Darwin Plus:
Overseas Territories Environment and Climate Fund
Annual Report

To be completed with reference to the "Project Reporting Information Note"
(https://darwinplus.org.uk/resources/information-notes)

It is expected that this report will be a **maximum of 20 pages** in length, excluding annexes)

Submission Deadline: 30th April 2023

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

Darwin Plus Project Information

Project reference	DPLUS165
Project title	Barcoding an Island: expanding genetic biomonitoring on Ascension
Territory(ies)	Ascension Island
Lead Partner	Ascension Island Government
Project partner(s)	University of Edinburgh, Natural History Museum
Darwin Plus grant value	£199,300.00
Start/end dates of project	1/7/22 – 1/7/24
Reporting period (e.g. Apr 2022-Mar 2023) and number (e.g. Annual Report 1, 2)	July 2022 – Mar 2023 Annual Report 1 (Y1Q2-Y1Q4)
Project Leader name	*Diane Baum*
Project website/blog/social media	n/a
Report author(s) and date	Melissa Morgan 28/04/23

1. Project summary

Identifying species is fundamental to biodiversity conservation. However, it is not easy. Modern molecular techniques offer a solution to this problem by providing species identification that is more accurate and efficient than standard taxonomic methods. Through this project, Ascension will enter the DNA age. The development of reference barcodes, analysis tools, and provision of training in DNA metabarcoding techniques will result in the Conservation Team on Ascension having the capacity to identify multiple species across a range of taxa.

2. Project stakeholders/partners

The project partners University of Edinburgh (UOE) and Natural History Museum (NHM) have been involved since the DPLUS165 project applied for funding. Project partners are involved in meetings where relevant to their expertise and are consulted on decisions regarding Outputs 1,2,3 and 4.

The project officer met with UOE partners in October before travelling to Ascension. The discussion involved the possible limitations to sequencing in a remote location, primer design, quality assurance steps, and the use of a Nanopore Minion device to allow for longer read sequences to be gathered.

Meetings with NHM to discuss the number of samples to be sent for barcoding and the timeframe for this process have been useful in keeping the project on track.

As the internet connection on Ascension can be somewhat unreliable, video call meetings are performed when there are major project milestones to discuss. Regular contact with project partners is maintained via email.

3. Project progress

3.1 Progress in carrying out project Activities

Progress for activities so far this year are as follows. Under Output 1, in which the aim is for DNA primers to be developed for the detection and identification of principal Ascension Island terrestrial and marine invertebrate species, there are three activities due to have begun.

Activity 1.1
Activity 1.1 to locate all verified invertebrate specimens from past and current studies and obtain a tissue sample from each was due to begin in Y1Q3. Invertebrate specimens are in the process of being catalogued and prepared to send to NHM (Annexe 4.1: Figure 1). Samples that are already located at NHM have been identified and a list sent to NHM. Some samples which were collected on Ascension but since moved off the island are in the process of being located and will hopefully be sent on to NHM for barcoding. There is a database cataloguing these specimens that can be updated with new entries.

Activity 1.2
Activity 1.2, to add genetic sample identification numbers to the Ascension Biodiversity database, is almost completed. Where any marine or terrestrial organisms have pre-published barcodes, the accession numbers have been added to the database (Annexe 4.1: Figure 2). Once samples are transported to NHM then the unknown barcode samples will be updated.

Activity 1.3
Activity 1.3 to begin barcoding or full sequencing of all samples collated in Activity 1.1 was due to begin in Y1Q4. This activity has not yet begun, talks are ongoing with NHM to determine the best method of sending samples for sequencing and should remain on track for the Y2Q4 completion date.

The remaining activities in Output 1 are not due to begin until later in the project.

Output 2 has the aim of training and allowing Ascension Island Government Conservation and Fisheries Directorate (AIGCFD) staff to carry out DNA extraction and metabarcoding.

Activity 2.1
Only Activity 2.1, to write and test protocols for DNA extraction and metabarcoding, was due to begin this year. There has been a small delay in completion of this activity due to shipment issues for sequencing reagents. All protocols up to the sequencing step have been tested (Annexe 4.1: Figure 3) and are working as expected. Sample tracking databases allow for the determination of which steps of the process each sample has progressed to (Annexe 4.1: Figure 4). Sequencing results should be ready for analysis by May 2023 (Y2Q1) and can be compared to sub-sample results which were sent to a verified sequencing laboratory.

Remaining activities in Output 2 are not due to begin until later in the project.

Output 3, to use Metabarcoding to identify terrestrial and marine invertebrate species as part of AIGCFD monitoring and biosecurity surveillance activities, is not due to be started until Y2Q2.

Activity 3.1
Progress is already being made as samples mentioned in activity 3.1 (AIGCFD staff collect monthly samples from 3 pitfall traps, 2 malaise traps, 3 inshore settlement panels, and 2 light traps over six months and preserve samples in ethanol) have already been collected and stored

for future sequencing. There has been some disruption due to difficulty reaching sampling locations and damage to some monitoring stations. However, as this is not due to begin until Y2Q2 there is time to adapt locations/sampling times to accommodate this.

Activity 3.4
A database of barcodes generated from Ascension samples and corresponding species matches from public databases is being kept to enable a final report to be drafted for biosecurity purposes as mentioned in Activity 3.4 (Annexe 4.1: Figure 5).

Output 4, to perform metabarcoding analysis on gut content samples to validate an isotope ecosystem model, was due to begin in Y1Q4. Indicator species from which to collect the gut contents have been decided and primer design is being discussed with UOE.

Activity 4.1
Indicator species have been selected which include multiple trophic levels and are obtainable. The species selected are, *Thunnus albacares* (Yellowfin Tuna), *Epinephelus adscensionis* (Rock Hind Grouper), *Gymnothorax moringa* (Spotted Moray), *Melichthys niger* (Black Triggerfish), *Caranx lugubris* (Black jack)*, Abudefduf saxatilis* (Sergeant Major), *Thalassoma ascensionis* (Ascension Wrasse), *Exocoetidae* (Galapagos shark), *Acanthocybium solandri* (Wahoo) and Exocoetidae (Flying fish).

Remaining activities in Output 4 are not due to begin until later in the project.

Activity 5.1
The final output of the project, to enable secondary school students on Ascension to understand how DNA biomonitoring techniques are carried out and their application for conservation, is not due to begin until Y2Q1. Activity 5.1 to encourage visits from students to visit the DNA lab has already begun with 16 students from the local school visiting the DNA lab and filtering eDNA samples in Feb 2023.

3.2 Progress towards project Outputs

Output 1
In Output 1, the aim is for DNA primers to be developed for the detection and identification of principal Ascension Island terrestrial and marine invertebrate species. Currently, the project is using previously published primers to allow for the detection and identification of Ascension Island species. Some samples have been extracted and are awaiting sequencing from terrestrial and marine invertebrate species. If any key species are difficult to identify using these primers then there is ample time left in the project to design more specific primers that amplify Ascension's species specifically. This output can be measured via the Ascension DNA sequencing database which contains details of the location a sample was taken from along with primers used and species detected.

Output 2
The second output of the project is to ensure AIGCFD staff can carry out DNA extraction and metabarcoding to ensure the longevity of barcoding on Ascension. Protocols have been written and tested on Ascension to allow for the continuation of DNA barcoding work once the project officer leaves post (Annexe 4.2: File 1 – PDF - Lab protocols Dplus165). Five staff members have been trained in how to do the start of the protocol from sample collection to DNA extraction of samples and training will continue to reach the end of the sequencing workflow during Y2. Staff members who are trained in each technique will be recorded in the DNA Training Spreadsheet (Annexe 4.1: Figure 6) to ensure that there is enough redundancy that staff can continue barcoding once the project officer leaves their post.

Output 3
Output 3 has the focus of using metabarcoding methods to identify terrestrial and marine invertebrate species as part of AIGCFD monitoring and biosecurity surveillance activities.

Although this output is not due to begin until Y2Q2 there are already settlement plate and light trap samples from the previous year alongside DPLUS135 terrestrial invert samples of interest that have been collected and are awaiting analysis (Annexe 4.1: Figure 7). As this Output is not due to begin until Y2 there is no measurement thus far of progress. Once sample collection starts then the Sample Collection Database and sequencing database will be the main reference for progress.

Output 4
The fourth output of the DPLUS165 project is to perform gut content analysis using metabarcoding techniques to validate an isotope-based ecosystem model. Indicator species have been selected, which are representative of multiple trophic levels and are assumed to be feasible for collection. These samples will be gathered over the next coming year to include a temporal and spatial range. Depending on fishing activity, 10 samples from 10 species may not be gathered and analysed on time; there is the possibility of having backup indicator species from the same trophic level if any prove difficult to catch.

Output 5
The final output of the project is for secondary school students on Ascension to understand how DNA biomonitoring techniques are carried out and their application for conservation. The Ascension Island MPA youth committee performed a visual assessment of species within a rockpool and then also collected 1L water samples for eDNA analysis. These will be included in the next eDNA library and results will be fed back to the school to show that eDNA has the possibility to detect species that are not always easy to spot visually. A count of the number of students who have performed DNA extractions and collected samples will be recorded and students will be asked what they have learned and enjoyed after the activity.

3.3 Progress towards the project Outcome

The project outcome is that Ascension has the on-island capability to identify species using DNA metabarcoding and the training materials and corporate memory to ensure this capacity is maintained in the long term. Progress is steadily ongoing despite some setbacks with reagent procurement and monitoring station locations. The first of the outcome indicators is not due to be achieved until Y2Q2 as there is a long setup time for this project before deliverables can begin. Indicators are adequate for measuring the intended outcome however none address the initial start of the project.

The first indicator of the project outcome is to have a developed reference library for all invertebrate species identified by previous taxonomic studies by Y2Q2. The reference library is being developed currently and all known invert species on Ascension with publicly available barcode sequences have been included in the reference database. The reference library will be completed once samples have been barcoded by NHM. Unless there are long delays to sample processing or shipping then this should be achieved in time.

Another indicator of the DPLUS165 project outcome is that a suite of primers will be designed and validated by UOE for the targeted metabarcoding of Ascension invertebrates. The project is currently using previously published primers and new specific primers cannot be designed until a DNA barcode reference library has been produced containing sequence information for known invertebrates. As progress on the reference library is running smoothly we see no reason for this indicator not to be achieved by the end of the project.

The third indicator to help determine progress towards the project outcome is that metabarcoding will be used routinely to identify species in AIGCFD's terrestrial invertebrate, gut content, settlement panel, and light trap monitoring programmes by Y3Q2. This is not applicable until Y3Q2. However, it is still a useful indicator and should help monitor progress toward the final outcome. There is no foreseen reason why this should not be achievable

A final indicator of the project is that long-term DNA extraction and metabarcoding capability should be established on Ascension through staff training and protocol creation by Y2Q2. Staff are being trained in the initial sample-gathering steps and sterile technique this year. In Y2 staff

will be trained in the entire workflow to ensure the longevity of DNA sequencing capabilities on Ascension. This is still an achievable goal for the project and training will be provided to as many staff as possible during the project duration. The only limitation to this is the space available in the Ascension Lab as it means only one person can be trained in practical lab techniques at any one time. Personal one-on-one training is taking place which may be more beneficial to staff long term.

It is very early in the project to assess progress towards achieving the outcome, but outputs are on track and nothing has changed in the project logic of how those outputs will lead to the outcome.

3.4 Monitoring of assumptions

The first assumption of the project is that AIGCFD can establish a functioning DNA laboratory on Ascension and train staff to undertake procedures and analysis. Alongside the cold chain for delivery of reagents being maintained.

The above assumption is correct and a functioning DNA laboratory has been established. There have been some setbacks with cold chain delivery of reagents with our shipping agent, Richard James, not storing items at 4 degrees or -20 degrees any longer. The project is currently attempting to test a new method of cold storage for these reagents before they are shipped to Ascension. Although the setback has caused minor delays in procuring reagents it should be remedied in time without causing any change to output time frames.

It was assumed that samples from past surveys (particularly DPLU021) were suitably preserved and would be shared by previous project partners where required. Contact has been made with previous project partners for DPLU021; however, information about the preservation and quality of all samples has yet to be obtained. Samples gathered for DPLUS135 are preserved in a way that is conducive to metabarcoding analysis.

Upon beginning the DPLUS165 project it was assumed that AIGCFD staff would be able to learn and execute procedures. Staff mostly have some previous experience in lab work and the Project Officer has experience in delivering training of required skills and protocols. There are no foreseen issues in training as it can be delivered on Ascension by the Project Officer.

As metabarcoding is such a well-published technique and has been proven useful in a number of studies, it was assumed that metabarcoding would successfully identify species in samples collected on Ascension. Thus far, pre-published primers have been sufficient for a wide range of species. If it becomes evident that there is any need for more specific primers to confidently identify Ascension's invertebrates then UOE have expertise in designing specific primers/ assays.

In order for the gut content analysis (Output 4) to be used as a comparison to the isotope model it was assumed that the Isotope model would be completed before the start of this project. The model has now been completed and can be used comparatively with metabarcoding analysis to assess the reliability of both methods.

Another assumption made for gut content analysis (Output 4) was that at least 10 samples can be collected from the 10 indicator species. Although sample collection from certain species is not as viable due to limitations on entering the water on Ascension, indicator species have been selected that are able to be acquired safely. There will be redundancy species built into the plan if other species are unable to be used.

A final assumption for the DPLUS165 project was that teachers at the school are supportive of adding DNA biomonitoring to the current curriculum. There is a good relationship existing with AIGCFD and the local school, some students have already been to visit the DNA lab and there are already DNA aspects to the student's curriculum.

4. Project support to environmental and/or climate outcomes in the UKOTs

The Ascension Island Biodiversity Strategy and Action Plan (2023) is a strategy that sets the objectives, principles and policies for the protection of Ascension's biodiversity. One of the priorities identified in this document is the need to address the lack of data on invertebrates and incorporate them into monitoring and conservation management programmes. This project, DPLUS165, is supporting this national commitment by increasing the taxonomic identification of marine and terrestrial invertebrates through DNA barcoding to create a sequence library for downstream metabarcoding capabilities. The data generated is also being made publicly available by uploading it to international databases. The development of the sequence library and protocols for rapid, on-site DNA testing will greatly improve the ability of Ascension Island to enact its Biosecurity Strategy and meet the objectives of the Biodiversity Strategy and Action Plan.

Ascension Island is also party to the Convention on Biological Diversity (CBD), a global framework for conserving genetic, species and ecosystem diversity while ensuring sustainable development and benefit sharing. It requires that signatories "7(b) Monitor, through sampling and other techniques, the components of biological diversity." This project directly supports this commitment. Through the development of a DNA lab, genetic barcoding, sampling protocols and metabarcoding workflows, this project is providing a system for generating accurate long-term biodiversity data that is economically sustainable.

5. Gender equality and social inclusion

Please quantify the proportion of women on the Project Board[1].	50%
Please quantify the proportion of project partners that are led by women, or which have a senior leadership team consisting of at least 50% women[2].	33%

The current percentage of the Conservation Department consists of 65% female employees and many of them will be able to increase their skills and knowledge by learning lab techniques and eDNA metabarcoding protocols. The training and opportunities stemming from this project will be fully inclusive, open to all staff regardless of gender. Much of the leadership roles in this project are also held by females, including the AIG project leads, the AIG Project Officer and the post-doctorate partner from UOE.

The outputs from this project will also be fully inclusive. The educational resources, publications and media generated will be accessible to everyone. Similarly, events and activities organised through the school or community groups will be designed to ensure no discrimination based on gender, religion, sexual orientation or disability.

6. Monitoring and evaluation

The monitoring and evaluation component of the project is on track with Outputs and Activities achieved as set out in the Project Logframe and Timetable. The Project Officer and direct Line Manager set deadlines for certain tasks to monitor and evaluate the project's progress against

[1] A Project Board has overall authority for the project, is accountable for its success or failure, and supports the senior project manager to successfully deliver the project.

[2] Partners that have formal governance role in the project, and a formal relationship with the project that may involve staff costs and/or budget management responsibilities.

the set timeline. To date, this has helped to track progress using the activities and outputs to direct work. The project incorporated SMART Indicators into its Outcome and Output Indicators to meet objectives set in the original Logframe. All data is recorded in the DPLUS165 master database (Annexe 4.2: File 2 – Excel – Master Database DPlus165).

7. Lessons learnt

This project has been ongoing for 9 months and so far, there have not been any major problems or learning opportunities. There have been minor setbacks with the shipping of lab-specific items however this was already expected and planned for in the timeframe of the project.

Planning sites for the collection of samples mentioned in activity 3.1 has proven to be difficult due to disruption of sampling locations and the lack of weather windows to get samples collected. The project team is currently looking at alternative sites for these sampling activities as backups should there be issues with the existing locations.

8. Actions taken in response to previous reviews (if applicable)

This is the first annual report for DPLUS165 so no previous feedback has been received.

9. Risk Management

Thus far there have been no new risks arising from the DPLUS165 project and the project design has not needed to be changed.

10. Other comments on progress not covered elsewhere

Acquisition of the Ascension Sampler to allow for 12 eDNA water filter samples to be taken at different depths and filtered in the water column to save time and remove contamination risks.

11. Sustainability and legacy

As the project is directly involved with AIGCFD, there is awareness of the Ascension's new sequencing capabilities within the government. Staff members of AIGCFD are being trained via the project officer in sterile handling techniques and best methods of sample collection to ensure a technical legacy is developed and the future of metabarcoding on Ascension is secure.

12. Darwin Plus identity

As the initial part of this project involves the setup and testing of sequencing protocols there has not been a large amount of data generated yet and as such no results to publicise and promote alongside the Darwin Plus identity.

The Dplus165 project has been ongoing for 9 months, during this time there have been a total of 5 social media posts that acknowledged the Darwin Plus Programme. The interaction with these posts included 358 likes and 14 shares across Facebook and Instagram. These interactions were mostly from people residing upon Ascension demonstrating there is a community interest in the project.

As Ascension has a number of Darwin Plus projects that began before DPLUS165 there is a pre-existing knowledge of the programme and it is recognised as being one of the main funders for conservation projects on the island.

13. Safeguarding

Has your Safeguarding Policy been updated in the past 12 months?		Yes/No
Have any concerns been investigated in the past 12 months		Yes/No
Does your project have a Safeguarding focal point?	Yes/No [*If yes, please provide their name and email]*	
Has the focal point attended any formal training in the last 12 months?	Yes/No [*If yes, please provide date and details of training*]	
What proportion (and number) of project staff have received formal training on Safeguarding?		Past: 66% [2] Planned: 0% [0]
Has there been any lessons learnt or challenges on Safeguarding in the past 12 months? Please ensure no sensitive data is included within responses. No		
Does the project have any developments or activities planned around Safeguarding in the coming 12 months? If so please specify. No		

Due to the scope of the work involved in DPLUS165 there is relatively little risk of safeguarding problems. Where activities involve the school or the MPA Youth Committee, there are always staff present who have received Safeguard training.

14. Project expenditure

Table 1: Project expenditure during the reporting period (1 April 2022 – 31 March 2023)

Project spend (indicative in this financial year	2022/23 D+ Grant (£)	2022/23 Total actual D+ Costs (£)	Variance %	Comments (please explain significant variances)
Staff costs				
Consultancy costs				
Overhead Costs				
Travel and subsistence				
Operating Costs				
Capital items				
Others (Please specify)				
TOTAL				

Table 2: Project mobilising of matched funding during the reporting period (1 April 2022 – 31 March 2023)

	Matched funding secured to date	Total matched funding expected by end of project
Matched funding leveraged by the partners to deliver the project.		
Total additional finance mobilised by new activities building on evidence, best practices and project (£)		

15. OPTIONAL: Outstanding achievements or progress of your project so far (300-400 words maximum). This section may be used for publicity purposes

I agree for the Biodiversity Challenge Funds Secretariat to publish the content of this section (please leave this line in to indicate your agreement to use any material you provide here).

File Type (Image / Video / Graphic)	File Name or File Location	Caption, country and credit	Online accounts to be tagged (leave blank if none)	Consent of subjects received (delete as necessary)
				Yes / No
				Yes / No
				Yes / No
				Yes / No
				Yes / No

Annex 1: Report of progress and achievements against logframe for Financial Year 2022-2023 – <u>if applicable</u>

Project summary	SMART Indicators	Progress and Achievements April 2022 - March 2023	Actions required/planned for next period
Impact Ascension acquires the long-term capacity to identify species in difficult taxa enabling a strategic reprioritisation of conservation efforts and increasing the efficacy of biosecurity surveillance and ecosystem analysis.		Ascension now has a fully functioning DNA Laboratory with the capabilities to sequence samples for biosecurity purposes on Island removing the need for costly sample shipping and unreliable cold chain transport options.	
Outcome Ascension has the on-island capability to identify species using DNA metabarcoding and the training materials and corporate memory to ensure this capacity is maintained in the long-term.	0.1 DNA barcode reference library developed for all invertebrate species identified by previous taxonomic studies by Y2Q2. 0.2 Suite of primers designed and validated for metabarcoding of Ascension invertebrates 0.3 Metabarcoding is used routinely to identify species in AIGCFD's terrestrial invertebrate, gut content, settlement panel and light trap monitoring programmes by Y3Q2. 0.4 Long-term DNA extraction and metabarcoding capability established on Ascension through staff training and protocol creation by Y2Q2.	0.1 – reference library is being created using publicly available barcodes 0.2 – not due to be completed this year 0.3 – not due to be completed this year 0.4 – although not due to be completed yet there has been progress made in training staff in sterile technique and sample collection	0.1 – When samples are sent to NHM for barcoding then these results will be merged with the reference library 0.2 – When initial sequencing results are generated if there is a need to improve species resolution then UOE will be consulted on primer design 0.3 – Samples will be gathered in the next year to allow for routine investigation of Ascensions Biodiversity 0.4 – Staff training will continue and involve the entire sequencing workflow by the end of next year
Output 1. DNA primers developed for detection and identification of principal Ascension Island terrestrial and marine invertebrate species.	1.1 All available tissue samples from verified terrestrial and marine invertebrates from Ascension collated by Y2Q2. 1.2 DNA barcoding undertaken on all species for which samples are available (up to a total of 800) by Y2Q4 1.3 Primers developed for all sampled species by Y2Q4.	Progress on this output is matching the expected timeframe with no anticipated delays. 1.1 – Samples are being collated and prepared– Y2Q2 completion of this process is plausible 1.2 – Once samples have been collated as stated in 1.1 then samples will be sent to NHM for barcoding 1.3 – Individual primers may not be needed if pre-published primers provide a good species resolution however if they are needed then there is time built into the project for primer optimisation	

Project summary	SMART Indicators	Progress and Achievements April 2022 - March 2023	Actions required/planned for next period
Activity 1.1: Locate all verified invertebrate specimens from past and current studies and obtain tissue samples from each.		Specimens are being located however some were not stored in conditions sufficient for DNA sequencing and thus cannot be used	Further specimens will be located and tissue samples obtained
Activity 1.2: Genetic sample identification number added to Ascension Biodiversity database.		Ascension Biodiversity database has had publicly available barcode accession numbers appended to species where available	Once samples have been barcoded at NHM barcodes will be published in public databases and appended to the Ascension Biodiversity database
Activity 1.3: Barcoding of all samples collated in activity 1.1 by NHM.		Cannot be completed until 1.1 is completed	Upon completion of 1.1 then samples will be sent to NHM and barcode sequences will be obtained
Activity 1.4: University of Edinburgh to develop a suite of primers that allow discrimination of species collated in output 1.1.		Cannot be completed until 1.1 is completed	Once barcodes have been established for species then primers can be developed to gain better species resolution if required
Output 2. AIGCFD staff able to carry out DNA extraction and metabarcoding	2.1 Protocols for DNA extraction and metabarcoding in Ascension's lab agreed between AIGCFD and University of Edinburgh by Y1Q4. 2.2 Ten AIGCFD staff trained in DNA extraction and metabarcoding delivered by Y2Q3. 2.3 QA of AIGCFD staff results undertaken by DNA Project Officer and University of Edinburgh by Y2Q3.	2.1 – Protocols for DNA extraction have been agreed upon between project partners 2.2 – 5 Staff currently trained in sample collection and sterile technique, next year progress will be made on training in molecular genetic techniques 2.3 – QA not due to be completed yet. Sampling strategy for QA yet to be determined.	
Activity 2.1: Write and test protocols for DNA extraction and metabarcoding.		Protocols have been written and tested by the project officer	Finalise method for Minion sequencing with UOE Finalise method for soil extractions
Activity 2.2: Create training reference documents and deliver practical training courses in DNA extraction and metabarcoding for ten members of AIGCFD.		5 members of staff trained in sample collection and sterile technique	Remaining staff members will be trained in entire sequencing workflow

Project summary	SMART Indicators	Progress and Achievements April 2022 - March 2023	Actions required/planned for next period
Activity 2.3: Metabarcoding results from AIGCFD staff quality assured by comparing with those from Project Officer and University of Edinburgh.		Results not yet obtained to be quality assured. Some samples sent for external validation and results obtained and analysed (Annexe 4.2 – File 3 – PowerPoint – Sequencing result analysis thus far DPlus165)	10% of DNA Extraction samples from each library will be sent to UOE for mirror workflow processing and comparison of results to validate the Ascension lab sequencing results
Output 3. Metabarcoding used to identify terrestrial and marine invertebrate species as part of AIGCFD monitoring and biosecurity surveillance activities.	3.1 Monthly samples collected and appropriately preserved over a six-month period from each of: 3 terrestrial pitfall traps; 2 malaise traps, 3 inshore settlement panels and 2 inshore light traps by Y2Q4. 3.2 Metabarcoding of all samples described above (180 samples in total including replicates) by AIGCFD staff by Y3Q2. 3.3 Any detections of newly- introduced species on Ascension's list of high priority biosecurity threats will be immediately passed to the Biosecurity Team and appropriate response action taken by Y3Q3. 3.4 Report summarizing species present in each sample and highlighting any new biosecurity threats produced by Y3Q4.	3.1 – Samples have already started being collected and sampling locations are being optimised. Inshore settlement plate samples have been recovered from 2 locations at three-month intervals (Aug 2022, Nov 2022, Feb 2023) 3.2 – When enough samples are gathered sample processing can begin 3.3 –Once sequencing progresses the Biosecurity team will be kept up to date on sequencing results and new species flagged via analysis 3.4 – Species found to be present via eDNA analysis will be recorded in the DPLUS165 Master Database which will be updated after every sequencing run. New species will be automatically highlighted and a reminder to email the biosecurity team will pop up on the database	
Activity 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.		Activity progressing with samples collected every 3 months from 2 settlement plate locations and every month from light trap locations	Discussion with DPLUS135 project officer to determine the best place for pitfall and malaise traps Light trap samples may be swapped to plankton samples due to difficulties with sampling

Project summary	SMART Indicators	Progress and Achievements April 2022 - March 2023	Actions required/planned for next period
Activity 3.2: Metabarcoding of samples collected in output 3.1 by trained AIGCFD staff		Not due to be completed this year	Once samples are gathered in appropriate numbers staff who have received training will perform metabarcoding
Activity 3.3: Pass any detections of high priority invasive species to AIG Biosecurity Team for response action.		Not applicable	If any high priority invasive species are detected the AIG Biosecurity team will be contacted immediately and samples can be sent for QA to partners to verify the invasive species presence
Activity 3.4: Produce summary report listing species detected in samples by metabarcoding.		List of all barcodes and matching species kept in DPLUS165 Master Database	Database will be kept updated with each sequencing run
Output 4. Gut content analysis using metabarcoding techniques undertaken to validate isotope-based ecosystem model.	4.1 Ten indicator species identified from different trophic levels of the isotope-based ecosystem model by Y1Q4. 4.2 Blocking primers developed for the indicator species where necessary by Y2Q2. 4.3 Gut content samples from a minimum of ten individuals of each indicator species (100 samples in total) analysed using metabarcoding and compared with standard morphological techniques by Y3Q3.	4.1 – Indicator species have been decided upon based on feasibility of collection through research fishing and donation from local fishers and are representative of multiple trophic levels 4.2 – Desktop study needed to determine if any prey species would also be affected by blocking primers (sequence similarity check) 4.3 – Now that indicator species have been determined, gut content samples can be collected throughout the year to avoid seasonal sample biases	
Activity 4.1: Select 10 indicator marine species for gut contents analysis.		Indicator species have been determined which include multiple trophic levels and are obtainable.	Completed
Activity 4.2: University of Edinburgh to develop blocking primers for the indicator species		Desktop study in progress to determine if any prey items have sequence similarity to host	Determine if blocking primers will negatively affect any prey items being sequenced

Project summary	SMART Indicators	Progress and Achievements April 2022 - March 2023	Actions required/planned for next period
			UOE to develop blocking primers and validate if needed
Activity 4.3: Conduct metabarcoding analysis on gut contents of ten individuals from each of the ten indicator species.		Not scheduled this year	Start sample collection to obtain samples from a wide range of locations/seasons
Activity 4.4: Conduct traditional gut content analysis on same samples and compare the results of the different methods in a report.		Not scheduled this year	Start sample collection at same time as 4.3
Output 5. Secondary school students on Ascension understand how DNA biomonitoring techniques are carried out and their application for conservation.	5.1 30 school students have visited the Ascension lab and extracted DNA in the classroom by Y3Q1.	5.1 – 16 school students have visited the Ascension Lab and filtered eDNA samples	
Activity 5.1: Organise school visits to the AIG DNA lab and lead practical lessons on DNA extraction.		Students have collected 1L water field samples from rockpools and visited the Ascension Lab to filter these samples.	Invite older students with an interest to perform DNA extractions on a range of samples (Filter Membrane, Settlement plate and Soil)

Annex 2: Project's full current logframe as presented in the application form (unless changes have been agreed)

Project Summary	SMART Indicators	Means of Verification	Important Assumptions
Impact: Ascension acquires the long-term capacity to identify species in difficult taxa enabling a strategic reprioritisation of conservation efforts and increasing the efficacy of biosecurity surveillance and ecosystem analysis. (Max 30 words)			
Outcome: Ascension has the on-island capability to identify species using DNA metabarcoding and the training materials and corporate memory to ensure this capacity is maintained in the long-term.	0.1 DNA barcode reference library developed for all invertebrate species identified by previous taxonomic studies by Y2Q2. 0.2 Suite of primers designed and validated for metabarcoding of Ascension invertebrates 0.3 Metabarcoding used routinely to identify species in AIGCFD's terrestrial invertebrate, gut content, settlement panel and light trap monitoring programmes by Y3Q2. 0.4 Long-term DNA extraction and metabarcoding capability established on Ascension through staff training and protocol creation by Y2Q2.	0.1 DNA sequence reference library. Sequences uploaded on international platform (BOLD). 0.2 Database of validated primers and their detection abilities. 0.3 Records of monitoring results. 0.4 Training records, skills assessment and results of QA process with Cefas.	AIGCFD can establish a functioning DNA laboratory on Ascension and train staff to undertake procedures and analysis. Cold chain for delivery of reagents is able to be maintained. Mitigation: AIGCFD have employed an experienced DNA researcher who is overseeing the establishment of the Ascension lab. University of Edinburgh will provide support throughout the project including the development of protocols, validation of methods and QA.
Outputs: **1.** DNA primers developed for detection and identification of principal Ascension Island terrestrial and marine invertebrate species.	1.1 All available tissue samples from verified terrestrial and marine invertebrates from Ascension collated by Y2Q2. 1.2 DNA barcoding undertaken on all species for which samples are available (up to a total of 800) by Y2Q4 1.3 Primers developed for all sampled species by Y2Q4.	1.1 Database of tissue samples. 1.2 Database of DNA barcodes 1.3 Database of primers including the detection capability of each assay	1.1 Samples from past surveys (particularly DPLU021) were suitably preserved and will be shared by previous project partners where required. Mitigation: partners have already been contacted and sharing of samples required by Ascension research permit. Many samples are already stored on Ascension and the DPLUS135 samples are being

			specifically preserved for this purpose. DNA can be extracted and barcoding conducted on the samples. Mitigation: samples are relatively recent and have been appropriately preserved. Amount of tissue stored exceeds that required for analysis.
2. AIGCFD staff able to carry out DNA extraction and metabarcoding	2.1 Protocols for DNA extraction and metabarcoding in Ascension's lab agreed between AIGCFD and University of Edinburgh by Y1Q4. 2.2 Ten AIGCFD staff trained in DNA extraction and metabarcoding delivered by Y2Q3. 2.3 QA of AIGCFD staff results undertaken by DNA Project Officer and University of Edinburgh by Y2Q3.	2.1 Copies of protocol documents including AIGCFD and University of Edinburgh sign off. 2.2 Record of training attendance and post training skills self-assessment and trainer assessment. 2.3 QA comparison report of results obtained by AIGCFD staff and Project officer/University of Edinburgh	AIGCFD staff are able to learn and execute procedures. Mitigation: Most AIGCFD staff have a background in biology and some experience of laboratory work.
3. Metabarcoding used to identify terrestrial and marine invertebrate species as part of AIGCFD monitoring and biosecurity surveillance activities.	3.1 Monthly samples collected and appropriately preserved over a six-month period from each of: 3 terrestrial pitfall traps; 2 malaise traps, 3 inshore settlement panels and 2 inshore light traps by Y2Q4. 3.2 Metabarcoding of all samples described above (180 samples in total including replicates) by AIGCFD staff by Y3Q2. 3.3 Any detections of newly-introduced species on Ascension's list of high priority biosecurity threats will be immediately passed to the	3.1 Photographs and records of sampling effort. Database of logged samples. 3.2 Output of metabarcoding analysis for all species. 3.3 Copy of results sent to Biosecurity Team. Biosecurity Response Record Form. 3.4 Copy of report.	Metabarcoding successfully identifies species in samples. Mitigation: Metabarcoding is a standard technique that has been used in such applications by University of Edinburgh, a partner in this project.

	Biosecurity Team and appropriate response action taken by Y3Q3. 3.4 Report summarising species present in each sample and highlighting any new biosecurity threats produced by Y3Q4.		
4. Gut content analysis using metabarcoding techniques undertaken to validate isotope-based ecosystem model.	4.1 Ten indicator species identified from different trophic levels of the isotope-based ecosystem model by Y1Q4. 4.2 Blocking primers developed for the indicator species where necessary by Y2Q2. 4.3 Gut content samples from a minimum of ten individuals of each indicator species (100 samples in total) analysed using metabarcoding and compared with standard morphological techniques by Y3Q3.	4.1 List of indicator species and diagram showing position in isotope-based ecosystem model. 4.2 Sequences of blocking primers for indicator species. 4.3 Report on gut content analysis showing species identified by metabarcoding and standard techniques.	Isotope model is completed before the start of this project. Mitigation: model is already advanced and forms part of a PhD project due to finish in 2022. At least 10 samples can be collected form the 10 indicator species. Mitigation: ease of sampling will be considered when selecting the indicator species.
5. Secondary school students on Ascension understand how DNA biomonitoring techniques are carried out and their application for conservation.	5.1 30 school students have visited the Ascension lab and extracted DNA in the classroom by Y3Q1.	5.1 Photographs of visits and student reports.	5.1 Teachers at the school are supportive of adding DNA biomonitoring to the current curriculum. Mitigation: Existing good relationship between Ascension school and AIGCFD. DNA is a topic within the school curriculum and visits will be schedules to tie in with planned teaching on the subject.

Activities (each activity is numbered according to the output that it will contribute towards, for example 1.1, 1.2 and 1.3 are contributing to Output 1)

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

1.2 Genetic sample identification number added to Ascension Biodiversity database.

1.3 Barcoding of all samples collated in activity 1.1 by NHM.

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

2.1 Write and test protocols for DNA extraction and metabarcoding.

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

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

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.

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

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

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

4.1 Select 10 indicator marine species for gut contents analysis.

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

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

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

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

Annex 3: Standard Indicators

Table 1 Project Standard Indicators

DPLUS Indicator number	Name of indicator using original wording	Name of Indicator after adjusting wording to align with DPLUS Standard Indicators	Units	Disaggregation	Year 1 Total	Year 2 Total	Year 3 Total	Total to date	Total planned during the project
DPLUS-A01	AIGCFD staff trained in DNA extraction and metabarcoding delivered	Number of staff members from AIGCFD (local stakeholders) will complete structured and relevant training	People	Gender: male and female Stakeholder group: AIGCFD Training typology: sample collection, sterile techniques and metabarcoding protocols	5			5	10
DPLUS-A03	Organisations on Ascension with the long-term capacity to identify species in difficult taxa enabling a strategic reprioritisation of conservation efforts and increasing the efficacy of biosecurity surveillance and ecosystem analysis.	One local organisation with improved capability and capacity as a result of the project.	Number	Local government	1			1	1
DPLUS-B01	Number of Protected Area Management Plans incorporating metabarcoding to identify terrestrial and marine invertebrate species as part of AIGCFD monitoring and biosecurity surveillance activities.	New Protected Area management plans available and endorsed	Number	Management plans for terrestrial and marine invertebrates will include metabarcoding	0			0	2
DPLUS-C01	Number of best practice protocols and reference materials for DNA extraction and metabarcoding	Best practice guides and knowledge products published and endorsed	Number	Typology: protocols	10			10	TBD

Table 2 Publications

Title	Type (e.g. journals, manual, CDs)	Detail (authors, year)	Gender of Lead Author	Nationality of Lead Author	Publishers (name, city)	Available from (e.g. weblink or publisher if not available online)

Checklist for submission

	Check
Different reporting templates have different questions, and it is important you use the correct one. Have you checked you have used the **correct template** (checking fund, type of report (i.e. Annual or Final), and year) and **deleted the blue guidance text** before submission?	Y
Is the report less than 10MB? If so, please email to BCF-Reports@niras.com putting the project number in the Subject line.	Y
Is your report more than 10MB? If so, please discuss with BCF-Reports@niras.com about the best way to deliver the report, putting the project number in the Subject line.	Y
Have you included means of verification? You should not submit every project document, but the main outputs and a selection of the others would strengthen the report.	Y
Do you have hard copies of material you need to submit with the report? If so, please make this clear in the covering email and ensure all material is marked with the project number. However, we would expect that most material will now be electronic.	N
If you are submitting photos for publicity purposes, do these meet the outlined requirements (see section 15)?	N/A
Have you involved your partners in preparation of the report and named the main contributors	Y
Have you completed the Project Expenditure table fully?	Y
Do not include claim forms or other communications with this report.	