Ecology and diversity of viruses in the Moorea coral reefs

Coral reefs are one of the most prominent examples of biodiversity on the planet. They offer critical habitat for thousands of different reef organisms, provide a natural barrier for coastal areas and also support local economies. Unfortunately, several decades of research have uncovered a startling fact: reefs are highly vulnerable and are undergoing dramatic declines in coral cover, species richness, and genetic diversity worldwide[1]. The causes of reef degradation are often elusive and are associated with the interacting effects of global changes (i.e., warming seas, ocean acidification), along with local stressors (i.e., overfishing, pollution, coral disease)[1,2]. One of the primary mechanisms causing coral decline in recent decades is the increase in prevalence and severity of coral diseases due to microbial pathogens[3]. However, little is known about the processes that alter dynamics, abundance and diversity of microbial assemblages on corals and how this translates into changes in disease prevalence and severity. It is critical to understand how anthropogenic stressors influence the dynamics coral-associated microbial assemblages and how these changes, in turn, alter the survivorship and recovery of corals. A recent hypothesis suggests that stressors such as nutrient enrichment may alter the dynamics and composition of coral-associated microbial assemblages, ultimately leading to declines in coral growth and survivorship[4-6].

Importance of the coral reef-associated microbial assemblages

Coral reefs are complex ecological systems in which various microbial organisms and viruses interact with each other and with the coral host. The coral's skeleton, tissue and mucus harbor an abundant and dynamic assemblage of marine microorganisms, including protists, bacteria, archaea, cyanobacteria, fungi, and viruses [3,7,8]. These coral-associated microbes exhibit great genetic and ecological diversity and may be important contributors to the overall health of the coral host[3,7,8]. It has been hypothesized that corals provide ecological niches and nutrients for the colonization of microbes[8]. In return, the coral-associated microbes provide nutritional by-products, protein, nitrogenous and sulfur compounds [8,9] as well as some essential vitamins for their coral hosts[7,8,10]. Some coral microbes may also provide protection from disease by preventing opportunistic infections by producing antibiotics or the occupation of available physical niches on coral colonies[7,8,10]. The best known coral-associated microbes are the microalgae in the genus *Symbiodinium* called "zooxanthellae" which provide the majority of the coral's energy needs[8]. Although it has become increasingly clear that coral-associated microbes are critical players in reef ecosystem processes and functions, still very little is known about the least-studied constituents of the coral-associated microbes, the viruses.

Importance of viruses in the oceans and coral reef ecosystems

The occurrence of viruses in aquatic environments has been acknowledged for many years[11,12], but it is only very recently that studies have identified viruses surrounding corals and in close association with corals and their microalgal symbionts[13-15]. Viruses are the most abundant, ubiquitous and diverse biological entities in the world's oceans. As obligate intracellular parasites, all forms of cellular life (plant, animal and microbial) are susceptible to viral infection. A vast majority of marine viruses are believed to infect primarily the microbial assemblages, and there is increasing evidence that viral-induced lysis of microbes contribute substantially to microbial mortality, diversity and evolution, with major impacts on the energy fluxes and global biogeochemical cycles in the world's oceans[11,12]. Few studies have found that viruses shift both across reefs and with proximity to coastal areas and corals themselves [16-18,]. Interestingly, studies reported elevated viral abundances occurring in seawater adjacent to diseased coral colonies and during massive spawning events[16,18].

Currently there are no known viral pathogens of corals. However, recent studies using molecular tools found herpes-like viral sequences rapidly increased when experimental corals were subjected

to abiotic stressors (temperature, pH and nutrient increases) [19]. Viruses might also have a beneficial role in coral health by controlling homoeostasis of the coral-associated microbes. For instance, viral infection of opportunistic pathogenic microbes might sustain the diversity and stability of the coral-associated microbial assemblages by selectively regulating the growth of microorganisms that would extensively proliferate due to a shift in environmental conditions[12].

Despite the growing literature on coral-associated microbes, very little is currently known about the ecological roles that viruses play on oligotrophic tropical reef ecosystems, and how they influence the coral associated microbial assemblages. These are particularly important questions to answer in regards to long-term coral reef ecosystem health and resilience.

Specifically, this proposed research aims to i) evaluate whether the coral reef ecosystem surrounding the island of Moorea harbors abundant, active and diverse marine viruses, ii) test whether changes in nutrient conditions (e.g. nutrient pollution) alter viral dynamics, infection activities and genetic structures in coral-associated microbial assemblages on corals, iii) determine whether certain viral subsets are associated with particular coral diseases. Overall this work aims to validate the hypothesis that viruses influence the health of corals in the Moorea reef ecosystem.

Hypotheses and experimental design

H1: Seawater surrounding coral reefs in Moorea harbors abundant, active and diverse marine viruses

Study sites - Sampling will be conducted to investigate the spatial shifts in the abundance, infection rates and genetic diversity of viruses in the coral reef ecosystem. Stations will be identified with the help of CRIOBE scientists. Samples will be collected in at least 3 habitat types (fringing reef, back reef, and fore reef) along seaward transects in 6 areas surrounding Moorea (Fig. 1).

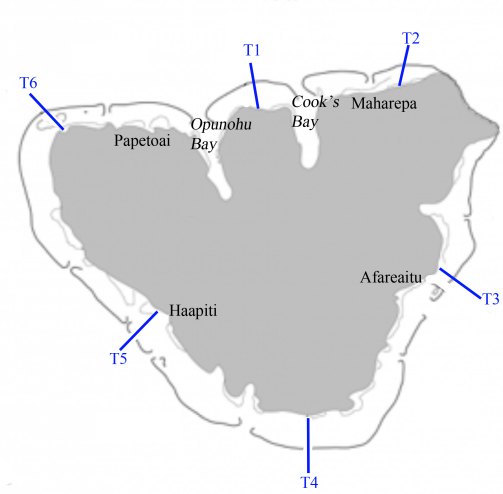

Figure 1: Map showing 6 seaward transects (blue lines).

At each station, samples will be collected at subsurface and just above the coral colonies using a combination of a Niskin bottle and sterile syringes. A small boat and SCUBA divers and/or snorkelers will be needed to conduct the sampling. Data for *in situ* temperature, depth and salinity will be collected at each station. Samples will be transported to the CRIOBE in coolers and aliquoted for the following purposes:

1) *Molecular approaches to explore diversity of viruses*: For each sample, 200 ml aliquots will be filtered successively through 0.22 µm and 0.02 µm filters (Millipore) to concentrate viral particles and their potential microbial hosts. Nucleic acids will be isolated from filters using DNA extraction kits. Extracts will be frozen and sent to Oregon State University (OSU) for molecular analysis.

Specific viral genes will be targeted for deep-amplicon using next-generation sequencers (e.g., 454 GS-FLX and/or Illumina technologies). Bioinformatic analysis will be conducted on the resulting sequences to infer the genetic richness and phylogenetic diversity of viral subsets.

2) *Quantification of viruses and their microbial hosts (bacteria, cyanobacteria and phytoplankton):* For each sample, 5 ml aliquots will be preserved, instantly frozen in liquid nitrogen and sent to OSU for quantification of viruses, bacteria, cyanobacteria and phytoplankton via flow cytometry. Resulting data will be correlated with environmental data to reveal what drives shifts in abundance of viruses and potential microbial hosts.

3) *Viral infection activities*: 500 ml aliquots of each sample will be filtered and then subjected to a series of experimental incubations in order to assess rates of viral-induced lysis of microbes. These results will indicate if viruses are important agents of microbial mortality, with possible consequences for biogeochemical cycling in the reef system.

4) *Nutrient analysis and chlorophyll a concentrations:* For each sample, 200 ml aliquots will be filtered for determination of Chl *a* concentrations and nutrient analysis (nitrogen, phosphorus). Frozen samples will be analyzed at OSU.

H2: Nutrient enrichment results in unique shifts in viral abundance, infection activities and diversity.

Methods- Small coral fragments from representative species (e.g., *Montastrea*, *Acropora*, *Porites*) will be collected and allowed to acclimatize for several days at a designated site on the reef. They will then be exposed to either nutrient enriched or ambient conditions. Each treatment plot will contain four replicates resulting in a total sample size of 8 per coral species. Nutrient enrichment will be created using a slow-release fertilizer (Osmocote) which has been used previously by our research group in the Caribbean and shown to have very limited diffusion rates and localized effects. This time series experiment will include sampling of the plots at several time points for up to 4 weeks. Samples will include seawater just above the corals as well as coral mucus and tissue. Samples will be processed as described in the H1 (section 1, 2, 3 and 4) to assess shifts in dynamics, infection activities and genetic diversity of viruses.

H3: The genetic structure of viral assemblages is different in diseased corals vs healthy corals.

Diseased corals will be identified based upon surveys and tissue/mucus samples will be collected, then filtered to further concentrate viruses and microbial cells, as described above in H1 section 1. Samples will be extracted, frozen and shipped to OSU for sequencing and bioinformatic analysis as previously described.

Intellecutal merit and boader impact:

This research will provide the first glimpse of viral ecology and diversity in the Moorea coral reef ecosystem, and generate unique data for the scientific community. Including viral components in coral reef ecological models will allow scientists to better predict the effects of anthropogenic stressors and natural threats to coral reefs and improve long-term reef management. In addition, because viruses could play a role in controlling proliferation of opportunistic microbial pathogens, this work could also be relevant to reef seawater quality. Furthermore, this work will provide a career development opportunity for a young French researcher from l'Île de La Réunion, who has a great interest in tropical reef systems.

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Appendix A: Proposal budget

1 x round-trip airfare from Portland (OR) to Moorea (include exceeding baggage allowance for personal and scientific gear)	1800 €
4 week accommodation at CRIOBE @ 40 €/day	1120 €
2 x viral DNA/RNA isolation kits (Qiagen)	300 €
Osmocote fertilizer	10 €
50 x 60 mL sterile syringes	40 €
100 x 10 mL sterile syringes	80 €
50 x Anotop syringe filters (0.02 μm)	200 €
50 x Anotop syringe filters (0.2 μm)	200 €
250 mL of RNA/DNA buffer	220€
50 x Whatman Microsep® centrifugal devices (30 kDa)	150 €
Lightweight galvanized metallic mesh roll	40 €
Fuel for boat and car operation	200 €
Large nails and steel bar for mounting the experimental plots	50 €
Total	4410 €

Jérôme Patrice Payet

Curriculum vitae

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Summary of my research areas

The unicellular organisms and viruses that constitute the microbial community are uniquely capable of catalyzing chemical transformations that establish the balance of vital nutrients in the sea. These nutrients are what ultimately determine the characteristics and quantity of marine life in the ocean from near shore habitats to the open ocean. The elucidation of the ecology of marine microbes is therefore essential to the understanding of the ocean and consequently the planet, especially in the context of the current exceptional rate of climate change. In this context, I have worked primarily with marine viruses, characterizing specific virus-host systems of ecological significance, describing the diversity of viral assemblages, and characterizing the relationship between viral dynamics and host community composition and population structure. Now I am applying molecular and ecological tools to elucidate these intricate virus-microbe relationships in coral-associated microbial assemblages.

Education

PhD in Oceanography **2012**
Dept. of Earth and Ocean Sciences, University of British Columbia, Vancouver, Canada
Thesis: Ecology and Diversity of Marine Viruses on the Canadian Arctic Shelf, Arctic Ocean
Supervisor: Prof. Curtis A. Suttle

Maîtrise en Oceanographie **2003**
Institut des Sciences de la Mer, Université du Québec à Rimouski, Québec, Canada
Thesis: Combined Effects of Ultraviolet-B Radiation and Dissolved Hydrocarbons on Natural Microbial Assemblages of the St-Lawrence Estuary
Supervisors: Dr. Emilien Pelletier & Dr. Serge Demers

Maîtrise de Biologie des Populations and des Écosystemes **2000**
Université de La Réunion, France (passed with distinctions)
Mémoire: Le blanchissement coralien et proliferation de microalgues toxiques
Supervisors: Prof. Chantal Conand & Dr. Jean Turquet

Licence de Biologie des Populations **1999**
Université de La Réunion, France (passed with distinctions)

DEUG B **1998**
Université de La Réunion, France (passed with distinctions)

Research Experience

Postdoctoral Fellow **since Feb 2012**

Oregon State University

with PI: Dr. Rebecca Vega Thurber

- Assessing abundance and diversity of coral-associated viruses in the Caribbeans
- Detection and isolation of viruses that infect coral symbionts

➢ Experimental reef structures were deployed and monitored for 3 months in the Florida Keys

PhD research work **2004 - 2011**

- Study of distribution of arctic marine viruses in relation to biotic and abiotic factors
- Detection of virus infection pathways and their ecological impacts on microbial mortality and biogeochemical cycles
- Study of phylogenetic diversity of viral subsets infecting arctic microbes

➢ Collected samples and conducted and conducted experiments for over 6 months on a icebreaker in the Arctic Ocean

MSc research work **2000-2003**

- Designed mesocosms to study the influence of dissolved hydrocarbons and ultraviolet-B radiation on natural marine microbial assemblages

Licence and Maîtrise research work **1999-2000**

- Study the extent of coral bleaching and colonization patterns of macroalgae harboring epiphytic harmful dinoflagellates implicated in ciguatera fish poisoning at two sites on the fringing coral reefs of Réunion Island. Artificial reef structures were deployed and monitored for 9 months.

Publications

Payet J.P. and Suttle C.A. (*in press*). To kill or not to kill: the balance between lytic and lysogenic viral infection is driven by trophic status in Arctic marine coastal waters. Limnology and Oceanography.

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Presentations

Payet J.P. and C.A. Suttle 2010. Genetic composition and diversity of marine viruses during a year-long study in the Southeastern Beaufort Sea, Arctic Ocean. ISME-13: Microbes – Stewards of a changing planet, Seattle WA, USA, August 22nd-27th (Oral)

Payet J.P. and C.A. Suttle 2009. Lysogenic versus lytic virus life strategies are strongly related to seasonal and spatial differences in productivity in Arctic Ocean coastal waters. ASLO Aquatic Sciences Meeting, Nice,

France, January 25th-30th (Oral)

Payet J.P. and C.A. Suttle 2007. Dynamics of marine viruses and their biogeochemical and ecological effects on the Canadian Arctic Shelf. 17th Evergreen International Phage Biology Meeting, Olympia, Washington, USA, August 12th-17th (Oral)

Payet J.P. and C.A. Suttle 2007. Environmental effects on the spatial and seasonal dynamics of marine viruses and bacteria in the Amundsen Gulf and Mackenzie Shelf. Canadian Arctic Shelf Exchange Study International meeting, Quebec QC, Canada, April 30th-May 2nd (Oral)

Payet J.P. and C.A. Suttle 2007. Seasonal variability of viral abundance and activity in the Canadian Beaufort Sea. ASLO Aquatic Sciences Meeting, summer meeting, Victoria BC, Canada, June 4th-9th (Oral)

Payet J.P. and C.A. Suttle 2006. Spatial and temporal variability of viral abundance and activity on the Canadian Arctic Shelf. Canadian Arctic Shelf Exchange Study International Meeting, Winnipeg MA, Canada, February 13th-16th (Oral)

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References

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