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

COMPONENT 2A - PROJECT 2A1

Knowledge, management, rehabilitation and beneficial use of coral ecosystems

May 2007

INTERNSHIP REPORT

DEVELOPMENT OF MULTISPECIFIC POSTLARVAL REARING APPROACH IN AQUARIUM

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CRISP

Coral Reef InitiativeS for the Pacific
Initiatives Corail pour le Pacifique

The CRISP programme is implemented as part of the policy developped 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 eco-systems 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 comprises three major components, which are:

Component 1A: Integrated Coastal Management and watershed management
- 1A1: Marine biodiversity conservation planning
- 1A2: Marine Protected Areas
- 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 ecosytems
- 2B: Reef rehabilitation
- 2C: Development of active marine substances
- 2D: Development of regional data base (ReefBase Pacific)

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

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COMPONENT2A

Knowledge, monitoring and management of coral reef ecosytems

PROJECT 2A-1 :
Postlarvae (fish and crustacean) capture and culture for aquarium trade and restoking

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 communitis 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 eco-tourism

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Financement :

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APPENDIX **I**

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I- Introduction and methodology

Within my formation of Agricultural Science and Engineering specialized in fisheries and aquaculture, I decided to make a 6-month internships from September 2006 to February 2007. It took place in Suva, Fiji islands. Pr René Galzin, EPHE-CNRS (Ecole Pratique des Hautes Etudes - Centre National de la Recherche Scientifique) UMR 5244, was my scientific supervisor, Gilles Lecaillon from Ecocean Inc. my technical supervisor.

I was working specifically on the development of a multispecific post-larval fish rearing approach in aquariums. This internship was part of the CRISP (Coral Reef Initiative for the South Pacific) program. Within component 2A named "Status of coral reefs and use of their resources", we aimed to answer the following questions:
- What are the advantages of a multispecific rearing?
- How to optimize it and transfer this knowledge to people in charge of post-larval fish farm?

For that, I had three objectives:
- learning and understanding a new technology: PCC (Post-larval Capture and Culture)
- developing a multispecific rearing approach
- through Ecocean, managing field experimentations and teams involved.

I will firstly introduce CRISP program, the sub-component and project my internship was part of, and also the PCC technology implemented in the post-larval fish farm in Suva. Then, I will detail and explain my results concerning the multispecific post-larval fish rearing approach. And in the last part I will explain the management aspects I was involved in and the prospects of this report.

1) What is CRISP program?

CRISP program is a South Pacific regional initiative, which "aims to develop a vision for the future of [coral reefs] 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), French overseas territories and Pacific Island developing countries" (Anonymous) [1]. It takes place in ten country islands, in which Fiji and three French territories in the South Pacific (see map in appendix 1). CRISP was initially designed by the French Development Agency (AFD - Agence française de développement) as part of an inter-ministerial effort beginning in 2002 (Anonymous) [1]. It currently associates many financial, financial and technical, technical and institutional partners (www.crisponline.net)

The project was technically launched in January 2005 through three components:
- component 1: Marine Protected Areas and Integrated Coastal Management
- component 2: Knowledge, Management, Rehabilitation and Development of Coral Ecosystems
- component 3: Institutional and Technical Support, Communication, Coordination and Extension

Each component was divided in sub-components. I was involved in sub-component 2A: Status of coral reefs and use of their resources; the coordinator is Professor René Galzin.

2) Sub-component 2A and people involved

2.1 Sub-component 2A, project 1

The goal of sub-component 2A is to improve knowledge, monitoring, management capacity and development of the resources of coral reefs to ensure sustainable development of these ecosystems.

Within this sub-component, my internship was part of project 1: Capture and economic use of post-larvae*. Pr R. Galzin is developing this project concerning post-larvae in cooperation with l'Ecole Pratique des Hautes Etudes - Centre National de la Recherche Scientifique (EPHE-CNRS), the French Development Agency (AFD), the University of South Pacific (USP) and a private French company Ecocean.

The studying area for this project is the capital of Fiji islands, Suva, located South East of Viti Levu, main island of Fiji. Project 1 began in October 2005 and will end in June 2007 concerning field experiment.

2.2 People involved in that project and places concerned

Pr R. Galzin, EPHE-CNRS is coordinating that project and Ecocean is his sub-contractor to manage the technical field by fishing post-larvae and transferring its PCC knowledge in sorting, identifying and rearing post-larval fish.

In late 2005, Ecocean designed a farm in the School of Marine Sciences (University of the South Pacific, Suva) to rear the harvested post-larvae (figure 1).

Different people are working in USP for the project 2A1:

- A French student, Julien Grignon, who is co-supervised by EPHE-CNRS and the University of South Pacific (USP). He needs post-larvae for his PhD which deals with restocking techniques in the natural environment. He will do some experiments in aquariums to prepare fish before restocking.
- A Fijian USP master student, Ms Shirleen Bala, working with J. Grignon on the optimisation of rearing conditions of these post-larvae before restocking.
- A Fijian USP technician, Mr Laisiasa Cavakiqaki. He has been employed by Ecocean since February 2006 and trained to identify and rear post-larval fish that are being caught.
- Gilles Lecaillon and Sven-Michel Lourié from Ecocean, based in France but coming in Suva three weeks every 2-3 months.
- And myself from September 2006 to February 2007.

Ecocean also employed fishermen to fish post-larvae in several sites in Suva area: Semi fished in "USP sites" (two different areas: Makuluva and Sand bank) and William fished in Muaivuso site (figure 1).

* Concerning demersal fishes, post-larval stage corresponds to the last part of the planktonic stage of larval phase when they colonize their habitat.

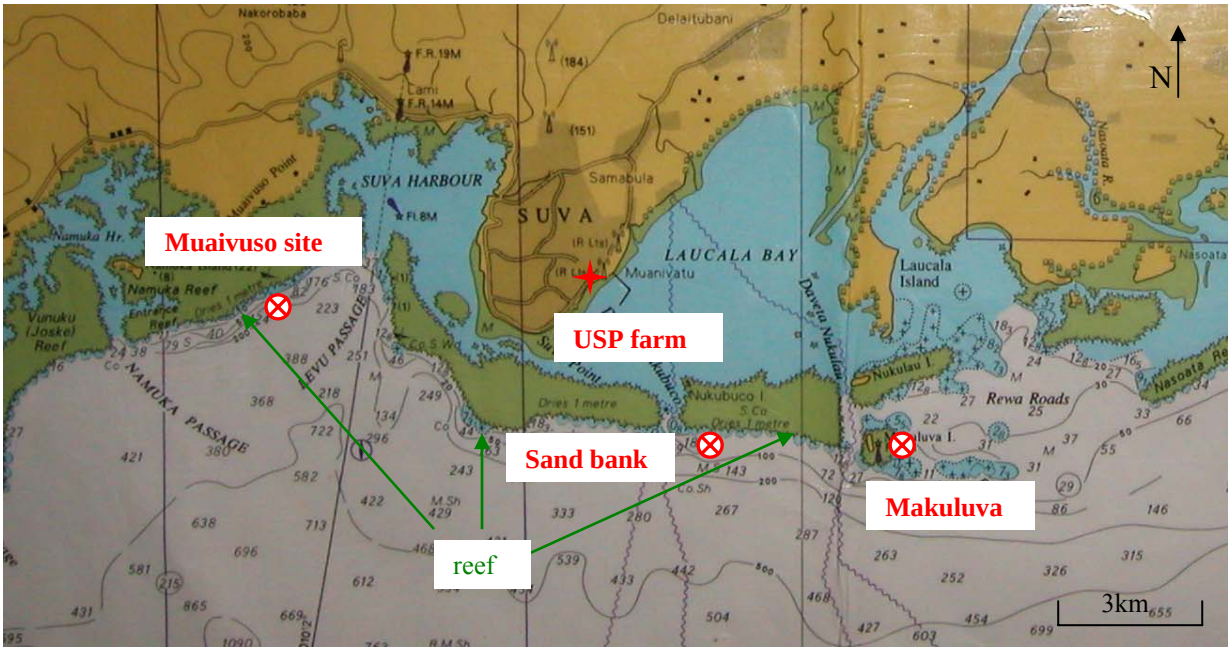


Figure 1: location of USP farm and fishing sites

3) Biological cycle of reef fish

Here is the biological cycle of coral reef fish:

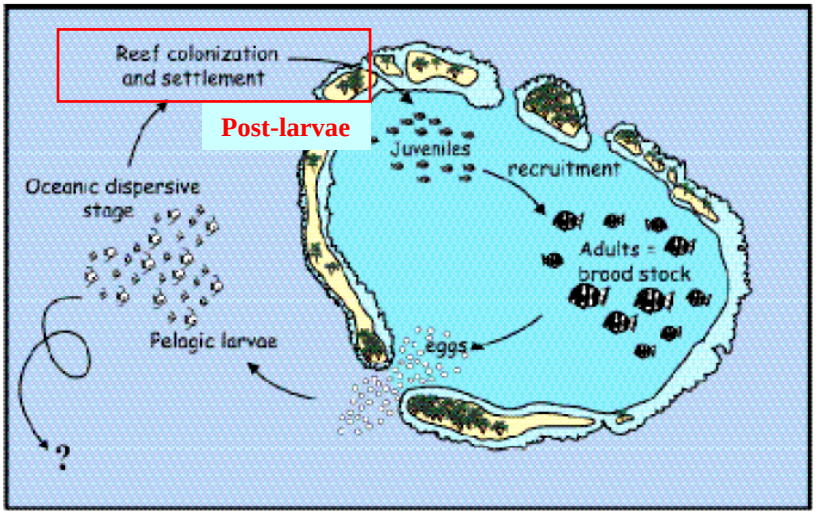


Figure 2: biological cycle of reef fish
Source: Ecocean

Recent studies have shown that most coral reef fish breed in reef areas (e.g. in the reef lagoon) and release their eggs into the open ocean to be carried by currents (Victor, 1986). The pelagic planktonic stage of the larvae then occurs in the open sea. Between 20 and 100 days later (Victor and Wellington, 2000) the larvae move to the surface, and in most cases actively swim back to nearby reefs utilizing the upper ocean currents.
This last part of the planktonic stage of reef fish is called 'colonization' (☐ on figure 2) and the larvae at this stage are termed post-larval. Depending on the year, month and moon cycle, millions of these post-larval fish return to their permanent habitat (Doherty and Williams, 1988; Dufour et al, 1996).
Unfortunately, more than 99% of these larvae will disappear within one week of returning to the reef area, mainly due to predation (Doherty et al, 2004; Planes et Lecaillon, 2001; Planes et al, 2002), physiological changes (the consequences of metamorphosis to become a juvenile) and coastal pollution.

4) PCC technology in Suva, Fiji

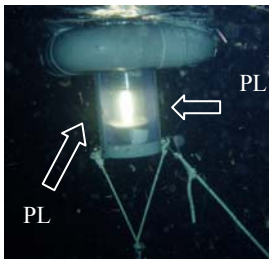
Worldwide, various larval collection techniques have been tested, including plankton nets, crest nets, and channel nets. However these techniques often have the drawback of wounding the animals during capture, as well as sometimes capturing unnecessarily large quantities of post-larval fish.
Ecocean Inc. has designed and patented a C.A.R.E (Collect by Artificial Reef Eco-friendly) system which only catches minimal amounts of post-larval fish, and does not harm or damage the captured fish or any other organisms in the collection area.
Ecocean PCC technology transfer technical assistance focuses upon:
1 catching live, healthy and uninjured post-larval fish
2 identifying and segregating species which could harm or consume one another
3 growing the fish; juveniles are saleable for marine aquarium trade or as pre-growth fingerlings for either future food fish grow-out, depending on species. They are also ideal for reef rehabilitation through restocking (Moana initiative, 2005).

4.1 Post-larval Capture

First step of PCC consists in catching post-larvae overnight. This first step was executed by local fishermen (Semi in USP sites, William in Muaivuso site).

As mentioned above, the chosen fishing device for CRISP project was C.A.R.E system. C.A.R.E is an illuminated artificial floating device which mimics a coral reef setting (figure 3).

Fishing occurs at night



Post-larvae (PL) and only post-larvae are attracted by the light and cover offered by the floating device, and upon arrival at the CARE trap they spontaneously dive into it, using its shelter to hide from predators.

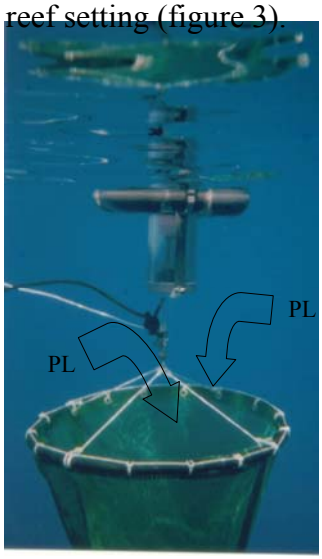


Figure 3: Semi, the fisherman, "deploying" a CARE, light of a CARE, CARE under water, Source: Ecocean

C.A.R.E collects a small percentage of post-larvae when they colonize their adult habitat (figure 2). By collecting a small percentage of these post-larval fish prior to their high mortality phase, the impact of collection on future fish stocks will be negligible (Bell et al., 1999; Lecaillon, 2004).
Moreover, the traps are laid well beyond the reef, so that they do not disturb reef ecosystems.

The following figure presents some statistics of post-larval fishing with CARE during my internship in Fiji.

FROM SEPT 2006 TO FEB 2007		
total of fishes caught (CARE)	8048	USP: 5939, Muaivuso: 2109
total of fishes available in aquarium	6948	USP: 5061, Muaivuso: 1887
total of available damsels	3491 : 50% of available fishes	
total of available apogons	2912 : 42% of available fishes	

number of fishes per CARE	USP	MUAIVUSO
sept	56	8
oct	48	
nov	37	17
dec		
jan	36	60
feb	33	

no fishing

number of fishing days	USP	MUAIVUSO
sept	11	5
oct	9	0
nov	13	10
dec	0	0
jan	14	9
feb	4	0
mean	10,2	8

Figure 4: statistics of 6-month fishing in Fiji

4.2 Description of USP fish farm

After post-larval collection, post-larval culture takes place in the USP farm.

The USP farm room is divided into two parts: the experimental part, which was designed by Julien Grignon and is under his responsibility and the rearing part, which was designed by Ecocean and is under its responsibility (figure 5). My internship dealt only with the rearing part to prepare the fish before being transferred inside experimental part.

Figure 5: rearing part of the USP farm
Source: Antonin Hubert and Sophie Vermond

There are nine 130L aquariums and four 7L aquariums in the farm so more than 1m^3 in glass aquariums. We also have four 1m^3 green cages but most of the time only one or two are used to rear juveniles (figures 5-6).

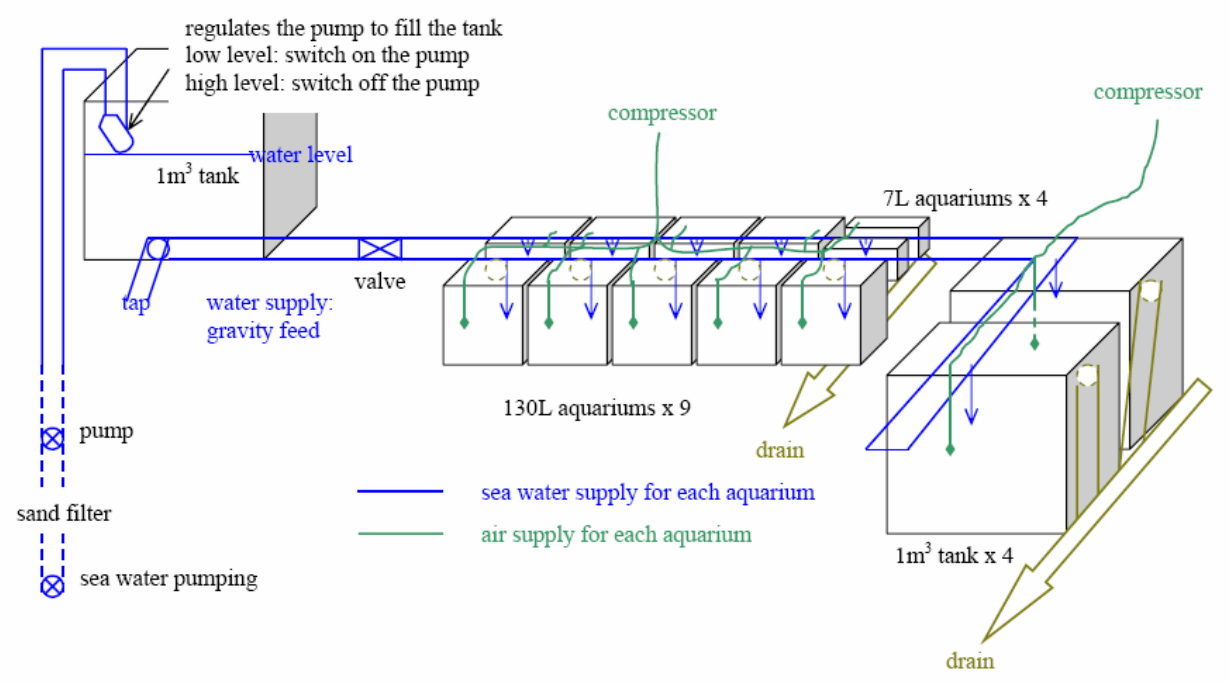


Figure 6: USP rearing farm scheme

Sea water is pumped at the end of the USP jetty (figure 7) by two big pumps (one working at each time). Then water is filtered by a sand filter (50µm mechanical filter) and stocked in a $10m^3$ tank outside. A small water pump brings filtered sea water from the storage tank to the $1m^3$ tank inside the farm. Water is continually delivered (gravity-fed) inside aquariums through water pipes coming from that $1m^3$ tank. The percentage of water renewing is 50% per hour maximum. Water goes out of each aquarium through a runoff connected to the drain. As strange as it may seem, all drains in USP (seawater labs, chemistry lab…) go directly into the sea.
This farm works as an open system.

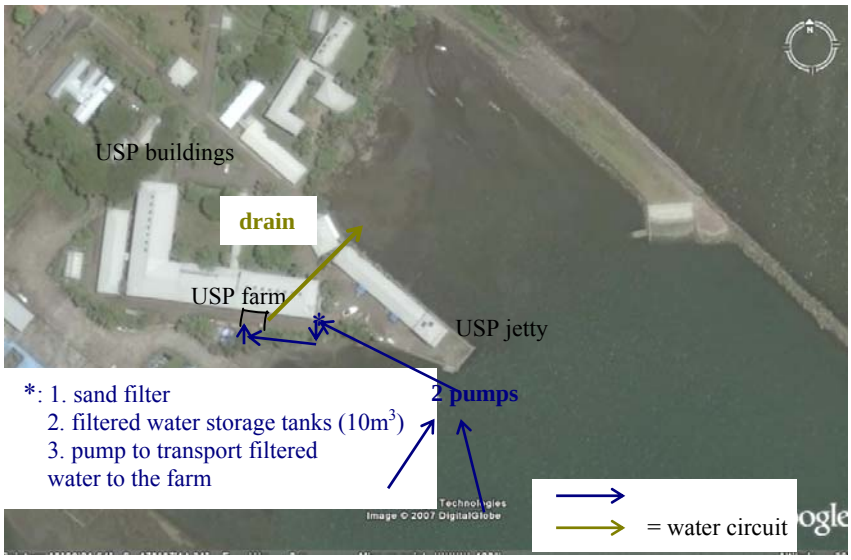


Figure 7: water circuit
Source: Google earth, 2007

In filter room, there is also a compressor that allows air delivering in each aquarium and green cage through air pipes connected to air stones.

4.3 Post-larval culture

Since the post-larval fish are already settled inside the trap after a night's fishing, harvesting the post-larval fish from the cod-end of the CARE is very easy. Fishers then simply pour them into coolers containing sea water, with oxygen supplied from small battery air pumps and bring them to land-based USP facility for sorting. Harvesting and transport are done as early as possible after sunrise (around 6-7:00am).

4.3.1 Reception, sorting, identification and recording of post-larvae

Reception

Coolers from fishermen are poured into Styrofoam boxes as a sorting table. We use one box per fisherman to count the number of post-larvae (PL) per fishing area. Immediately after, we add filtered seawater and aeration in sorting table.

Sorting

Post-larval size ranges from 6mm for a Napoleon wrasse up to 50mm for larger families such as surgeonfish, squirrelfish and filefish (Moana initiative, 2005).

We use a big aquarium net to group PL together inside the box. It is important to keep them into water. Then we use a small glass cup to sort the post-larval fish one by one into 1,5L dedicated containers filled with filtered seawater. We combine fish species with similar habits and keeping predators separate to avoid predation. When container is full enough, PL are counted and poured into aquariums

Pictures: Antonin Hubert and Sophie Vermond

Identification and recording

Books and PL behaviour will help identify PL. Moreover, a visual quick identification guide is provided by Ecocean enabling people to sort the PL quickly, thereby minimizing stress, as this is a critical stage in the process.

A torch helps sorters to see colours, small spots and fins shape. A typical example is *Plectroglyphidodon lacrymatus* PL; their blue spots can be seen only with a torch.

We record in the farm hand book the species and the number of PL per species we have collected per fishing site.

NB: Some post-larvae may die during the night or during the transport back to the farm, this mortality is named DOA (Dead On Arrival). DOA must also be recorded in the farm book because it is part of the CPUE (Catch Per Unit Effort). This explains the difference between the number of fishes caught and the number of fishes available on figure 4 page 9.

When fishermen bring morning catch, sorting PL must be the priority. Sorting should be done carefully to minimize stress but as quickly as possible.

4.3.2 Initial feeding

Post-larvae are fed three times a day: breakfast around 8am, lunch around 1pm and dinner around 5pm (last feeding must be done before dark). We feed them with artificial food (dry granules) and live food (brine shrimps).

Granules are complete feedstuff for sea fish mainly made of fish flour and oil, and vegetable proteins (see precise composition in appendix 2). The food we used is produced in France by a fish food specialist, specialized on larval feed. In Fiji we had four sizes of granules (300-450µm Ø, 500-700µm Ø, 1mm Ø and 2mm Ø). These sizes are fitted to the needs of the different species and size of their mouth openings. When predators like groupers and snappers arrive in the farm, we directly start feeding them with 500-700µm Ø granules. But all other species are firstly fed with 300-450µm Ø pellets.

We prepare live food everyday. Every morning we put brine shrimps (*artemia*) eggs in a conical tank (figure 8) filled with filtered seawater and with strong aeration at the bottom of the tank. If needed, seawater is heated to reach 28°C (if temperature is too low eggs do not hatch correctly).

Figure 8: conical tank where *artemia* eggs are hatching

Twenty-four hours later, eggs have hatched. We then separate eggs which have not hatched from young brine shrimps. Young brine shrimps are kept in a bucket full of filtered seawater with aeration. We feed post-larvae for the three meals of the day with these young brine shrimps. Depending on species, brine shrimps are the major feed for days or weeks.

During a day, the global amount of food that we give to post-larvae (PL) decreases from breakfast to dinner. That limits risk if there is a problem overnight when nobody is there. Moreover, fish has less time to eat before dark.

For capacity building Ecocean has written a series of technical handouts which covers the following steps essential to managing a post-larval collection and rearing facility: collection, sorting, feeding, sanitary aspects, and packing. Feeding process is thus detailed in these handouts (appendix 3).

We used this *artemia* hatching method at the beginning of my internship but the hatching was not very good (50% or even less). So we decided to use another method to decapsulate eggs before putting them in hatching tank. We have tested it several times and hatching rate was very good (80-90%) so we implemented this new *artemia* Standard Operating Procedure (SOP) as follow.

SOP : ARTEMIA

➢ **Starting artemia collection**

- put 1g of eggs per litre of water (10g per artemia tank) in a container with one litre of freshwater and strong aeration
- after one hour add 25cL of pure bleach without removing aeration
- don't let the bleach more than 5 minutes

N.B.: that will decapsulate eggs but if eggs are in contact with bleach for too long time it will kill them

- pour the container on the filter and rinse well with freshwater (to remove bleach)
- put the eggs in the artemia tank with aeration and filtered seawater

➢ **Sorting artemia**

- the morning after, switch off aeration
- wait for the alive artemia to go down the conical tank
- connect the pipe to the bottom of the tank and open the valve to let the tank content flow on the filter
- close the valve before letting the floating dirts (eggs capsules) flow
- put the filter content in a container with freshwater
- wait for 5-10min so that alive artemia are separated from non-hatched eggs
- siphon alive artemia with a small pipe on the filter
- put alive artemia in a bucket with seawater and strong aeration
- fill the container with freshwater again, wait and siphon left over alive artemia
- put alive artemia in the bucket with seawater and quite strong aeration

➢ **Clean *artemia* tank and start process again for the day after**

N.B.: Everything that was in contact with artemia (filter, containers, pipes…) must be rinsed with freshwater, stay for a while in bleach and be rinsed with fresh water again.

Figure 9: Standard Operating Procedure for *artemia* hatching

4.3.3 Water pumping

Everyday or two days we have to pump seawater. At high tide we fill the $10m^3$ storage tank. This task is apparently easy but it can really be crucial for fishes. Indeed in such countries as Fiji it can rain heavily for several days, thus the salinity of seawater drops (from 30‰ to 13‰). We had this problem and we could not avoid decreasing salinity inside the aquariums. But post-larvae could survive the low salinity (20‰ and even 16‰). Nevertheless, recurrent variations of salinity may stress them and thus affect their resistance to diseases (Marques and Pozet, personal communication). Consequently it is important to manage water pumping in order to maintain quite stationary salinity inside rearing tanks (check weather forecasts, measure salinity where seawater is pumped, consider the differences between low tide and high tide…).

4.3.4 Cleaning

We siphon aquariums everyday or every two days to remove food which has not been eaten and faeces. That prevents proliferation of bacteria in the bottom of the tank.

If some fishes die, we remove them and record them in the farm book as mortality (different from DOA).

To prevent the spreading of an eventual disease, we bleach and then rinse the material which has been used inside aquariums (scoop nets…) or material which has been in contact with *artemia*. Floor of the farm is also bleached every evening.

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II- Biological, ecological and economic characteristics of reared species as part of the rearing approach

My internship focused on the culture of post-larvae and its optimisation. I was concerned by the rearing set-up only to prepare fish to be transferred into experimental set-up.
As I explained before, the first step of PCC consists in catching post-larvae overnight. Ecocean has taught me the different steps from reception of PL to their rearing in aquariums. They have taught me their experience about multispecific post-larval fish rearing. Then my goal was to formalise and possibly improve multispecific rearing approach and rearing procedures.

To deal with the multispecific rearing approach, thus the compatibility of different families and genus of fish we have caught and reared, we characterised the fish using twelve biological, ecological and economic parameters: aggressiveness, *artemia* dependence, diet, fragility, gregarity, growth rate, interest for aquarium trade, length of post-larvae, mobility in the wild, risk while siphoning, sensibility to diseases and water agitation.
We considered fifty-three families and genus (most common reef fish families and genus) and we characterised each family/genus with an intensity ladder based on Ecocean team's experience (from 1=lower to 4=higher) for each of these twelve parameters as follow:

aggressiveness			
very docile	docile	aggressive	very aggressive
1	2	3	4
artemia dependance			
<1month	1< <3months	3< < 6months	> 6months
1	2	3	4
diet			
corallivorous	herbivorous	zooplankton feeder	predator
1	2	3	4
fragility			
not fragile	a little bit fragile	fragile	very fragile
1	2	3	4
gregarity			
isolated	in pair	small group	school
1	2	3	4
growth rate			
- -	-	+	++
1	2	3	4
aquarium trade value /landed price			
low (<1€)	medium (1< <3€)	expensive (3< <7€)	very expensive (>7€)
1	2	3	4
length of post-larvae			
<1cm	1< <3cm	3< <5cm	>5cm
1	2	3	4
mobility in the wild			
territorial	sedentary	mobile	very mobile
1	2	3	4
risk while siphoning			
very low	low	high	very high
1	2	3	4

sensibility to diseases			
very low	low	high	very high
1	2	3	4
water agitation: current, aeration strength			
very calm	calm	choppy	very choppy
1	2	3	4

Figure 10: twelve parameters to characterize reef fish families and genus

I keep working on these parameters that characterise the fifty-three families and genus. We aim to sort these families and genus into different compatibility groups. Inside a group, families and genus could be reared together.

Some of these twelve parameters are more important than others in the sorting process of the families and genus. The first and more important parameter in this sorting process seems to be the length of post-larvae. It would be the first level of sorting.
We now have to determine the second, third and fourth most important chosen parameters. Then Excel reorganises successively the list of families and genus in a descending sort for the most important parameters we defined:
first level of sorting: descending sort on the length of post-larvae
second level of sorting: descending sort on the second most important parameter
…
With this process, we aim to sort the families and genus in a dozen of compatibility groups.

During this internship, I used some of these twelve parameters as different approaches in multispecific post-larval fish rearing (*artemia* dependence, aggressiveness, growth rate, sensibility to diseases).

III- Development of multispecific post-larval fish rearing approach

1) Weaning approach and *artemia* dependence

1.1 Weaning process

Weaning process can be explained by following scheme:

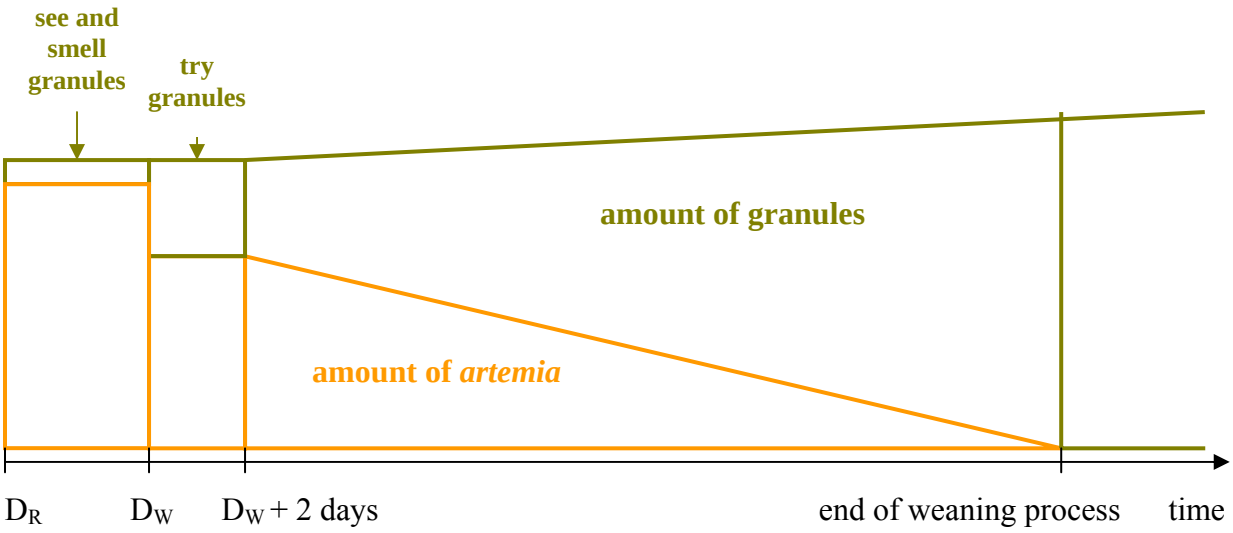


Figure 11: scheme of weaning procedure

D_R = reception day of PL
D_W = weaning day

We give to new incoming post-larvae a small amount of granules every meal since they arrive in the farm (D_R) so that they progressively get used to artificial food by seeing it and smelling it.

They finally try granules (D_W = weaning day). The time taken varies from a few days for species like damsels to a few months for butterfly fishes. Between D_R and D_W, it is very important to give properly *artemia* to post-larvae because this live food is the only thing they eat.

Two days after D_W, we can consider granules as part of their diet. Thus, we start decreasing the amount of live food we give them. And finally, we only give them granules. That corresponds to the end of the weaning process; they are completely weaned and do not depend on live food anymore. The time taken to be completely weaned depends on the species.

1.2 *Artemia* dependence

The time taken to be completely weaned is linked to the *artemia* dependence. This dependence depends on the species. Some are more *artemia* dependent than the others. This is an important parameter that characterises the families and genus in our table (figure 10, page 16). Species can be classified as follow (the time ladder corresponds to the ladder in figure 10: four groups: < 1 month, 1 month $\leq \leq 3$ months, 3 months $\leq \leq 6$ months, > 6 months):

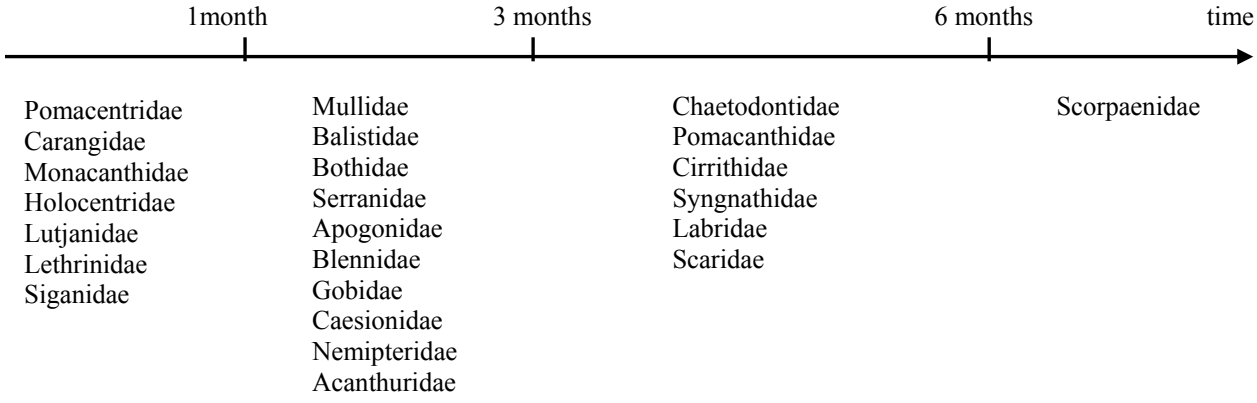


Figure 12: artemia dependence for coral reef families

1.3 Importance of multispecific rearing in weaning process

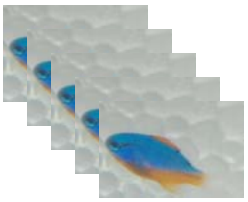
I studied the importance of rearing different species together in the weaning process. It has been demonstrated that "learning enables fish to modify their foraging behaviour in response to a fluctuating environment" (Kieffer and Colgan, 1992). Yet some experiments suggested that "individual differences in learning persist across learned feeding tasks" (Kieffer et al., 1991). And then there is some social transmission of learning. Thorpe (1963) defined observational learning, which is a mechanism of social transmission, as "the copying of a novel or otherwise improbable act or utterance for which there is clearly no instinctive tendency". And eating artificial food is a novel act for which there is clearly no instinctive tendency.

I made an experience on two species (*Chromis viridis* and *Chrysiptera taupou*) from the same family (Pomacentridae, damsels) with the same post-larval size range (around 1cm). I firstly reared several *Chromis viridis* and several *Chrysiptera taupou* separately (one species per tank) and then both species in the same aquarium. I observed the following thing:

Only *Chromis viridis* in a tank
$D_W = D_R + 7$ days

Only *Chrysiptera taupou* in a tank
$D_W = D_R + 2$-3 days

+



=> *Chromis viridis* are weaned after 4-5 days

Chromis viridis are weaned faster if reared with *Chrysiptera taupou*. That is probably a case of observational learning.

The same was also observed with snappers (genus *Lutjanus*).

Rearing different species of butterfly fish (genus *Chaetodon*) may also be interesting. *Chaetodon auriga* and *Chaetodon citrinellus* start eating granules quite fast (D_W = 1-2 weeks) but some other *Chaetodon* species are strict corallivorous. If reared together, *Chaetodon auriga* and *Chaetodon citrinellus* can encourage strict corallivorous species to try and eat artificial food.

Multispecific post-larval fish rearing could reduce the weaning age of some species due to an observational learning.

That is what observation tends to prove but many more behavioural experiments have to be done to confirm the observational learning process for many more post-larval species.

2) Sensibility to diseases

During my internship in Fiji, we encountered a pathological problem. The first symptoms were observed at the beginning of October. Fishes were rubbing their head, probably their gills, against the bottom of the tanks. They seemed to jump on the bottom. They were not doing it continually but more and more often with time. Then we noticed that these symptoms appear 10-15 days after arrival of post-larvae in the farm. Firstly, two or three fishes show these symptoms, then, three or four days later, around ten fishes are rubbing. Fishes start dying around ten days after appearance of the symptoms. But not all of them die and they keep eating correctly.
"The fish is constantly bathed in potential pathogens, including bacteria, fungi and parasites. […] Suboptimal water quality, poor nutrition, or immune system suppression generally associated with stressful conditions allow these potential pathogens to cause disease." (Francis-Floyd, 2005).

2.1 Identification of the disease

We firstly thought it was a parasitic problem since rubbing is a feature of parasites. So we treated with potassium permanganate (efficient against almost all parasites and easy to find in Fiji). But post-larvae do not tolerate this medicine well. It seems to be too aggressive for their fragile gills. They do not seem to tolerate copper sulphate (15ppm) well neither.

So then, we treated them with Praziquantel, which is said to be efficient against worms' life cycle (also destroy their eggs) and not aggressive for fishes. Indeed it was well tolerated (2ppm) by fishes for several days. Nevertheless, fishes were still rubbing immediately after putting them back in a totally cleaned farm when treatment over.

Some fishes also had fin rot (fins seem to be totally bitten). So we treated them with a broad-spectrum antibiotic, Furazone green, 5ppm, 6 hours per day during 5 days. Post-larval fish tolerated well this treatment and their global condition seriously improved. Nevertheless, it apparently had no effect on rubbing.

Many autopsies have been done, especially to look for Trichodine, and nothing abnormal was noticed.

This disease is not identified yet but Ecocean keeps working on its identification and prevention with specialists: a histological analysis is to be done in France to check the presence of internal parasites and a microbiological analysis has already been done; the Institute of Applied Sciences (USP) analysed a dead sick *Chrysiptera leucopoma*. Two different bacterial

strains were observed and *Pseudomonas spp.* was identified. This bacteria is an indicator of bad quality water (personal communication, Fouré, 2007). Indeed we pump seawater in a polluted urban area (Suva area). We are close to the biggest river in Fiji whose polluted water tends to go towards our pumping area due to currents and wind. Moreover, the waste water of the farm is not treated and goes into USP drains. All these drains go directly into the sea, not so far from the pumping station. So me may pump contaminated water.

All fish specialist we met, say that the filtration system in USP is not efficient because there is no UV. Moreover, they told us that a lot of pathological strains may grow up in the sand filter.
I also detailed all the observations, hypothesis, actions we made and the results day by day in a table (appendix 4) to help us analyse this disease with fish specialists in France.

So we have a disease in the USP farm due to bad quality water pumped in Suva area. Nevertheless, to prevent and minimize this disease, we wrote and enforced a Standard Operating Procedure for zootechnic implementation in the USP farm. It deals with cleaning (bleaching material and floor, regular cleaning of the pipes and the water storage tanks…), daily handlings and preventive treatments (more details are available in appendix 5).

2.2 Differences in species resistance to diseases

Because of this problem, several experiments were done during my internship.

We were wondering if fishes, once contaminated and showing the symptoms of pathology, would be able to recover without any treatment. So we put about ten really sick fishes (could not swim correctly anymore, had bitten fins…) of different species in a 130L clean aquarium without any treatment. We just fed them correctly. After a few days, they had recovered: even *Stegastes* could swim correctly, condition of their fins improved… But unfortunately they got sick again quickly.

We noticed that *Stegastes* have always been the first ones to be sick (rubbing against the bottom of the tank, bitten fins…). So we have been thinking about considering this species as a good indicator of the first signs of pathology. That means that if we notice some *Stegastes* having the symptoms of a disease, then we can treat all other tanks preventively and "save" them before they get contaminated or start dying.
Stegastes are the least resistant to the disease but some species are quite sensitive too, that is the case of *Chrysiptera leucopoma*. On the contrary, Lethrinidae is the only family which has never got contaminated, or if contaminated they resist the disease because they have never shown any symptom and they did not die.

Thus, in term of capacity-building, and because pathology is part of PCC, we (technicians and students) have learnt a lot concerning the sensibility to disease of different post-larval fish species and medicine we can use to treat post-larvae.

3) Post-larval fish compatibility considering growth and aggressiveness

In a multispecific rearing process, the most important thing is to determine which species can be reared together, all of them growing correctly.

3.1 A growth variability depending on species reared together

Behaviour of post-larvae is checked each time we feed them (three times a day). We check if all fishes eat correctly, if they are active and if they swim correctly. Furthermore, every

morning, two hours after the feeding, I checked each aquarium with a torch to see if fishes react to light, if they are in good conditions (no bitten fins, not skinny, normal colour, no bulging eyes…) and if fishes are almost all the same size. Mortality is recorded everyday and also the cause of mortality if known. Dead fishes are removed from aquariums and were also measured and weighted.

By observing post-larval behaviour and evolution everyday with that "behaviour checking" method (eat, react, swim, checking with torch) I noticed that one month old *Chrysiptera taupou* were smaller (35% smaller) and really skinny (three times less heavy) if reared with *Stegastes* than reared with *Chrysiptera leucopoma*. Indeed, *Stegastes* are territorial and aggressive fishes so *Chrysiptera taupou* may have been attacked by *Stegastes*. *Stegastes* must also have prevented them from acceding food.

Thus *Chrysiptera taupou* cannot be reared with *Stegastes*. That is an example that shows that we must deal with different behaviours (especially aggressiveness) of different species reared if we want all of them to grow correctly.

3.2 Apogonidae: a particular "non interesting" case

During my internship, 94% of fish we have reared were Apogons (family Apogonidae) and damsels (family Pomacentridae). So I focused my experiments on these two families.

Apogonidae must be reared alone because they are quite aggressive predators but their post-larvae are not very long (between 1 and 3cm) so they cannot be reared with predators which have big post-larvae. Moreover, they are difficult to rear: although they are weaned quite fast, they start dying after the first rearing month (several per day). Most probably because the diet granules+artemia does not fit them anymore.
They are cryptic fishes that eat at night so they may not like their rearing conditions (in a glass aquarium without anything to hide and not fed at night). Anyway, these fishes are valuable neither for aquarium trade, nor as food fish. So we finally decided not to use space in glass aquariums to rear them but to keep them all in a cage inside a $1m^3$ tank as live food for predators.
Nevertheless, some species such as *Apogon lineatus* can be useful for experiments such as restocking (Montebon, personal communication).

3.3 Optimisation of damsels rearing

We have tested the compatibility of the different species of damsels that we have encountered, considering the twelve chosen parameters described on figure 10 page 16. If in a tank with several species:

- no fish had bitten fins
- all or almost all fishes ate and grew correctly
- and maximum one or two fishes died per week,

then I considered that rearing these species together was fine.
Here are some suggestions concerning an optimised multispecific rearing of damsels.

	Abudefduf sp.	*Chromis lepidolepsis*	*Chromis margaritifer*	*Chromis ternatensis*	*Chromis vanderbilti*	*Chromis viridis*	*Chrysiptera biocellata*	*Chrysiptera leucopoma*	*Chrysiptera taupou*	*Dascyllus aruanus*	*Dascyllus reticulatus*	*Dascyllus trimaculatus*	*Plectroglyphidodon dickii*	*Plectroglyphidodon lacrymatus*	*Plectroglyphidodon leucozonus*	*Plectroglyphidodon phoenixensi*	*Pomacentrus bankanensis*	*Pomacentrus coelestis*	*Pomacentrus pavo*	*Pomacentrus vaiuli*	*Stegastes sp.*
Abudefduf sp.	+	-	-	-	-	-	P	P		-	-	-	P	P	P	P	P	P	P	P	P
Chromis lepidolepsis	-	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Chromis margaritifer/iomelas	-	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Chromis ternatensis	-	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Chromis vanderbilti	-	+	+	+	+	+	P	P	+	+	+	+	+	+	+	+	P	P	P	P	-
Chromis viridis	-	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Chrysiptera biocellata	P	-	-	-	P	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Chrysiptera leucopoma	P	-	-	-	P	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Chrysiptera taupou	P	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Dascyllus aruanus	-	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Dascyllus reticulatus	-	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Dascyllus trimaculatus	-	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-	-	-	-
Plectroglyphidodon dickii	P	-	-	-	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Plectroglyphidodon lacrymatus	P	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Plectroglyphidodon leucozonus	P	-	-	-	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Plectroglyphidodon phoenixensis	P	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Pomacentrus bankanensis	P	-	-	-	P	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Pomacentrus coelestis	P	-	-	-	P	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Pomacentrus pavo	P	-	-	-	P	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Pomacentrus vaiuli	P	-	-	-	P	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	P
Stegastes sp.	P	-	-	-	-	-	P	P	-	-	-	-	P	-	P	-	P	P	P	P	+

+ = always match - = no compatibility (aggressiveness, diet, length of PL)
P = may be reared together, depending on the size and the number of PL and also the space available in the farm and aquariums

Figure 13: compatibility of damsels' species

NB: In this table compatibility means optimising growth. So "-" does not mean that if we put species together, one will eat the other, but it means that if we put them together one will not grow correctly (less or no food access because of the other species…).

So basically we can consider three rearing groups:

Compatibility groups	**Characteristics**	**Genus/species**
Small damsels	small PL ≈ 1-1,5cm	*Chromis spp.*
	docile PL	*Chrysiptera taupou*
	quite fragile PL	*Dascyllus spp.*
Big damsels	bigger PL ≈ 2-2,5cm	*Chrysiptera spp. (except Chrysiptera taupou)*
	less docile PL	*Plectroglyphidodon spp.*
	less fragile PL	*Pomacentrus spp.*
Aggressive damsels	grow fast	*Abudefduf spp.*
		Stegastes spp.

Figure 14: compatibility groups of damsels and their characteristics

Aggressive damsels can possibly be reared with big damsels' group during the first couple of weeks but must rapidly be sorted.

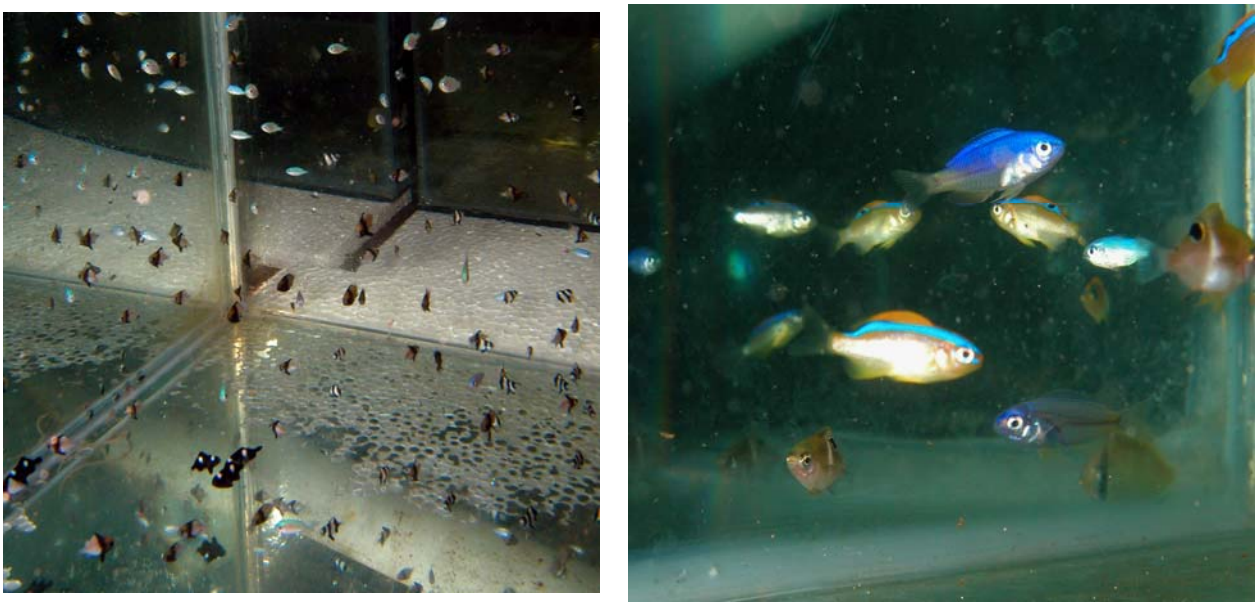


Figure 15: a 130L aquarium of "small" damsels (*Chromis viridis, Dascyllus spp.*) and a 130L aquarium of "big" damsels (*Chrysiptera leucopoma, Pomacentrus coelestis, Chrysiptera biocellata, Plectroglyphidodon leucozonus*)
Source: Gilles Lecaillon

These suggestions concern post-larvae when they arrive in the farm. But within the first month or even the first weeks, the different species reared together may not grow the same way (for example, *Chrysiptera leucopoma* grow faster than other "big damsels") and their behaviour also changes (for instance, *Dascyllus* become more and more aggressive) so aquariums must be sorted so that all individuals keep growing correctly.

Once you know which species can be reared together, you have to decide the fish density in aquariums.

4) Density in a multispecific post-larval fish rearing

Density is a key parameter in aquaculture. In intensive fish culture systems, increasing density is a means of optimizing productivity. However, high density may be a cause of poor growth and it often increases the incidence of disease.
If the density is too high, weakest fishes will not access food so they will become weaker and weaker and will finally die if they are not separated from bigger and stronger fishes. “Lower growth rates of subordinate fish compared with dominants have been recorded in many studies (Li and Brocksen, 1977; Abbott and Dill, 1989; Pottinger and Pickering, 1992; Sloman et al., 2000)” (Bolasina et al., 2006). Density also affects territoriality. "Inter-specific aggression is probably the single largest source of damselfish mortality" (Anonymous) [2].
“High stocking density produces crowding stress; this effect has been described in different species” (Bolasina et al., 2006). And “virtually any extrinsic stress, including shipping, crowding, poor water quality and inadequate nutrition may predispose an ornamental fish to bacterial disease” (Lewbart, 2001). And if a disease is contagious, the more the aquarium is crowded, the more the disease spreads quickly.
So what is the optimal rearing density, the density that optimises productivity without reducing growth rate too much, without increasing incidence of disease?

4.1 An experience made on *Stegastes*

To answer that question, I began an experiment. I tried to rear some *Stegastes sp.* with quite high density (234 fishes in 130L aquarium, corresponds to 1,8fishes/L) and compared their behaviour, their growth, their survival rate versus another "regular density" aquarium (110 *Stegastes sp.* and 19 other fishes mostly damsels, corresponds to 1fish/L).

In high density aquarium, everything was fine the first ten days, even if [NH_3] was a bit higher (0,5mg/L) than in regular density aquarium. But then, lots of *Stegastes* had bitten tails and there was huge mortality (almost all after 3 weeks). Some also had bitten tails in regular density aquarium and three *Stegastes* died. So we stopped the experiment and treated the regular density aquarium.

But at this time the first symptoms of a disease appeared. Fishes had strange behaviour: they were rubbing against the bottom in both aquariums. So we cannot know if huge mortality in high density aquarium was due to too high density, disease or both.

4.2 Density suggestions

To help us to suggest a density for damsels' compatibility groups I defined previously, I measured and weighted almost all dead fishes (mortality or DOA).
Here are these results concerning damsels PL:

Compatibility groups	**Genus/species**	**Mean PL length**		**Mean PL weight**	
		Per genus/species	**Per group**	**Per genus/species**	**Per group**
Small damsels	*Chromis viridis*	1cm	1,2cm	0,03g	0,05g
	Dascyllus sp.	1,3cm		0,07g	
	Chrysiptera taupou	1,4cm		0,04g	
Big damsels	*Chrysiptera sp. (except Chrysiptera taupou)*	1,8cm	1,8cm	0,12g	0,10g
	Plectroglyphidodon sp.	1,6cm		0,08g	
	Pomacentrus sp.	2,1cm		0,11g	
Aggressive damsels	*Abudefduf sp.*	2cm	2,1cm	0,3cm	0,26g
	Stegastes sp.	2,2cm		0,22g	

Figure 16: mean PL length and weight of damsels' compatibility groups

Considering Ecocean experience, here are the density suggestions for these groups of damsels.

Compatibility group	**Density (number of fishes per litre)**	**Density (number of fishes per 130L tank**	**Biomass per 130L tank**	**Density (grams per litre = kg/m^3)**
Small damsels	2,5	325	16g	0,12g/L
Big damsels	2	260	26g	0,2g/L
Aggressive damsels	1,5	195	51g	0,4g/L

Figure 17: density suggestions for damsels' compatibility groups

NB: These suggestions concern only post-larvae when they arrive in the farm. So the tanks must be sorted at the end of the first 3-4 weeks since these suggestions will not be right anymore at this time.

Density depends on the water renewing in the tanks and also the amount of food given to the fish. The suggestions we made are right for 5 to 50% per hour of water renewing in an open-system and for 100% per hour in a close-system.

For comparison, the maximal density allowed for sea brass and sea bream organic aquaculture is 25kg/m^3. And the density used in extensive shrimp aquaculture is around 0,1kg/m^3.
In every instance, fish behaviour has to be carefully checked everyday and density may have to be readjusted depending on that daily observation and also depending on local situation.

IV- Capacity building process and prospects

1) Managements aspects and capacity building process

The third objective of my internship, apart from multispecific rearing approach, was to work with Ecocean to manage the project in Fiji. In fact, Ecocean manager cannot stay for all project duration in Fiji so part of my internship responsibility was to help them on the field.

As a CRISP trainee working with Ecocean, I have been taught the PCC technology. This learning was a capacity building process.
One objective of CRISP program and specifically to Ecocean is to transfer this new technology to local communities in the South Pacific. One of my goals was to take part of this capacity building process in Suva.

Working with Fijian people (Laï and Shirleen) everyday in the farm is a major phase of capacity building; we share theoretical, technological and practical knowledge. Ecocean brings its experience and tools concerning fishing, post-larval identification and rearing, farm managing and PCC technology in general. Fijian people bring their knowledge fitted to local context and practices.

But the first step of PCC is post-larval capture so the first and very important step of capacity building involves the fishermen. We have to explain them the best as we can why PCC can be really interesting for them: new source of income, increasing number of fishes by restocking juveniles so more fishes to fish mid-term or even short-term…Because they get really involved in that new technology only if they can find new advantages.
I was in charge of the fishermen on the field. We had some difficulties working with them but during my internship, Semi has fished for 51 nights in USP sites and William has fished for 24 nights in Muaivuso. And 8048 post-larvae have been fished.

The second step of capacity building is: local people become autonomous so that they can manage PCC on their own. That is required for PCC to be sustainable in Fiji, socially speaking. Laï and I were in charge of making daily decisions in the farm under Ecocean instructions. But the goal was to let him make the decisions as much as possible and I tried to egg on his initiatives.

Capacity building process is still in progress but the Fijian technician, Laï, is already autonomous and can manage post-larval sorting, identification and rearing. He is now an important person in this capacity building process in Fiji.

2) Prospects

Based on Ecocean experience and my daily observations in the USP farm, we formalized the weaning approach in a multispecific post-larval fish rearing and made some suggestions concerning the compatibility and density of different species of coral reef post-larval fish we have reared.
In this report, the suggestions mostly concern damsels' species (family Pomacentridae).

But I will keep working on the compatibility and density aspects within the multispecific post-larval fish rearing approach with Ecocean. We will focus on the compatibility of most common reef families and genus characterized by the twelve biological, ecological and economic

parameters I described in chapter II. We aim at generalizing the suggestions we made in this report to the fifty-three most common reef families and genus.
We will also work on heath management with fish disease specialists to write down a prophylactic and therapeutic Standard Operating Procedure. We also consider developing a general health kit and providing each new post-larval farm with it.

These compatibility, density and health management procedures would be tools for capacity building.

Conclusion

During my 6-month internship within CRISP 2A1 in Suva, Fiji, I discovered a new technology thanks to EPHE/CNRS with technical participation of Ecocean: Post-larval Capture and Culture (PCC), and I focused on the development of multispecific post-larval fish culture approach. Indeed, I started working on the weaning in the USP multispecific post-larval farm and also on damsels fish compatibility and density. Moreover, we started dealing with health management in such multispecific post-larval farm.

PCC means access to a new post-larval resource which is easily achievable and Ecocean provides the tools for capacity building. PCC potential is now evaluated in Suva area within CRISP program and capacity building is still in progress. My internship was part of this capacity building process. Furthermore, Ecocean and I keep working on the multispecific post-larval fish culture approach to generate new tools for this process.

PCC is being tested in Suva area but this technology is also suitable for use in other places in Fiji and by other South Pacific islands as an additional economic, conservation and restocking resource.

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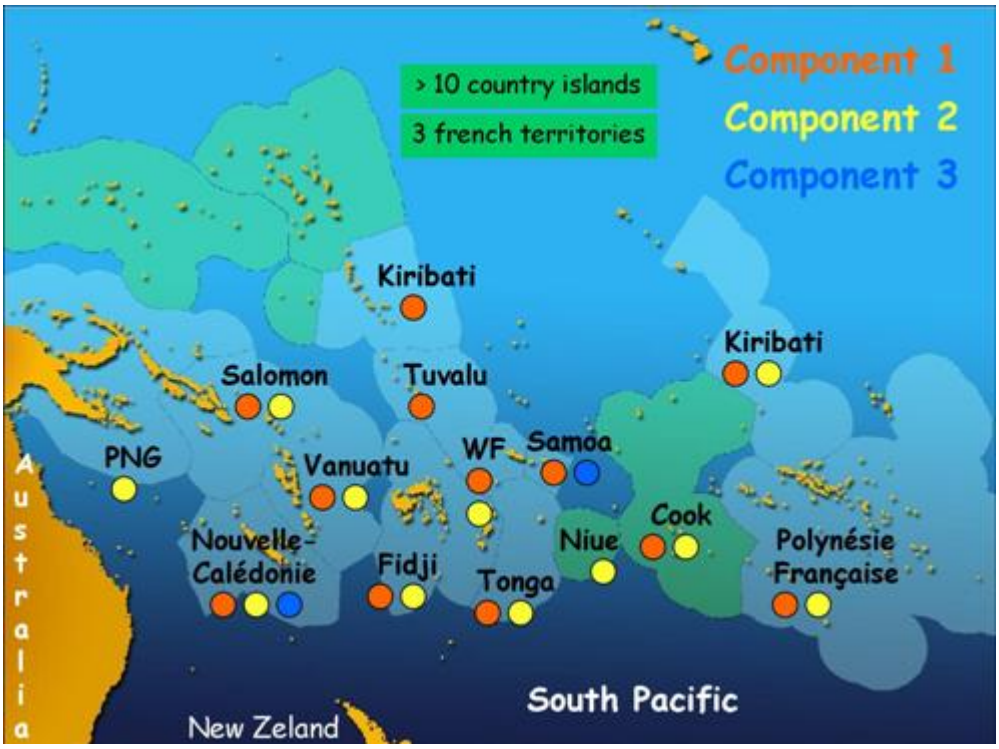
Appendix

Appendix 1: Coral Reef Initiative for the South Pacific (CRISP)



CRISP in South Pacific islands
Source: http://www.crisponline.net/Home/tabid/36/Default.aspx

Appendix 2: Food APHYMAR 1 (APHYTEC)

Ingredients
Fish and vegetable proteins, products of legume seeds, fish oil improved with EPA, DHA, mineral and vitamin compounds, without genetically modified organism, preservatives or colouring agents.
Mean analysis
Protein: 53%, fat: 15%, mineral salts: 8%, fibres: 2,5% max, moisture: 10%
Additives (per kg)
A: 20 000U.I., D(3): 2 000 U.I., E: 200mg, C: 400mg stabilized and coated.
Other vitamins: B1, B2, B6, B9, B12, H, K1, PP, meso-inositol, iodine.

Store in a dry place, not sun-exposed but ambient temperature.

Appendix 3: Ecocean technical handouts: feeding process

NFWF – Facility Management MANUAL

FEEDING PROCESS:

Overall issue: feeding is very important, even crucial to fish survival, growth and domestication. Take your time and do it right!

1-General issue:

- Clean hands with bleach and rinse with freshwater before feeding the fish.
- 3 feeds/day,
  - Breakfast between 6 and 7 am, it can be earlier.
  - Lunch between 11 am and 12 pm
  - Diner between 4:30 to 6 pm (before dark)
- Each tank must receive dry food first, only after you will give the artemias and/or other fresh food like shrimp, small fish.
- If a tank needs various granule sizes, always start with the biggest size one.
- Don't give too big granules or too much granules => it waste the water and increase ammoniac level.
- Keep the granules dry inside a sealed reservoir, in order to avoid bacterial development or rat's intrusion.
- If possible, keep the food in refrigerated temperature.
- Switch OFF air blower to have calm water during feeding time, but maintain the water distribution.
- During the feeding, the feeder has to watch the fishes to see if they are eating or not. Sometimes, the fish comes, takes the granule but spits it out immediately. This is not good.
- Never scare the fish, especially before feeding. Avoid tank/aquarium invasion, or manipulation, don't stand up too close to the aquarium/tank.
- For all kinds of food, try to put the food away from the drain when feeding.
- Clean extra food and shits minimum 30mn to 1h after feeding.

Overall issue: always feed the targeted fish with the biggest granule he cans eat. To help feeding, keep some weaned fishes to train the news arrivals on eating!

2-Detailed steps:

Granules

- The size of the chosen granule is directly related to the size of the fish mouth.
- Don't give too big granule, risk of choking, dirt the water.
- For example, if there are 20 fishes in one tank, do not give all the food, say 100 granules, at the same time. The fish will eat 1 granule after one. The best is to give them 5 times 20 granules. This is, of course, an example.
- Minimum duration for the feeding: 30 min. The feeder needs to come back many times in the same tank.
- Maintain few minutes between each feeding to let the fish eat slowly.
- Theoretically, no granule must touch the bottom of the tank. In some cases, it happens because we have to give too much food in one tank to give the chance to the fish to reach the granule. Also, some are eating even if granules are on the bottom. Siphon the extra food 1h after feeding.

• If there are plenty of fishes in one tank/aquarium, split the feeding in each corner of the tank/aquarium, and one in the middle.
• If you just let the granules fall from your fingers, the granules will float for a few minutes. If you want them to fall down immediately (for bottom fish), give a small force throw.

Live animal

• Artemias nauplii (optimum hatch after 24h in seawater with airstone at 28°Celsius)
  - Close air stone 15 to 20 min before harvesting.
  - Avoid at the maximum to harvest shells and non-hatched eggs (if the airstone is placed in the middle of the bottle, no shell should sink).
  - Take the small mesh net to filter the artemias from the artemias tank.
  - "Clean" artemias nauplii with freshwater for few minutes (mini 5 min, maxi 20 min). This will kill lots of germs.
  - That is the moment to separate shell/egg from alive artemia, if necessary.
  - Throw away the freshwater and put them back into seawater before feeding.
  - Clean all the instrument in contact with artemias.
  - *Advice*: you can split in two cups your artemias to facilitate the feeding (more chance, more control).
  - Clean with bleach the artemia tank and rinse it.
  - Restart a new artemia tank for next day (seawater and air). 1 table spoon/tank.

Overall issue: most of the artemia are for the new post-larvae, for tiny fishes (1 to 2 weeks) and hight value fishes. When a species is in growing phase don't give them artemias anymore!

• Small fish:
  - Some species can eat live small fish (mostly predators). If small fishes are available (small pelagic if caught alive or recently dead, cardinalfishes, small damselfishes... with no commercial value) give 1 alive prey per fish once a day.
  - If possible, give the same number of prey as the number of predators, and give all the prey (=food) at the same time.

Rq: To keep the wild behavior of the certain predator fish (grouper, snapper, jacks...), and if the target is restocking, some small no value pelagic fish can be added in the tank.

• Small shrimp/krill

During PL collection, small shrimp/krill/crabs can be caught overnight. This zooplankton can be use to feed the predators. The fish that can eat this zooplankton must have big mouth.

Overall issue: alive krill (shrimp) trapped in care feeds predator < 1 inch, the young pelagic can be given as food to predator > 1 inch.

Others:

<u>Dead fish/shrimp: for bigger fish, almost juvenile, they can eat piece of dead fish or shrimp:</u>

• Cut small piece of trash fish before feeding
• Leave all the dangerous part of the fish/shrimp (like big scale, roster, spine….)
• Remove skeleton when feeding is over.

<u>Algae: some herviror need algae to avoid nutrients lack</u>

• Green ulva, filamentous algae can be given to herbivores.

Appendix 4: disease monitoring

DATE	OBSERVATIONS	HYPOTHESIS	ACTIONS	RESULTS
13 to 15 SEPT 06	huge mortality (100) among 2 tanks (5 and 6) of Apogon		bacterial treatment SERA	by the 15th almost all dead so 2 tanks bleached
16 SEPT to 1st OCT 06	more or less skiny damsels die regularly in tank 7	disease	treatment for 2 days	keep dying
	22 sept 1 dead *Chromis viridis* has bitten fins			
26 SEPT to 5 OCT 06	period of heavy rain so low salinity (13 outside, 20-21 inside tanks = minimum for one day)			
2 OCT 06	11 dead damsels (tank 7) of different sizes, not skiny	cause would be low salinity		
3 OCT 06	62 damsels + 1 blennie dead in tank 7			rare left over alive fishes removed, tank 7 bleached
4 to 8 OCT 06	14 dead *Stegastes* in tank 6 with high density (234 in 120L), many have bitten tail	cause would be high density	8 OCT: sorting	still huge mortality
9 to 10 OCT 06	127 dead *Stegastes* in two tanks 6 and 7 after sorting	sorted too late or cause of death was not density		
10 OCT 06	strange behaviour: rubbing on the bottom and against each others in *Stegastes* tanks	disease due to parasite	treatment for 2 days malachite green + 2 spoons SERA anti ectoparasite	still huge mortality in *Stegastes* tank 6, bit of improvement in tank 7
11 OCT 06	same strange behaviour in a damsel tank but without any mortality		treatment malachite green + anti ectoparasite	no mortality
OCT 06			observation of gills under microscope, careful observation of many fishes dead and about to die, many researchs on internet	nothing except on one fish: small orange points
		cause of the strange behaviour: parasite probably monogeans maybe Lernea		
15 OCT 06	30 more dead in tank 7		fishes treated with potassium permanganate (PP) 500ppm in hospital tank, 20 minutes bath	5 dead in the afternoon after the treatment and one the day after

16 OCT 06	strange behaviour observed in many tanks even in the experimental rack	parasite would have contaminated all the tanks	treatment with PP 500ppm directly in all tanks after siphoning and reducing the water level by half	after 20 minutes, fishes started dying => loss of around 1300 fishes
				improvement of the situation for survivors
27 OCT 06	2 dead *Chrysiptera leucopoma* in tank 1 where "new" caught fishes are put from 17 OCT to date			
28 OCT 06	3 dead *C. leucopoma* and 39 *Stegastes* in tank 1		sorting: *Stegastes* left in tank 1 and all other damsels put in a new tank 7	still big mortality
29 OCT 06	11 dead damsels in tank 7	parasite would still be in the tanks or "come again"	careful observation under microscope	no parasite observed
		may not be a parasite		
	34 more dead *Stegastes* in tank 1		10 alive fishes removed and tank bleached	
30 OCT 06	6 more dead damsels in tank 7	may not be a parasite	NH3 et pH checked: ok antibacterial treatment (one day)	treatment efficient: no more mortality
4 NOV 06	strange behaviour observed again in *Stegastes* tank and other damsels tanks and even in tanks which survived the strong PP treatment		treatment with PP 0,5ppm directly in tanks for	improvement of their behaviour but treatment damages the gills so mass mortality occured due to the treatment
MID NOV 06			contact fish specialists by mails	they think the cause of the strange behaviour is probably a parasite
1 DEC 06	one dead fish is pink	probably a bacterial problem	antibacterial treatment 5ppm	
4 to 8 DEC 06		parasite or its eggs must be hard to kill and/or may stay in the water system	"clean-up campaign": treatment praziquantel 2ppm of all fishes during 3 days 1/2, cleaning of all the water system with high concentration of PP, bleach, cleaning of all aquariums and all farm (walls, table, water and aeration pipes changed)	all treated fishes put back in clean aquariums but still have the strange behaviour 111 fishes died during the treatment and the transfer
from 8 DEC 06			enforcement of a SOP concerning sanitary conditions (siphoning, cleaning of pipes and tanks, footbath…)	
12 DEC 06	still strange behaviour	another praziquantel treatment may be needed to kill all parasites and their eggs	praziquantel treatment all tanks 2ppm mixed with artemia for lunch	

13 to 15 DEC 06	15 dead *Stegastes* in one tank, tail bitten, cannot swim correctly (head down)	would be bacterial problem: tail rot, which could be secondary infection after parasite	treatment furazone green 6ppm during 5 days	fishes whose fins were totally "bitten" kept dying but general improvement of the condition of fishes
20 DEC 06	fishes have bitten fins in many other tanks		treatment furazone green 6ppm first on one damsels tank and as everything was ok same treatment for all tanks for 4 days	improvement but we had to stop the treatment after 4 days because fishes could not "bear" the treatment anymore
4 JAN 07	some fishes have tail rot in several tanks		same treatment furazone green in tanks concerned by tail rot	improvement, no mortality
	no more furazone green			
23 JAN 07		would be good to make preventive antibacterial treatment before transferring fishes in experimental rack	test of organic antibiotic (melafix) on Apogon during 4 hours	Apogon ok
24 JAN 07			treatment melafix during 2 days	no problem
late JAN 07	strange behaviour observed again on "old" fishes and new ones (arrived 1 or 2 weeks before)			
24-25 JAN 07			treatment paraguard 2 days in *Chaetodon* tank	don't rub against the bottom of the tank anymore
30-31 JAN 07			treatment paraguard 2 days in 2 damsels tanks	
early FEB 07	big mortality every day in experimental rack, some fishes have bitten fins			
8 FEB 07	received furazone green		treatment all tanks furazone green 5ppm, 5 days, 6 days for 4 damsel tanks	general improvement (less mortality and even no mortality some days) but some fishes are still rubbing
9 FEB 07	one *Chrysiptera leucopoma* dead in the night has "white stuff" under the skin in different locations on the body (pelvian fins, mouth…)		microbiological analysis of these "white stuff"	one fungus and three bacterial strains were found

Appendix 5: Sanitary SOP in the USP farm

Standard Operating Procedure (SOP) for zootechnic implementation concerning the 2A13 team component into the USP farm:

(1) Laboratory floor to be cleaned with hypo-chlorite bleach daily (evening), and floor left to dry completely overnight (subject to no annoyance from chlorine smell caused to IAS staff in offices opposite Lab)
=> RESPONSIBILITY - ECOCEAN staff
=> RESSOURCE PERSON: SV, Lai, Shirleen, JG

(2) During tank-cleaning, siphon hoses are to be directed into a bucket rather than allowed to drain onto the floor, and contents of bucket to be disposed-of directly into suitable drainage outlet from lab. A white bucket is made for
=> RESPONSIBILITY - ECOCEAN staff
=> RESSOURCE PERSON: SV, Lai, Shirleen, JG

(3) Outlet drain pipes from aquaria overflows to be bleached each month.
=> RESPONSIBILITY - ECOCEAN staff
=> RESSOURCE PERSON: SV, Lai, Shirleen, JG

(4) Increase backwash of multi-media filter to twice weekly. Fill a form with name and date to follow the backwash process.
=> RESPONSIBILITY - SMS hatchery staff
=> RESSOURCE PERSON: SV, Lai, Shirleen, JG, ALFRED, DEEPAK

(5) Foot bath to be maintained at both doors to CRISP Lab.
=> RESPONSIBILITY - ECOCEAN staff;
=> RESSOURCE PERSON: SV, Lai, Shirleen, JG.

(6) As preventive deasease, do a weekly treatment of fish with medecine **formalin** and a monthly treatment of fish with Praziquantel (0,2mg/L).
RQ: Only when necessary, Furazone treatment will be made on locally aquarium at a normal concentration and time.
RESPONSIBILITY - ECOCEAN staff
RESSOURCE PERSON: SV, Lai, Shirleen, JG

(7) Chlorinate delivery pipes and header tank of both set up system every month.
RESPONSIBILITY - EPHE students
RESSOURCE PERSON: Shirleen, JG, SV and Lai

(8) All the material in direct contact with artemia (eggs or brine shrimp) must be bleached after use.
RESPONSIBILITY - ECOCEAN staff;
RESSOURCE PERSON: SV, Lai, Shirleen, JG.

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