Project Proposal

Aims, Significance and Expected Outcomes

The goals of this project are:
Goal 1: To generate baseline genomic data for the limestone-boring alga *Ostreobium*.
Goal 2: To trial procedures for molecular probing of limestone-boring communities.
These goals are two aspects of my overarching vision to develop a research program that will increase our knowledge of the diverse and important roles of limestone-boring algae in coral reef functioning and resilience.

Significance: Coral reefs are increasingly under threat because of the combined effects of rising sea surface temperatures, ocean acidification and nutrient load [1,2]. Calcified corals, which form the principal structural component of coral reefs, are a close symbiosis of many organisms. As is the case in most ecosystems, the microbial flora associated with corals dominates many processes, including energy acquisition, nutrient cycling, calcification and disease [3,4]. The coral itself forms a thin layer of tissue over its calcified skeleton [3]. Symbiotic algae harbored within this tissue, known as zooxanthellae, provide the coral with nourishment in the form of photosynthetic products and also facilitate skeleton formation [5]. In the limestone skeleton beneath the coral tissue, a layer of boring algae is found [6]. These algae penetrate the coral skeleton by chemical dissolution of $CaCO_3$. A green alga, *Ostreobium quekettii*, usually dominates the boring algal flora [6,7].

Ostreobium has two important functions in the coral reef ecosystem [7]. First, it facilitates coral survival during bleaching events. Coral bleaching is a stress-induced event that is occurring at increasing frequency due to global climate change [8]. When coral bleaching occurs, zooxanthellae are expelled from the tissue, leaving many corals to die if no other source of food becomes available. But in the absence of zooxanthellae, more light reaches the underlying layer of *Ostreobium*, boosting its photosynthesis and growth [9]. A portion of the photosynthate is transferred from *Ostreobium* to the coral [10,11], likely extending the time it can survive without zooxanthellae. A second key function of boring algae is bioerosion. *Ostreobium* is the main agent of carbonate dissolution in coral skeletons and contributes greatly to reef bioerosion [12]. Furthermore, boring algae form an important food source for other key bioeroders like sea urchins and parrotfishes [13]. As such, *Ostreobium* plays a major role in the calcification-decalcification balance of the coral reef and is key to understanding how it will develop (or dissolve) under climate change and ocean acidification.

A problem with microbial communities in general, and certainly those living within solid substrata like a coral skeleton, is that they are difficult to observe and study. So, despite their tremendous importance, limestone-boring microbial communities are poorly characterized in terms of their taxonomic and physiological diversity, although a recent study does show that multiple species of *Ostreobium* exist in a Red Sea coral species [14]. Similarly, we are only starting to scrape the surface when it comes to understanding how the boring algal flora responds to disturbances that affect coral reef resilience (e.g. global warming and ocean acidification) [4,15,16]. Environmental sequencing approaches offer solutions to these problems [4]. They permit investigating different aspects of a complex microbial assemblage in a culture-independent manner. Metabarcoding can be used to rapidly assess the taxonomic diversity in an environmental sample. Metagenomics focuses on the pool of DNA in an environmental sample and yields insight in the possible activities of a microbial community (i.e. all genes combined). Finally, metatranscriptomics, in which environmental mRNA is sequenced, is used to eavesdrop on microbial physiology and ecology – it tells us which genes are active under particular circumstances. For these approaches to work effectively, good genomic baseline information needs to be available and protocols need to be optimized. The goals of this project address these two issues.

Expected outcomes: At the conclusion of this project we will have an annotated draft genome sequence of *Ostreobium quekettii* that will inform us about the function of this organism in the coral reef and forms the foundation of future metagenomic/transcriptomic work. It will also yield a set of optimized procedures for diversity assessment as well as metagenomic and metatranscriptomic investigations of microbial communities in coral skeletons. These data will form the basis for a competitive grant application in 2013 or 2014 as described above. The results do not just offer perspectives for future research; they are important in their own right and will be published. I anticipate 3 papers will come out of this project: a high-impact paper describing the *Ostreobium* genome and two lower-impact papers, i.e. a primer note and an article describing the metabarcoding findings.

Research Plan, Methods, Techniques and Proposed Timing

Research Plan and Collaboration: The research plan focuses on two areas that correspond to the two goals. These actions will be carried out in parallel. For the first goal, a cultured *Ostreobium* isolate will be sequenced using a combination of whole genome shotgun sequencing and transcriptome sequencing, followed by computational assembly, annotation and analysis of the results. The second goal consists of a series of small-scale experiments to trial protocols that permit probing the taxonomic, genomic and physiological diversity of field-collected samples. I will collaborate with the team of Prof. Debashish Bhattacharya, a leading researcher in the field of algal genomics (Rutgers University, USA). The project will also contribute to the learning of a PhD student (Vanessa Marcelino), who will in turn advance the goals of the project through her thesis work.

Summary of Methods and Techniques: For Goal 1, a culture strain of *Ostreobium* will be grown in artificial seawater. Several clones of the same strain will be grown for different purposes. A first clone will be grown under what I will define here as "default conditions" (ESAW medium, 23ºC, 5 $\mu E\ m^{-2}\ s^{-1}$ white light, 12-12h light/dark cycle). Substantial amounts of biomass will be grown under default conditions for DNA extraction and whole-genome shotgun sequencing. To obtain sequences of expressed genes, messenger RNA (mRNA) will also be extracted and sequenced. To achieve the highest possible level of gene discovery, some clones will be subjected to shock treatments (dark, temperature, salinity, nutrients) as described for other algae [17]. The mRNA isolated from these treatments as well as the default treatment will be normalized and pooled prior to sequencing. A second set of transcriptomes aims to identify genes associated with the biology of the organism and its responses to relevant environmental conditions. For this, *Ostreobium* will be allowed to grow in sterilized $CaCO_3$ substrate to examine genes involved in carbonate dissolution. Additional experiments involve growth under different light conditions to mimic coral bleaching, under enhanced nutrient loads, and under increased levels of CO_2 to mimic ocean acidification.

DNA and RNA extraction will follow protocols that have been extensively tested on green algae [18]. To achieve approximately 100× coverage of the genome (the diploid genome size of *Ostreobium* is ca. 500 Mb), three lanes of an Illumina GIIx flowcell will be used, yielding > 40 Gb of sequence. Different insert sizes (0.3, 2 & 5 kb) will be used to improve the assembly of the short reads [19]. The pooled normalized RNA will be sequenced in the fourth lane of the flowcell and the barcode-tagged non-normalized RNA libraries (i.e. the second set of transcriptomes) in the fifth and sixth lanes.

For Goal 2, DNA and RNA will be extracted from five environmental samples. For metabarcoding, three partial genes will be PCR amplified (*tuf*A, 16S rDNA and 23S rDNA) with tagged fusion primers suitable for high-throughput sequencing. The extraction and PCR protocols will be optimized prior to sequencing. Following extraction using optimized protocols, a DNA and an RNA library will be constructed from a single environmental sample to trial metagenome and metatranscriptome sequencing, respectively. A combined library of all experiments under Goal 2 will be sequenced on the seventh lane of the Illumina flowcell.

Timeline:

- Months 1-2: extract DNA and trial PCR for environmental samples
- Month 2: extract DNA/RNA for metagenome and metatranscriptome trial
- Months 1-3: grow culture strain under different conditions
- Months 3-4: obtain DNA/RNA from cultures
- Months 5-6: sequencing, assembly pipelines, automated annotations
- Months 7-12: manual annotation, data analysis and publication

Role of each Researcher in the Proposed Project

Chief Investigator – **Dr Heroen Verbruggen**. My expertise includes evolutionary biology of algae and bioinformatics. I have experience in growing algal strains, second-generation sequencing and data analysis, and have published on *Ostreobium* [7,20]. I will provide intellectual direction for the project, supervise the research assistant and graduate student, and conduct computational analyses. My extensive network of collaborators can advise on any troubleshooting that may have to be done.

Associate Investigator – **Prof. Dr Debashish Bhattacharya** (Rutgers Univ.) has a long-standing interest in algal and protist evolution and genomics. When the Bhattacharya lab moved to Rutgers University in 2009, it established a fully equipped genome facility with a second-generation Illumina GAIIx DNA sequencer and high-performance computing clusters. This is the only algal evolution and genomics lab to have in-house high-throughput sequencing facilities, giving it an advantage over all other such labs, and making it an excellent partner for this project. Bhattacharya's team will facilitate the sequencing, read assembly and automated gene detection and annotation.

PhD Student – Miss **Vanessa Marcelino** has a background in evolutionary biology and macroecology of frogs and algae. She will commence her PhD in the Verbruggen lab in the first semester of 2013. She has already trialed sample preservation techniques, DNA extraction and PCR of coral skeleton samples. She will execute the diversity assessment and environmental genomics aspects of the study. She will be trained to analyze high-throughput sequencing data and contribute to data analysis and publication.

The casual **Research Assistant** (grade 1) will be responsible for the experimental work under the direction of the CI. I will source a research assistant with prior experience with algal culturing and molecular biology techniques, who will be able to work semi-independently.

References

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