The Rufford Small Grants Foundation
Final Report

Congratulations on the completion of your project that was supported by The Rufford Small Grants Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Anak Agung Gede Indraningrat
Project title	Assessing impact of depths on biodiversity of sponges and corals in Curaçao
RSG reference	17660-1
Reporting period	9 July 2015 – 31 July 2016
Amount of grant	£5000
Your email address	anak.indraningrat@gmail.com
Date of this report	4 August 2016

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Collecting sponge species along depth gradient			√	We collected 63 sponge individuals from three different depths (0-30, 30-60 and 60-90 m). Molecular identification via Cytochrome Oxidation I (COI) barcoding PCR has confirmed that 34 individuals of two sponge species (*Agelas sventres* and *Xestospongia muta*) were found along depth gradient. Ten samples were identified as *Corallistes typus* which are typical deep sponges (>100 m). The remaining of samples were identified as *Niphates* sp.
Cultivation of sponge-associated bacteria			√	We prepared six cultivation media to isolate sponge-associated bacteria. Based on morphology characteristics, we have isolated 225 bacterial colonies.
16S rRNA Illumina Miseq library preparation and sequencing			√	DNA of the 63 sponge samples was isolated and amplified using 515f and 806r primers to target 16S rRNA V4 region. These 16S rRNA gene amplicons were sequenced by the Illumina Miseq sequencing platform.
Diversity of sponge-associated bacteria along depth gradient		√		At the moment we are analysing 16S rRNA Miseq data to fully study (dis)similarity and diversity of bacterial communities along depth gradient of our sponge samples.
Identification of bioactive compounds from bacterial		√		We are now working on establishing methods to screen bioactivity of sponges and their associated microbes

isolates				against microbial pathogens. After establishing a screening method, we aim to apply this knowledge to screen bioactivity from the isolated sponge-associated bacteria.
Collecting corals along depth gradient	√			Sampling corals was initially planned. However, we cancelled this plan since sampling corals required a special permit and we had limited info on corals diversity along depth gradient. Therefore, in this study we focused mainly on sponges.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

It was initially planned to sample sponges from three different regions on Curaçao Island. On the site, executing this initial plan was not possible since a ship is required to transport the submersible vehicle, the Curasub. Sampling, therefore, was focused in front of Substation Curaçao and no additional cost to transport the Curasub is required. The adjustment of plan did not affect our main objective to collect sponges along depth gradient.

During cultivation experiment, we planned to incubate agar plates in 30^{o}C for 2 months since we would like to accommodate slow-growing sponge-associated bacteria. However, this plan did not run smoothly since the plates had already dried out after 30 days and therefore had to stop.

3. Briefly describe the three most important outcomes of your project.

a. Collection and identification of sponges along depth gradient.
In this study our main objective is to assess whether the deeper–mesophotic-reefs can serve as a refuge for shallow sponges. Sponges are highly correlated with their microbes and therefore we specifically want to study diversity of sponge-associated bacteria of the same sponge species along depth gradient. To achieve this purpose it is crucial to have at least one sponge species in a sufficient number (>five individuals) that can be found along depth gradient. In this project, we managed to sample and identify sponge individuals of four different species that spread along depth gradient. Using these samples, we are running 16S rRNA analysis of microbes associated with sponges and soon we will be able to confirm whether sponges along depth gradient share a similar microbial diversity.

b. Cultivation of sponge-associated bacteria along depth gradient
We finished cultivation experiment to isolate sponge-associated bacteria from sponge samples we have collected. In total we obtained around 4000 bacterial colonies during the cultivation experiment and from this number we selected 225 colonies based on different morphological characteristics. Screening bioactivity of these sponge-associated microbes are now in progress. Isolation of active isolates will provide a basis to explore important compounds that can be derived for human health purposes.

c. Microbial profiling of sponge-associated microbes
Next generation sequencing provides valuable information to access diversity of microbes inside sponge's tissue. We had prepared 16S rRNA library amplicons using Illumina Miseq platform and we just received back sequences data of our samples. After completing the analysis, we expect to get a better insight on the effect of depth in diversity of microbial community in sponges. In a bigger frame, this information will provide an indication to what extend mesophotic area can act as a refuge for shallow water sponges.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

The Curacao Sea Aquarium and CARMABI (Caribbean Research and Management of Biodiversity), are the two important stakeholders on Curaçao that had supported this project by providing technical support such as lab facilities, submarine and equipment. Outputs of this project will give more insight on the resilience of sponge in mesophotic area which further can be applied for improving ecosystem conservation and policy on Curaçao

5. Are there any plans to continue this work?

We plan to expand our study by analysing microbial diversity of sponges along depth gradient from different islands of Caribbean. These samples have been collected by our collaborators and we expect to receive sequence data of these samples in the end of year 2016. Combining microbial data from our study and study of our collaborators, we expect to gain more insight about sponges in mesophotic area.

6. How do you plan to share the results of your work with others?

- We have presented this project in two symposia as follows:

  a. 3rd WUR PhD Symposium 2016 "Diversity in Sciences". A symposium of PhD students in Wageningen to share and to discuss their works.
  b. 1st AcroporaNet symposium. Acropora is a Dutch platform for scientists conducting fundamental and applied research into tropical marine biology.

- We received media attention from the Dutch Radio programme Vroege Vogels who broadcasted our sampling trip underwater. Vroege Vogels is a Dutch Radio programme focuses on nature and conservation. Summary and recording of the programme (in Dutch) can be found in this link: http://vroegevogels.vara.nl/Fragment.150.0.html?tx_ttnews[tt_news]=374525&cHash=fbfc4284936366bb939271f254d7029f
- Wageningen University highlighted the field trip we had in their website.
- We submitted an abstract to the 1st International Conference in Marine Biology (COMBI) 31 October – 1 November 2016 in Bali, Indonesia.
- Two research papers from this project are being prepared to publish in peer-reviewed journals

7. Timescale: Over what period was the RSG used? How does this compare to the anticipated or actual length of the project?

The RSG funds were used for period of 12 months starting from July 2015 to July 2016. The period used of this grant is in line with the anticipated period of time.

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Transportation, Accommodation, Food	1800	2027.26	(-) 227.26	Extra expenses for local transports as several time I have to rent taxis for processing samples to the lab at Carmabi
Equipment	940	295.08	(+) 644.92	It was planned to rent SCUBA gears and to buy a Go Pro Hero4 black camera. We did not spend money on these items because they were provided by the Curacao Sea Aquarium
Chemicals	1570	1883.32	(-) 313.32	Extra expense to buy 20 bars of dry ices for transporting sponge samples and 80 bottles (@100 mL) of distilled water
Shipping equipment and chemicals	690	794.34	(-) 104.34	Extra luggage was purchased for transporting samples to the Netherlands.
Total	5000	5000	0	

Kurs in July 2015: £1 Pound sterling = €1, 3848

9. Looking ahead, what do you feel are the important next steps?

It is important to continue research based on results we have obtained in this project. The next important step is to fully analyse metadata of sponge-associated bacteria in our samples and to screen bioactivity from isolated sponge-associated bacteria.

10. Did you use the RSGF logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

Yes. RSGF was acknowledged in every project presentation I had e.g. WUR PhD symposium, Acroporanet symposium, and internal PhD meetings of Laboratory Microbiology. Acknowledgement of RSGF will be mentioned in two scientific papers I am currently working on and international conferences I am planning to participate in the upcoming 2 years.

11. Any other comments?

We would like to thank RSG for providing financial support to this project and we hope to receive a more support from RSG for our projects in the future.