EUROPEAN UNION OF AQUARIUM CURATORS

REPORTING FORM

FOR CONSERVATION PROJECTS FUNDED IN

2022

1 TITLE OF PROJECT	Development of sustainable livelihood and upscaling reef restorations in Palau
2 NAME OF APPLICANT	Dr Jamie Craggs
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DATE OF REPORT:	25th July 2024

PLEASE SEND YOUR REPORT TO ISABEL KOCH, SECRETARY-GENERAL OF EUAC
(ISABEL.KOCH@WILHELMA.DE)
AND COPY TO
Max Janse: m.janse@burgerszoo.nl; Brian Zimmerman: bzimmerman@bzsociety.org.uk

3 LOCATION OF PROJECT (REGION & COUNTRY)
Western Pacific Ocean, Micronesia - Palau

4 PROJECT START AND END DATES:
March 2024 – March 2025

5 PROJECT CO-ORDINATOR, ADDRESS AND INSTITUTIONAL AFFILIATION
(IF DIFFERENT FROM APPLICANT)

6 PROJECT TYPE
(TICK ANY COMPONENTS THAT APPLY)

☑ BIOLOGICAL/ECOLOGICAL RESEARCH
☐ VETERINARY/CONSERVATION MEDICINE
☐ ANIMAL WELFARE
☑ CAPTIVE BREEDING
☑ RE-INTRODUCTION/RE-STOCKING/TRANSLOCATION
☐ HUMAN-WILDLIFE CONFLICT
☐ EDUCATION/PUBLIC AWARENESS
☑ TRAINING/WORKSHOPS
☐ COMMUNITY-BASED/SOCIAL POLICY
☐ ECOTOURISM/SUSTAINABLE DEVELOPMENT
☑ SUSTAINABLE USE
☐ WARDENING/LAW ENFORCEMENT
☐ PROTECTED AREAS MANAGEMENT
☐ EX SITU PROJECT ONLY
☐ OTHER: _____

7 FOCAL SPECIES (COMMON AND SCIENTIFIC NAME)

Collector sea urchin, *Tripneustes gratilla*
Hard coral - *Acropora digitifera*

8 IUCN RED LIST STATUS (OR OTHER THREAT LISTING) OF FOCAL SPECIES

Collector sea urchin, *Tripneustes gratilla* - NO

Hard coral - *Acropora digitifera* – YES NT

APPENDIX

Collector sea urchin, *Tripneustes gratilla* - NO

Hard coral - *Acropora digitifera* – Appendix II

9 PROJECT BACKGROUND

Anthropogenic driven climate change is causing significant loss of biodiversity in coral reef habitats resulting in a global decline. This has led some researchers to suggest human intervention, through active restoration, is increasingly important. However, reef restoration efforts, particularly utilizing genetically diverse sexual coral propagation, need up-scaling to have ecologically meaningful impact. Coral post-settlement survival bottlenecks, partly due to competitive benthic algae interactions, are a significant cause of coral mortality. Recent research shows that survival and growth of sexually propagated corals is enhanced when newly settled spat are co-cultured with ex situ cultured juvenile sea urchins, *Mespilia globulus* (Craggs *et al*., 2019). This leads us to speculate that co-culturing urchin species may have significant mariculture potential. For example, the Collector sea urchin *Tripneustes gratilla* is consumed in countries across SE Asia and Oceania. Anecdotal evidence from Palauan fishers suggest that Collector sea urchins have declined due to overharvesting, highlighting a need to develop urchin mariculture to replace wild harvesting. While Palau's reefs are still relatively diverse, the global decline in coral reef health has led to increased interest in reef restoration technology. Co-culturing could boost coral production for reef restoration, and generate an alternative local sustainable livelihood through urchin farming.

10 WAS THE OVERALL PROJECT PURPOSE FULFILLED?

The aquarium staff at Palau International Coral Reef Center (PICRC), both Palauan and international, were trained on phytoplankton culturing, sea urchin larval rearing and sexual coral propagation techniques.
Urchin larvae have been reared to settlement, although not in numbers great enough to trial co-culturing with coral juveniles.

11 WHAT OBJECTIVES WERE MET?

Mariculture and reef restoration capacity were developed through facility development at PICRC – specifically in reference to the new culturing facility.

Training was provided on all aspects of spawning and rearing urchins, the culturing techniques of their larval stage microalgae food source, and methods to enhance settlement from planktonic to benthic life stages and grow–out

Full training in sexual reproductive modes of broadcast spawning corals and culturing methods, including in vitro fertilization techniques, embryo rearing settlement and grow-out, for restoration, were provided to PICRC members of staff.

Outreach with the broader local community, including schools, to provide a vital link between the research and its applied output in the future.

WHAT OBJECTIVES WERE NOT MET?

Trial of in situ co-culturing coral juveniles (*Acropora digitifera*) with the Collector sea urchin (*Tripneustes gratilla*). Whilst *T. gratilla* have been successfully spawned and reared, this will need to be repeated approximately 2 months prior to *A. digitifera* spawning in 2025 to trial co-culturing.

12 WHAT PROJECT ACTIVITIES WERE UNDERTAKEN?

Preliminary work undertaken at The Horniman to further develop spawning and rearing methods for *Tripneustes gratilla* to maximise juvenile output. Five different rearing vessels were trialed, these were: MoLaRS (Modular Larval Rearing System), 5L bowls with fine mesh to allow water exchange, 200L static cone, 20L static cone and flat sided kreisel with air repeater (as developed by Martin Moe, "Diadema Culture Manual" 2022). Three species of phytoplankton were trialed as food sources for developing urchin larvae – Tisochrysis lutea, Chaetoceros calcitrans and Rhodomonas salina.

Collection and spawning of sea urchin broodstock in Palau.

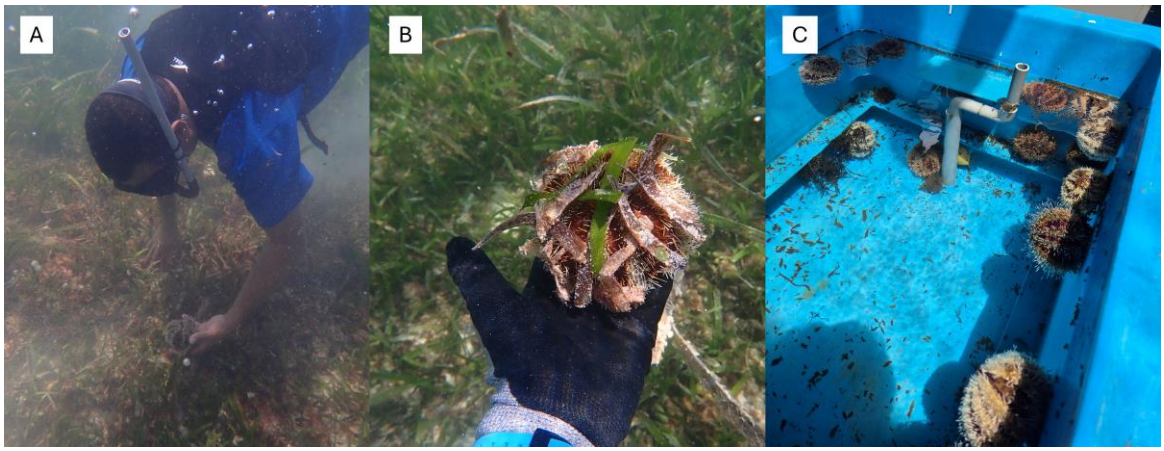

Figure 2. A) Aquarium Supervisor, Asap Bukurrou, collecting *T. gratilla* broodstock in seagrass habitat **B)** Healthy, full grown urchins were found, although very low abundance **C)** broodstock holding system at PICRC, the broodstock were fed a mix of wild collected macroalgae species.

A trial for collecting wild sea urchin larvae by placing artificial settlement media at two locations near PICRC. Pre-conditioned settlement media (cultured with the benthic diatom Nitzschia sp.) was tested against media conditioned in aquarium tanks (natural biofilm) at two locations; one located very close to PICRC aquarium and one in a nearby seagrass bed.

Figure 3. A) Wild sea urchin recruitment trial in seagrass bed and **B)** off of PICRC dock. **C)** Recycled dry slope ski matting was conditioned with a benthic diatom (Nitzschia sp.) in an 30L tank before being placed at the two sites for 2 weeks.

Construction of a new phytoplankton and sea urchin larval rearing facility, including:

- Temperature controlled room
- High power grow lights with removable shading
- Filtered air source (0.5 micron) with CO2 injection equipment
- Filtered tap and seawater
- Seawater sterilisation station
- Space to culture up to eight 3L vessels for each of six different species of phytoplankton and ten 20L cones for urchin larvae (individually temperature controlled)
- Cleaning area for culture vessels

Figure 4. A) Lighting PAR was tested before instalaltion to confirm density of lights and spacing was sufficient. **B)** and **C)** Newly constructed custom phytoplankton culturing rack fitted with high power LED lights.

Figure 5. A) Separate air supply pumps for phytoplankton and urchin larvae rearing air ring mains to allow for CO2 injection only on phytoplankton supply. Both supplies 0.5 micron filtered with reusable filters. **B)** Tisochrysis lutea being cultured, younger cultures have custom light shades to avoid light stress, as cultures become more dense, and hence self-shading, the light shades are removed to maximise growth.

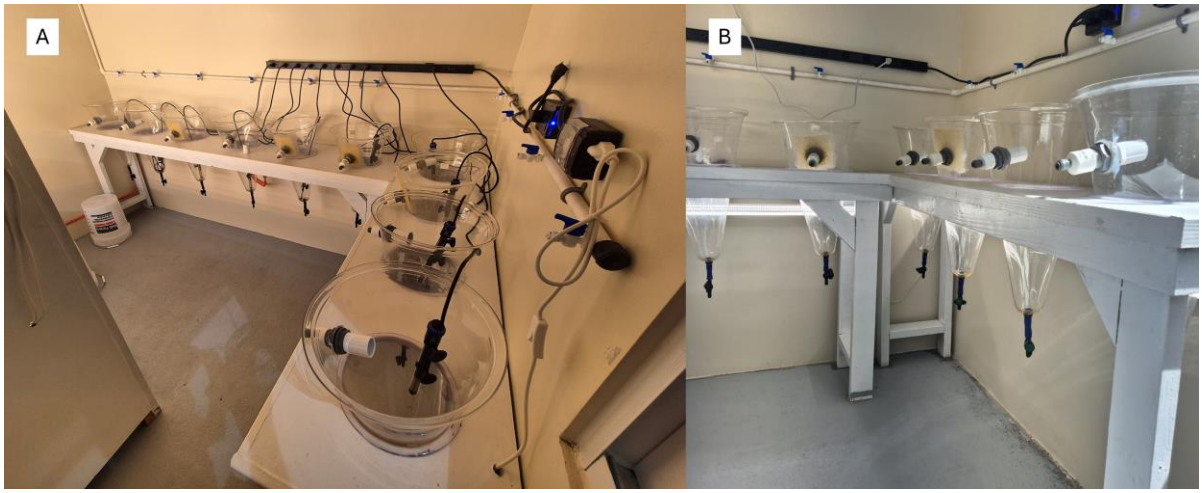

Figure 6. A) Sea urchin larval rearing setup with air ring main and individual heaters. **B)** LED lights fitted under rearing cone table, these can be swtiched on to encourage a biofilm to develop on the inside surface of the cones to provide a food source for newly settled urchins.

Aquarium staff at PICRC were trained on the use of the new lab equipment and algae culturing techniques, urchin husbandry, coral spawning, rearing and ongoing care.

13 WHAT OUTCOMES WERE ACHIEVED DURING THE COURSE OF THE PROJECT? IF THIS WAS AN EX SITU PROJECT ONLY, WHAT WERE THE BENEFITS TO THE SPECIES EX SITU AND IN SITU?

Construction of a microalgae culturing and sea urchin larvae rearing systems to accommodate the project work. As a result of this new facility, Palau Aquarium is now culturing 5 different species of phytoplankton, including *Isochrysis spp., Chaetoceros sp.,* and some local strains of *Zooxanthellae* and *Nitzschia sp.* (Fig. 7). The phytoplankton is being used to condition giant clam broodstock and to feed clam and coral juveniles.

Sea urchin broodstock was successfully collected for conditioning and spawning *ex situ*.

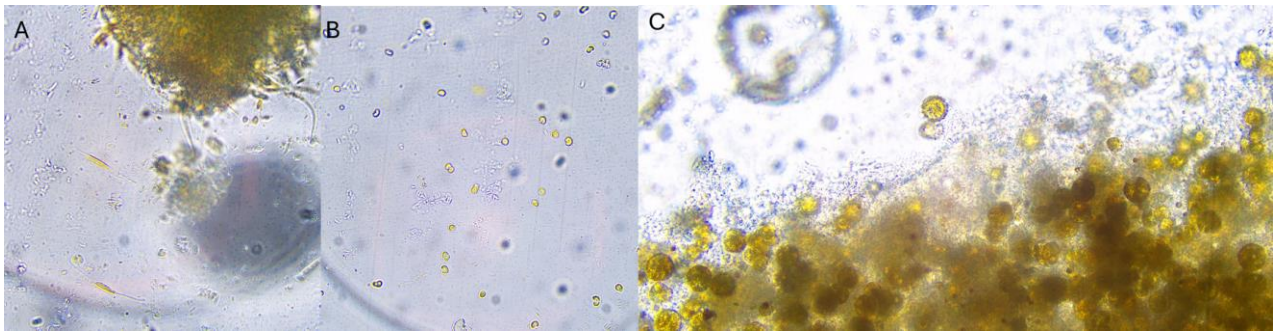

Figure 7. Microscope photos of **A)** Nitzschia sp. grown from a swab of a benthic biofilm on one of the giant clam raceway tanks, **B)** Isochrysis sp. grown from a seawater sample taken from the PICRC dock and **C)** Zooxanthellae cells taken from a giant clam mantle.

Immediately after collection of broodstock on March 26th 2024, spawning was observed, with 2 individuals releasing sperm and 2 individuals releasing eggs. The eggs were fertilised and stocked at 3 embryos/mL in 3 separate 20L cones. However, this occurred prior to the construction of the culturing lab and with no temperature control or phytoplankton culture, no larvae were found at day 5 post spawning.

Spawning was then attempted a further four times, where each time one individual released sperm, suggesting the induction method was appropriate, but no individuals released eggs. It was hypothesised this could indicate either a poor diet or strict seasonality in spawning period.

On October 2nd 2024, PICRC aquarium team successfully spawned both male and female *T. gratilla*. Sperm from 3 individuals was collected, pooled and used to fertilise eggs from 3 urchins. Overall fertilization rate was poor (30%), yielding 362,083, 90,000 and 195,611 fertilised embryos from each urchin, respectively.
Embryos were stocked in 9 of the 10 cones in the newly constructed culturing lab. Cones were stocked at 1, 3 and 9 embryos mL-1 in triplicate. Each triplicate cone was stocked with eggs from just 1 individual.
Larvae were given a daily water change of 50% culture volume each day, with 1µm filtered seawater. Feeding of 15mL Isochrysis galbana was provided every other day, based on culture water turbidity. All cultures were maintained at 29°C using aquarium thermostat heaters.
Surviving larvae were counted on 16th October (14 days post fertilization).

At this time, survival was as follows:

Cone number	Initial number stocked	Count on 16th October
2	7,000	2,800
4	21,000	16,100
9	63,000	7,700
10	63,000	22,400

(Cones not listed had negligible survival.)

Losses are possibly due to inconsistent feeding, as the food provided was not adjusted to match the density of urchins in the cones, combined with power cuts leading to a lack of aeration.

To date, newly settled urchins have been seen in the rearing cones (Figure 8), suggesting phytoplankton

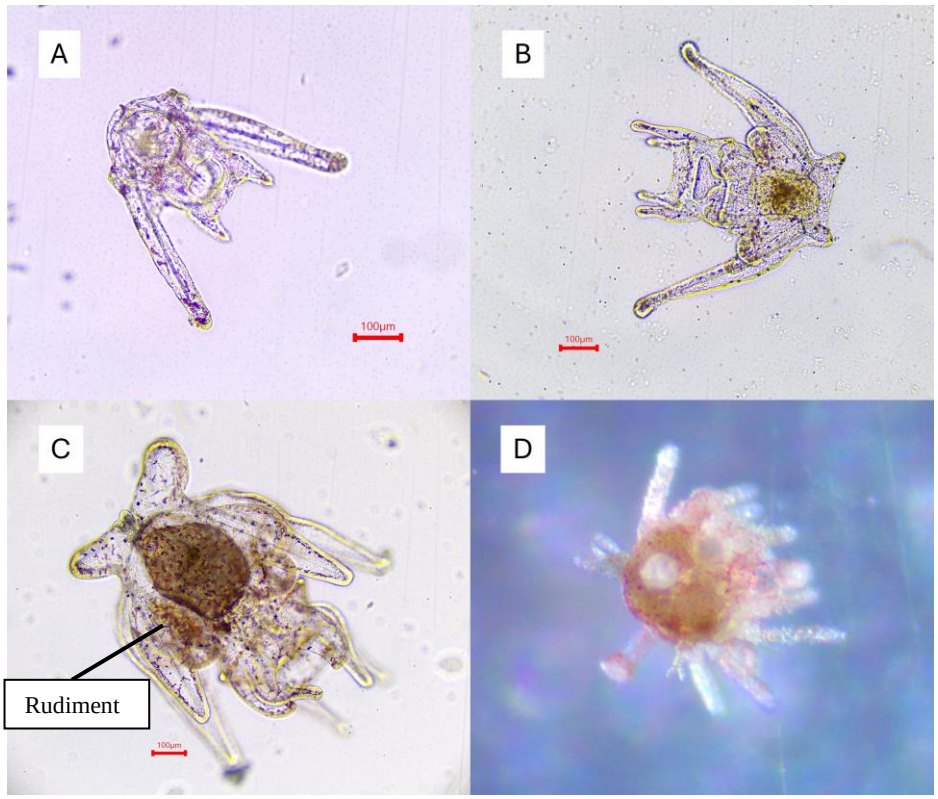

Figure 8. *T. gratilla* pluteus at **A)** day 7, **B)** day 12 and **C)** day 19 post spawning. Rudiment development can be clearly seen in **C)**. **D)** shows *T. gratilla* shortly post settlement.

ARE ANY ONGOING?

The Palau Aquarium team are continuing to work on improving *T. gratilla* rearing methods and The Horniman continues to support this remotely. Once reared in sufficient numbers, they will conduct a co-culturing trial with both *A. digitifera* and with multiple giant clam species.
After this we can statistically analyse any increase in coral production as a result of co-culturing approach compared to current methods of Coralassist team.
When sufficient numbers of *T. gratilla* have been cultured a trial of *in situ* coral and sea urchin grow out approaches will take place.

DID ANY EXPECTED OUTCOMES FAIL?
N/A

14 DID LOCAL PEOPLE/COMMUNITIES PARTICIPATE IN THE PROJECT? IF SO, WHO WERE THEY, HOW MANY PARTICIPATED AND WILL CONTINUED CONTACT BE MADE?

The project supported existing staff members at PICRC, both Palauan and international, who will provide an important link between researchers and the community.

The project has had further impact through existing engagement community programs run at PICRC including running a practical phytoplankton culturing class with students from Palau Community College (PCC)

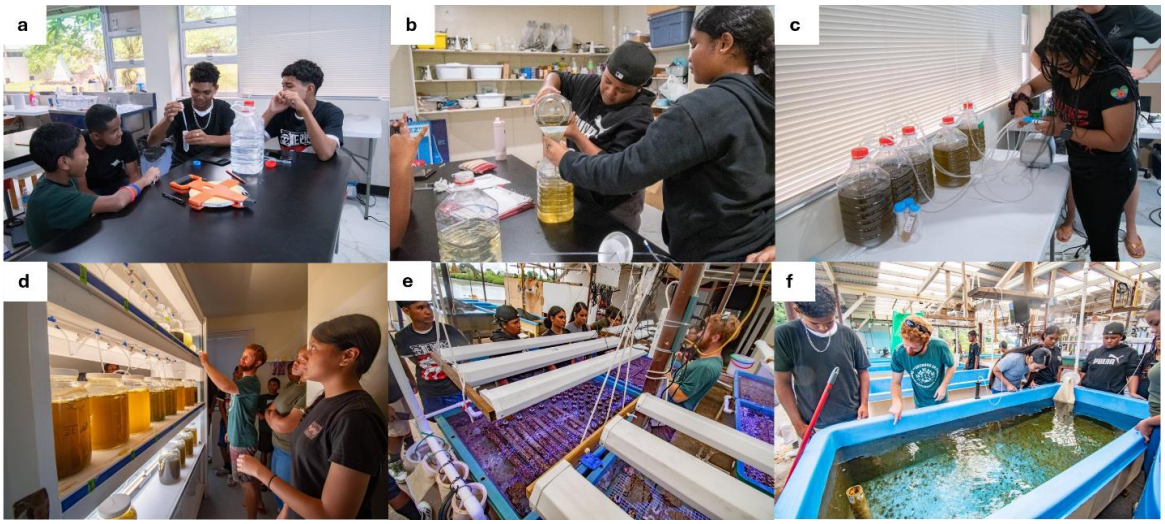

Figure 9. Students from PCC **A)** Preparing media, **B)** Inoculating media with phytoplankton, **C)** Connecting aeration and lights, **D)** Visiting the culturing lab, **E)** Feeding coral recruits and **F)** Feeding giant clam juveniles.

IF THERE WAS COLLABORATION WITH ANOTHER EUAC MEMBER OR AQUARIUM PLEASE PROVIDE DETAILS ONTHE COLLABORATION.

N/A

15 DID THE GOVERNMENT OF THE HOST COUNTRY RECEIVE INFORMATION ON THE PROJECT'S RESULTS?

PICRC have a close working relationship with the Palauan government and provide updates on ongoing projects. The president visited PICRC whilst the main stage of the project was undergoing and showed interest in the project.

16 HOW DID THE RELATIONSHIP WITH OTHER NGOS WORK? WERE THERE ANY ISSUES?

There has been a longstanding relationship between Horniman Museum and Newcastle University and Newcastle University and PICRC. The project has helped build a strong relationship between The Horniman and PICRC which hopefully will continue to grow. There were no issues to report.

17 TOTAL PROJECT BUDGET AND EXPENDITURE (IN EUROS)
Submitted budget: 22721,00 €
Total spend: 22438,95 €

18 AMOUNT OF MATCHING FUNDS SPENT:
12438,95 €

19 AMOUNT SPENT FROM EUAC FUNDS:
10000,00 €

Horniman Museum	7752,97 €
Newcastle University	1303,63 €
PICRC	3382,35 €
EUAC	10000,00 €

20 EXPENDITURE BREAKDOWN (IN EUROS)

TRAVEL	2458,98 €
SALARIES	5882,35 €
ACCOMMODATION	2127,06 €
EQUIPMENT	10,439,74 €
COMMUNICATION	1176,47 €
MISCELLANEOUS (PLEASE DETAIL)	354,35 €
TOTAL	22438,95 €

21 PUBLICATIONS PRODUCED AS A RESULT OF THE PROJECT

Publications are in the process of being written up and EUAC will be kept updated as this progresses.