Darwin Plus Main & Strategic: Final 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: no later than 3 months after agreed end date.

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

Darwin Plus Project Information

Scheme (Main or Strategic)	Main
Project reference	DPL165
Project title	Barcoding an Island
Territory(ies)	Ascension Island
Lead Organisation	Ascension Island Conservation and Fisheries Department
Project partner(s)	University of Edinburgh (UOE) Natural History Museum (NHM)
Darwin Plus Grant value	£199,300
Start/end date of project	1/7/22 – 31/3/25
Project Leader name	Tiffany Simpson
Project website/Twitter/blog etc.	NA
Report author(s) and date	Melissa Morgan (PO) Feb 2025

1 Project Summary

The DPLUS165 Barcoding an Island project aimed to enhance the understanding and conservation of Ascension Island's biodiversity by applying DNA barcoding techniques to document and identify terrestrial and marine species. The project sought to build a comprehensive genetic reference library to aid in biodiversity monitoring, inform conservation efforts, and support environmental management strategies. The development of reference databases, analysis tools and provision of training in DNA metabarcoding techniques has resulted in the Conservation Team on Ascension having the capacity to identify multiple species across a range of taxa without the need to rely on external taxonomic experts.

2 Project Partnerships

The two project partners involved in this project were the Natural History Museum (NHM) and the University of Edinburgh (UOE). These partnerships were necessary for the advice and expertise that each of the organisations could add to the project. Project partners were consulted with during the planning and original application phase and as such had input into final indicators and outputs.
The final report was led by AIGCFD due to their direct involvement in the project throughout its duration. Both UOE and NHM reviewed this document before it's submission.

Working with partner agencies is particularly tough when projects are based in remote locations however regular contact was maintained with UOE via emails, in person training and video calls. The partnership with NHM proved to be more challenging, as locating the historic samples took longer than anticipated. NHM's broad scope of work meant that delays in receiving samples, beyond what was originally agreed in the logframe, require them to reallocate time from other projects. This ultimately caused delays that were beyond the control of both AIG and NHM. Please see Annexe 9_NHM_latesample_email.pdf as evidence of when samples from the South Atlantic Environmental Research Institute (SAERI) were shipped to NHM (4/4/25) which was outside the scope of the project. See also the reply from NHM regarding when sampling is due in a new timeline.
Relationships between AIGCFD, UOE and NHM will continue after the projects end. There are still scientific papers to be published involving work from this project (Annexe 4 – paper draft feasibility study). It is hoped as AIGCFD continues to progress its DNA research and that both partners will continue to be valued sources of guidance.

3 Project Achievements

3.1 Outputs

The project aimed to achieve several key outputs as outlined in the logframe. Below is an assessment of progress made towards each output, challenges encountered, and how these were addressed. Outputs were achieved in a timely manner for everything except Output 1 due to reliance on project partners and external collaborators.

Output 1.1 to locate all verified invertebrate specimens from past and current studies and obtain a tissue sample from each proved to be more difficult than expected due to the far-reaching range of historical samples that were removed from Ascension Island.The process was slowed down due to reliance on external collaborators, whose response times and processing capacity did not align with the project's timeline. Continuous follow-ups and engagement with collaborators helped improve the process, though delays remained a limiting factor. Please see Annexe 9 for evidence of late sample shipping and correspondence on delays to project.

The addition of genetic sample ID's to the Ascension Biodiversity Catalogue (Output 1.2) was mostly achieved with all species present in the database at the start of the project being checked for pre-existing sequence data from three different gene regions 12s, 18s and COI. Any pre-existing sequence for these regions and species had accession numbers added to the database to ensure they are easy to source in future if needed (Annexe 6 – DPLUS165 master database tab 1a). For species with no current sequence data attempts were made to locate historic samples that may have been stored in a manner allowing for DNA preservation. Some samples are still pending barcoding (see output 1.3 section for further details) and have yet to be added to the database. The project team continues to track the barcoding process, and outstanding samples will be added as soon as they are processed (Annexe 9 – email correspondence NHM).
As mentioned above, delays to Output 1.1 caused a knock on in delays to Output 1.3 with NHM struggling to fulfil their involvement in the project which was to provide barcode sequences for all samples from 1.1.
Due to these delays the alternative timeline for barcoding specimens was as follows and should have been completed by projects end. Specimens of 50 beetles gathered during the DPLUS135 to be processed and sequenced by end of Feb. Barcoding of 130 marine invertebrate samples gathered historically and also from settlement plates from DPLUS165 project to be processed with data back by the end of Feb. Genome skimming of 5 *Discophallus* crickets to provide all barcode area data by late March. Another 100 marine specimens from the DPLUS021 project are expected to arrive at NHM by mid-March and will be processed by mid-April. Unfortunately, these outputs are currently still outstanding despite ongoing correspondence with NHM.
The project was able to continue with barcoding due to the use of universal primers for regions such as 18S and COI. The pre-existing sequence information for most species within the Ascension Biodiversity Catalogue meant that species identifications were possible for the most part, with only a few endemic or unknown species being classified to Family or Genus level. Due to the new timeline for barcoding data from NHM, the University of Edinburgh did not have time to create and develop a suite of primers to allow discrimination of the species collated in Output 1.1 (Output 1.4). The project team deemed that this step was unnecessary due to the species

discrimination achieved by the universal primers for both bony fish and marine invertebrates (see Annexe 6 – DPLUS165 Master Database Tab 2 for primer sequences). For species that proved difficult to identify using a single subset of primers, they were run with generic COI primers and a more specific primer set (see Annexe 8 – preliminary studies - mussel sequencing section references the use of both COI and 18S primers to enable better identification).
Importantly, the use of pre-existing universal primers enhances the contribution of this project to the global scientific community. Universal primers target conserved gene regions used widely in biodiversity research, such as 18S rRNA and COI, enabling the resulting sequences to be directly comparable and integrable with global databases like GenBank and BOLD. In contrast, custom-developed primers tailored for a narrow set of species often yield sequences that are not standardized or broadly comparable, limiting their utility beyond the immediate study. By using universal primers, the project ensures that its sequence data can be accessed and used by researchers worldwide, promoting interoperability, reproducibility, and future comparative analyses.
Output 2 focused on the development, refinement, and quality assurance of metabarcoding protocols prior to their use in staff training. This objective was met in full and on schedule, with no delays. Output 2.1, which involved writing and testing protocols for DNA extraction and metabarcoding, was completed by Year 1, Quarter 4. See Annexe 2 for protocols document. These protocols have since been expanded to include workflows for both Illumina iSeq and Nanopore MinION platforms. The incorporation of Nanopore sequencing enables the Ascension Island Government team to process longer DNA fragments (>300 bp), enhancing taxonomic resolution and enabling access to more comprehensive reference databases, particularly for the COI barcode region.
Training materials, as outlined in Output 2.2, were finalised by Year 2, Quarter 1 (see Annexe 3 for training manual document provided to staff). However, delivery of training continued throughout the full project duration due to high staff turnover within AIGCFD (see Annexe 6 – DPLUS165 Master Database Tab 3). This adaptive approach ensured continuity and sustainability of the metabarcoding workflow beyond the project's end. Staff were trained to different levels depending on their roles, with some learning the full workflow and others focusing on sample collection and preliminary processing.
Output 2.3 was for data quality assurance, this was provided by both the University of Edinburgh and Curtin University, institutions with a proven track record of producing high-quality, publishable metabarcoding data. In both cases, species detection concordance exceeded 95% when compared with results from the Ascension DNA lab (see Annexe 6 – DPLUS165 Master Database Tab 7). Additionally, contamination levels in the container laboratory were comparable to those observed in larger academic facilities, confirming that the lab environment was sufficiently sterile to support reliable metabarcoding workflows. Newly trained staff were given samples that had been previously sequenced by the project officer and as such could be quality checked against a known list of species to determine if outputs were a similar quality level. Trainees typically had slightly higher levels of contamination but were still able to resolve the same species list as the project officer.

The third output of the DPLUS165 project focused on the collection of samples to support biodiversity assessments and monitor non-native species around Ascension Island. The table below outlines the sampling efforts initially proposed and the actual outputs achieved under Output 3.1. For a more detailed list of samples please see Annexe 6 – DPLUS165 Master Database Tab 4 Sample Database and Tab 0 for a summary.
All survey types—except for the settlement plates—exceeded their original sampling targets. The deployment of settlement plates proved challenging, as standard practice requires fixing them to the seabed, which could not be carried out due to health and safety restrictions preventing AIGCFD staff from diving. As an alternative, settlement arrays were attached to mooring lines in the main shipping bay. However, this method left the arrays vulnerable to damage from rope chafing during heavy swell conditions. As a result, at least six arrays were lost and unrecoverable. This issue has informed future design improvements to increase durability and reduce loss.

Across all methods, the project successfully collected and processed a large number of environmental and biological samples. A total of 60 pitfall trap samples and 60 Malaise trap samples were collected—far exceeding the original targets of 18 and 12, respectively—although

not all traps yielded specimens suitable for sequencing. Plankton sampling also surpassed expectations, with 36 samples gathered during full and new moons over an 18-month period, compared to the original plan of 12. Settlement plate sampling almost met the target of 18 arrays, despite some losses due to early deployment and extension of the sampling window. Additional sampling initiatives expanded the project's reach, including: 100 water eDNA samples from 10 sites around the island; 28 water and 24 plankton samples collected through the Citizen Science yacht programme; hull inspections yielding 2 water eDNA samples and 6 individual specimens; and 20 terrestrial samples (10 soil, 8 plant, and 2 well water) targeting plant pathogens. In response to barcoding evidence of *Phytophthora* species in pitfall samples, a supplementary study was launched to investigate its presence and spread. Furthermore, 4 samples were taken from visibly sick fish to support the first investigation into possible pathogenic agents affecting marine life around Ascension. For these supplementary studies on plant and fish pathogens please see Annexe 8.

Type	Expected	Actual	Notes
Pitfall	3 traps monthly for 6 months = 18	15 traps every 3 months for 12 months = 60	*not all traps contain specimens so sampling effort and samples sequenced are not equal
Malaise	2 traps monthly for 6 months = 12	15 traps every 3 months for 12 months = 60	*not all traps contain specimens so sampling effort and samples sequenced are not equal
Settlement	3 panels monthly for 6 months = 18	3 settlement arrays every 3 months for 18 months = 18	*Some settlement panels were lost due to bad swell – design was adapted to prevent this in future
Plankton	2 tows monthly for 6 months = 12	2 tows during full and new moon for 18 months = 36	**not all sequenced by projects end – awaiting reagents and visual processing first
Additional Samples			
Water eDNA screening	NA	10 sites around island every 6 months for 1 year (5x1L duplicates) = 100 samples	
Citizen Science water eDNA and Plankton	NA	7 yachts provided 28 water and 24 plankton samples	
Hull inspection eDNA and specimens	NA	2 hull eDNA 1L water samples gathered, 6 single specimens from hull gathered	A known invasive species was suspected to be on the hull of a supply vessel
Soil and plant pathogen samples	NA	10 samples from soil 8 plant samples	Discovery of *Phytophthora sp.* in some pitfall

		2 other (well water)	barcoding led to larger study to determine species ID and delineate its spread
Fish Pathogen screening	NA	4 samples from sick fish	Fish around ascension cyclically present with an illness but it has not before been known what the causative agent is or its effects on public health

With regard to Activity 3.2, trained members of AIGCFD staff have conducted metabarcoding on samples collected through Activity 3.1. As a result of increased sampling effort beyond the original target of 160 samples (18 pitfall, 12 malaise, 18 settlement plates, 12 plankton tows and 100 fish gut contents), a total of 463 samples have been processed and sequenced via metabarcoding to date, a 189% increase in output. See Annexe 6 Tab 0 for a summary of libraries run and samples sequenced. This includes a broad range of terrestrial and marine samples. Sampling efforts are ongoing as part of AIGCFD's continued commitment to long-term biosecurity surveillance, and additional samples will be processed routinely as they are collected.

The supplementary marine biodiversity eDNA sampling was carried out in support of the Ascension Marine Protected Area (MPA) Management Plan objectives, particularly the need for improved baseline data across the wider MPA. This included areas of the MPA not accessible to the AIGCFD team due to distance offshore. To address this, a citizen science programme was established prior to the start of this project to leverage the sailing routes of visiting yachts. These vessels provided water and plankton samples from remote areas of the MPA, enabling broader spatial coverage and enhanced biodiversity surveillance. Additional sampling activities—including the collection of soil, plant, fish, and hull samples—were initiated at the request of AIGCFD staff in response to emerging biosecurity concerns. These included the detection of plant pathogens such as *Phytophthora spp*., investigation of potential fish pathogens, and hull fouling risks linked to priority invasive species, see Annexe 8. Together, these efforts have contributed significantly to Ascension Island's ability to detect, monitor, and respond to biosecurity threats using DNA-based tools.

Activity 3.3 was to pass on detections of high priority invasives to biosecurity teams for action, please see Annexe 6 – DPLUS165 Master Database Tab 5 species detections and species highlighted in red. These were passed onto the AIG biosecurity team for discussions on responses. Where relevant, as in the case of mussel species detected on a ship hull, this information was also passed onto neighbouring UKOTs (Annexe 8 – Mussel report) with shared shipping routes to enable them to begin monitoring and training on species ID for divers carrying out hull inspections. Due to the lack of baseline data for certain taxonomic groups it was not known if species had been on Ascension for a long period of time or if they had recently arrived. This project will now be setting a baseline for any new species detections.

Activity 3.4 was to produce a summary report listing species detected by metabarcoding, this is provided in the form of Annexe 1. The report is a summary, however, more detailed results containing sequences can be found in Annexe 6 – DPLUS165 Master Database Tab 5. In total 12S primers detected 182 species, 18S primers detected 377 species and COI primers detected 420 species. Of these, 640 (65% of total species detected) were not previously recorded in biodiversity catalogues, reflecting the gap in knowledge on plankton, marine invertebrate assemblages and deep sea species.

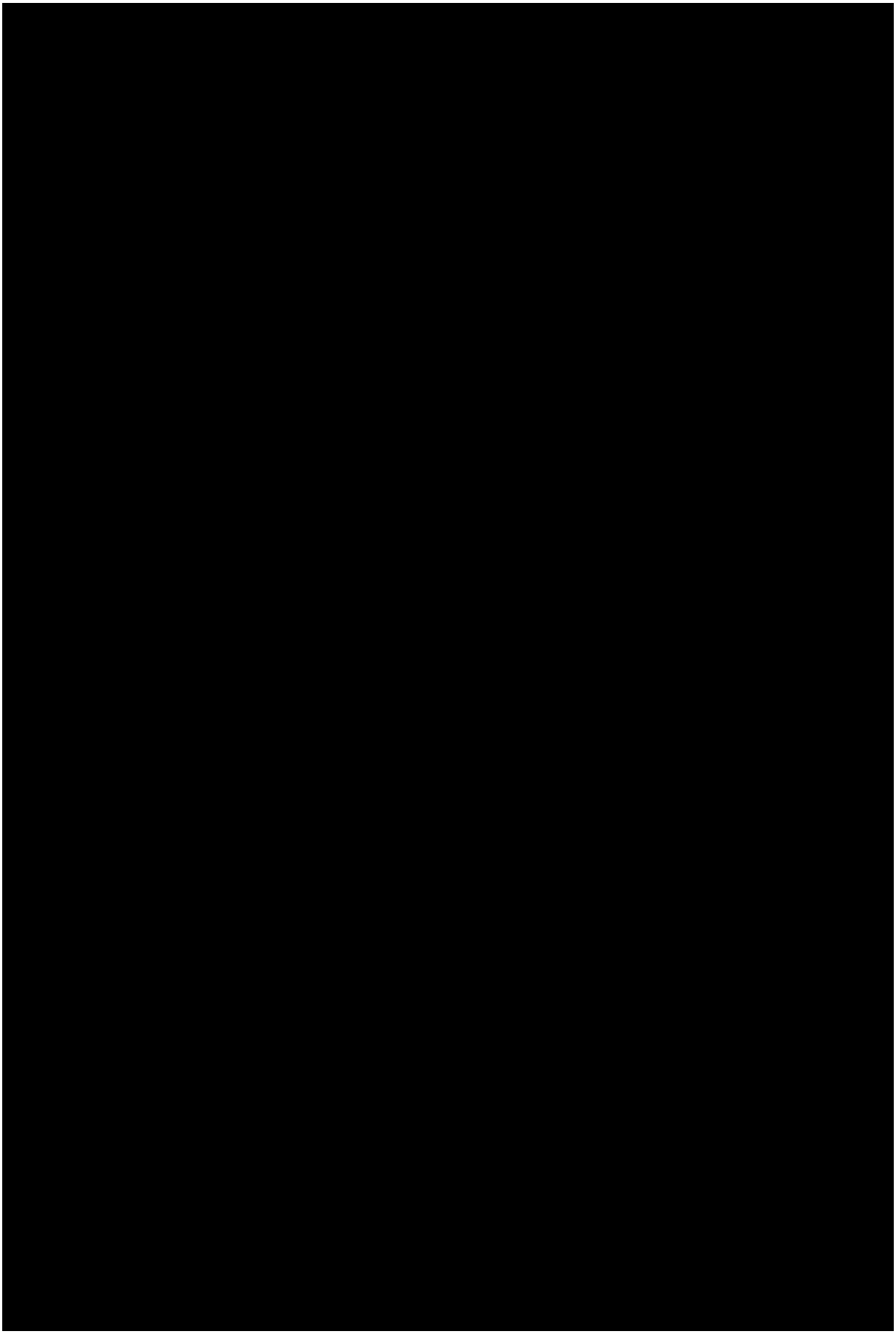
As a brief summary of data collected over the course of the project, DNA-based techniques enabled the addition of hundreds of previously undocumented species to the Ascension

Biodiversity Catalogue (ABC). While the ABC was already robust for visually identifiable shallow-water species, it lacked representation of cryptic taxa, sessile invertebrates, zooplankton, and deep-sea organisms. DNA screening allowed detection without the need for direct observation.
12S primers, targeting vertebrates, detected over 57 new fish species. These were mostly deep-sea taxa such as lanternfish (*Myctophidae*), bristlemouths, viperfish, and pearlsides. These are key components of mesopelagic food webs, typically missed in traditional surveys. A few epipelagic and reef-associated species, including puffers were also recorded.
18S primers (Uni18S), used across a range of sample types—plankton tows, settlement plates, sediments—yielded broad taxonomic coverage and detected over 350 new species to Ascension. In total, plankton samples alone revealed over 90 new detections, including 22 copepod species and 16 dinoflagellates, along with krill, amphipods, jellyfish, and chaetognaths. These reflect a rich and productive pelagic zone with primary producers (diatoms, algae) and mid-trophic consumers (copepods, lanternfish). Fish larvae were also identified, aiding ichthyoplankton visual identification efforts. Settlement plates yielded over 150 new species, showcasing diverse early-stage biofouling communities. Sessile invertebrates (sponges, tunicates, bryozoans, barnacles) dominated, accompanied by mobile taxa like amphipods, polychaetes, and nematodes. The presence of chlorophytes, diatoms, and haptophytes indicates active biofilm development in sunlit waters, while protistan and microbial diversity—including parasitic taxa—reflects complex ecological interactions on submerged substrates. Many 18S sequences matched uncultured or unclassified eukaryotes in public databases, highlighting the potential for reanalysis as reference libraries improve.
COI primers targeting general eukaryotic diversity added over 250 marine and terrestrial species to the ABC. Gut content analysis via COI revealed over 40 new marine taxa, illustrating predator diets rich in midwater cephalopods, crustaceans, and mesopelagic fish. Plankton samples processed with COI yielded over 30 new additions, echoing the 18S results and providing a detailed snapshot of primary producers, consumers, and vertically migrating micronekton. Settlement plate analysis with COI contributed over 60 new marine species. These data reflect shallow subtidal biofouling communities characterized by high sessile invertebrate diversity and active colonization of hard substrates.

A comprehensive list of Ascension's terrestrial invertebrate community was produced as part of DPLUS135; this list has been further built upon by molecular sequencing techniques and continued invertebrate screening during the DPLUS165 project. As such, over 30 species have been added to the list. The majority of the species added are either due to species misidentification which has been rectified by molecular techniques (*Monomorium pharanosis* / *Monomorium sahlbergi*) or the addition of microscopic taxa / nematodes to the database.

Output 4 was to analyse the gut contents of 10 marine indicator species and compare this to the classic visual method of gut content analysis. The indicator species chosen were selected from a wide range of trophic levels to allow for a larger picture of food webs in the Ascension nearshore environment. Annexe 6, tab 8 details the species, primer selection and assumed trophic level. COI primers were selected for species thought to have a diet consisting more of marine invertebrates, 12S primers were selected for higher trophic level feeders to highlight bony fish. Preliminary studies were performed to determine if 12s or COI primers provided the best prey to host resolution for those in-between levels.

[redacted]



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3.2 Outcome

The outcome of this project was to ensure 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. This has been achieved and all materials and training (Annex 3 for training booklet, Annex 6 tab 3 for training database) needed for DNA metabarcoding by long term AIGCFD staff is in place. This includes DNA barcode reference libraries for terrestrial invertebrates (COI region), marine invertebrates (18s region) and bony fish (12s region) (indicator 0.1 Annex 6 tab 1a, 1b). These reference databases are also in the format of FASTA files used in metabarcoding workflows; however these are exceptionally large files with large quantities of sequence data. A summary of them is provided as mentioned above and actual reference databases can be provided upon request via wetransfer link. The DNA metabarcoding workflow has been in use routinely since Y2Q4 of the project and has been carried out in its entirety via multiple different AIGCFD staff members, again evidence is provided in Annex 6 showing number of samples sequenced and number of staff members trained (indicator 0.3 and 0.4). Although the outcome has been fully achieved, the already strained capacity constraints placed on AIGCFD staff means they may not have the time needed to complete full metabarcoding on all samples and as such sampling methods may need to be scaled back now that a good baseline has been set.

3.3 Monitoring of assumptions

The monitoring of project assumptions was performed during each review cycle unless previously flagged as a possible problem. The main assumption that caused delays to the project was 1.1 which assumed that “Samples from past surveys (particularly DPLU021) were suitably preserved and will be shared by previous project partners where required.” Unfortunately, the delivery of the DPLUS021 samples from the Falkland Islands to NHM was delayed until Y3Q4 due to lack of staff capacity to process and record these samples prior to shipment. Workarounds were put into place to mitigate this, and pre-published data was utilized by the project team for barcodes for most species. Please see Annex 9 for the emails detailing the late delivery of samples and re-established time lines on data output.

Another of the assumptions that caused delays to the project was 0.1 “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”. The set-up of the lab and training of staff was not an issue, however the availability of reagents and consumables due to COVID-19 lab manufacturing setbacks caused some delays. The largest delay was with frozen and cold reagent shipment. Before the project started, an agreement was made with AIG’s shipping provider that they would be able to transport cold and chilled goods. However, after new policies at the shipping company it was decided that liability for cold chain items would no longer be allowed. This caused delays to ordering in all three of the financial years of the project. Alternative arrangements were put into place involving AIG staff hand carrying items on flights in cold bags and the purchase of 5-day cold storage boxes for air freight once the Ascension runway was operational again.

The assumptions in outputs 2 and 3 were fine and there were no issues with any of these as staff were able to learn and execute metabarcoding and this successfully identified species listed in reference databases.
The assumption that the isotope model would be finished by 2022 was valid and the PhD project did finish but there were additional samples still to be analyzed; as such any data regarding the backup of the isotope model is under embargo until it is published (Orrell et al. unpublished).
As the gut content analysis was not due to be completed until Y3Q3 this did not pose a delay to the project. There were issues with being able to catch and sample the Ascension wrasse which was originally selected as an indicator species – AIGCFD staff decided it was unnecessary to use clove oil in an entire interlinked rockpool system for the sake of catching 10 Wrasse. Due to the fact that Sergeant Majors and Black triggerfish occupy the same trophic niche it was decided that the Ascension Wrasse would be swapped out with the Silky Shark as data from this could help another existing Darwin project the DPLUS161.
Output 5 held the assumption that teachers at the local school would be receptive to adding aspects of DNA Biomonitoring to the curriculum – all activities and outreach events were received warmly by staff at the school and engagement with the wider community via social media posts was also positive. In total over 44 posts were shared across both Facebook and Instagram reaching 17,124 people and accumulating 1,522 likes, 28 comments and 14 shares. (see Annex 11 for some examples of social media posted during the project)

4 Contribution to Darwin Plus Programme Objectives

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

The Barcoding an Island project (DPLUS165) has made a significant contribution to the long-term strategic conservation of Ascension Island's natural environment through the development of a genetic reference library for the island's terrestrial and nearshore biodiversity. By providing a foundational resource for species identification and monitoring, the project has strengthened local capacity to make evidence-based management decisions and respond to emerging environmental threats.
This project directly supports strategic goal 4 outlined in Ascension Island Government's Biodiversity Action Plan (*"4. There are no new introductions of invasive, non-native species and the impacts of those already present are reduced."*) by providing fast species ID's on any possible non-natives that are introduced. The Ascension Island Biosecurity Strategy point 7.2.3 involves the monitoring of key sites around Ascension for non-native introductions. The strategy states *"Effective surveillance monitoring will require species-level identification to distinguish between high risk species and those already present"*. The DPLUS165 project has helped to provide a more accurate baseline for those species that are already present and enables AIGCFD staff to check the updated Biodiversity database for information on what species may or may not be new introductions, this enables more accurate and efficient detection of invasive species. The integration of DNA barcoding into ongoing environmental monitoring programmes enhances the ability to detect cryptic, rare, or morphologically ambiguous species that might otherwise go unnoticed, particularly in early stages of invasion or ecosystem change.
In addition, the investment in training and infrastructure has built long-term capacity within the AIGCFD. Local staff have received hands-on experience in molecular techniques and data analysis, ensuring that the skills and knowledge gained remain within the Territory. The establishment of a sequencing pipeline on-island also reduces reliance on external laboratories, lowering costs and turnaround times for future biodiversity assessments.
The project's outputs also feed into global databases, ensuring Ascension's unique biodiversity is represented and accessible to the international scientific community. This promotes collaboration and supports wider regional and global conservation efforts, particularly in the face of climate change and biodiversity loss.
In summary, the project has created a robust scientific and institutional platform for the ongoing protection and understanding of Ascension Island's natural heritage, ensuring a legacy that extends well beyond the project's duration.
The project has helped Ascension Island make progress toward its obligations under several multilateral environmental agreements extended to the UKOTs, including the Convention on Biological Diversity (CBD) Article 7 (Identification and Monitoring): through the barcoding of

species and monitoring via eDNA, improving knowledge of native and invasive biodiversity and article 8(h) (Invasive Species): by building tools for early detection and rapid response systems.

4.2 Gender Equality and Social Inclusion (GESI)

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	

While the primary focus of Barcoding an Island was biodiversity conservation and capacity building, gender and inclusion considerations were incorporated from the outset. The project was delivered on Ascension Island, where the local conservation workforce is small but diverse in terms of gender and nationality. The project was designed to be inclusive, ensuring opportunities for technical training were available to all staff, regardless of gender or background.

Project activities were delivered within an inclusive team culture. Training workshops in molecular techniques and data analysis were open to all members of the Conservation and Fisheries Directorate, and efforts were made to encourage participation from individuals who had not previously engaged in lab-based or technical work. Of those trained in barcoding and sequencing methods, 58% were women, including early-career staff who had not previously worked with molecular tools or in laboratory environments. Social media posts highlighting women in STEM were posted under relevant hashtags #womeninSTEM to ensure the widest reach possible towards women interested in careers in science. Please see Annexe 11 for social media post detailing the reach of this post.

5 Monitoring and evaluation

The singular change request submitted was one involving the change in the projects start and end date due to delays in the announcement of funding. The projects start was transferred from 01/06/2022 to 01/07/2022 to enable time to prepare for the start of the project. This also meant a follow-on effect of the end date changing from 31/01/2025 to 28/02/2025. There was no reason to change either the log frame as the time change was only a month and did not fall into different quarters.

The project was monitored using the Log frame timelines, indicators and means of verification by the project lead and project officer, these proved to be sufficient for the monitoring of the project. These results were monitored quarterly and any items that did not meet the project timelines were referred to project partners where applicable for mitigation. The addition of Darwin reports occurring every 6 months allowed for a larger more in-depth review of project progress, however feedback on these was often delayed and not timely enough to rely on for mitigation.

6 Lessons learnt

The *Barcoding an Island* project delivered significant outcomes, particularly in technical innovation and capacity building in a remote UK Overseas Territory. However, it also faced several challenges that have generated important lessons for future Darwin Plus projects. These lessons span technical and logistical issues and can inform continuous improvement across the wider Darwin Plus programme.

Things that worked well include, local capacity building which was highly effective after trained personnel were put in position to give hands on, in-person training to pre-existing and new staff members. The multi-partner collaboration between NHM, UOE and AIGCFD helped to foster and further develop strong lasting partnerships. The integration of DNA based monitoring activities into the wider scope of work for AIGCFD even outside the scope of the DPLUS165 project is the most important outcome. It has allowed AIGCFD staff to increase knowledge in areas such as plant and marine pathogen analysis, citizen science projects, plant pest identifications and dietary analysis.

Lessons learnt focused mainly around logistical delays involving cold chain shipment as many shipping companies refuse to take responsibility for cold items due to potential delays and storage requirements. Fortunately, with the Ascension Island runway coming back into service with UK based flights during the project, this was mitigated with the purchase of cold chain shipping boxes that keep reagents cool for up to 5 days. Any future Darwin projects depending on sequencing in remote territories should have carefully planned out cold chain delivery pathways to ensure this is not a limiting step. There were also logistical issues with the delivery of lab equipment and consumables; however, this was mostly due to the scarcity of sterile equipment after the COVID-19 pandemic and, as such, is unlikely to affect any future projects.

Another major point to be considered when planning to undertake sequencing in remote UKOTs is the need for a reliable and fast internet connection – this can be mitigated by pre-downloading a reference database (typically 300GB) and using that for the duration of any projects. However, the downfall of this is that reference databases are being updated all the time and obtained sequences will not be run against the most updated database.

7 Actions taken in response to Annual Report reviews

No recent feedback on reports from final financial year – other feedback was responded to in the Y2 final year report. All review feedback was shared between project partners and responded to in Y2 annual report.

8 Risk Management

There were no new risks in the final 12 months of the project. Of the risks which were previously accounted for only the delay in sourcing external samples continued to prevent progress on Output 1. As mentioned in section 3, the project team were able to use generic COI, 12s and 18s primers to perform biodiversity monitoring and did not require the development of more Ascension specific primers.

9 Scalability and Durability

The Barcoding an Island project (DPLUS165) has gained significant visibility and support within Ascension Island. Over the course of the project, we engaged the local community through outreach events, and school sessions to promote awareness of the island's unique biodiversity and the importance of genetic barcoding in conservation. Collaboration with the wider AIG and local stakeholders ensured that project activities were well-integrated into the island’s ongoing environmental initiatives. Regular updates were shared via local media (e.g., the Islander) and social media platforms, which helped maintain a strong public profile. See Annex 11 for social media posts.

The most enduring achievements of the project include the development of a genetic reference library for Ascension Island species, the training and capacity building of local staff, and the

integration of barcoding techniques into conservation monitoring workflows. These outcomes are likely to persist because they have been embedded within local workplans and into official Biosecurity and nature reserve monitoring plans. The skills developed will continue to be applied across future conservation activities. Additionally, the generated barcode data will be made openly available after results from delayed output 1 have been received, ensuring their utility for global researchers and future biodiversity assessments.
The intended sustainable benefits remain valid and relevant. The goal of increasing local capacity for molecular biodiversity monitoring has been achieved, and the benefits continue through trained staff, established protocols, and ongoing collaboration with UK-based institutions. No major changes have been made to the original sustainability plan, although greater emphasis is now being placed on integrating genetic data into environmental decision-making and long-term species monitoring plans.
Following the conclusion of Darwin Plus funding, trained project staff have transitioned into other roles within the AIG Conservation and Fisheries Directorate although this is still largely dependent on Darwin funding via other projects. The molecular equipment and resources purchased during the project remain in use on-island and are being incorporated into the Directorate's routine biodiversity and biosecurity monitoring work. Continued collaboration with UK academic partners will support further development of local capacity.
As part of our open access plan, all barcode sequences generated will have been submitted to public databases such as BOLD and GenBank (although approval of this may run overdue from the projects final timeline), with metadata clearly linked to Ascension Island specimens. Project reports and protocols have been shared with stakeholders and made available online where possible. Scientific outputs will also be published in open access journals after manuscript writing and approval to ensure maximum visibility and accessibility.
The project has contributed to increased recognition of the value of molecular tools in biodiversity conservation and invasive species management on Ascension. While no formal policy changes have been enacted, the inclusion of continued genetic monitoring in both marine and terrestrial protected area management plans shows the project will have contributed to longer term monitoring of Ascension's biodiversity.

10 Darwin Plus Identity

This project has been promoted on both the AIGCFD Facebook and the Ascension Island MPA Facebook and Instagram channels. On all occasions the DPLUS165 code has been mentioned and where photos/videos were included the DPLUS logo was shown clearly. See annexe 11 for details.

A total of 1,522 likes, 28 comments and 14 shares were achieved with the 44 posts throughout the project's lifetime. The posts were reported to have reached an audience of 17,124 people.

The project was recognised as a distinct project forming part of AIGCFD's wider work. As Darwin Plus has been a contributor to the AIGCFD programme for many years the community are familiar with the concept and most are aware of the help Darwin Plus provides, both financially and capacity building wise, for conservation on Ascension Island.

This project has also been promoted internationally through presentations at conferences, meetings with stakeholders and cross territory training activities. In all cases, the Darwin Plus Initiative has been credited as being a funder and supporter of DPLUS165 and other associated projects.

11 Safeguarding

[redacted]

[redacted]

12 Finance and administration

12.1 Project expenditure

[redacted]

Project spend (indicative) since last Annual Report	2024/25 Grant (£)	2024/25 Total actual Darwin Plus Costs (£)	Variance %	Comments (please explain significant variances)
Staff costs	[redacted]			

Project spend (indicative) since last Annual Report	2024/25 Grant (£)	2024/25 Total actual Darwin Plus Costs (£)	Variance %	Comments (please explain significant variances)
Consultancy costs	[redacted]			
Overhead Costs				
Travel and subsistence				
Operating Costs				
Capital items				
Others				
TOTAL	£63,550	59240.55		

Staff employed (Name and position)	Cost (£)
Melissa Morgan – Project Officer	[redacted]
Kirsten Harper UOE Postdoc	
TOTAL	

Consultancy – description and breakdown of costs	Other items – cost (£)
TOTAL	

Capital items – description	Capital items – cost (£)
TOTAL	

Other items – description	Other items – cost (£)
AIG DNA Lab Consumables UoE DNA Lab Consumables Freight	[redacted]
TOTAL	

12.2 Additional funds or in-kind contributions secured

Matched funding leveraged by the partners to deliver the project	Total
Fieldwork travel and subsistence (AIGCFD)	[redacted]
Staff Costs (AIGCFD, TS and CM)	

NHM (Estates and indirects / Overheads)	[redacted]
TOTAL	[redacted]

Total additional finance mobilised for new activities occurring outside of the project, building on evidence, best practices and the project	**Total (£)**
TOTAL	

12.3 Value for Money

The Barcoding an Island project represented strong value for money across all key areas: economy, efficiency, and effectiveness. By using DNA barcoding as the primary method for species identification, the project achieved a far greater volume and breadth of identifications than would have been possible through traditional taxonomic methods alone.

Hiring expert taxonomists to identify the wide range of invertebrates, algae, and other poorly documented taxa on Ascension Island would have required substantial financial resources. In addition to consultant fees, there would have been significant operating costs associated with collecting, preserving, and shipping specimens internationally, along with lengthy timeframes for return of results. The molecular approach allowed for rapid, standardised, and cost-effective identification of hundreds of species, many of which would otherwise remain unclassified.

The project maximised efficiency by training local staff in molecular techniques, reducing reliance on off-island expertise and creating long-term capacity for future biodiversity monitoring. Investments in equipment and training have continued utility beyond the life of the project, offering long-term benefits at no additional cost to funders.

Delivering projects in remote UKOTs such as Ascension Island presents unique logistical and financial challenges. Shipping delays, higher costs for materials, and limited access to specialist support all impact the budget. Despite these constraints, the project managed resources carefully and adapted plans to ensure delivery of key outcomes within budget.

Overall, DPLUS165 delivered excellent value for money by achieving wide-reaching biodiversity documentation, building local capacity, and creating a lasting scientific and conservation legacy through cost-effective, scalable methodologies.

13 Other comments on progress not covered elsewhere

14 OPTIONAL: Outstanding achievements of your project (300-400 words maximum). This section may be used for publicity purposes.

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 the life of the project

Project summary	Progress and achievements
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.	The project contributed to biodiversity conservation by generating the first genetic reference library for Ascension Island, improving species identification and monitoring. It also supported local capacity building, enabling on-island molecular analysis for sustainable biodiversity management. This empowers the community to make informed conservation decisions and supports equitable, long-term environmental stewardship.
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.	
Outcome indicator 0.1 DNA barcode reference library developed for all invertebrate species identified by previous taxonomic studies by Y2Q2.	Achieved and completed: Annex 6 tab 1a, 1b
Outcome indicator 0.2 Suite of primers designed and validated for metabarcoding of Ascension invertebrates	Completed - Specific primers were not needed as pre-developed generic primers worked to resolve Ascension's invertebrates and have the benefit of having large reference databases already. Primers for both COI and 18s were validated for marine and terrestrial invertebrates please see annexe 6 tab 2 for a list of primers
Outcome indicator 0.3 Metabarcoding used routinely to identify species in AIGCFD's terrestrial invertebrate, gut content, settlement panel and light trap monitoring programmes by Y3Q2.	Completed - Metabarcoding used on a total of 783 samples through the project Annexe 6 tab 0
Outcome indicator 0.4 Long-term DNA extraction and metabarcoding capability established on Ascension through staff training and protocol creation by Y2Q2.	Achieved and completed – staff training database Annex 6 tab 3 QA figures from Curtin samples Annex 6 tab 7
Output 1 DNA primers developed for detection and identification of principal Ascension Island terrestrial and marine invertebrate species.	

Output indicator 1.1 All available tissue samples from verified terrestrial and marine invertebrates from Ascension collated by Y2Q2.	Completed but was delayed – see Annex 9 for evidence and details on when samples were shipped
Output indicator 1.2 DNA barcoding undertaken on all species for which samples are available (up to a total of 800) by Y2Q4	Not completed – still awaiting results to be provided by NHM, hoped to be completed within 3 months of project finishing. See Annex 9 for timeline details.
Output indicator 1.3 Primers developed for all sampled species by Y2Q4.	Completed by using generic primers, database of primers (Annex 6 tab 2) and species they detect (Annexe 6 tab 5)
Output 2. AIGCFD staff able to carry out DNA extraction and metabarcoding	
Output indicator 2.1 Protocols for DNA extraction and metabarcoding in Ascension's lab agreed between AIGCFD and University of Edinburgh by Y1Q4.	Completed see Annexe 2 Protocols PDF
Output indicator 2.2 Ten AIGCFD staff trained in DNA extraction and metabarcoding delivered by Y2Q3.	Completed see Annex 3 for training materials and Annex 6 tab 3 for Training database
Output indicator 2.3 QA of AIGCFD staff results undertaken by DNA Project Officer and University of Edinburgh by Y2Q3.	Completed - QA undertaken by Curtin university as pre-existing arrangement was in existence. UOE also performed QA on gut content samples during blocking primer development. See Annex 6 tab 7 for QA results
Output 3. Metabarcoding used to identify terrestrial and marine invertebrate species as part of AIGCFD monitoring and biosecurity surveillance activities.	
Output indicator 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.	Completed - Annexe 6 tab 4 for sample database (detailed) see Annex 6 tab 0 for summary of number of samples of each type

Output indicator 3.2 Metabarcoding of all samples described above (180 samples in total including replicates) by AIGCFD staff by Y3Q2.	Completed - Annexe 6 tab 0 for summary of sequencing libraries run and tab 4 for sample database and tab 5 for species results. Total of 783 samples run.
Output indicator 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.	Completed and ongoing - Annexe 6 tab 5, species highlighted in red were possible invasives and were passed along to relevant teams. See Annex 8 for Mussel report shared with St Helena regarding possible shared invasives.
Output indicator 3.4 Report summarising species present in each sample and highlighting any new biosecurity threats produced by Y3Q4.	Completed please see Annexe 1 – DPLUS165 results report. Report will be shared with any collaborators upon request.
Output 4. Gut content analysis using metabarcoding techniques undertaken to validate isotope-based ecosystem model.	
Output indicator 4.1 Ten indicator species identified from different trophic levels of the isotope-based ecosystem model by Y1Q4.	Completed see Annexe 6 tab 8 for the list of indicator species chosen. Note Ascension wrasse swapped for silky shark due to ease of sampling and usability of data. Species in red were not used.
Output indicator 4.2 Blocking primers developed for the indicator species where necessary by Y2Q2.	Completed. Primers developed for Grouper, Moray, Sharks and Wahoo. Annex 7 shows a report regarding the development of these.
Output indicator 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.	Completed - Annex 5 provides a report on gut content analysis showing species identified by metabarcoding and visual standard techniques.
Output 5. Secondary school students on Ascension understand how DNA biomonitoring techniques are carried out and their application for conservation.	
Output indicator 5.1 30 school students have visited the Ascension lab and extracted DNA in the classroom by Y3Q1.	Completed – See Annex 6 tab 14 for a list of visits to DNA lab and outreach activities. Also see Annex 10 and 11 for photos of outreach activities and social media posts.

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.			
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.
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.	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
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 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.
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 summarising species present in each sample and highlighting any new biosecurity threats produced by Y3Q4.	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.
Output 4 Gut content analysis using metabarcoding techniques undertaken	4.1 Ten indicator species identified from different trophic levels of the isotope-based ecosystem model by Y1Q4.	4.1 List of indicator species and diagram showing position in isotope-based ecosystem model.	Isotope model is completed before the start of this project.

to validate isotope-based ecosystem model.	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.	4.2 Sequences of blocking primers for indicator species. 4.3 Report on gut content analysis showing species identified by metabarcoding and standard techniques.	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.
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 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)			

Table 1 Project Standard Indicators

Please see the Standard Indicator Guidance for more information on how to report in this section, including appropriate disaggregation. N.B. The annual total is not cumulative. For each year, only include the results achieved in that year. The total achieved should be the sum of the annual totals.

DPLUS Indicator number	Name of indicator	If this links directly to a project indicator(s), please note the indicator number here	Units	Disaggregation	Year 1 Total	Year 2 Total	Year 3 Total	Total achieved	Total planned
DPLUS-A01	Number of people in eligible countries who have completed structured and relevant training *Please see Annexe 6 tab 3 for gender details and training received	Indicator 2.2	People	Trained	6	7	4	17	*Round 10 started before standard indicators were required so none planned
DPLUS-B01	E.g. Number of new or improved habitat management plans available and endorsed *number of management plans including genetic analysis as a tool MPA Biosecurity Strat MPA monitoring evaluation and research strategy Bat Cave NR		Number	New/Improved	2	0	1	3	X
DPLUS-A03	Number of local or national organisations with enhanced capability and capacity		Number	Organisations	1	0	0	1	X
DPLUS-C08	Number of Media related activities.		Number	Talks Given	1	2	2	5	X
DPLUS-C08	Number of Media related activities		Number	Social media posts	10	14	20	44	X

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, scheme type of report (i.e. Annual or Final), and year) and **deleted the blue guidance text** before submission?	X
Is the report less than 10MB? If so, please email to BCF-Reports@niras.com putting the project number in the Subject line.	X
Is your report more than 10MB? If so, please consider the best way to submit. One zipped file, or a download option, is recommended. We can work with most online options and will be in touch if we have a problem accessing material. If unsure, please discuss with BCF-Reports@niras.com about the best way to deliver the report, putting the project number in the Subject line.	
If you are submitting photos for publicity purposes, **do these meet the outlined requirements (see section 14)?**	X
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.	X
Have you provided an updated risk register? If you have an existing risk register you should provide an updated version alongside your report. If your project was funded prior to this being a requirement, you are encouraged to develop a risk register.	X
Have you involved your partners in preparation of the report and named the main contributors	X
Have you completed the Project Expenditure table fully?	X
Do not include claim forms or other communications with this report.	