Biodiversity Challenge Funds Projects
Darwin Initiative, Illegal Wildlife Trade Challenge Fund, and Darwin Plus

Half Year Report

It is expected that this report will be a **maximum of 2-3 pages** in length.

If there is any confidential information within the report that you do not wish to be shared on our website, please ensure you clearly highlight this.

Submission Deadline: 31st October 2024

Please note all projects that were active before 1 October 2024 are required to complete a Half Year Report.

Submit to: BCF-Reports@niras.com including your project ref in the subject line.

Project reference	*This should be the project reference provided in your offer paperwork and **not** your application number*
Project title	Barcoding an Island: expanding genetic biomonitoring on Ascension
Country(ies)/territory(ies)	Ascension Island
Lead Organisation	Ascension Island Government
Partner(s)	University of Edinburgh (UOE), Natural History Museum (NHM)
Project leader	Tiffany Simpson
Report date and number (e.g. HYR1)	*HYR3*
Project website/blog/social media	*n/a*

1. Outline progress over the last 6 months (April – September) against the agreed project implementation timetable (if your project started less than 6 months ago, please report on the period since start up to end of September).

Although we are not looking for specific reporting against your indicators, please use this opportunity to consider the appropriateness of your M&E systems (are your indicators still relevant, can you report against any Standard Indicators, do your assumptions still hold true?). The guidance can be found on the resources page of the relevant fund website.

During the 6-month period of April 2023 to September 2024, the DPLUS165 project has been continuing to meet objectives set out in the log frame. Unfortunately, due to delays with project partners and collaborators, Output 1 is behind schedule as mentioned in the previous annual report. All other Output streams are progressing as expected. Mitigation of the delays to Output 1 are being put in place and primers (COI, 12s and 18s) that are used in a high number of other studies are being used to identify the presence of organisms to as specific a taxonomic level as possible.

1.1 Locate all verified invertebrate specimens from past and current studies and obtain tissue sample from each.

Progress – behind schedule – Unfortunately there has still been little progress on this objective but positive news in the form of SAERI samples being located and prepared to send to NHM means this should be completed before project end.

1.2 Genetic sample identification number added to Ascension Biodiversity database.

Progress – behind schedule – Due to issues mentioned in 1.1 some samples still do not have a genetic sample identification number; they have been located but no barcodes have been generated as of yet.

1.3 Barcoding or full sequencing of all samples collated in activity 1.1 by NHM or commercial laboratory. Protocols will follow those required for inclusion within the Darwin Tree of Life database.

Progress – behind schedule – Samples from settlement plates were transported to NHM for barcoding and identification however these samples have yet to be processed by project partners due to commitments to other projects. It is hoped that these will be analysed alongside the samples from SAERI mentioned in output 1.1 and samples from the DPLUS135 terrestrial invertebrate project.

1.4 University of Edinburgh to develop a suite of primers that allow discrimination of species collated in output 1.1.

Progress – behind schedule – Due to delays with the other activities in Output 1 these primers will likely not be developed in time for the end of project. It is likely that the discrimination of the species from output 1.1 will be possible with the primers that are currently in use on Ascension. Should any of the specimens not be picked up via the universal primers then it is hoped a close working relationship with partners at the University of Edinburgh would allow for development of more specific primers after project end.

2.1 **Write and test protocols for DNA extraction and metabarcoding**

COMPLETED – all protocols have been written and tested including extra protocols for the use of the Nanopore Minion Sequencer.

2.2 **Create training reference documents and deliver practical training course in DNA extraction and metabarcoding for ten members of AIGCFD.**

**COMPLETED – ** Reference documents and protocols for staff to follow have been completed and were attached in previous annual reports. Training has been completed with key members of staff to ensure the longevity of DNA barcoding on Ascension. As of the writing of this report 17 members of staff have been trained in some aspect of the DNA workflow.

2.3 **Metabarcoding results from AIGCFD staff quality assured by comparing with those from Project Officer and University of Edinburgh**

**COMPLETED – ** Matched samples have been run through the workflow by both newly trained staff and the project officer with satisfactory results showing the staff training is adequate to allow for AIGCFD staff members to perform DNA metabarcoding independently if they have time outside of current job roles. External laboratories in UOE and Curtin University have also validated results to ensure the workflow on Ascension is providing results to a similarly high level as these institutions.

3.1 AIGCFD staff collect monthly samples from 3 pitfall traps, 2 malaise traps, 3 inshore settlement panels and 2 light traps over six month period and preserve samples in ethanol.

COMPLETED AND ONGOING – As previously mentioned slight changes have been made to sampling strategy and methods to ensure safety of staff. Light trapping is no longer a viable option on Ascension due to strong swells and destruction of sampling equipment – the project lead and officer have decided to gather samples from plankton tows which should provide the same information as a light trapping sample. Work on this output began early and as such there are over 1 year of plankton and settlement panel samples. Every 3 months since Sept 23, Pitfall traps and malaise trap sample gathering is performed at 15 locations, increasing the sampling effort that was suggested in the activity. The below table shows the activity's expected sampling effort and the actual sampling effort.

Type	Expected	Actual	Notes
Pitfall	3 traps monthly for 6 months = 18	15 traps every 3 months for 12 months = 60	*not all traps contain specimens so sampling effort and samples produced are not equal
Malaise	2 traps monthly for 6 months = 12	15 traps every 3 months for 12 months = 60	*not all traps contain specimens so sampling effort and samples produced are not equal
Settlement	3 panels monthly for 6 months = 18	3 settlement arrays every 3 months for 18 months = 18	
Plankton	2 tows monthly for 6 months = 12	2 tows during full and new moon for 18 months = 36	

On top of the increased sampling efforts, 1L seawater eDNA samples are being collected from 10 shallow water areas of the MPA around Ascension every 6 months for biomonitoring (current total of 150 samples) alongside samples from citizen scientists on board yachts travelling in the wider MPA that AIGCFD vessels cannot access (total 44 samples).

3.2 Metabarcoding of samples collected in output 3.1 by trained AIGCFD staff

COMPLETED AND ONGOING – As mentioned in Activity 2.3, trained members of AIGCFD staff have been performing metabarcoding on samples obtained in Activity 3.1. Due to the higher amount of sampling effort when compared to the expected number of samples, 219 have been sequenced via metabarcoding. As sampling is continuing as part of the AIGCFD's commitment to biosecurity, more samples will be run periodically.

3.3 Pass any detections of high priority invasive species to AIG Biosecurity Team for response action.

ONGOING – As of Oct-24 there have been 6 species highlighted to the AIG Biosecurity team. Of these, 3 were mussel species that were collected from the hull of a shipping vessel which visits both Ascension and St Helena. Samples were collected as part of a joint OT effort and identified as

- *Mytilus sp.*

- *Semimytilus algosus*
- *Choromytilus meridionalis*

Both *Mytilus sp.* and *Semimytilus algosus* were highlighted in the horizon scanning exercise by Roy et al. 2019 as having the potential to cause significant impacts to biodiversity. Monitoring equipment in the form of a settlement rope has been deployed in Clarence Bay and will be checked monthly for any settlement of mussels.

Other identified biosecurity threats include the plant pathogen *Phytophthora sp*. and the ant species *Monomorium sahlbergi.* Further increased sampling for both of the species was undertaken to better understand the location and possible impact of them on the island's biodiversity. The Cotton Aphid (*Aphis gossypii)* was also identified and brought to the attention of the AIG Biosecurity team however upon further investigation it seems this species was found on Ascension Island prior to it being highlighted in the horizon scanning exercise.

3.4 Produce a summary report listing species detected in samples by metabarcoding

ONGOING – Although this is not due to be completed until the project end a comprehensive record is kept updated with sequences and species after each sequencing library is run.

4.1 Select 10 indicator marine species for gut contents analysis

COMPLETED – species have been selected from different trophic levels.

4.2 University of Edinburgh to develop blocking primers for the indicator species

ONGOING – behind schedule – Collaborators at UOE have been given list of indicator species and samples of gut content DNA from each species have been sent to allow for primer testing to go ahead. Primers are currently under development and will be on Ascension before the end of the project to allow for the gut content sequencing to be undertaken.

4.3 Conduct metabarcoding analysis on gut contents of ten individuals from each of the indicator species

ONGOING – All samples except for the Ascension Wrasse have been collected and are awaiting sequencing. Preliminary sequencing was undertaken on a subset of samples to assess the percentage of host reads compared to prey item reads to determine if blocking primers are necessary.

4.4 Conduct traditional gut content analysis on same samples and compare the results of the different methods in a report

ONGOING – As expected, a traditional analysis of gut contents yields very few species identifications due to lack of taxonomic expertise; however all samples with identifiable prey items were catalogued. The gut contents of smaller fish species such as the Sergeant Major, Black Triggerfish and Wrasse are non-identifiable visually and as such cannot be used to draw comparisons to barcoding.

5.1 Organise school visits to the AIG DNA lab and lead practical lessons on DNA extraction.

COMPLETED - Students have visited the AIG DNA lab and conducted sample gathering and filtering steps. Visits to the school to highlight the work of the DPLUS165 project have been undertaken and students taking part in the Marine Biology qualification have expressed interest in learning further lab techniques. To further build on the outreach aspect of the project a small portable lab will be taken to the school before the end of the project to enable a larger number

of students the opportunity to do DNA extractions - size limitations in the DNA laboratory currently make it difficult to have lessons with more than 3 people at a time.

2. Give details of any notable problems or unexpected developments/lessons learnt that the project has encountered over the last 6 months. Explain what impact these could have on the project and whether the changes will affect the budget and timetable of project activities.

There have been no notable problems over the past 6 months and delays to Output 1 were highlighted in the second annual report. The success of the DPLUS165 project mean that the detection of invasive species leads to the need for further increased sampling efforts. These biosecurity samples must take priority over others due to the need to determine quickly the exact species and location of biosecurity threats on Ascension to allow for informed management decisions. As such there may be certain outputs such as Gut Content analysis that are not prioritised due to the non-urgent nature of the samples.

3. Have any of these issues been discussed with NIRAS and if so, have changes been made to the original agreement?

Discussed with NIRAS:	Yes/ No
Formal Change Request submitted:	Yes/ No
Received confirmation of change acceptance:	Yes/ No

Change Request reference if known: *If you submitted a financial Change Request, you can find the reference in the email from NIRAS confirming the outcome*

4a. Please confirm your actual spend in this financial year to date (i.e. from 1 April 2024 – 30 September 2024)

Actual spend:

4b. Do you currently expect to have any significant (e.g. more than £5,000) underspend in your budget for this financial year (ending 31 March 2025)?

Yes ☐ **No** ☒

4c. If you expect and underspend, then you should consider your project budget needs carefully. Please remember that any funds agreed for this financial year are only available to the project in this financial year.

If you anticipate a significant underspend because of justifiable changes within the project, please submit a re-budget Change Request as soon as possible. There is no guarantee that Defra will agree a re-budget so please ensure you have enough time to make appropriate changes to your project if necessary. Please DO NOT send these in the same email as your report.

NB: if you expect an underspend, do not claim anything more than you expect to spend this financial year.

5. Are there any other issues you wish to raise relating to the project or to BCF management, monitoring, or financial procedures?

N/A

6. Please use this section to respond to any feedback provided when your project was confirmed, or from your most recent annual report. If your project was subject to an Overseas Security and Justice Assistance assessment please use this space to comment on any changes to international human rights risks, and to address any additional mitigations outlined in your offer letters. Please provide the comment and then your response. If you have already provided a response, please confirm when.
Discussion with BCF admins regarding delays to Output 1 can be facilitated if deemed necessary, the project can continue without sequences produced by NHM due to ample data in pre-existing reference databases however the lack of input in the project from NHM should be addressed. Hopefully barcoding of historic specimens can still be completed before the projects end date.

Checklist for submission

For New Projects (i.e. starting after 1st April 2024)	
Have you **responded to any additional feedback** (other than caveats) received in the letter you received to say your application was successful which requested response at HYR (including safeguarding points)? You should respond in section 6, annexes other requested materials as appropriate.	
If not already submitted, have you attached your **risk register**?	
For Existing Projects (i.e. started before 1st April 2024)	
Have you responded to **feedback from your latest Annual Report Review?** You should respond in section 6, annexes other requested materials as appropriate.	Y
For All Projects	
Include your **project reference** in the subject line of submission email.	Y
Submit to **BCFs-Reports@niras.com**.	Y
Have you **clearly highlighted any confidential information** within the report that you do not wish to be shared on our website?	Y
Have you reported against the most **up to date information for your project?**	Y
Please ensure claim forms and other communications for your project are not included with this report.	Y