Progress Report Template 2016

Tetiaroa Holistic Reef Replenishment project

Leonardo DiCaprio Foundation

Mid-Term Progress Report 2016

1. Overview

The ***Tetiaroa Holistic Reef Replenishment*** project is a conservation initiative that employs an ecosystem perspective in which corals, and juvenile fish and invertebrates are simultaneously restocked into Tetiaroa's lagoon. This holistic approach increases the success of the lagoon replenishment as successful recruitment of fish and invertebrate populations depends directly or indirectly on healthy coral communities.

In this first year of this project, we are:

1) selecting i) two degraded reefs of interest for coral restoration, ii) two sites for fish and invertebrate larval capture, iii) four key coral species for transplantation efforts, and iv) identifying all fish and invertebrate species captured as larvae to be released at the restoration sites.

2) assessing the suitability of restoration sites by successfully developing coral gardens using asexual coral reproduction (with high survival rate of transplanted coral fragments called nubbins from different coral species of interest) and by successful capture of a high diversity and abundance of fish and invertebrate larvae.

3) rearing fish and invertebrate larvae in open-system aquaria at the Tetiaroa Society's Ecostation.

4) identifying thermally tolerant and sensitive corals *in situ* during the El Niño 2016 event that will be used for the next step of the project; growing resistant coral nubbins in coral gardens and rearing resistant larvae via sexual reproduction.

Challenge for the project: El Niño event of 2015-2016

The major El Niño of 2015-2016 was a real challenge for our Holistic Reef Replenishment project. It was predicted that this El Niño event could be equal to or worse than events that have occurred at any time in the past (16% of the world's coral communities bleached and died during the last major bleaching event in 1998). For this reason we delayed the beginning of this project.
As expected, the 2015-2016 El Niño event led to massive bleaching events worldwide due to abnormally high seawater temperatures. In French Polynesia, the level of bleaching was

moderate, and only certain coral genera displayed bleaching signs. For example, in the lagoon of Tetiaroa, bleaching was observed mainly for the coral *Acropora hyacynthus* living near to the barrier reef, and for certain species of the genera *Pocillopora* and *Montipora*.

Despite the moderate level of bleaching in Tetiaroa, the abnormally high seawater temperature recorded in the lagoon generated a challenging environment for the health of coral nubbins. The first objective of this project was to use asexual reproduction to produce a stock of 3-5 cm coral fragments ("nubbins") from adult colonies that are maintained and grown in coral gardens. After fragmentation corals are generally extremely sensitive to environmental changes, and during the peak of the warming event of April 2016, certain species of coral maintained in the gardens showed signs of bleaching and partial mortality, and the project was interrupted in order to avoid any additional mortality of coral nubbins.

However, the coral bleaching event observed in Tetiaroa lagoon for several species of corals generated an unique occasion to identify thermally tolerant coral colonies, also called "winner colonies" that will be use in the next steps of the restoration project to generate a "winner reef" to face global warming in the future years. For that purpose, 70 colonies of *Acropora hyacinthus* (from 2 sites) and 30 colonies of *P. damicornis* (from 1 site) of bleached (loser) and unbleached (winner) types have been tagged, geo-located and sampled in the lagoon of Tetiaroa for further molecular analyses. The monitoring of these coral colonies is currently underway and sampling will be performed in the next months to investigate the resilience of these corals. This coral sampling effort initiated during this warming event creates the opportunity to discuss with international researchers the potential for future collaboration. We have currently identified Dr. Ruth Gates and Dr. Hollie Putnam from the Hawaii Institute of Marine Biology (University of Hawaii), and are discussing with other scientists that expressed interest in the project. The development of such international collaboration in the coming year of the project will enhance its worldwide visibility but will definitely contribute to increase our understanding on thermal coral resistance and the transfer to offspring using molecular approaches that will focused on the coral host as well as on their associated organisms (i.e. zooxanthellae and bacteria), known as key contributors to the coral health/survival.

One of most surprising and encouraging findings from this project results from the development of coral gardens in the field. Tables of PVC were deployed *in situ* in order to grow coral nubbins in different sites and we observed that small fish species were attracted by the coral garden experiments.

2. Activities & Outcomes

2.a. Activities undertaken and completed & preliminary results

REEFS SELECTION FOR RESTORATION & FISH-INVERTEBRATE LARVEA CAPTURE

- *Characterization of degraded reefs for restoration*

One of the most relevant criteria to characterize the health state of coral reefs is the consideration of the coral cover. So far, we have selected two reef sites for our restoration effort, each characterized by less than 10% coral cover (**Fig. 1**). **Reef A** is characterized by low coral diversity made up of primarily of the massive coral *Porites*. **Reef B** is an isolated reef patch harboring a higher diversity of coral species but with a low coral abundance (<10%).

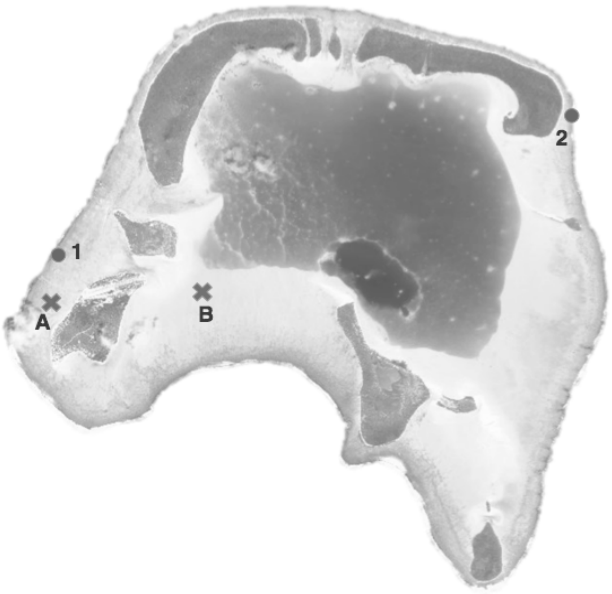

Figure 1 : Location of the selected reefs in Tetiaroa : A and B for restoration, and 1 and 2 for fish and invertebrate larval capture.

Surveys of fish communities of these sites identified at least 17 fish species: Damelfish, Surgeonfish, Blenny, Triggerfish, Parrotfish, Pufferfish, Jackfish, Butterflyfish, Wrasse, Emperor, Snapper, Mullet, Cardinalfish, Sandperch, Goatfish, Angelfish and Gobby. Additional surveys will be regularly conducted (every 3 months) to monitor fish abundances as coral restoration efforts proceed. Further analyses will provide information about the preferential habitat of particular fish species among corals, and other substrate types (sand, coral rubble or algae). In order to restore these two reefs, the second objective of our work is to select coral species of interest and to identify the most suitable site for fish and invertebrate larval capture using the crest net technique.

- *Characterization of reefs for larval capture*

Between November 2015 and February 2016, the *Tetiaroa Holistic Reef Replenishment* fish team identified three suitable sites around Tetiaroa to collect fish larvae on the reef using crest nets: one site on the west coast and one site on the north-east coast. Whatever the wave orientation (north, east or south-west), crest nets can be deployed on one of the two sites to capture larval fishes and invertebrates. Since February 2017, we successfully captured marine larvae at least 10 days per month.

▪ *Coral species selection*

Three corals species *Acropora hyacinthus*, *Pocillopora verrucosa* and *Pavona cactus* were selected for the restoration project because they belong to the main coral genera from Pacific (Fig. 2). The choice of these three species was reinforced by the fact that they were not observed in any of both selected reefs A and B, described above (Fig. 1). For each selected coral species, coral fragments were prepared to be directly translocated into coral gardens.

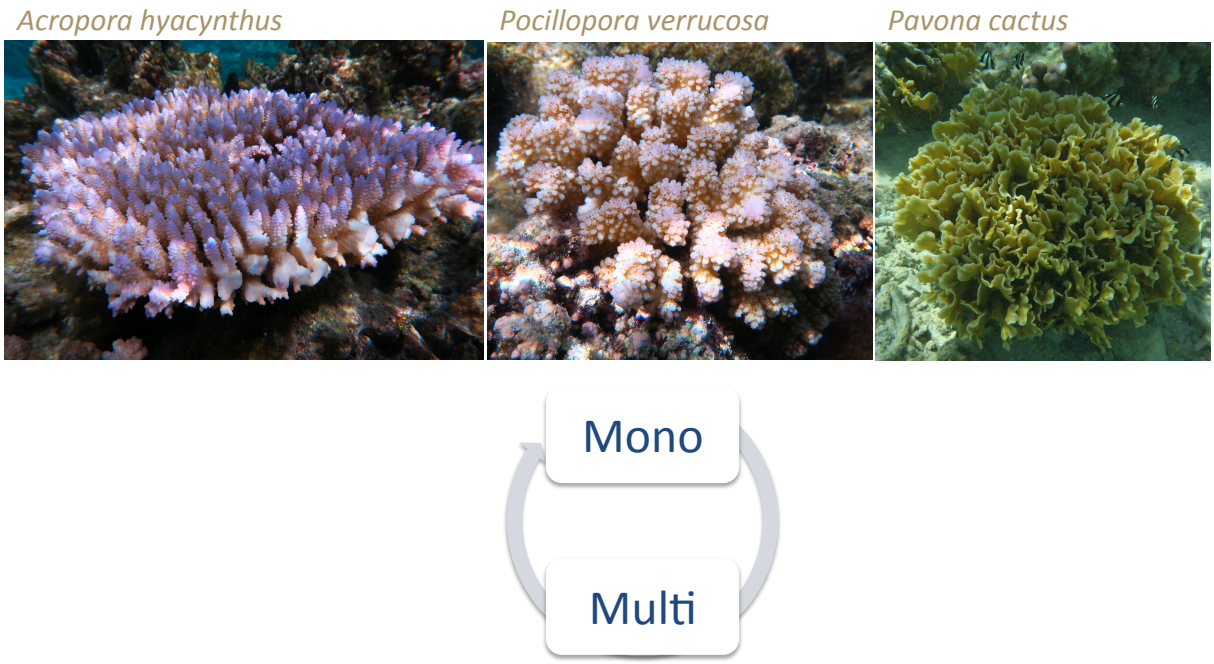

Figure 2 : The three coral species selected for the restoration effort and different strategies tested for testing the success of transplantation.

▪ *Creation of coral gardens & strategies to increase the fragmentation success*

One experimental design was established in Tetiaroa in order to test the most relevant strategies for fragmentation success. First we want to test the **effect of the environment on the first growing stages of coral nubbins**. Two different types of coral gardens are being compared: 1) areas with metallic coral tables that represent an artificial support, and 2) areas with degraded pinnacles and/or rocks used as natural substrate, already inhabited with others organisms that could act as competitors (e.g. algae) or as promoters. Second, we want to **evaluate the influence of the coral arrangements** (Fig. 2): either exclusively composed of coral nubbins from the same species (i.e. mono-specific assemblage), or composed with coral nubbins belong to different species (i.e. multi-specific assemblage mixing different coral species).

In total, 8 colonies per coral species have been collected and split into 36 nubbins: 24 for a deployment on coral tables and 12 for a deployment on natural degraded pinnacles. Into each selected reef A and B, we have deployed three small tables (56 x 70 cm) for testing the mono-specific assemblage (each small table contained fragment from a unique coral species) and one intermediate table (112 x 126 cm) for testing the multi-specific assemblage (mix of nubbins from the 3 different coral species). A total of 576 and 288 nubbins have been generated and are currently maintained on coral tables and on degraded pinnacles, respectively.

The comparison of success between these different strategies is evaluated through biological attributes, such as the coral nubbins survival and growth rates. Due to the slow coral growth of the genera selected, quantitative data regarding coral growth will be obtained every 6 months. Nevertheless, it revealed that despite corals deeply suffered from increasing sea surface temperatures (observation of bleaching signs), all coral nubbins are currently alive which insures the head start for reef restoration. In addition, the **preliminary results highlighted that the fish predation of coral nubbins directly fixed on degraded pinnacles was higher than those maintained in coral gardens.** With that consideration of a highest survival and growth inside the coral gardens, we are planning in the second phase of this project to keep and increase the use of the coral table phase for all coral nubbins to limit predation and increase survival and growth.

A second experimental design for testing the fragmentation success was also established. Coral growth is generally slow, as mentioned before, and we are currently testing a novel approach of coral fusion with the objective of enhancing coral growth rates and sexual maturation earlier than with the conventional fragmentation technic. Our preliminary results show that in less than two months, isolated coral nubbins fuse together. This experiment will be maintained until November 2016, until spawning time of *A. hyacinthus* to decipher whether such technique allow coral to reach sexual maturity sooner. Based on this positive results observed for the fusion experiment, we will apply this new technique in Tetiaroa in order to boost coral growth and hopefully generate mature nubbins in less than 4 years.

- *Selection of bleaching-resistant corals*

During the coral bleaching event caused by 2016 El Niño, we tagged coral colonies of *Acropora hyacinthus* (N=70) and *Pocillopora damicornis* (N=30) displaying bleaching (loser or sensitive corals) and those not displaying bleaching (winner or resistant corals) signs. For each coral colony, small fragments (0.5-1 cm^3) were sampled and conserved in ethanol 90%. To date, these coral colonies are still being monitored to follow their recovery from thermal stress and other sampling (as previously described). This sampling will allow a better understanding of coral bleaching mechanisms and processes through molecular analyses to determine whether coral bleaching alters maternal transfer of corals.

FISH AND INVERTEBRATE LARVAL CAPTURE

▪ *Description of the crest net*

Different methods have been used for sampling early and late larval stages of reef fish in the field (e.g. light trap, crest net, net either towed or dropped in the water column). Although no one technique supersedes all others, crest nets (Fig. 3) have interesting potential for extended monitoring of larval supply: *i)* fish larvae are caught just before reef recruitment, yielding the most accurate measure of larval supply; and *ii)* the turbulence of the surf zone reduces the potential for net avoidance, inducing a wide range of species to be sampled with great efficiency.

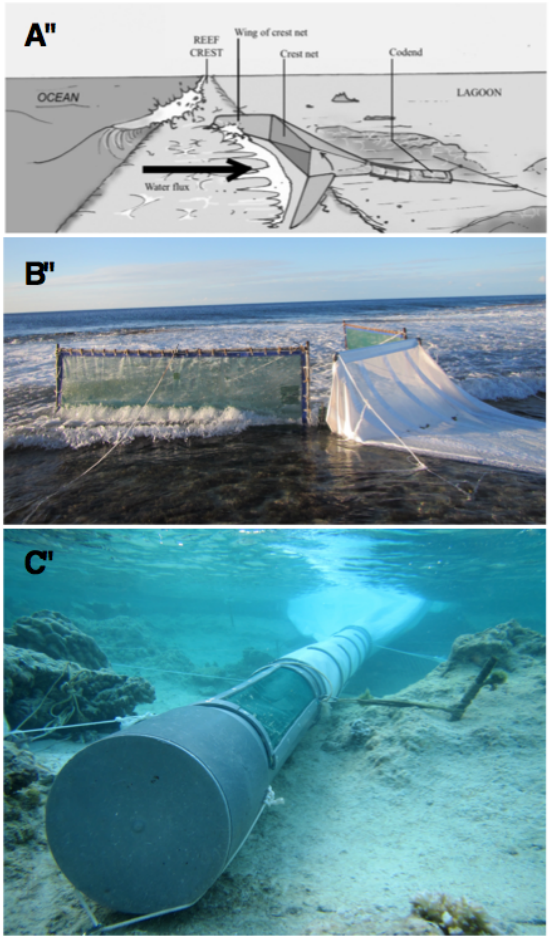

Figure 3 : Capture of coral reef fishes and invertebrate larvae with the crest net technic

The net (5.0 m long) had a rectangular mouth (2.0 m wide, 1.5 m height) and was made of a 1 mm mesh (Fig. 3 A, B), which was fine enough to retain the majority of incoming fish larvae before reef recruitment. The mouth of the net was open to the offshore. Two hinged panels (2.0 m long, 1.5 m height) of 0.7 mm mesh enlarged the mouth area of the net to 4 m (Fig. 3 A, C). The crest net was divided into two chambers: the mouth where larvae entered and the cod-end where the larvae are captured. The whole structure was fastened secured by steel cables, which were bolted firmly onto the reef-rock to prevent the net from being swept away during times of strong current. The cod-end was attached to the net at dusk (6 pm) to minimize the accumulation of debris in the net during the day when few larvae are usually captured and was cleared of catches at dawn (6 am).

▪ *Capture of fish and invertebrate larvae*

Since February 2016, fishing sessions with the crest net during the night have been processed every month around the new moon. In total, more than 250 marine larvae have been captured, belonging to more than 51 species (Fig. 4). Many of the captured larvae belong to species absent or rarely observed from the selected reefs A and B. The preliminary results showed a variability of larvae species captured by night and/or by month. Some of these species more commonly captured are surgeonfish *Acanthurus triostegus* or groupers *Epinephelus merra*

in comparison with some other species rarely such as the goatfish *Parupeneus multifasciatus*. The general low abundance and diversity of fish and invertebrate larvae observed in February 2016 compared with March and April may be explained by the period of full moon, close to 100% of moon visibility (Table 1). This expected result confirms that during period of full moon the larvae colonization is limited, otherwise they would be too much exposed to the predators. With that consideration, it convinces us to **concentrate fish activities efforts for the next months during nights characterized with low percent of moon visibility**.

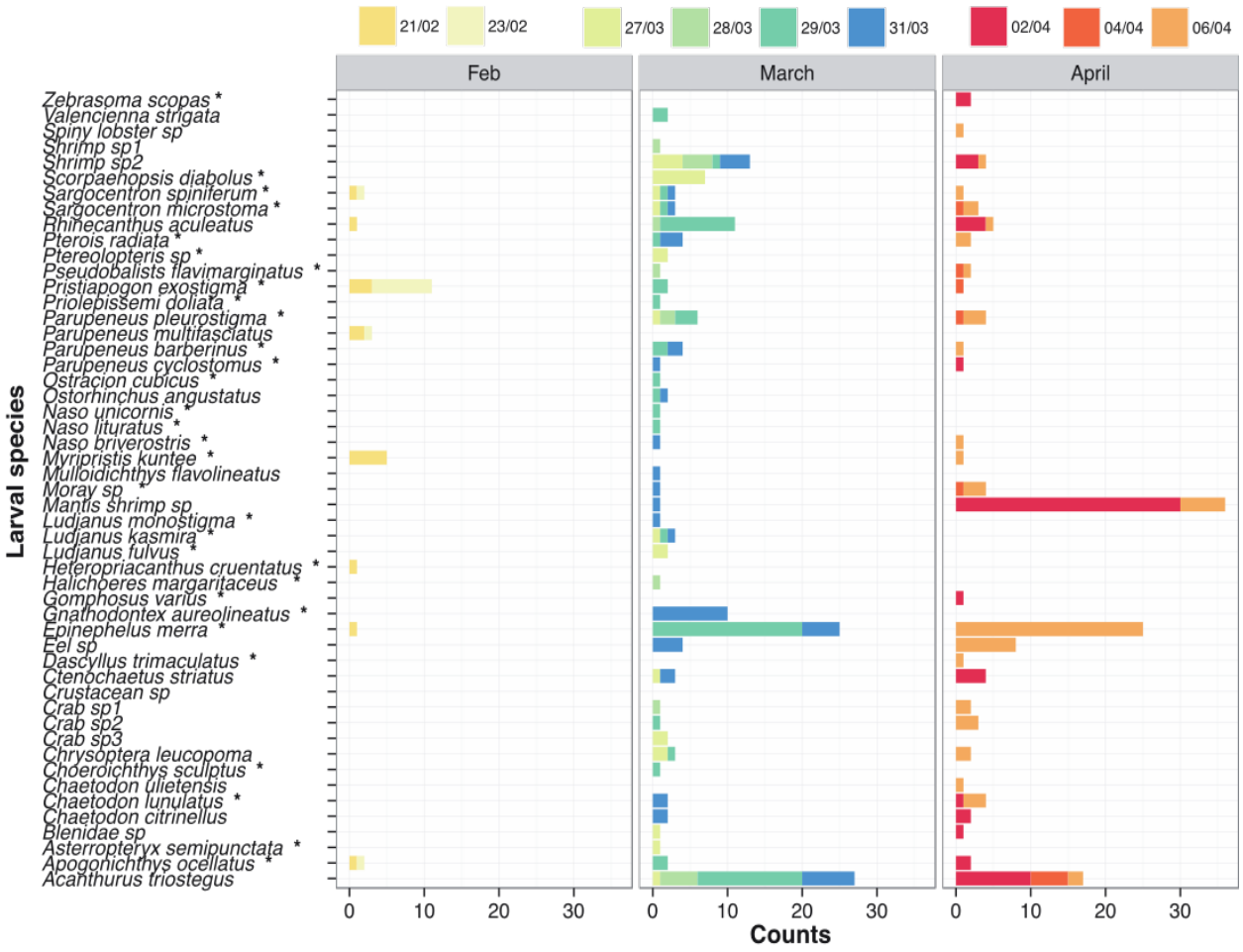

Figure 4 : Capture of fish and invertebrate larvae from February to April 2016. (*) represent species not observed on the selected restoration reefs A and B.

- *Fish and invertebrate larvae rearing*

Once fish and invertebrate larvae are caught with crest net, they are kept between 1 to 3 months until they reach juvenile stages. Rearing is conducted in tanks of 60, 120 and 500 liters of water set up at the Ecostation, and/or in cages set up in Tetiaroa lagoon. The larvae are fed with shrimp the first week and then granules to the juvenile stage. This **rearing** step of the PCC

project (Post-larval Capture and Culture) **was already successfully** established with several larvae species, which have displayed **high survival and growth rates**. However, due to the breakdown of water pump at Tetiaroa, we did not have water in the aquaria at the Ecostation. So, we had to release quickly marine larvae in Tetiaroa lagoon early in April 2016.

The next step (from May to December 2016) will be to define the best sampling protocol to rear marine larvae in order to improve the efficiency of replenishment in Tetiaroa lagoon.

2.b. Core learnings and shifts in strategy identified, if relevant

CORAL RESTORATION

Due to the coral bleaching event caused by the El Niño event, some of the predicted actions and strategies were shifted and/or delayed. However this event was a unique opportunity to identify resistant *vs*. sensitive coral species and/or colonies and to create in the future of a sustainable reef resistant to temperature changes (e.g. with coral colonies better suited to cope with climate change). Moreover experiments on sexual reproduction will be performed on the selected coral colonies to assess the resistance of offspring to thermal stress to generate 'winner' lineages.

▪ *Stand-by of the coral associated exosymbionts experiments*

Initially we planned to investigate whether the presence of associated exosymbionts (i.e. crabs and shrimps) was beneficial for nubbins survival and resistance, but this experiment suffered from the El Niño event. Indeed, to accomplish that, the coral species *Pocillopora damicornis* was selected, due to the systematically association with exo-symbionts. However, through this experiment during the El Niño event, we revealed the high sensitivity of this species to the thermal stress, given the low proportion (< 20%) of healthy coral nubbins (Fig. 3A) *vs*. the high proportion (~80 %) of bleached (Fig. 3B) and/or partially dead (Fig. 3C) coral nubbins. In consequence, most of the exosymbionts escaped from the bleached coral nubbins, likely due to the degradation of both nutritional resources (loss of coral zooxanthellae that generate more than 90% of energetic requirements for corals) and vital space.
This observation was quite surprising since generally the most sensitive genera to rising seawater temperature is *Acropora*, so we were not expecting bleaching of *Pocillopora damicornis* nubbins.

Due to this observation, and the current thermal stress occurring in Tetiaroa, we decided to suspend the preparation of *P. damicornis* nubbins until the observation of coral resilience of both pre-existing nubbins and coral colonies located in the lagoon occurred.

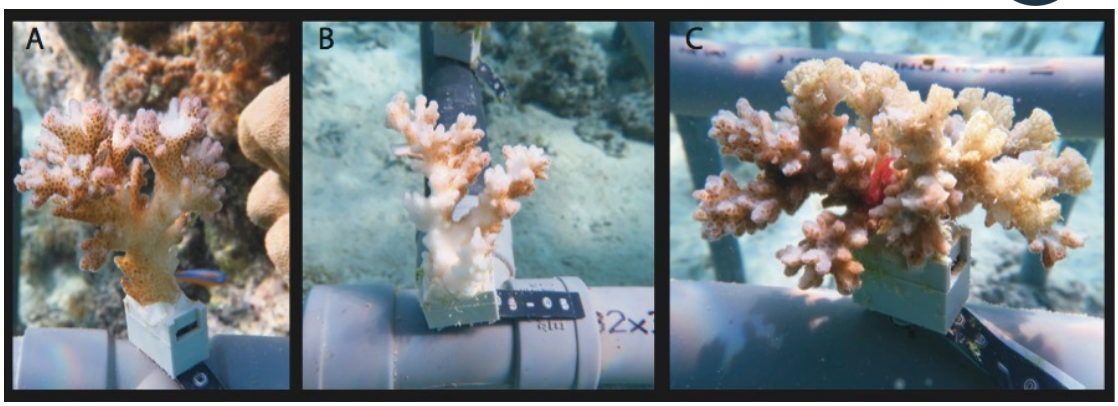

Figure 6 : Different health states of *Pocillopoara damicornis* nubbins during the coral bleaching event in Tetiaroa : (A) healthy, (B) bleached and (C) partially dead with an associated crabs into the branches.

▪ *Delay for coral nursery establishment*

The establishment of coral nursery, based on the coral sexual reproduction, was initially planned for the first phase of the Holistic Replenishment project. However, as an additive consequence of the El Niño event, the success for sexual reproduction of corals was seriously altered, with very low survival rates and null recruitment capacities of coral larvae. In this context, the elaboration of coral larvae nursery was not feasible. However, the monitoring of resistant *A. hyacinthus* colonies previously described will provide a supplementary advantage for success of the coral nursery, that should be performed during the next spawning period expected between September to November 2016. This highlighted the relevance of the current coral bleaching event, which will allow us to create a coral nursery composed with coral winners.

FISH AND INVERTEBRATE LARVAL

The first difficulty was to find two sites to set up crest net at Tetiaroa without having an impact of the Brando guests. With some fruitful discussions with the Direction of The Brando, we found easily the two sites. The discussion with the Direction of the Hotel is excellent. The fish team gave two conferences for the guests, one conference for the Hotel and Tetiaroa Society employees, and one conference for the direction staff of hotel to fit our project as much as possible with the requirement of a 5 stars' hotel.

The second problem was due to a breakdown of water pump at Tetiaroa in April. So, we did not have water in our aquaria to continue the rearing of fish and invertebrates larvae at Tetiaroa Society. Thus, we had to quickly release the marine larvae in the lagoon. At present, this problem is resolved, and so we are capturing some new marine larvae.

3. STORIES FROM THE FIELD

A picture book was performed to illustrate and explained main steps of the restoration project (attached file).

4. MONITORING & EVALUATION

See Table of Grant Evaluation Chart

5. CAPACITY

No changes to report.

6. BUDGET

The Tetiaroa HolIstic Reef Replenishment project is co-funded by Biotherm and Mission Blue, that cover the costs for the fish and invertebrate larvae approach. The funding provided by the LDF is devoted for the coral restoration.

Tableau 1 : Summary of costs for the coral restoration project

Mission-Functionning	Designation	Depenses
Coral replenishment	Flight Paris-Papeete	3 541 $
	Transport Moorea-Papeete	115 $
	Field consumable (table, glue epoxy, etc.)	2 741 $
	Tetiaroa equipment (bicycles)	376 $
	Lab consumable	1676 $
	Water-proof camera	860 $
	Post-doc salary (Feb-June 2016)	9 518 $
	Ecostation Tetiaroa Society (boat, facilities, food)	2 205 $
TOTAL		***21 032 $***

How are your fundraising efforts tracking? If your efforts are lagging, what changes or additions will you make to your fundraising strategy?

Tetiaroa Society has opened a Visitor Center Office in The Brando resort where guests can learn about Tetiaroa Society, and hired a Chief Scientist (Dr. Hannah Stewart, PhD, University of California Berkeley) to oversee and facilitate the scientific and conservation initiatives of Tetiaroa Society and collaborate with her colleagues from around the world. This interaction with resort guests and the international scientific community is creating fundraising opportunities through donations from guests and via funding from government grants and conservation

foundations.

Have you been able to leverage your LDF grant to attract investment in your organization or project?
One aspect of the Holistic Reef Replenishment Project, fish replenishment, has received seed funding from a cosmetics company through Sylvia Earle's Mission Blue Foundation. By combining philanthropic donations from The Brando guests, support from the Leonardo DiCaprio Foundation, and leveraging funds via University and Government Agencies, we combine a grassroots effort with international political will a model for the future of caring for our planet.

7. SUPPLEMENTARY INFORMATION

No further information to report at this time.

Grant Evaluation Chart Template

THREE-FIVE YEAR OUTCOMES:

ACTIVITIES	OUTCOMES (for grant period)	OUTCOME INDICATORS	OUTCOME BASELINES	OUTCOME PROGRESS (to be completed at reporting)	ULTIMATE OUTCOMES
STRATEGY ONE: DEVELOP A CORAL GARDEN FOR NUBINS AND CORAL REPRODUCTION					
Build coral tables	5 Tables with nubins growing	Number of nubins growing on tables	Process protocol for culture of nubins	Survival and growth of nubins	10 tables deployed on 2 selected reefs with nubbins growing
Build aquarium to collect coral reproduction	Aquarium system with filter to collect coral larvae	Success of controlled reproduction	Process protocol for controlled reproduction in aquarium	Abundance of coral larvae spawned	Elaboration of filters and systems for coral larvae capture
Set recruitment tiles	Substratum for coral larvae to settle	Number of recruits settled on tiles	Process protocol for culture of recruits	Density of coral recruits on tiles	Planed in next step
STRATEGY TWO: DEVELOP COLLECTION RAISE OF NEWLY RECRUITING FISH AND INVERTEBRATES					
Build and set up crest nets of the reef	Deployment of 2 crest nets	Species diversity and abundance collected	Process protocol for fish & invertebrates collection	Durability of crest net collection and overall collection abundance	2 crest nets operational
Develop aquarium facilities to raise fish & some invertebrates in aquarium	Fish & invertebrates raised for 2-3 months and ready to realize	Number of fish & invertebrates raised for 2-3 months and ready to realize	Process protocol for fish & invertebrates culture in aquarium	Abundance of fish recruit ready to release	Rearing protocol operational
STRATEGY THREE: ESTABLISH SUBSTRATING TO BE FEED WITH CORAL, INVERTEBRATE AND FISH					
Restoration of a degraded reef	Isolated vs. poorly diverse reef	Survival of coral nubbins growing on the selected sites	Process protocol for direct fixation of grown coral nubbins		Coral nubbins growing on the selected sites
Release raised new recruits from crest nets	Raised fish new recruits	Number of fish & invertebrates released	Process protocol for fish & invertebrates release	Number of fish & invertebrates released	In progress