

Analysis of Variance (size-at-age vs positions)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITION$</td>
<td>45.077</td>
<td>2</td>
<td>22.538</td>
<td>1.382</td>
<td>0.251</td>
</tr>
<tr>
<td>Error</td>
<td>113074.006</td>
<td>6933</td>
<td>16.310</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Least squares means

<table>
<thead>
<tr>
<th>POSITION$</th>
<th>LS Mean</th>
<th>SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>8.289</td>
<td>0.084</td>
<td>2288</td>
</tr>
<tr>
<td>M</td>
<td>8.172</td>
<td>0.084</td>
<td>2325</td>
</tr>
<tr>
<td>N</td>
<td>8.367</td>
<td>0.084</td>
<td>2323</td>
</tr>
</tbody>
</table>
Appendix 4: GLM analysis output of size-at-age of *C. viridis* at the three positions F, M and N.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Parameter</th>
<th>Standard error</th>
<th>t</th>
<th>Signification</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>SIZE_AT_AGE00</td>
<td>Constant</td>
<td>.011</td>
<td>266.442</td>
<td>.000</td>
<td>2.994</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.016</td>
<td>.037</td>
<td>.970</td>
<td>-.031</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.016</td>
<td>-1.619</td>
<td>.113</td>
<td>-.059</td>
</tr>
<tr>
<td>SIZE_AT_AGE05</td>
<td>Constant</td>
<td>.032</td>
<td>103.807</td>
<td>.000</td>
<td>3.275</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.045</td>
<td>.802</td>
<td>.427</td>
<td>-.054</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.046</td>
<td>-1.845</td>
<td>.113</td>
<td>-.179</td>
</tr>
<tr>
<td>SIZE_AT_AGE10</td>
<td>Constant</td>
<td>.099</td>
<td>41.679</td>
<td>.000</td>
<td>3.928</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.138</td>
<td>1.371</td>
<td>.178</td>
<td>-.089</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.143</td>
<td>-.945</td>
<td>.350</td>
<td>-.422</td>
</tr>
<tr>
<td>SIZE_AT_AGE15</td>
<td>Constant</td>
<td>.159</td>
<td>33.015</td>
<td>.000</td>
<td>4.936</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.222</td>
<td>1.742</td>
<td>.089</td>
<td>-.132</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.229</td>
<td>-.690</td>
<td>.494</td>
<td>-.621</td>
</tr>
<tr>
<td>SIZE_AFT_SET00</td>
<td>Constant</td>
<td>.192</td>
<td>44.240</td>
<td>.000</td>
<td>8.123</td>
</tr>
<tr>
<td></td>
<td>[POSITION=2]</td>
<td>.268</td>
<td>-.246</td>
<td>.807</td>
<td>-.606</td>
</tr>
<tr>
<td></td>
<td>[POSITION=3]</td>
<td>.277</td>
<td>.926</td>
<td>.360</td>
<td>-.302</td>
</tr>
<tr>
<td>SIZE_AFT_SET05</td>
<td>Constant</td>
<td>.195</td>
<td>47.929</td>
<td>.000</td>
<td>8.950</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.287</td>
<td>1.559</td>
<td>.127</td>
<td>-.132</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.297</td>
<td>-.172</td>
<td>.864</td>
<td>-.649</td>
</tr>
<tr>
<td>SIZE_AFT_SET10</td>
<td>Constant</td>
<td>.214</td>
<td>47.516</td>
<td>.000</td>
<td>9.720</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.297</td>
<td>.102</td>
<td>.919</td>
<td>-.570</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.307</td>
<td>1.488</td>
<td>.144</td>
<td>-.163</td>
</tr>
<tr>
<td>SIZE_AFT_SET15</td>
<td>Constant</td>
<td>.236</td>
<td>46.088</td>
<td>.000</td>
<td>10.419</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.329</td>
<td>.365</td>
<td>.717</td>
<td>-.544</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.340</td>
<td>1.472</td>
<td>.149</td>
<td>-.186</td>
</tr>
<tr>
<td>SIZE_AFT_SET20</td>
<td>Constant</td>
<td>.268</td>
<td>43.445</td>
<td>.000</td>
<td>11.106</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.373</td>
<td>.553</td>
<td>.583</td>
<td>-.547</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.386</td>
<td>1.356</td>
<td>.182</td>
<td>-.256</td>
</tr>
<tr>
<td>SIZE_AFT_SET25</td>
<td>Constant</td>
<td>.293</td>
<td>42.287</td>
<td>.000</td>
<td>11.779</td>
</tr>
<tr>
<td></td>
<td>[POSITION=F]</td>
<td>.407</td>
<td>.674</td>
<td>.504</td>
<td>-.547</td>
</tr>
<tr>
<td></td>
<td>[POSITION=M]</td>
<td>.421</td>
<td>1.318</td>
<td>.195</td>
<td>-.295</td>
</tr>
</tbody>
</table>
Appendix 5: ANOVA analysis for back-calculated size-at-age (at stages) of wild fish by positions.

### Analysis of Variance (for 25 days after hatching)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITION$</td>
<td>4.616</td>
<td>2</td>
<td>2.308</td>
<td>3.499</td>
<td>0.033</td>
</tr>
<tr>
<td>Error</td>
<td>88.381</td>
<td>134</td>
<td>0.660</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bonferroni Adjustment.

Matrix of pairwise comparison probabilities:

<table>
<thead>
<tr>
<th>Postn</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F)</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(M)</td>
<td>0.117</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>(N)</td>
<td>1.000</td>
<td>0.049</td>
<td>1.000</td>
</tr>
</tbody>
</table>

### Analysis of Variance (at settlement)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITION$</td>
<td>8.544</td>
<td>2</td>
<td>4.272</td>
<td>5.230</td>
<td>0.006</td>
</tr>
<tr>
<td>Error</td>
<td>109.445</td>
<td>134</td>
<td>0.817</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Bonferroni Adjustment.

Matrix of pairwise comparison probabilities:

<table>
<thead>
<tr>
<th>Postn</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F)</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(M)</td>
<td>0.005</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>(N)</td>
<td>0.124</td>
<td>0.845</td>
<td>1.000</td>
</tr>
</tbody>
</table>
Appendix 6: GLM analysis output for *C. viridis* size-at-age versus percentage of sand cover. C1 = <50% and C2 > 50% sand substrate.

<table>
<thead>
<tr>
<th>Dependant Variable</th>
<th>Parameter</th>
<th>Standard Error</th>
<th>t</th>
<th>Signification</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIZE_AT_AGE00</td>
<td>Constant</td>
<td>.018</td>
<td>166.131</td>
<td>.000</td>
<td>2.989, 3.063</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE05</td>
<td>Constant</td>
<td>.020</td>
<td>-1.014</td>
<td>.316</td>
<td>-0.059, 0.020</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE10</td>
<td>Constant</td>
<td>.054</td>
<td>61.467</td>
<td>.000</td>
<td>3.235, 3.455</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE15</td>
<td>Constant</td>
<td>.058</td>
<td>-.371</td>
<td>.713</td>
<td>-.140, 0.096</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE20</td>
<td>Constant</td>
<td>.163</td>
<td>26.409</td>
<td>.000</td>
<td>3.969, 4.625</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET00</td>
<td>Constant</td>
<td>.175</td>
<td>-.953</td>
<td>.346</td>
<td>-.519, 0.186</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET05</td>
<td>Constant</td>
<td>.262</td>
<td>21.661</td>
<td>.000</td>
<td>5.138, 6.193</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET10</td>
<td>Constant</td>
<td>.311</td>
<td>31.204</td>
<td>.000</td>
<td>8.117, 9.347</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET15</td>
<td>Constant</td>
<td>.334</td>
<td>31.193</td>
<td>.000</td>
<td>9.073, 10.327</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET20</td>
<td>Constant</td>
<td>.339</td>
<td>31.446</td>
<td>.000</td>
<td>9.987, 11.355</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET25</td>
<td>Constant</td>
<td>.399</td>
<td>1.403</td>
<td>.168</td>
<td>-1.364, 0.245</td>
</tr>
<tr>
<td></td>
<td>[sand_C=1]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[sand_C=2]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 7: GLM analysis output for *C. viridis* size-at-age at water depths. Where C1 =<1.0m, C2= 1.1-1.2m and C3= 1.3-1.8m.

<table>
<thead>
<tr>
<th>Dependant Variable</th>
<th>Parameter</th>
<th>Standard Error</th>
<th>t</th>
<th>Signification</th>
<th>95% confidence interval</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE00</td>
<td>Constant</td>
<td>.014</td>
<td>208.452</td>
<td>.000</td>
<td>2.977 - 3.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.017</td>
<td>.341</td>
<td>.735</td>
<td>-.029 - .041</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.020</td>
<td>.049</td>
<td>.961</td>
<td>-.038 - .040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE05</td>
<td>Constant</td>
<td>.041</td>
<td>80.082</td>
<td>.000</td>
<td>3.223 - 3.389</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.049</td>
<td>1.051</td>
<td>.299</td>
<td>-.048 - .152</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.056</td>
<td>-.429</td>
<td>.961</td>
<td>-.038 - .040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE10</td>
<td>Constant</td>
<td>.127</td>
<td>32.687</td>
<td>.000</td>
<td>3.897 - 4.410</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.152</td>
<td>.348</td>
<td>.730</td>
<td>-.254 - .360</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.172</td>
<td>-.604</td>
<td>.961</td>
<td>-.451 - .243</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE15</td>
<td>Constant</td>
<td>.207</td>
<td>25.996</td>
<td>.000</td>
<td>4.972 - 5.809</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.248</td>
<td>.022</td>
<td>.982</td>
<td>-.496 - .507</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.281</td>
<td>-.643</td>
<td>.549</td>
<td>-.747 - .386</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AT_AGE20</td>
<td>Constant</td>
<td>.228</td>
<td>39.531</td>
<td>.000</td>
<td>8.538 - 9.457</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.273</td>
<td>-2.104</td>
<td>.041</td>
<td>-1.124 - 0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.308</td>
<td>-1.666</td>
<td>.103</td>
<td>-1.135 - 0.108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET00</td>
<td>Constant</td>
<td>.228</td>
<td>43.699</td>
<td>.000</td>
<td>9.493 - 10.412</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.273</td>
<td>-2.627</td>
<td>.012</td>
<td>-1.267 - 0.166</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.308</td>
<td>-1.673</td>
<td>.0102</td>
<td>-1.138 - 0.106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET05</td>
<td>Constant</td>
<td>.246</td>
<td>44.291</td>
<td>.000</td>
<td>10.393 - 11.385</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.294</td>
<td>-2.937</td>
<td>.005</td>
<td>-1.549 - 0.271</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.333</td>
<td>-1.608</td>
<td>.115</td>
<td>-1.207 - 0.136</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET10</td>
<td>Constant</td>
<td>.269</td>
<td>43.819</td>
<td>.000</td>
<td>11.235 - 12.320</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.322</td>
<td>-3.076</td>
<td>.004</td>
<td>-1.640 - 0.341</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.364</td>
<td>-1.814</td>
<td>.077</td>
<td>-1.395 - 0.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET15</td>
<td>Constant</td>
<td>.304</td>
<td>41.567</td>
<td>.000</td>
<td>12.033 - 13.261</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.364</td>
<td>-3.013</td>
<td>.004</td>
<td>-1.834 - 0.363</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.412</td>
<td>-1.839</td>
<td>.073</td>
<td>-1.589 - 0.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIZE_AFT_SET20</td>
<td>Constant</td>
<td>.330</td>
<td>40.789</td>
<td>.000</td>
<td>12.810 - 14.144</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=1]</td>
<td>.396</td>
<td>-3.068</td>
<td>.004</td>
<td>-2.013 - 0.415</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[Water_Depth_C=2]</td>
<td>.447</td>
<td>-1.818</td>
<td>.076</td>
<td>-1.716 - 0.089</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 8: GLM analysis output of size at age of *C. viridis* grouped in different densities. Where C1 =<50 indiv.m⁻², C2 =50-100 indiv.m⁻², C3 = >100 indiv.m⁻².

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Parameter</th>
<th>Standard error</th>
<th>T</th>
<th>Signification</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td>SIZE_AT_AGE00</td>
<td>Constant</td>
<td>.008</td>
<td>369.235</td>
<td>.000</td>
<td>2.995</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.019</td>
<td>-.002</td>
<td>.998</td>
<td>-0.038</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.019</td>
<td>-.707</td>
<td>.483</td>
<td>-0.052</td>
</tr>
<tr>
<td>SIZE_AT_AGE05</td>
<td>Constant</td>
<td>.024</td>
<td>141.285</td>
<td>.000</td>
<td>3.286</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.055</td>
<td>.523</td>
<td>.604</td>
<td>-.082</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.055</td>
<td>-1.345</td>
<td>.186</td>
<td>-.185</td>
</tr>
<tr>
<td>SIZE_AT_AGE10</td>
<td>Constant</td>
<td>.072</td>
<td>57.857</td>
<td>.000</td>
<td>4.039</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.169</td>
<td>-.231</td>
<td>.818</td>
<td>-.050</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.169</td>
<td>-1.000</td>
<td>.323</td>
<td>-.011</td>
</tr>
<tr>
<td>SIZE_AT_AGE15</td>
<td>Constant</td>
<td>.117</td>
<td>46.225</td>
<td>.000</td>
<td>5.178</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.273</td>
<td>-.752</td>
<td>.456</td>
<td>-.379</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.273</td>
<td>-.894</td>
<td>.377</td>
<td>-.795</td>
</tr>
<tr>
<td>SIZE_AT_AGE20</td>
<td>Constant</td>
<td>.147</td>
<td>45.659</td>
<td>.000</td>
<td>6.407</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.342</td>
<td>-1.095</td>
<td>.280</td>
<td>-1.065</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.342</td>
<td>-1.735</td>
<td>.466</td>
<td>-.942</td>
</tr>
<tr>
<td>SIZE_AFT_SET00</td>
<td>Constant</td>
<td>.132</td>
<td>64.083</td>
<td>.000</td>
<td>8.213</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.308</td>
<td>.228</td>
<td>.821</td>
<td>-.552</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.308</td>
<td>1.583</td>
<td>.121</td>
<td>-.134</td>
</tr>
<tr>
<td>SIZE_AFT_SET05</td>
<td>Constant</td>
<td>.133</td>
<td>70.211</td>
<td>.000</td>
<td>9.086</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.310</td>
<td>-.038</td>
<td>.970</td>
<td>-.638</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.310</td>
<td>1.983</td>
<td>.054</td>
<td>-.011</td>
</tr>
<tr>
<td>SIZE_AFT_SET10</td>
<td>Constant</td>
<td>.142</td>
<td>71.713</td>
<td>.000</td>
<td>9.892</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.331</td>
<td>-.140</td>
<td>.889</td>
<td>-.714</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.331</td>
<td>2.583</td>
<td>.013</td>
<td>.187</td>
</tr>
<tr>
<td>SIZE_AFT_SET15</td>
<td>Constant</td>
<td>.155</td>
<td>70.716</td>
<td>.000</td>
<td>10.655</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.361</td>
<td>-.395</td>
<td>.695</td>
<td>-.872</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.361</td>
<td>2.656</td>
<td>.011</td>
<td>.230</td>
</tr>
<tr>
<td>SIZE_AFT_SET20</td>
<td>Constant</td>
<td>.175</td>
<td>66.899</td>
<td>.000</td>
<td>11.384</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.409</td>
<td>-.334</td>
<td>.740</td>
<td>-.962</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.409</td>
<td>2.618</td>
<td>.012</td>
<td>.245</td>
</tr>
<tr>
<td>SIZE_AFT_SET25</td>
<td>Constant</td>
<td>.191</td>
<td>65.182</td>
<td>.000</td>
<td>12.081</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=1]</td>
<td>.446</td>
<td>-.169</td>
<td>.867</td>
<td>-.975</td>
</tr>
<tr>
<td></td>
<td>[DENS_C=2]</td>
<td>.446</td>
<td>2.655</td>
<td>.011</td>
<td>.284</td>
</tr>
</tbody>
</table>
Appendix 9: ANOVA analysis for back-calculated size-at-age of wild and cultured fish

Analysis of Variance (15 days after hatching)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_C$</td>
<td>1.536</td>
<td>1</td>
<td>1.536</td>
<td>5.004</td>
<td>0.027</td>
</tr>
<tr>
<td>Error</td>
<td>44.202</td>
<td>144</td>
<td>0.307</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Variance (for 25 days after hatching)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_C$</td>
<td>11.894</td>
<td>1</td>
<td>11.894</td>
<td>17.546</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>97.620</td>
<td>144</td>
<td>0.678</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Variance (at settlement)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_C$</td>
<td>14.723</td>
<td>1</td>
<td>14.723</td>
<td>15.868</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>133.607</td>
<td>144</td>
<td>0.928</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Variance (10 days after settlement)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_C$</td>
<td>5.299</td>
<td>1</td>
<td>5.299</td>
<td>5.147</td>
<td>0.026</td>
</tr>
<tr>
<td>Error</td>
<td>87.521</td>
<td>85</td>
<td>1.030</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Variance (60 days after settlement)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_C$</td>
<td>21.274</td>
<td>1</td>
<td>21.274</td>
<td>4.699</td>
<td>0.042</td>
</tr>
<tr>
<td>Error</td>
<td>90.555</td>
<td>20</td>
<td>4.528</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Variance (70 days after settlement)

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum-of-Squares</th>
<th>df</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>W_C$</td>
<td>20.661</td>
<td>1</td>
<td>20.661</td>
<td>5.847</td>
<td>0.032</td>
</tr>
<tr>
<td>Error</td>
<td>42.404</td>
<td>12</td>
<td>3.534</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 10: Picture of different traps designed to collect post larval reef fish.

Light trap with the arrow showing the opening from which larvae enters the trap. 
(Source: Lecaillon and Lourie 2007)

Crest net setup on the reef crest at Navutulevu Village (Fiji). 
(Source: Lecaillon and Lourie 2007)

Eco-ocean designed C.A.R.E net with light and net attached. 
(Source: Lecaillon and Lourie 2007)