Darwin Plus Main: 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 2024

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 2023-Mar 2024) and number (e.g. Annual Report 1, 2)	April 2023 – Mar 2024 Annual Report 2
Project Leader name	*Tiffany* [redacted]
Project website/blog/social media	n/a
Report author(s) and date	Melissa [redacted] 22/04/24

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.

Ascension Island is remote and lacks easily accessible taxonomic expertise. Through this project, Ascension has entered 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 through the duration of this DPLUS165 project. 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 attended a two-week training session with UOE partners from 29th Jan 2024 - 9th Feb 2024 to learn methods relating to the Nanopore Minion sequencing 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 are scheduled to happen as this output is currently running behind. As the internet connection on Ascension can be somewhat unreliable, video call meetings are performed when there are major project milestones to discuss. Contact with project partners is maintained via email otherwise.

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. Reference databases have been created with species already in the Ascension Biodiversity Catalogue (Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 1a Marine and 1b Terrestrial).

Activity 1.1 - DELAYED
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 and be completed by Y2Q2.This is delayed due to reliance on outside collaborators who have access to samples that were collected on Ascension and were subsequently moved off island. The remainder of Output 1 is also delayed. Attempts are being made to speed up the collection of samples from SAERI to be sent onwards to NHM. We expect the samples will be delivered by May 2024. NHM and UOE have been briefed about the delays to this output.

Activity 1.2 - DELAYED
Activity 1.2 to add a genetic sample identification number to the Ascension Biodiversity Database is delayed due to issues stated above in Activity 1.1. This was due to be completed in tandem with Activity 1.1.

Activity 1.3 – DELAYED
Activity 1.3 was due to begin in Y1Q4 and end Y2Q4. The barcoding or full sequencing of all samples from Activity 1.1 has as of Y2Q4 yet to have any data produced by NHM for either Terrestrial or Marine Invertebrate specimens. A meeting will be organized to discuss these delays and establish a clear timeframe to make up for the lack of data.

Activity 1.4 - DELAYED
Activity 1.4 for UOE to develop a suite of primers to allow for discrimination of species collated in Output 1 is also delayed due to lack of progress in getting samples sent to NHM for barcoding. UOE have advised on the best universal and pre-published primers that should allow discrimination of expected species but these cannot be checked against the verified invertebrate

specimens until Activity 1.3 has been completed. (See Annexe-DPLUS165-4.2.4 DPLUS165 Master Database, sheet 2 for primer sequences)

Under Output 2, the aim is to develop protocols for metabarcoding on Ascension and train AIGCFD staff members to undertake these protocols.

Activity 2.1 – COMPLETED
Activity 2.1 to write and test protocols for DNA Extraction and Metabarcoding has been completed. Methods for both the Illumina ISeq and the Nanopore Minion have been tested and are confirmed to be working from external validation. (See Annexe-DPLUS165-4.2.1 Lab Protocols)

Activity 2.2 – DELAYED
Activity 2.2 to create reference training documents and deliver a practical training course on DNA extraction to 10 members of AIGCFD staff is ongoing – this was due to be completed by Y2Q3 however issues with the shipment of reagents has meant that hands on training was not possible. It is hoped that once a shipment from the UK arrives in May 2024 the remainder of staff can undergo practical hands on training. Training resources have been created and the PO hopes to give a talk to brief all staff on the workflow steps even if they don't get hands on training. (See Annexe-DPLUS165-4.2.2 DNA training manual, and Annexe-DPLUS165-4.2.3 DNA Training PPT)

Activity 2.3 – COMPLETED
Activity 2.3 to quality assure results from AIGCFD staff with those of the project officer and University of Edinburgh has been performed. AIGCFD staff have performed all steps prior to sequencing and these have been sequenced alongside the same samples which were processed by the project officer. There were no discrepancies in species detected but higher than usual levels of contamination occurred from human sources. Extra training will be provided to further stress the importance of maintaining sterility. There has also been external validation from Curtin University who have undertaken sequencing of 320 samples using the ISeq. (See Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 4 Sample Database for information on samples run in these libraries)

Activity 3.1 – COMPLETED
Activity 3.1 to collect monthly samples from terrestrial locations: 3 pitfall traps, 2 malaise traps, (See Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 10 invert biosecurity for information on samples collected). There was also the aim of collecting marine samples from 3 inshore settlement panels and 2 light traps over a 6-month period. (See Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 4 Sample Database for information on samples gathered). Samples are continuing to be gathered for biomonitoring purposes however as mentioned in previous reporting light trapping has been replaced with plankton tows due to safety concerns over light trapping locations.

Activity 3.2 – ONGOING
The metabarcoding of samples mentioned in 3.1 is ongoing and due to be completed by September 2024. Thus far sequencing has been performed on all sample types to verify methodology and primer output. Each metabarcoding library is recorded in the Annexe-DPLUS165-4.2.4 DPLUS165 Master Database summary page and species detected are listed in sheet 5)

Activity 3.3 – ONGOING
Any detections of high priority invasive species will be passed onto the AIG Biosecurity team for response action. As of yet there have been no high priority species detections (see list of these species in Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 1c) but new to Ascension ant species have been detected and increased sampling is being undertaken around the island to determine the spread of these ants. Collaborators from previous Darwin projects relating to invertebrates on Ascension have been briefed and are involved in next steps alongside collaborators from FERA.

Activity 3.4 – NOT YET DUE
The production of a summary report detailing species detected via metabarcoding is not due to begin until Oct 24. The continual addition of new species detections and sequences to the DPLUS165 master database will allow for the report to be easily compiled. (See Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 5 Species Detections)

Activity 4.1 – COMPLETED
As stated in previous reports the 10-indicator species have been selected to represent different trophic levels.

Activity 4.2 – DELAYED
Activity 4.2 for UOE to develop blocking primers has been delayed as it was due to be completed by Sept 2023. The delays are due to the need to ensure the Nanopore Minion sequencer was working on Ascension to enable COI sequencing of longer fragments than the Illumina Iseq can allow, this has now been completed. This will give a better resolution of lower trophic level gut contents. Preliminary checks are underway currently to determine which of the indicator species require blocking primer development and are due to be designed and validated by September 2024 to allow for Activity 4.3 to be completed by Y3Q3. (See Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 8 Indicator species blocking primers)

Activity 4.3 – ONGOING
Conducting metabarcoding on ten individuals from the ten species selected in Activity 4.1 was not scheduled to begin until April 2024 however thus far 4 Galapagos shark samples and one Tuna and Wahoo sample have been barcoded in preliminary tests to check for host DNA percentage in sequencing outputs. There has also been opportunistic analysis done on 4 Silky shark cloacal swab samples. While these are not one of the 10 indicators species they were samples taken alongside the DPLUS161 project and provide a good example of further applications that can be achieved as a result of this research. (Annexe-DPLUS165-4.2.4 DPLUS165 Master Database sheet 9 Gut Content Samples)

Activity 4.4 – ONGOING
A Subset of samples collected for gut content analysis are being analysed using traditional gut content analysis and visual checks these will eventually be compared to the sequencing outputs in a report.

Activity 5.1 – ONGOING
Outreach with the local school as part of this Darwin Project has been ongoing for the past year with visits to the DNA lab by students undertaking a marine biology course and younger students collecting eDNA samples from rockpools. Onion skin DNA extraction lessons are being discussed with the school to be incorporated into the genetics component of the curriculum as any more complex extractions require the use of the DNA lab which is limited to only 3 persons at a time.

3.2 Progress towards project Outputs

The aim of Output 1 is for the development of DNA primers to detect and identify key 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. We recognise that these primers may not provide a high enough taxonomic resolution to identify endemic species with high confidence and may only resolve to genus level. Unfortunately, there have been significant delays to this output due to difficulties in obtaining samples from external collaborators (marine invertebrate) or specimens needing to be verified by taxonomic experts before being sent to NHM for barcoding (terrestrial invertebrates). There is still time in the project to obtain barcodes for these samples and re classify sequencing data to a higher taxonomic level once reference databases have been updated. If any species are not able to be identified using pre-published primers, development of more Ascension species specific primers can be undertaken and incorporated into pre-existing protocols before the end of the project. Overall this output is still achievable by project end however a large amount of it depends upon the collaboration of external sources.

The Second Output has the aim of developing metabarcoding protocols and providing training to AIGCFD staff to ensure longevity of DNA Biomonitoring on Ascension. This output is mostly completed and all protocols have been written and tested for both the Illumina Iseq and Nanopore Minion (Annexe-DPLUS165-4.2.1 Protocols). To date 13 staff members have been trained in sterile collection methods, lab safety and sample preparation. Five of these staff members have also been trained in carrying out DNA extractions and a further two of these have progressed to sequencing library preparation. Due to high staff turnover within AIGCFD four of the thirteen members who have received training have now left the department. Training will focus on staff members who are likely to remain at AIGCFD for longer than the project to ensure longevity of these methods. This output remains achievable by project end.

Output 3 is focused on the collection of samples for biomonitoring and the subsequent sequencing and analysis of these. This output is progressing well and more samples than required have been collected thus far. The metabarcoding of these samples is ongoing and results are continuously being analysed for possible harmful species detections to pass onto the Biosecurity team. New to Ascension species have been detected but are not classed as harmful, however with guidance from collaborators at FERA a more in-depth survey protocol is being developed to determine the spread of these species using both visual and DNA analysis.

The gut content analysis to be undertaken in Output 4 is progressing well and should be completed on time. To date there have been 91 gut content samples collected from the 10-indicator species along with 18 opportunistically gathered samples from other species. The University of Edinburgh is in the process of developing blocking primers for these samples however this is slightly delayed when compared to the Log Frame. The initial design of the blocking primers is due to begin soon and the validation of primers is expected to be completed by September. This delay is due to the wait on getting a Nanopore Minion sequencing device to allow for longer DNA barcode regions to be sequenced. The delays should not impact the progress of this Output as results are not due until December 2024.

The aim of Output 5 is to perform outreach activities and ensure that the local school benefit from the presence of the DNA lab and expertise on island. Visits to the DNA lab are somewhat limited due to the small space and three-person restriction. Some of the outreach activities include fieldwork visits with the Marine Protected Area Youth Committee to gather eDNA samples from rockpools and three Marine Biology students visiting the DNA lab. It is hoped that a DNA extraction lesson can be done at the school if it fits into the curriculum.

3.3 Progress towards the project Outcome

The hopeful outcome of this DPLUS165 project is to ensure Ascension has the on-island capability to identify and monitor species using DNA metabarcoding. This will be done via the production of training materials and on island training to ensure capacity is built up and maintained long term. As of Y2 there has been steady progress towards these aims with sequencing outputs and reference libraries completed. There have been setbacks to certain aspects of the project due to issues such as reagent shipping and historic sample procurement however the outcomes should still be achievable by project end.

The first indicator is to have a reference library developed for all invertebrate species identified by previous taxonomic studies. Unfortunately, due to difficulties in accessing these samples this indicator has not been achieved. Efforts have been made by the project officer to develop the most in-depth reference databases possible without this specimen information, meaning some endemic marine invertebrate species are identified only to genus level and not species level until samples can be sent to NHM and barcoded. Terrestrial invertebrate samples from DPLUS135 have taken longer than expected to be verified by taxonomic experts and as such cannot yet be barcoded. Some of these samples are already located at NHM and ideally will be barcoded as soon as possible.

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. New specific primers cannot be designed until a

DNA barcode reference library has been produced containing sequence information for known invertebrates. Although this is delayed we anticipate that these primers may not be needed as previously published primer sets may provide enough taxonomic resolution.

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 metabarcoding has been performed on all of the previously mentioned samples and is being utilised as a method of biodiversity monitoring and data is being passed onto Biosecurity teams where relevant.

The 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 were trained in the initial sample-gathering steps and sterile techniques in Y1. During Y2 staff have been 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 two persons can be trained in practical lab techniques at any one time.

As of completion of Y2 it is still within the project reach to achieve all outputs however delays to certain aspects of the project (mainly output 1) that are out of the project lead and partners hands could lead to the delay in publishing of verified specimen barcodes.

3.4 Monitoring of assumptions

Assumption 1: 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.

Comments: A fully functioning DNA laboratory has been set up and established on Ascension. Cold chain delivery of reagents is always difficult to such remote locations but attempts to mitigate the risk of items defrosting with the use of cold chain shipping boxes that maintain temperature for up to 5 days has been put into place.

Assumption 2: Samples from past surveys are suitably preserved and will be shared by previous project partners.

Comments: There has been little to no progress with this assumption since the previous year. Contact has been made with previous project partners for DPLU021 and specimens will be transported to NHM by May 2024. Specimens from the DPLUS135 project are located at NHM and with various taxonomic experts for ID before barcoding can be performed.

Assumption 3: AIGCFD staff are able to learn and execute procedures

Comments: The vast majority of AIGCFD staff have prior experience in some aspects of lab work. Training is continually being provided to different staff members by the project officer however high levels of staff turnover and the time needed for training mean it is difficult to teach enough members of staff the entire sequencing workflow. There will be multiple staff members trained in the whole sequencing workflow before the projects end.

Assumption 4: Pre-published primers will successfully identify species in samples collected on Ascension.
Comments: Thus far pre-published primers are providing enough taxonomic resolution to successfully identify species around Ascension.

Assumption 5: An isotope model is already developed for Ascensions species and can be used as a comparison to gut content analysis.
Comments: The model has now been completed and can be used comparatively with metabarcoding analysis to assess the reliability of both methods.

Assumption 6: At least 10 samples can be collected from the 10-indicator species
Comments: 50% of the indicator species have had over ten samples collected and it is expected that the remainder will have 10 samples by Y3Q3.

Assumption 7: Teachers at the school are supportive of adding DNA biomonitoring to the current curriculum.
Comments: There is a good relationship existing between AIGCFDs outreach officer and the local school. Recently a Marine Biology qualification has been added to the curriculum showing the interest of the school and pupils in areas relating to this project. Teachers are supportive of students gaining as much experience as possible.

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 will 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 (GESI)

Please quantify the proportion of women on the Project Board[1].	57%
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%

GESI Scale	Description	Put X where you think your project is on the scale
Not yet sensitive	The GESI context may have been considered but the project isn't quite meeting the requirements of a 'sensitive' approach	
Sensitive	The GESI context has been considered and project activities take this into account in their design and implementation. The project addresses basic needs and vulnerabilities of women and marginalised groups and the project will not contribute to or create further inequalities.	X
Empowering	The project has all the characteristics of a 'sensitive' approach whilst also increasing equal access to assets, resources and capabilities for women and marginalised groups	
Transformative	The project has all the characteristics of an 'empowering' approach whilst also addressing unequal power relationships and seeking institutional and societal change	

The current percentage of the Conservation Department consists of 70% 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 lead, 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 Log frame and Timetable. The Project Officer and Project Lead set deadlines for certain tasks to monitor and evaluate the project's progress against 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 Log frame. All data is recorded in the DPLUS165 master database

[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.

(Annexe 4.2.4 – Master Database DPlus165). Project partners are kept up to date with meetings and results from the project.

7. Lessons learnt

This project has been ongoing for 2 years, thus far there have not been any major problems or learning opportunities other than the importance of securing a reliable cold chain which has been mostly resolved as of April 2024. The importance of securing this cold chain should be noted by others who wish to undertake DNA work in UKOT's as it could cause major delays if this were not sorted before the start of projects. Due to Ascensions remote nature most other OT's have more reliable transport links and as such this would not pose as much of an issue.

There have been some setbacks due to reliance on outside collaborators to access specimens however it is hoped that DPLUS021 specimens will reach NHM by May 2024.

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

One comment raised in the previous year's review is to determine if the cold-chain lab reagents supply issue is resolved, and if the solution is sustainable long term. The cold chain supply issue has been navigated and items are being shipped in frozen however these are all hand carried when members of staff or visiting scientists leave/return to Ascension on flights from the UK. Five-day cold shipping boxes have been purchased and items are delivered to a collaborating lab in Oxford for freezer storage. It is hoped by the end of May 24 that airfreight bookings will become a realistic way to send these cold shipping boxes and reagents without the need for personnel to hand carry items making it more sustainable long term.

The award letter mentioned several points that were not highlighted in previous documents. One of these was concern about the availability of a suitably skilled project officer to be recruited for the project. A project officer with the relevant skillset was recruited and arrived on Ascension in November 2022.

The comments on how and where sampling will take place are addressed as mentioned in the below table. Please see Annexes 4.1.1 and 4.1.2 for maps detailing marine and terrestrial sampling sites.

Task	Description	When	Sites/Samples	Reason
Water Sampling	1L water samples	Every 6 months	10 different sites 5 x 1L samples	Biodiversity
Marine Invasives	Settlement plates	Every 3 months	3 sites in Clarence Bay 4 plates on each	Biosecurity
Gut Contents	Gut Contents	2023-2024	10 indicator species 10 samples of each	Biodiversity
Terrestrial Invasives	Ground and Air traps	Every 3 months	15 sites each with ground and air traps	Biosecurity

The fast turnover of staff on Ascension was mentioned as a concern for longevity of DNA based research and training. The project team are confident that staff will stay long enough for Ascension to benefit from their training and that knowledge transfer will be a continual process. Training documents and presentations made by the project officer that are specific to work carried out on Ascension are available for all AIGCFD staff to access.

A comment on the development of the relationship with UOE to enhance the sustainability and exit strategy of the project made good points. The working relationship between the University of Edinburgh and AIGCFD is very positive and discussions at a time closer to the project end about ongoing technical support or training will be prioritised.

One concern raised was to confirm whether the Ascension Island Government funding support for the future of the DNA lab is approved. As mentioned in the exit strategy of the original application, costs associated with the future operation of the lab and purchase of consumables

will come from the AIGCFD core budget as well as being incorporated into new research projects as required. Some costs will also be offset by the savings against standard methods.

Another comment on the award letter is that samples from past surveys will be used (if suitable) but new sample collecting is only described in the log frame. This detail is missing in the methodology and it seems there is to be little inshore marine sampling. Annexe 4.2.1 contains a detailed overview of all protocols and collection methods.

With regards to strengthening the outreach of the project to inform other UKOTs of the project results and future applications. The project officer gave a brief presentation at the Blue Belt 2024 Symposium to representatives of UKOT's to highlight the projects progress thus far and encourage stronger collaboration amongst UKOTs using eDNA for biomonitoring. The project lead also highlighted the use of DNA monitoring in the larger scheme of MPA management. (see Annexe 4.2.6 – Blue Belt Symposium 2024 DPLUS165 presentation).

Regarding strengthening the log frame the project has progressed through more than half of its lifetime and as such, large-scale changes to the log frame could cause confusion. Additions to log frame queries will be stated below and referred back to by the PO when relevant.

Comment 1: Indicator 0.2 should be timebound

This indicator should be done by Y3Q2 as it will be validated at the same time as completion of metabarcoding mentioned in activity 3.2.

Comment 2: Indicator 2.2 could be clearer on capacity building benefits, rather than simply completion of training, and include gender disaggregated targets.

All AIGCFD staff should be briefed on sterile sample collection methods to enable teams to benefit from DNA barcoding should they require it for other projects. Long term staff members should be given hands on training with DNA extraction and PCR based methods to ensure longevity of DNA work on Ascension. Key staff members should be given training on the entire sequencing and analysis workflow to ensure that there are no knowledge gaps once the projects exit strategy is executed. Training databases should make note of gender of trainees.

Comment 3: Indicator 3.3 could be reformulated to be clearer what exactly will be measured and how.

Any detections of newly-introduced species on Ascension's list of high priority biosecurity threats (records of these species to be kept in DPLUS165-MasterDatabase) will be immediately passed to the Biosecurity Team and appropriate response action taken as required. Appropriate action will be highly species specific and driven by knowledge from outside experts who have dealt with the species previously. Delineating the extent and distribution of these high priority species and monitoring will be increased either by more intensive sampling routines or targeted qPCR primer development. As the requirements for sampling is so species dependant we cannot be more specific on measurement methods in the log frame.

Comment 4: Indicator 4.2 could be more specific and measurable;

Blocking primers developed for the indicator species where necessary by Y2Q2. In order to make this more specific and measurable the goal of these primers is to ensure that no more than 40% of reads from any sample are host derived. If samples are sequenced without blocking primers and fall under the 40% threshold then blocking primers are not necessary.

Comment 5: Indicator 5.1 should also be gender disaggregated and better able to measure achievement of Output i.e. "understanding".

All students from relevant age groups within the school will be invited to undertake DNA extraction practical lessons in the school laboratory. A record of gender %'s in these classes will be recorded. A small exercise of hands up with multiple choice questions will be done with the class before and after the practical lesson, an increase in correct answers will be classed as a success.

9. Risk Management

There have been no new risks arising during the previous 12 months and the risks that were mentioned in the risk register (cold chain shipping) have been mitigated.

10. Sustainability and legacy

The project officer has been steadily increasing the capacity on Ascension to ensure other staff members are able to carry out the sequencing workflow. Effort is being made to ensure all staff members who show an interest in DNA work are offered some training.
Recently the PO and PL attended a Blue Belt Symposium in London, during which the PO presented on DNA metabarcoding as a technique for biosecurity and biomonitoring and the PL highlighted the use of this work in the wider MPA management plan. Other UKOT's expressed an interest in the work and a DNA steering group was proposed as an idea.
The PO also went to do training at the UOE and discussed options for an increasing scope of work and standardised protocols across other OT's especially as UOE partners are also involved in eDNA sampling on Pitcairn.

The intended sustainable benefits of this project are still valid and it is hoped this will provide the tools and skills for DNA extraction and metabarcoding to become mainstreamed into the work of AIGCFD. Protocols that have been produced through this project and the training of AIGCFD staff means there will be a reservoir of knowledge and experience that can be passed on and retained even with the relatively high turnover rates seen within AIGCFD.
The training documents and protocols can be shared with other UKOT's interested in performing similar research and wanting to increase DNA biomonitoring capacity.

11. Darwin Plus identity

Efforts to publicise this Darwin plus have been made on a variety of social media platforms including highlighting staff training and results. On each of these posts engagement has been positive and Darwin plus and Biodiversity challenge funds logos have been used or have been tagged in the posts (Annexe 4.2.7). The project has been highlighted as a distinct project with a clear identity under the scope of the Darwin Plus initiative. There have also been public notices sent out by AIG to highlight the progress with the project and engage the local community (Annexe 4.2.7).

The project officer gave a presentation on the DPLUS165 project at the Blue Belt 2024 symposium in London (Annexe 4.2.6) and a presentation highlighting all marine work on Ascension (including this project) was given by Tobias Capel in St. Helena further strengthening ties between the sister UKOT's.

As Darwin plus funds a significant amount of conservation work on and around Ascension there is a wide understanding of its benefit to Ascension within the community.

12. Safeguarding

Has your Safeguarding Policy been updated in the past 12 months?		Yes/No
Have any concerns been reported 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: 100% Planned:
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		
Please describe any community sensitisation that has taken place over the past 12 months; include topics covered and number of participants. None		
Have there been any concerns around Health, Safety and Security of your project over the past year? If yes, please outline how this was resolved. None		

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.

13. Project expenditure

Table 1: Project expenditure during the reporting period (1 April 2023 – 31 March 2024)

Project spend (indicative) in this financial year	2023/24 D+ Grant (£)	2023/24 Total actual D+ Costs (£)	Variance %	Comments (please explain significant variances)
Staff costs	[redacted]			Slight underspend in project officer salary due to utilities being shared
Consultancy costs				
Overhead Costs				
Travel and subsistence				Slight overspend due to delayed booking of overseas accommodation
Operating Costs				Slight underspend due to decrease in software price
Capital items				
Others: Sample Shipping Consumables Consumables NHM Consumables UOE				Underspend in consumables due to difficulties in purchasing and shipping reagents and dangerous goods.
TOTAL	**70,700**	**59580**		

Table 2: Project mobilised or matched funding during the reporting period (1 April 2023 – 31 March 2024)

	Secured to date	Expected by end of project	Sources
Matched funding leveraged by the partners to deliver the project (£)	[redacted]		AIG
Total additional finance mobilised for new activities occurring outside of the project, building on evidence, best practices and the project (£)	-	-	-

14. Other comments on progress not covered elsewhere

Samples are being gathered by Citizen Scientists travelling on yachts on route from St Helena to Ascension to allow for monitoring of wider areas of the MPA that are inaccessible to AIGCFD staff due to vessel limitations. See annexe 4.1.3 for a photo of locations samples have been collected from. Results from this will give a better understanding of the species that use the travel corridor from St Helena to Ascension and a good picture of the wider MPA's biodiversity.

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 to edit and use the following for various promotional purposes (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 including description, country and credit	Social media accounts and websites 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 2023-2024

Project summary	Progress and Achievements April 2023 - March 2024	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.	Ascensions DNA laboratory is continuing to produce results for biomonitoring and biosecurity purposes, these results are being used to determine more in-depth monitoring and screening methods.	[redacted]
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.	0.1 – reference library created using publicly available barcodes for COI, 18s and 12s.	Once historic samples are barcoded reference libraries will be updated.
0.2 Suite of primers designed and validated for metabarcoding of Ascension invertebrates	0.2 – publicly available primers being used	Once historic samples are barcoded primers can be designed to highlight these species if current primers don't provide enough taxonomic resolution
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.	0.3 – Ongoing metabarcoding is producing these results	Continue metabarcoding of samples
0.4 Long-term DNA extraction and metabarcoding capability established on Ascension through staff training and protocol creation by Y2Q2.	0.4 – all training protocols and PowerPoints up to date and available for staff members. All teams briefed on sterile sample collection methodology. DNA extraction training ongoing.	Continue training of new staff and ensure key individuals are trained in entire workflow for long-term stability of metabarcoding on ascension
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.	1.1 – delayed due to issues obtaining samples from external collaborators	Gather samples from SAERI and send to NHM for barcoding. Follow up with NHM regarding

		terrestrial invert samples from DPLUS135
1.2 DNA barcoding undertaken on all species for which samples are available (up to a total of 800) by Y2Q4	1.2 – delayed due to 1.1	Once samples collated and sent to NHM get barcodes produced ASAP
1.3 Primers developed for all sampled species by Y2Q4.	1.3 – delayed due to 1.1	If current primers do not resolve species set up meeting with UOE to design new primers
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.	2.1 – Protocols all verified and working. Addition of Nanopore Minion sequencing protocols.	Completed
2.2 Ten AIGCFD staff trained in DNA extraction and metabarcoding delivered by Y2Q3.	2.2 – Training has continued from Y1 and all staff have been briefed on sterile sample collection. Key staff are in the process of learning the entire workflow.	Continue with training and ensure key staff members can perform the entire eDNA pipeline from sample collection to analysis
2.3 QA of AIGCFD staff results undertaken by DNA Project Officer and University of Edinburgh by Y2Q3.	2.3 – QA of results done by PO and Curtin University.	Continue to get QA of sequencing performed to ensure accuracy of 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.	3.1 – samples continually being collected from all methods	Continue with sample collection
3.2 Metabarcoding of all samples described above (180 samples in total including replicates) by AIGCFD staff by Y3Q2.	3.2 – metabarcoding ongoing with over 90 samples analysed currently due to increased scale of sampling and 15 terrestrial invertebrate locations (every 3 months) See annexe 4.1.4 and 4.1.5 for details of samples collected and analysed	Continue with metabarcoding
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.	3.3 – new species to ascension are passed on to the biosecurity team to determine if they pose a threat and if increased sampling is needed	Pass on any newly detected species to Biosecurity team

3.4 Report summarizing species present in each sample and highlighting any new biosecurity threats produced by Y3Q4.	3.4 – not due to be completed thus far but detailed records kept of all sequencing results to ensure report is easy to compile	Continue keeping detailed databases with sequencing results
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.	4.1 – completed in Y1	Completed
4.2 Blocking primers developed for the indicator species where necessary by Y2Q2.	4.2 – Discussions with UOE taking place waiting on preliminary results of gut content analysis to determine if primers are necessary	If any preliminary samples are more than 40% host reads then primer design to be completed by Sept 2024
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.	4.3 – Gut content samples are still being gathered should be completed by Y3Q3	Gather remaining samples and compare standard and DNA techniques
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 – School students of different ages have visited the DNA lab	Plan a DNA extraction lesson and ask the school when this fits into the curriculum best

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 Biosecurity Team and appropriate response action taken by Y3Q3.	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.

	3.4 Report summarizing 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 Sample collection and sterile techniques	13 Sample Collection 5 DNA extraction Metabarcoding protocol 2		13	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	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		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	14		14	TBD

Table 2 Publications

Title	Type (e.g. journals, best practice manual, blog post, online videos, podcasts, 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)

Library Type	Library Name	Primer	Samples analysed
Water eDNA	Curtin-01-12s	MiFish	121
Marine Invert	Curtin-02-18s	18s	73
Water eDNA	Curtin-02-12s	MiFish	126
Marine Invert	ASCN01-18s	Uni18s	6
Terrestrial Invert	ASCN02-COI	Leray-Lobo (COI)	10
Marine Gut Content	ASCN02-COI	Leray-Lobo (COI)	10
Marine Invert	ASCN02-18s	Uni18s	2
Terrestrial Invert	ASCN03-COI	Leray-Lobo (COI)	53
Total Samples Analysed:			401

Table 4.1.5 – A summary of samples analysed thus far on and around Ascension.

Annexe 4.2 Attached files in ZIP folder

Annexe-DPLUS165-4.2.1 – PDF - Lab protocols Dplus165 (additional Nanopore protocol attached from previous annual report)
Annexe-DPLUS165-4.2.2 – PDF – DNA Training Manual
Annexe-DPLUS165-4.2.3 – PDF - DNA training PPT
Annexe-DPLUS165-4.2.4 – Excel – Master Database DPlus165
Annexe-DPLUS165-4.2.5 – PDF – DNA results Y2
Annexe-DPLUS165-4.2.6 – PDF – Project Officers presentation for Blue Belt Symposium
Annexe-DPLUS165-4.2.7 – PDF – Outreach Materials DPLUS165