EAZA Terrestrial Invertebrate Taxon Advisory Group

Best Practice Guidelines

for Desertas land snails

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EAZA Preamble

Right from the very beginning it has been the concern of EAZA and the EEPs to encourage and promote the highest possible standards for husbandry of zoo and aquarium animals. For this reason, quite early on, EAZA developed the “Minimum Standards for the Accommodation and Care of Animals in Zoos and Aquaria”. These standards lay down general principles of animal keeping, to which the members of EAZA feel themselves committed. Above and beyond this, some countries have defined regulatory minimum standards for the keeping of individual species regarding the size and furnishings of enclosures etc., which, according to the opinion of authors, should definitely be fulfilled before allowing such animals to be kept within the area of the jurisdiction of those countries. These minimum standards are intended to determine the borderline of acceptable animal welfare. It is not permitted to fall short of these standards. How difficult it is to determine the standards, however, can be seen in the fact that minimum standards vary from country to country. Above and beyond this, specialists of the EEPs and TAGs have undertaken the considerable task of laying down guidelines for keeping individual animal species. Whilst some aspects of husbandry reported in the guidelines will define minimum standards, in general, these guidelines are not to be understood as minimum requirements; they represent best practice. As such the EAZA Best Practice Guidelines for keeping animals intend rather to describe the desirable design of enclosures and prerequisites for animal keeping that are, according to the present state of knowledge, considered as being optimal for each species. They intend above all to indicate how enclosures should be designed and what conditions should be fulfilled for the optimal care of individual species.

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In memoriam of: Tamara Canalejas, who played a key role in Bristol Zoological Society's involvement with these snails, and is keenly missed by those who knew and worked with her.

Summary

- These 4 snail species are all Critically Endangered in the wild and subject to ongoing reintroduction efforts- biosecurity is crucial for any holders of these species.
- The tiny size of hatchlings and young juveniles provide husbandry challenges that can be overcome through the use of specific equipment
- These snails are undemanding in terms of their housing and diet, although adjustment in keeping methods is needed as institutional population sizes grow.
- Following seasonality in temperature and humidity designed to mimic conditions in the Desertas Islands enables breeding and the creation of robust, healthy populations of snails suitable for reintroduction
- There are a number of knowledge gaps still around these species and several aspects of their behaviour & ecology; all holders are encouraged to collect as much data as is feasible to improve our understanding of these little-studied snails.

Contents

Section 1: Biology and field data 5

Biology 5

1.1 51.2
51.3
81.4
91.5 Conservation status/Zoogeography/Ecology
10

1.5.1 Distribution 12

1.5.2 Habitat 14

1.5.3 Population 16

1.5.4 Threats to Wild Populations 16

1.5.5 Conservation Status 18

1.6 Diet and feeding behaviour 19

1.7 Reproduction 19

1.7.1 Developmental Stages to Sexual Maturity 19

1.7.2 Age of Sexual Maturity 20

1.7.3 Seasonality of Cycling 21
1.7.4 Clutch Size 21
1.8 Behaviour 21
1.8.1 Activity 21
1.8.2 Predation 21
1.8.3 Sexual Behaviour 22
Section 2: Management in Zoos and Aquariums 23
2.1 Enclosure 23
2.1.1 Boundary 23
2.1.2 Substrate 24
2.1.3 Furnishings and Maintenance 25
2.1.4 Environment 30
2.1.5 Dimensions 31
2.2 Feeding 33
2.2.1 Basic diet 33
2.2.2 Special dietary requirements 33
2.2.3 Method of feeding 34
2.2.4 Water 35
2.3 Social Structure 35
2.3.1 Sharing Enclosure with Other Species 35
2.4 Breeding 35
2.4.1 Mating 35
2.4.2 Egg Laying 35
2.4.3 Incubation & hatching 37
2.4.4 Development and Care of Young 37
2.4.5 Population Management 39
2.5 Behavioural Enrichment 39
2.6 Handling 39
2.6.1 Individual Identification and Sexing 39
2.6.2 General Handling/ Catching 40
2.6.3 Transportation 41
2.7 Veterinary Considerations for Health and Welfare 42
2.8 Recommended Research 45
Section 3: Acknowledgements, References and Appendices 46

3.1 Acknowledgements 46

3.2 References 46

3.3 Appendices 47

Appendix I : seasonality charts for Desertas species at Chester Zoo 47

Appendix II: Marking methodology for marking *Discula* and *Geomitra* for reintroductions 50

Appendix III: Pre-reintroduction health screening protocol for *Partula* snails 52

Section 1: Biology and field data

Biology

1.1 Taxonomy

- Order: Stylommatophora Schmidt, 1855
  - Family: Geomitridae Boettger, 1909
    - Subfamily: Geomitrinae Boettger, 1909
      - Genus: *Geomitra* Lowe, 1852
        - Species: *Geomitra grabhami* Wollaston, 1878
        - Species: *Geomitra coronula* Lowe, 1852
      - Genus: *Discula* Lowe, 1852
        - Species: *Discula lyelliana* Lowe, 1852
    - Family: Gastrodontidae Tryon, 1866
      - Genus: *Atlantica* Ancey, 1887
        - Species: *Atlantica calathoides* Lowe, 1863

All these species have congenerics found outside the Desertas Islands, and several other *Discula* are also endemic to the Desertas (e.g. *D. tetrica*, *D. cameroni*), but as the *ex-situ* breeding programme focuses on these four Desertas Island endemic species, congeners are not listed here.

Common Names:

English: collectively known as the Desertas Land Snails, the individual species do not have their own common names.
French: Collectively called 'Escargots des Desertas', the individual species do not have their own common names in French.
Portuguese: No current common names. A survey will be done in 2025 to try to find a common name for the species.

1.2 Morphology

Discula lyelliana: The largest of the four species. A sample of mature captive-bred adults of this species had an average shell diameter of 14.3mm, an average shell height of 7.2mm, and an average weight of 0.7g. The shell of this species varies from khaki to chestnut brown, with darker markings around the outer whorl, and with an attractive dark brown stripe on the underside (Figs. 1 & 2). Very

large, fully mature specimens tend to be a darker, almost reddish brown, and can reach up to 16mm in shell diameter and 0.8g in weight. This species has a smooth, moderately flattened shell.

Figure 1. Adult *D. lyelliana* withdrawn into shell, showing (left) upper side and (right) the underside of the shell, showing the dark brown stripe and thickened lip of the aperture (Imogen Newens-Hill).

Figure 2. (left) Juvenile *Discula lyelliana* under the microscope (Anaëlle Loiseau), (right) Colour variation in juveniles of *Discula lyelliana* (Katie Kelton).

Geomitra grabhami: The two *Geomitra* species in the *ex-situ* breeding programme have a smaller, rougher shell, of a more uniform and less rich shade of brown (Fig. 3). This is typically a fairly dark brown but can be paler, especially if the periostracum (the thin outer membrane coating the shell) has been grazed upon by their tankmates (see section 2.7). A sample of mature captive-bred adult *G. grabhami* had an average shell diameter of 7.7mm, an average shell height of 5.0mm, and an average weight of 0.11g.

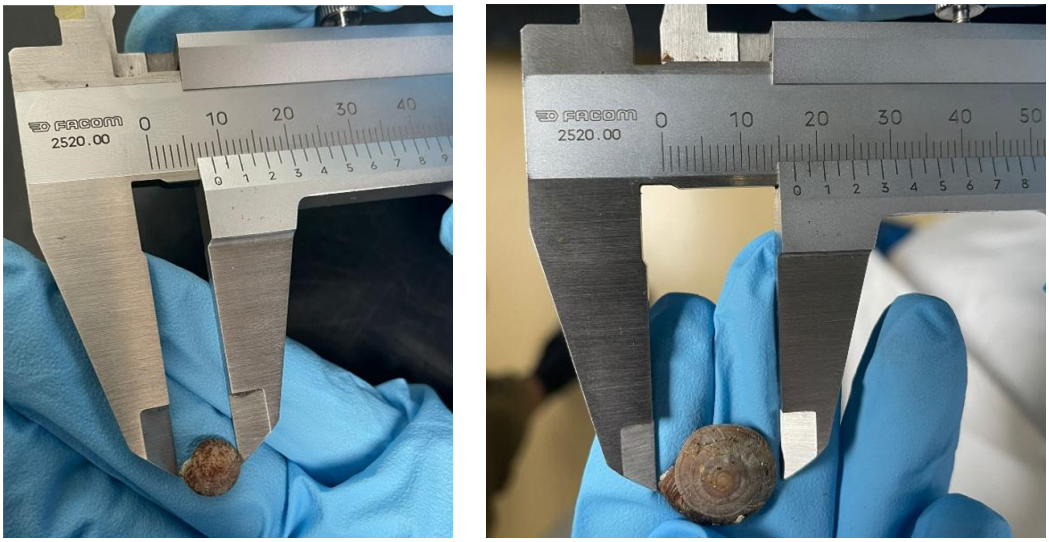

Figure 3. Measuring method for the diameter of *Geomitra grabhami* on the left and *Discula lyelliana* on the right (Anaëlle Loiseau).

Geomitra coronula: Colouration the same as described above for *G. grabhami*. A sample of mature captive-bred adult *G. coronula* had an average shell diameter of 7.6mm, an average shell height of 4.8mm, and an average weight of 0.11g (Fig. 4). The morphology of both these *Geomitra* species is exceedingly similar, and any institution keeping both species must take extreme care to never mix up and intermingle the two species (see section 2.8, further research).

Figure 4. *Geomitra coronula* active in its enclosure (Katie Kelton).

Both *G. grabhami* and *G. coronula* also show a large degree of variation in shell height, with flattened individuals measuring 4.1mm and pyramid-shaped individuals measuring 6.1mm both being recorded within the same group of *G. grabhami* (Fig. 5).

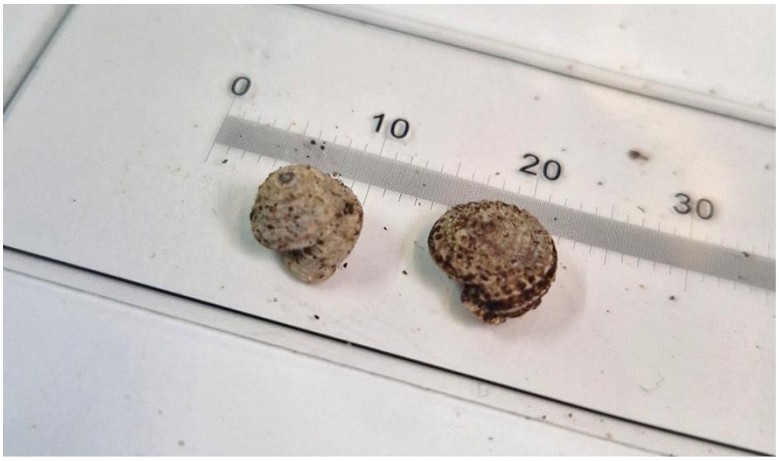

Figure 5. Morphological diversity among mature adult *G. grabhami*. Scale is in mm (Kieran Richardson).

Atlantica calathoides: An extremely flattened species, with a flat but highly textured shell of a fairly uniform pale yellow-grey colour (Fig. 6). The species' shell is translucent on the underside, and the animal's soft body can be seen moving within. A sample of mature adult captive-bred *A. calathoides* had an average shell diameter of 8.1mm, an average shell height of 3.3mm, and an average weight of 0.14g.

Figure 6. *Atlantica calathoides* on an artificial plant (Imogen Newens-Hill).

1.3 Physiology

Like all land snails, the shells of these species are formed predominantly of calcium, and covered by the periostracum, an outer layer of living tissue which grows as the shell grows and protects the shell from corrosion. It is this which can be damaged by snails grazing on their tankmates for extra calcium (see section 2.7).

The tribe Geomitrini, to which the genera *Discula* and *Geomitra* belong, contains all the Madeiran and Azorean native Geomitridae species (Brozzo et al., 2020). All these endemic snails share some common traits in their genital anatomy. Most land snails have a calcareous 'love dart' which is used

to transfer sperm to a potential mate, by piercing their soft tissue and injecting the sperm (Fig. 7). In Geomitrini, the calcareous dart has been lost and the dart sac which holds the sperm has been transformed into a hollow tube, called the appendicula (Brozzo et al., 2020).

Figure 7. (left) *Geomitra grabhami* with penis everted (Katie Kelton) and (right) *Discula lyelliana* with penis everted (Imogen Newens-Hill).

A. calathoides is quite distantly related to the other three project species, being placed in the family Gastrodontidae, not the family Geomitridae (Cameron et al., 2013). One of the most distinctive features of *Atlantica*'s physiology is its very large umbilicus, the depression in the centre of the shell on the underside. This means *Atlantica* have a very low body weight compared to other snails of the same shell dimensions (Cameron et al., 2013).

1.4 Longevity

Data on longevity in these species is very limited; some of the wild-collected founders are still alive in captivity, as are many F1 generation animals.

Discula lyelliana: Most of the wild-collected founder *D. lyelliana* lived for 13-27 months after collection as adults, which, coupled with an average time to maturity of 16 months based on captive-bred specimens, gives an estimated typical longevity for this species of 29-43 months, or between 2 years 5 months and 3 years 7 months. Some particularly long-lived individuals will live upwards of 4 years. Wild data for *D. lyelliana* suggests an average lifespan of 2 years and 7 months. The oldest individual collected in the wild was 3-4 years old. The last wild-collected founder individual of *D. lyelliana* died in March 2025, 3 years and 9 months after being collected on Deserta Grande as an adult.

Geomitra grabhami: The last of the founder *G. grabhami* individuals (Fig. 8) was found deceased in March 2023, just over 21 months after being collected on Deserta Grande as an adult. Several F1 *G. grabhami* that were housed individually to investigate their longevity had an average total lifespan of 2 years, 4 months and 17 days (Newens-Hill, pers. obs.). In the wild, the average lifespan for *G. grabhami* is 1 year and 9 months.

Geomitra coronula: There is presently no data on the longevity of *G. coronula.* This is the most recently brought into the *ex-situ* programme of the four species, and the least observed in the wild.

Atlantica calathoides: *A. calathoides* has a calculated average lifespan in the wild of 4 years and 6 months. The oldest individual collected in the wild was 6 years & 1 month old (Teixeira, pers. obs.).

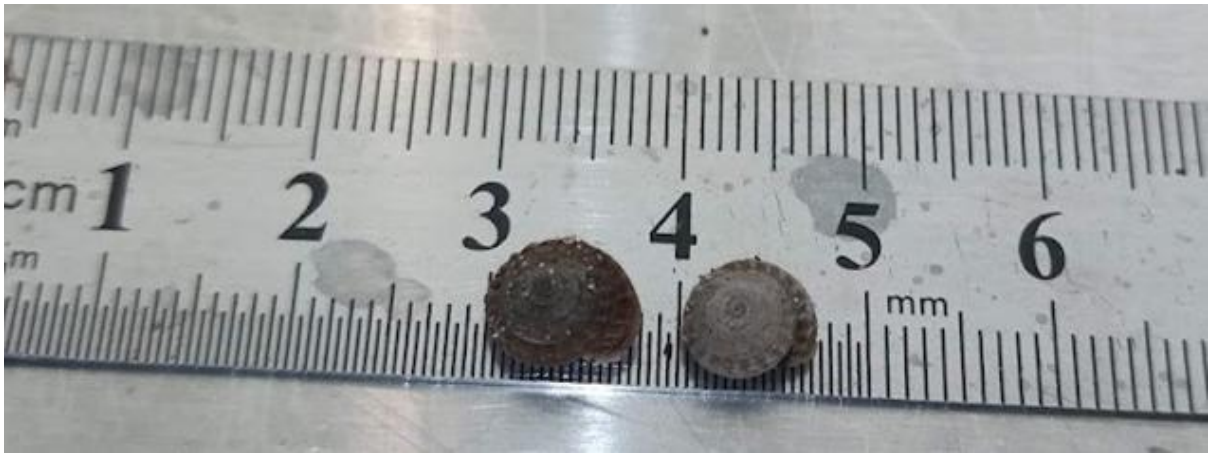

Figure 8. Comparison of an F1 generation captive-bred *Geomitra grabhami* (left) and a wild-collected founder individual (right) (Tamas Papp).

Field data

1.5 Conservation status/Zoogeography/Ecology

The Desertas are a small group of islands to the southeast of the main island of Madeira (Fig. 9). Last connected to the Madeiran main island around one million years ago, the Desertas are comprised of the islands of Ilhéu Chão, Deserta Grande and Bugio, going from north to south. Deserta Grande is the largest, at about 10 km^2, Bugio is about 3 km^2 and the smallest, Ilhéu Chão, is around 0.5 km^2. The islands are long and narrow, with steep cliffs surrounding central ridges, with the highest points on Deserta Grande reaching around 460m above sea level (Gonçalves et al., 2021).

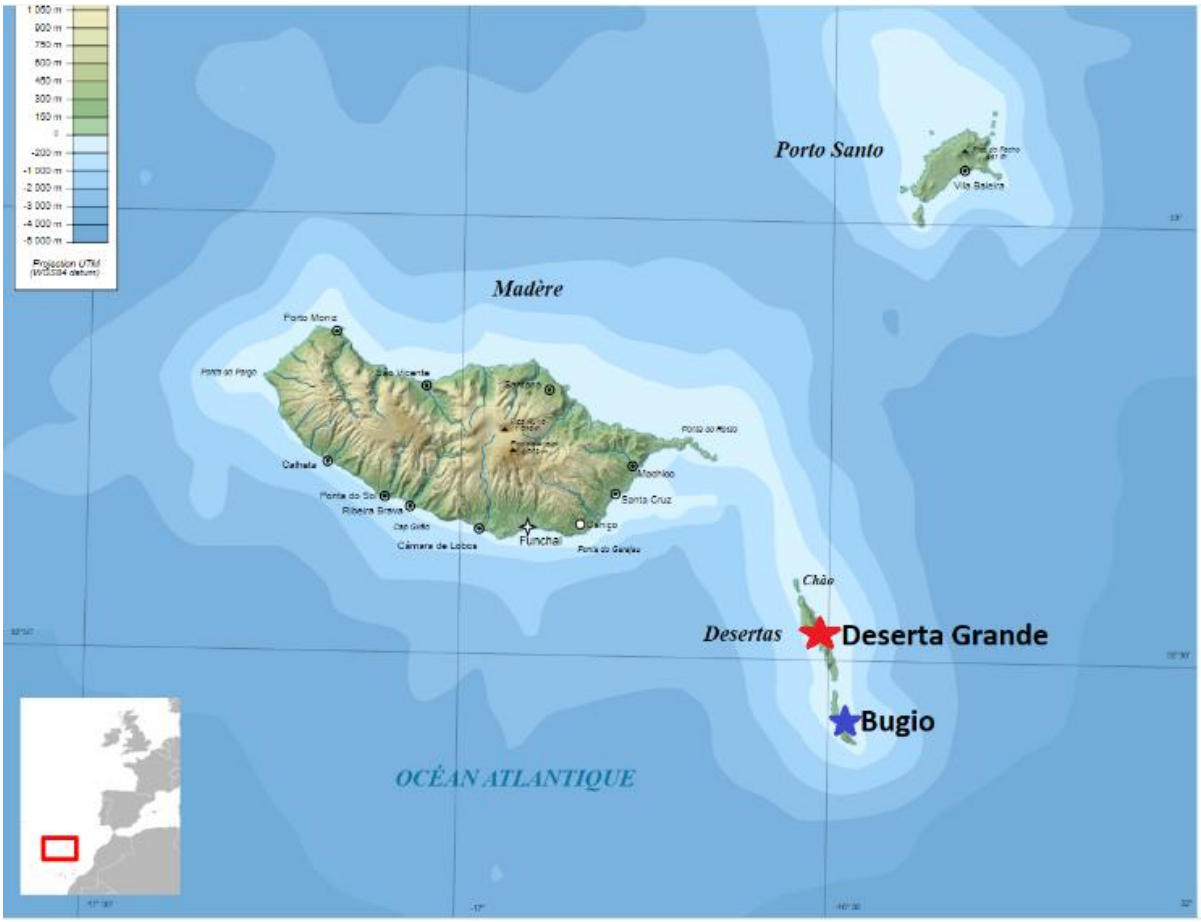

Figure 9. Map of Madeira, highlighting the Desertas, with inset showing wider location in the Atlantic. All the snail species these guidelines focus on are currently restricted to Deserta Grande, with three having previously been found on Bugio as well. From the creative commons map "Madeira topographic map-fr" under CC BY-SA 3.0-licence (https://creativecommons.org/licenses/by-sa/2.0/de/legalcode).

Looking at both fossil deposits and living assemblages, there are around 55 land snail species and subspecies that are believed to be native to the Desertas islands (Cameron et al., 2021). Of these, 23 are no longer living in the Desertas, and 18 of those are believed to be globally extinct (Teixeira et al., 2019; 2022). Thus, the fossil record suggests just under half of the snail taxa once extant there have been lost from these islands. While it is impossible to say for certain the causes of extinction of many of the fossil taxa, with some possibly having gone extinct due to natural climatic shifts prior to human arrival, many species have undoubtedly been driven extinct more recently by human influences, including habitat loss and the introduction of invasive species. Of the 34 species and subspecies considered endemic to the Desertas, 12 are presumed extinct (Teixeira et al., 2022). The four species which are the subjects of these guidelines are all endemic to the Desertas Islands. All four had been known historically from specimens from Deserta Grande but had not been recorded alive for decades and were thought to be extinct. Three of the four (*D. lyelliana, G. coronula* and *A. calathoides*) are additionally known from fossil remains on Bugio, the neighbouring island within the Desertas group (Teixeira et al., 2019; 2022).

1.5.1 Distribution

The four focal species of these guidelines all have very small remnant ranges on Deserta Grande, each with a single tiny population still extant.

Discula lyelliana: Survives in a very restricted area on the west side of Deserta Grande, living under rocks in a disturbed habitat at an elevation of 350m (Cameron et al., 2021; D. Teixeira pers. comm.). Fossil shells of this species are known throughout Deserta Grande except for the north and far south of the island and from southern Bugio (Teixeira et al., 2019; 2022). *D. lyelliana* is also subject to ongoing reintroduction efforts on Bugio (see Section 1.5.5).

Geomitra grabhami: known from Fajã Grande, in the east of Deserta Grande, where it lives between 25-40m above sea level in a rocky habitat among lichens (Teixeira et al., 2018a). Fossils of this species are known from two sites at the north end of Deserta Grande, where they are abundant, but are not found anywhere else within the Desertas Islands, nor elsewhere in Madeira (Teixeira et al., 2022); it is possible the species has always been endemic to the northern portion of Deserta Grande. A molecular study to clarify the species' taxonomy is ongoing.

Geomitra coronula*:* Lives in a very similar habitat to *G. grabhami*, under rocks among lichens, but its sole surviving population is at Pedregal, in the northwest of Deserta Grande, at 200-250m above sea level (Teixeira et al., 2018b). Fossils of this species are known from sites throughout Deserta Grande apart from the sites at the north end of the island where *G. grabhami* shells are abundant and are also found throughout the island of Bugio (Teixeira et al., 2022). The lack of overlap in fossil sites of the two closely related *Geomitra* species could suggest some kind of competitive exclusion. *G. coronula* is also subject to ongoing reintroduction efforts on Bugio (see Section 1.5.5). A molecular study to clarify the species' taxonomy is ongoing.

Atlantica calathoides: When it was rediscovered, *A. calathoides* was thought to only survive in two very small populations on Deserta Grande, in the northwest and southwest of the island (Cameron et al., 2021). After a severe drought from 2012 to 2017, changes in vegetation succession were observed in the habitat of the northern population. The native bracken fern, *Pteridium aquilinum*, which provided shelter and maintained controlled humidity levels for the subpopulation, has been replaced by the endemic plant *Siderites candicans*. This succession has led to habitat loss for the species, resulting in a decline in their population, and the northwestern population has presumably since gone extinct, having last been recorded in 2018 (Teixeira et al., 2023; Teixeira pers. comm. 2024). Fossils of this species are known from southern Bugio and throughout Deserta Grande, where it seemed to be common in the past (Teixeira et al., 2022).

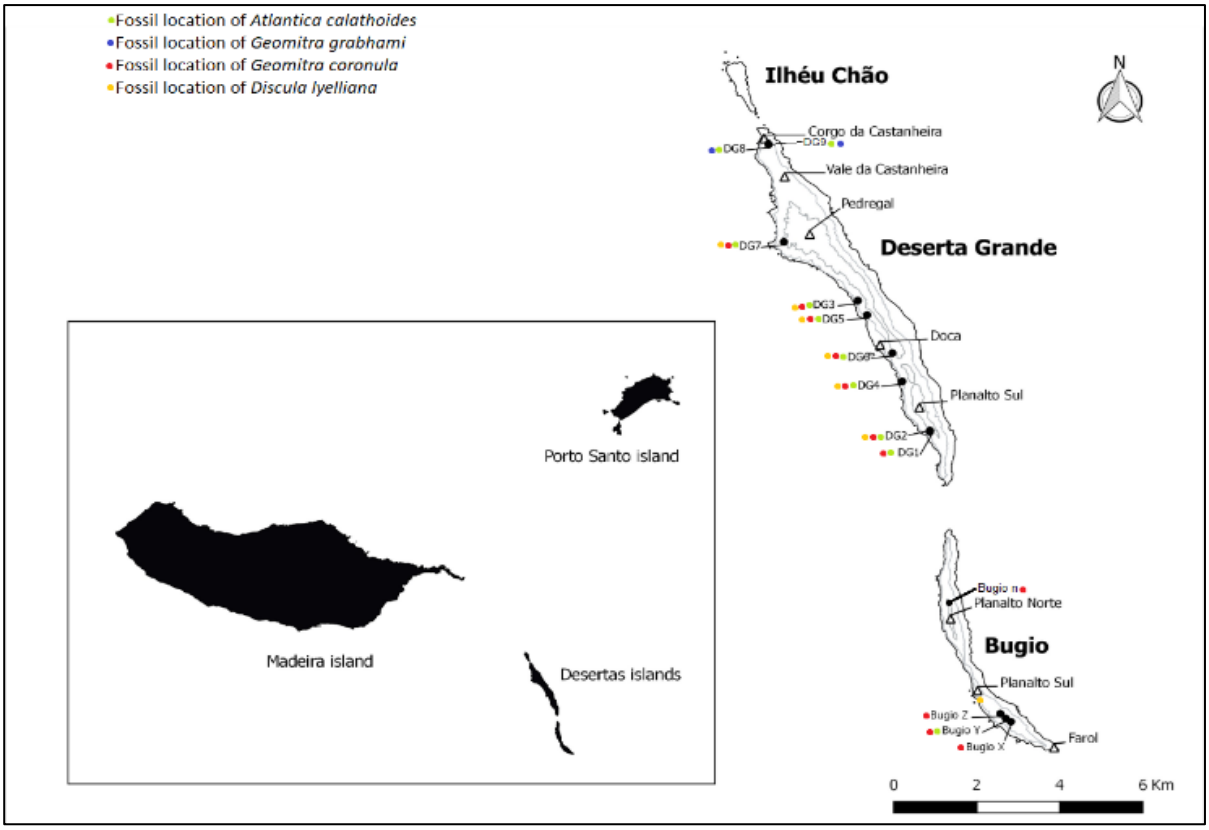

Figure 10. Map of the Desertas Islands showing fossil sites, with recorded species listed next to the marker for each site. Map originally from Teixeira et al., 2019, modified and with data added from Teixeira et al., 2021.

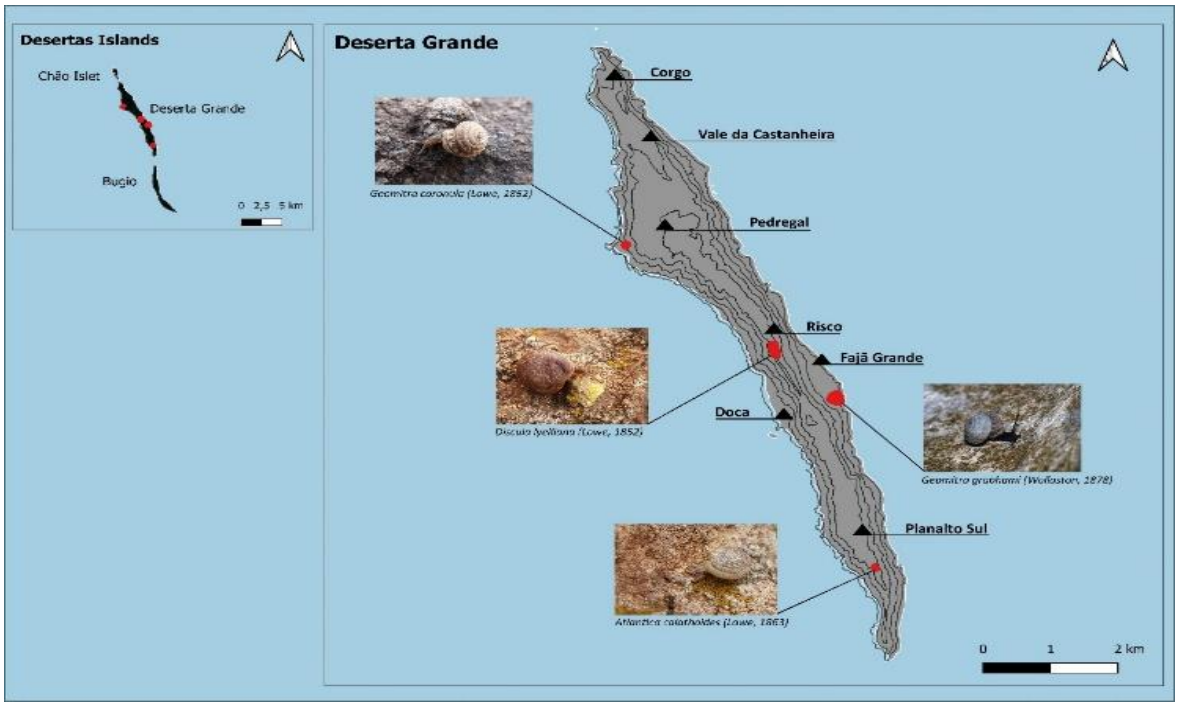

Figure 11. Map of Deserta Grande, showing locations of rediscovered relict populations of each species. From north to south: *Geomitra coronula*, *Discula lyelliana*, *G. grabhami* and *Atlantica calathoides*. From Teixeira et al., 2023.

1.5.2 Habitat

It is hard to say for certain what the species' natural habitat preferences are, as the vegetation of the island has been extensively altered since the islands were first colonised by humans in the 15th Century. It is thought that the initial vegetation of the Desertas prior to human settlement may have been dry laurel forests, like those found on south-facing slopes of Madeira's main island, though records are scarce, and it is hard to piece the initial ecosystem together from what now remains (Ramos, 2020). In the past, the Deserta Islands had native forests, and some specimens of those times remain in the island in small pockets of refuge, such as the laurel species *Apollonias barbujana*. The fossil record shows that these snail species were all more widely distributed in the past (Teixeira et al., 2022). This implies their current habitats may not be representative of optimal conditions for these species. This is a widely recorded phenomenon in other species living in remnants of much wider historical ranges (Fisher, 2011).

Discula lyelliana: Found under rocks in disturbed, scrubby habitat, at 350m elevation above sea level, being associated with the common fern (*Pteridium aquilinum*) (Fig. 12).

Geomitra grabhami: Found under rocks in scree near the base of cliffs, at 25 to 40 meters of elevation.

Geomitra coronula: Found in rocky scree areas at 200 to 250 meters above sea level, again living under rocks or associated with the purple false brome (*Brachypodium distachyon*) (Fig. 13).

All three of the above species are closely associated with lichens, which they feed upon, as well as other organic sources such as leaves and even animal carcasses.

Atlantica calathoides: Occurs in deep ravines or other landscape features with an accentuated slope (Fig. 14). It seems to be strongly associated with the presence of the native fern species *Pteridium aquilinum,* being found in the leaf litter, on the callus of young ferns, or under nearby rocks. The sister species of *A. calathoides* has its type locality at the Ribeiro Frio, in the heart of the native forest of Madeira, the Laurissilva Forest, suggesting *A. calathoides* may have originally inhabited similar habitat on the Desertas prior to environmental degradation.

Substrate examined from the *D. lyelliana* reintroduction site consists of 15% sand and 85% plant matter, including wood splinters, grass seeds and decomposed organics. It is presumed the sand is very mineral rich as it comes from a volcanic region. Reintroduced populations of *D. lyelliana* and *G. coronula* on Bugio are associated with large boulders among scrubby vegetation, with a thick crust of lichens covering them (Fig. 15).

Figure 12. Habitat of remnant population of *Discula lyelliana* on Deserta Grande (Dinarte Teixeira).

Figure 13. Habitat of remnant population of *Geomitra coronula* on Deserta Grande. Flowering plant in the lower right is *Echium plantagineum* (Dinarte Teixeira).

Figure 14. Habitat of remnant population of *Atlantica calathoides* on Deserta Grande. Vegetation is composed of *Pteridium aquilinum* and *Sideritis candicans* (Dinarte Teixeira).

Figure 15. General habitat at the release site for reintroduced *D. lyelliana* and *G. coronula* on Bugio, and a released & marked *D. lyelliana* on one of the boulders (Gerardo Garcia).

1.5.3 Population

All of these species have very small wild populations, varying between 200 mature individuals (*D. lyelliana* and *G. grabhami*) and less than 50 mature individuals (*A. calathoides* and *G. coronula*) (D. Teixeira pers. comm.). It is possible these populations are slightly larger, as when collecting individuals of *G. grabhami* for the *ex-situ* breeding programme, 81 snails were collected from the population, which had been previously estimated to number less than 50. However, any populations will still be very small, even if slightly over the estimated size, and are all still at imminent risk of extinction. Fossils of both *Geomitra* species and *A. calathoides* are noted to be abundant at the locations where they are found, suggesting that they would originally have been a major component of the snail communities on Deserta Grande and Bugio (Teixeira et al., 2022).

1.5.4 Threats to Wild Populations

The primary threats to the snails on Deserta Grande come from the impacts of two invasive species introduced by humans; overgrazing of native vegetation by feral goats (*Capra hircus*) and predation by house mice (*Mus musculus*).

Goats were introduced as a food source in the 15th Century but turned feral and proliferated across the Desertas (Fig. 16). Their overgrazing of the islands, coupled with historical habitat clearance for agriculture and forestry (neither of which proved successful in the long term), mean that most of the natural vegetation has disappeared from the Desertas (Ramos, 2020). The loss of vegetation not only deprives the snails of potential food and limits the availability of the damp retreats needed by molluscs, but also makes the environment more uniform, removing the range of niches and microclimates required to support such a diverse community of land snails. Overgrazing has further exacerbated the pressure on the snails by also increasing erosion and the frequency of rockfalls (Teixeira, 2017; Teixeira et al., 2018a).

Figure 16. Feral goat (*Capra hircus*) grazing on the limited vegetation on Deserta Grande (Gerardo Garcia).

It is unknown when mice were introduced to the Desertas, as their introduction was entirely accidental, but it is possible the snails only became a significant component of their diet after the loss of much of the natural vegetation removed other food sources that they were dependent upon. The gnawing incisors of a mouse are quite capable of breaking through a snail shell to eat the soft body of the mollusc inside and prior to their arrival, there were no terrestrial mammalian predators inhabiting the Desertas, making the endemic snails highly vulnerable to their predation (Garcia et al., 2021).

An additional invasive species present on the islands is the Argentine ant (*Linepithema humile*). This generalist species is a voracious feeder and has been shown to have a highly detrimental impact on endemic invertebrates in other island ecosystems (Wetterer et al., 2009). At this stage, it is unclear if this species is impacting endemic snails in the Desertas and, if so, how severe that impact may be. Based on empirical observations from the Ilhéu Chão between 2012 and 2017, the species is responsible for the mortality of a small percentage (15-20%) of the central population of the endemic Chão snail species *Plebecula anaglyptica* (D. Teixeira, pers. comm. 2025).

The snails have several natural predators which are native to the Desertas. The endemic Carabid ground beetles *Eurygnathus latreillei wollastoni* and *Scarites abbreviatus desertarum* (Fig. 17) are known to predate on snails, as does the non-native Staphylinid beetle *Ocypus olens.* The endemic wall lizard (*Teira dugesii mauli*) also predates the snails' egg clutches and juvenile specimens (D. Teixeira pers. comm.). It is assumed that some breeding and visiting birds, notably common blackbirds (*Turdus merula*) and yellow-legged gulls (*Larus michahellis*) do as well (Garcia et al., 2021). Recent reports indicate that gulls patrol the *G. coronula* population area during the night, feeding on the specimens hanging on the stiff brome (*Brachypodium distachyon*) (D. Teixeira, pers. comm. 2025).

Figure 17. The Carabid beetle *Scarites abbreviatus,* native to the Desertas Islands and among the snails' natural predators (Gerardo Garcia).

Due to anthropogenic climate change, the frequency of rainfall is declining in the Desertas, and the duration of droughts is increasing, and is predicted to continue to increase (Teixeira, 2017; Teixeira et al., 2018b). Not only does this make conditions less hospitable to terrestrial molluscs, it also exacerbates the increase in erosion caused by overgrazing, by making the ground more friable (Teixeira et al., 2018a).

1.5.5 Conservation Status

All four of the focal species of these guidelines are assessed as Critically Endangered (CR) on the IUCN Red List and based on the ongoing species reassessment, will remain as such (D. Teixeira. pers. comm.). All were previously believed to be extinct, having not been seen alive for many decades prior to their rediscoveries. *A. calathoides* was rediscovered in 2008 and listed as CR in 2017 (Teixeira, 2017). *G. grabhami* was rediscovered in 2012, and *G. coronula* in 2013; both were listed as CR in 2018 (Teixeira et al., 2018a; b). *D. lyelliana* was listed as Critically Endangered, Possibly Extinct in 2011 (Seddon, 2011); this was prior to its rediscovery in 2017, and the ongoing species reassessment will reflect this situation (D. Teixeira pers. comm.). The Critically Endangered status has been applied to all species because of the very small population sizes and distribution ranges, and ongoing threats from invasive species in the wild presumably driving further declines.

In November 2024 the first reintroduction to Bugio took place, with 900 *G. coronula* and 650 *D. lyelliana* from the *ex-situ* conservation populations released into suitable habitat on Bugio. Subfossil evidence shows both these species previously occurred on the island but were presumably extirpated

by the same human impacts that drove the species to near extinction on Deserta Grande (Teixeira et al., 2022). Goat and mouse eradication from Bugio has paved the way for these reintroductions.

1.6 Diet and Feeding Behaviour

There is little known about the wild diet of these species, and therefore more research is needed. All the species are detritivores, feeding on a wide range of dead organic materials, including fallen leaves and even animal carcasses. *G. coronula*, *G. grabhami* and *D. lyelliana* are found associated with lichens in the wild (Teixeira et al 2018a; b; Garcia et al., 2021), but it is uncertain if they feed on them. Observations in captivity seem to confirm that at least in the case of *D. lyelliana*, they will feed on lichens. *A. calathoides* is associated very strongly with ferns but again, it is uncertain if this is due to the species utilising them as a food source or as shelter (Teixeira, 2017). The range of food items eaten in captivity suggests they are fairly generalist feeders (Prince, pers. obs.).

1.7 Reproduction

1.7.1 Developmental Stages to Sexual Maturity

Within the *ex-situ* breeding programme, the life stages of hatchling, juvenile, and adult can be referred to, with large juveniles that are not yet fully adult potentially able to be split out as subadults. In both *Geomitra* species and *D. lyelliana*, fully mature adults can be distinguished by a thickened lip around the aperture of the shell (Fig. 18). *A. calathoides* does not develop a thickened lip, so differentiating the different age classes is done solely based on size. Hatchlings are defined as the smallest, mostly unpigmented, newly hatched individuals. Juveniles are individuals that do not yet have the thickened lip of the shell aperture, in *Geomitra* or *Discula*, or, in *Atlantica*, have not yet reached 7mm shell diameter.

Figure 18. Adult *G. grabhami* under a microscope, showing the thickened lip used to distinguish fully mature adults in these taxa (Tamas Papp).

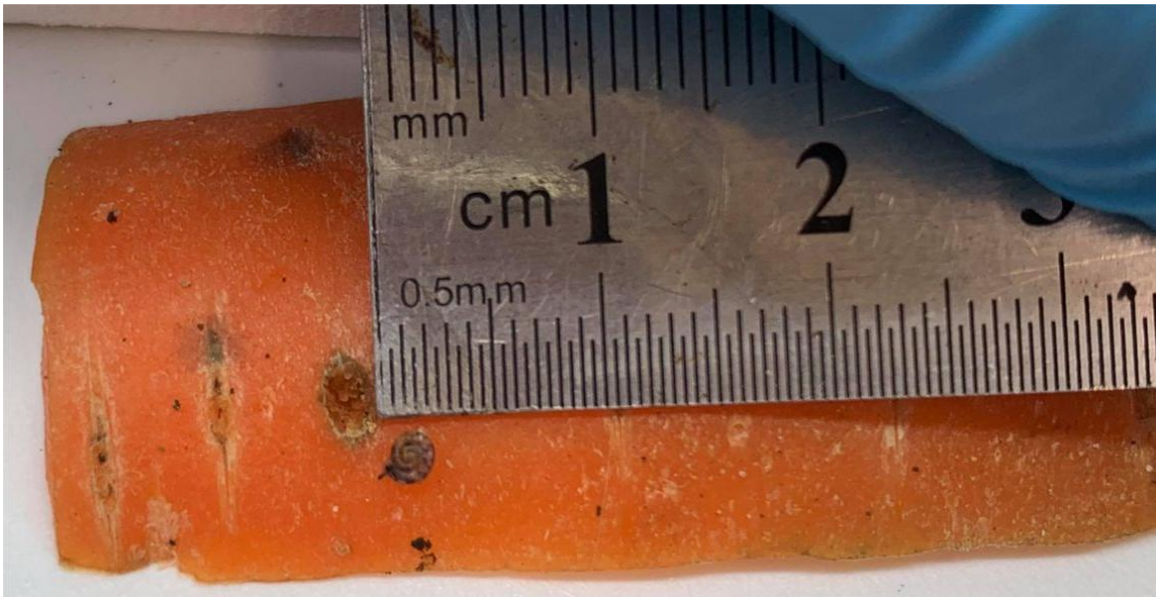

Figure 19. Juvenile *D. lyelliana* on a slice of carrot, with a shell diameter of approximately 2mm (Heather Prince).

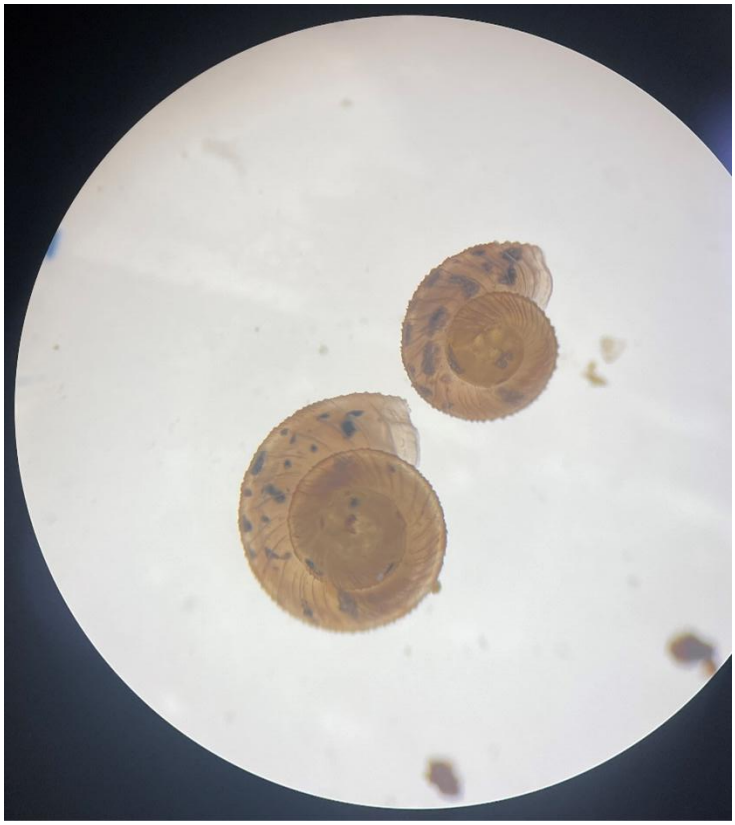

Figure 20. Two hatchling *G. grabhami* viewed under a microscope. Note how the rough texture of the shell is visible even at this size (Anaëlle Loiseau).

1.7.2 Age of Sexual Maturity

Discula lyelliana*: D. lyelliana* in the *ex-situ* programme have taken an average of 16 months to reach maturity, with variation of a couple of months.

Geomitra grabhami: Very variable maturation rate, with individuals reaching full adult size in as little as six months but some other individuals taking over a year to mature.

Geomitra coronula: Very variable maturation rate, with individuals reaching full adult size in as little as four months but some other individuals taking over a year to mature.

Atlantica calathoides: According to captive observations, *A. calathoides* seems to take around 14 months to mature, this is the subject of further investigation.

1.7.3 Seasonality of Cycling

In the wild, the snails have two breeding seasons: one between September and November; the second occurring between January and March. They are much less active in the warmer, drier summer months. This is supported by the majority of the breeding in the *ex-situ* population taking place between October-February in *G. grabhami*, *G. coronula* and *D. lyelliana*. However, there are various exceptions (see sections 2.4 and 2.8 below). *A. calathoides* is the least studied with regards to seasonality; anecdotal captive observations have alluded to a preference in breeding in cooler temperatures around November to February.

1.7.4 Clutch Size

Discula lyelliana: This species lays the largest egg clutches, typically numbering in the 50s; one clutch counted contained 55 eggs, which averaged 1.4mm in diameter.

Geomitra grabhami: Lays fairly small clutches of eggs, observed to number 8-9 eggs on average. These eggs are large compared to the snail's body size, measuring 0.95mm across.

Geomitra coronula: Lays a similar number of eggs to *G. grabhami*, with an average clutch size of 9-10 eggs, and a range from 6-15 (Papp, pers. obs.).

Atlantica calathoides: Clutch size and number of clutches laid warrants further investigation. Initial observations suggested a clutch size of 2-3 eggs, but this has increased with adjustments to the seasonality offered (Newens-Hill, pers. obs.). Similar to *G. grabhami & G. coronula*, these eggs are quite large compared to the adult snail.

The small clutch sizes of *Atlantica* and *Geomitra* may be a factor in their apparent decline from former abundance, which is suggested by the fossil record (Teixeira et al., 2022). Producing less offspring reduces recruitment and this is one potential contributor to why they seem so susceptible to factors such as predation by introduced house mice.

1.8 Behaviour

1.8.1 Activity

Discula lyelliana: The most active species, often seen moving around the enclosure and climbing the sides throughout the day. Activity levels are particularly high immediately after spraying. Wild observations are limited but activity may be more limited during the day to under rocks and in fissures between boulders. Reintroduced *D. lyelliana* on Bugio are capable of travelling across areas of open ground in between suitable rocky habitat, during wet periods and/or at night (Teixeira, pers. comm.).

Geomitra grabhami: Less active, with most staying under the rocks in the enclosure during the day but can sometimes be seen feeding or moving over the substrate, particularly after spraying. Both *Geomitra* species will be found above the surface, on the sides of the tank and décor, during the day, but not active. Their activity above ground increases overnight.

Geomitra coronula: Identical activity patterns to those described for *G. grabhami* above. Reintroduced *G. coronula* on Bugio have been recorded climbing up vegetation on Bugio, possibly to feed or collect moisture from condensed droplets (Garcia, pers. obs.).

Atlantica calathoides: Predominantly nocturnal, however diurnal activity increases at cooler temperatures. As temperatures increase to 18°C diurnal above-ground activity decreases and the snails remain active under rocks and leaves. Nocturnal activity above ground persists above 18°C, indicating a possible shift to nocturnality associated with warmer temperatures. (Newens-Hill, pers. obs.).

1.8.2 Predation

See section 1.5.4 for details of native & introduced predators the species face in the Desertas islands.

1.8.3 Sexual Behaviour

All four species are presumed to be hermaphroditic, as almost all land snails are. Self-fertilisation has been confirmed in *G. coronula* by isolating an individual from a very young age. This individual snail was still able to lay viable eggs which hatched. Mating in the wild may occur after rain or other sources of high humidity, such as seasonal fog, and may occur predominantly at night. Mating is very rarely observed in the *ex-situ* populations. Mating is often preceded by penile eversion (Fig. 7). In the few observed instances of mating, it has occurred on the glass sides, mesh, or Foamex lid of the vivarium, rather than on the substrate or rocks (Figs. 21-23). One pair of *D. lyelliana* observed mating (Fig. 22) continued for 10 minutes after the initial observation, so mating may be a rather prolonged process.

Figure 21. Mating pair of *Geomitra grabhami* (Adam Button, Bristol Zoo).

Figure 22. Mating pair of *Discula lyelliana* (Adam Button, Bristol Zoo).

Figure 23. Mating pair of *Geomitra coronula* (Imogen Newens-Hill).

Section 2: Management in Zoos and Aquariums

2.1 Enclosure

2.1.1 Boundary

Adults of these snails can be maintained in simple glass or acrylic tanks, as long as these are escape-proof. Hinge-door glass tanks, which are popular for some reptiles and invertebrates, are not advisable due to the risk of small snails being crushed when the door is opened. Several different styles of tanks have been used, but all are accessed through lids that are lifted completely off, rather than hinged doors (Figs. 24-25). The lids of each tank were made in-house. The lids need to fit perfectly to prevent tiny hatchlings from getting stuck or crushed. Each tank has minor differences in dimensions, so the lids were custom made to fit each tank. Lids are made of Foamex, with a hole in the middle and fine mesh glued over it, to improve ventilation.

The best method of rearing the hatchlings of all four Desertas species is in plastic tubs. These allow the easy censusing of juveniles while also allowing appropriate humidity and temperature to be maintained. Once the juveniles begin to grow too large for the tubs (this is judged by how frequently the sides and lid need cleaning), they are upgraded to the adult tank setups.

In the event of pest outbreaks, such as free-living detritivores mites (see section 2.7), an extra barrier can be employed by putting the tanks above a water bath. This can be achieved by placing a tray of water containing a small amount of detergent/washing-up liquid, at a concentration of around 10ml per litre and a depth of a few centimetres under each tank. The tank is then elevated above the water on some sort of rack to ensure it does not adversely impact the temperature or humidity for the snails themselves. This water barrier prevents the mites from colonising the tank, allowing each tank to be isolated and any invertebrate infestations to be dealt with without the risk of recolonisation.

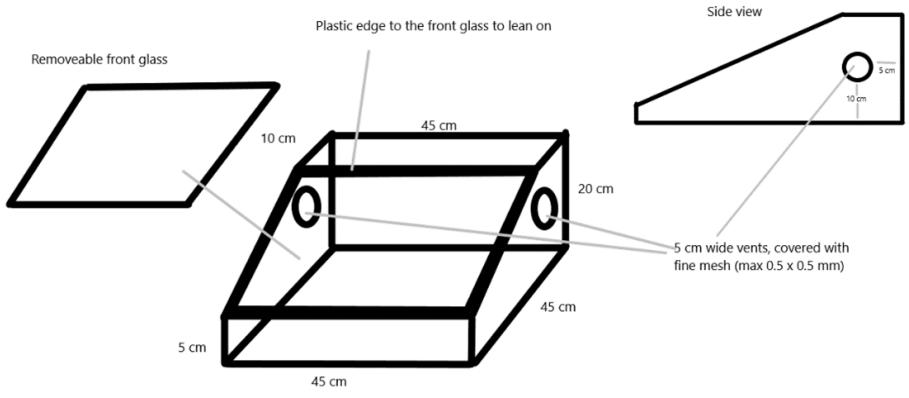

Figure 24. Labelled diagram of the *D. lyelliana* tank shown in Fig. 25 (Heather Prince).

Figure 25. An enclosure for adult & juvenile *D. lyelliana*. Note the fully removable lid (Heather Prince).

2.1.2 Substrate

A substrate of ¾ soil or coir fibre and ¼ sand should be used. This ratio has proven to be optimal for providing sufficient humidity and drainage for these species, with the lowest mortality rates. Other substrates such as topsoil tend to compact, preventing the snails from burrowing or laying egg clutches. Substrate depth should be between 3-5cm to allow s nails to burrow and lay their eggs, while making it easy to find small hatchlings. Substrate should be changed once per month (see section 2.1.3 below).

In larger, planted tanks, substrate depth needs to be deeper to give space for the plant roots, and should also be sloped to prevent waterlogging of substrate. Live planted tanks with a maximum substrate depth of approximately 15cm at the back of the tank, sloping down to around 5cm at the front (Fig. 26) have proved successful.

Figure 26. Planted tank for *G. coronula*, showing the sloped substrate. Plants are *Brachypodium sylvaticum* and *Crithmum maritimum* (Imogen Newens-Hill).

2.1.3 Furnishings and Maintenance

These snails do not require complex or intricate furnishings. Food, a feeding plate, cuttlebone for calcium and a few rocks for shelter will be sufficient to maintain a colony (see Fig. 27). Limestone-based rocks are preferred as the snails will then also graze on the surface of the rocks themselves to meet their calcium needs. Rocks must either be securely placed on the floor of the enclosure or, if stacked, ensured to be completely secure and stable, to prevent unstable rocks slipping and falling, which risks both crushing or injuring snails and damaging the glass. It has been observed that *G. grabhami and D. lyelliana* prefer to have the rocks raised slightly above substrate level by stacking them as they use this to hide underneath.

If introducing any plastic or stone furnishings, as well as tubs used to house juveniles, to the keeping area, they should be soaked in F10 or Safe4 for 24 hours and then rinsed in filtered water before being used in the snail enclosures, in order to ensure no breaches of the biosecurity. After they are being used for the snails, when cleaning tanks, furnishings can be cleaned with an animal-safe disinfectant such as Safe4, F10 or any similar product. These should be sprayed with the disinfectant, left for ten minutes, then rinsed off with filtered water before being reintroduced to any snails. The same method can be used to disinfect glass tanks. For F10, veterinary recommendation is to use a 1:500 dilution.

Figure 27. An enclosure for a group of adult *Discula lyelliana*, featuring (**a**) food items, (**b**) cuttlebone, (**c**) limestone pieces and (**d**) a Perspex feeding plate with *Partula* diet (from Garcia *et al*., 2021).

Additionally, plastic plant pots provide an alternative form of shelter to Desertas land snails and have proven to be popular refuges with *D. lyelliana* and *G. grabhami* (Fig. 28). Dark-coloured plastics seem to be preferred by the snails. Artificial plant leaves have also been used with success as refuges for *D. lyelliana* (See Fig. 25 above). Larger tanks can be furnished in much the same way, but with a greater number of cover items (Fig. 29). Alternatively, the use of larger tanks can allow for the creation of naturalistic live-planted tanks with deeper substrate (Fig. 30). Plants selected for such setups include *Crithmum maritimum* (native to the Desertas Islands) and *Brachypodium sylvaticum* (structurally very similar to other *Brachypodium* species which are native to the Desertas Islands). To ensure biosecurity is maintained, care must be taken when sourcing plants that they haven't been used in a terrarium before. Best options are those from large commercial growers that are grown in coir, which can easily be washed off roots. The now bare-rooted plant should be soaked in plenty of water, so it is fully hydrated. Use a weak solution Safe4 or other similar disinfectant (10% concentration) to sterilise the roots, at least to kill any surface pathogens and then leave in for 24 hours before rinsing off with filtered water. The plant can then be planted into the snail substrate. Plants should be watered directly in addition to enclosure spraying for the snails, and this should vary across the year according to the seasonality chart (Appendix I). Use of live plants is encouraged, particularly in groups of snails that are to be released, with the aim of more closely mimicking the wild habitat (Fig. 31), provided that the biosecurity protocols described above are adhered to. *G. coronula* will also graze upon *Brachypodium* species in their tanks (Newens-Hill, pers. comm.), more closely mimicking their wild diet.

The remnant population of *A. calathoides* on Deserta Grande is associated with leaf litter under common bracken ferns (*Pteridium aquilinum*) in the wild, and leaf litter should be added to *A. calathoides* enclosures to replicate this. Leaf litter must be treated through freezing (for 24 hours)

and/or thorough disinfection (soaked in 10% disinfectant solution for 24 hours) to ensure biosecurity is maintained. As the leaf litter is a potential food item for the snails, it must then be soaked in filtered water for 24 hours to prevent the snails ingesting trace amounts of disinfectant. The other Desertas species in the *ex-situ* programme can also be offered leaf litter as furnishings, but in smaller quantities, and are less associated with it than *A. calathoides*.

Figure 28. A group of *Geomitra grabhami* sheltering inside a black plastic plant pot (Katie Kelton).

Figure 29. Furnishings in a larger tank, consisting of multiple rocks, artificial leaves, and plastic pots (Imogen Newens-Hill).

Figure 30. A more naturalistic setup of a large tank, furnished with live plants and large rocks (Tamas Papp).

While attempting to recreate the wild conditions these snails experience is desirable, it is important to note that due to their small size, particularly as juveniles, extensive furnishings can make finding snails and conducting accurate headcounts very difficult. Therefore, it is recommended to keep furnishings for hatchlings to a minimum, only using small pieces of rock, which, if it contains calcium, the snails will graze on as well as sheltering under, and a fine substrate (see Fig. 32). Hatchlings have been kept with small pieces of rock taken from soil samples from Deserta Grande. These are primarily tuff, an ash-derived volcanic rock. Hatchlings will also use food items and pieces of cuttlebone as shelter.

Figure 31. *Geomitra coronula* resting among *Brachypodium* stems in a planted tank, as this species does in the wild. (Imogen Newens-Hill)).

Figure 32. A petri dish for rearing hatchlings of *Geomitra grabhami*. Note the hatchlings visible on the cuttlebone on the left. These setups are suitable only for a maximum of 20 hatchlings (Katie Kelton).

A consistent directional flow of management routine should be maintained when servicing multiple enclosures of Desertas land snails at once. This ensures that any spread of pathogens/ parasites between terraria can be easily identified and isolated if necessary. Full tank maintenance, including a full substrate change and cleaning of the glass, should take place once per month. Snails should be carefully searched for and counted off all the habitat furniture items and out of the substrate, then placed to one side in a tub or petri dish. Substrate should be removed from the tank and put to one side in a sealed tub for up to 12 weeks, to allow any eggs in the substrate to hatch. This should be a quick visual check for any emerging hatchlings daily, when the tanks are being serviced unless the tank is being population managed and further hatchlings are not desired, in which case the substrate can be frozen immediately (see section 2.4.5). After fresh substrate has been added and the cover items replaced, snails should be placed back into the tank in petri dishes on damp paper towel (Fig. 33). and allowed to disperse on their own. This also allows the keeper to check for mortality.

Figure 33. A freshly-cleaned tank of *Atlantica calathoides*, showing snails placed back into the tank in petri dishes to allow them to disperse (Imogen Newens-Hill).

2.1.4 Environment

Seasonality mimicking the natural conditions in the Desertas Islands is essential to ensure animals for the reintroduction project remain adapted to their natural conditions and will also allow institutions to better plan the breeding and capacity of their facilities, as environmental cues trigger breeding. See Appendix I below for seasonality charts showing monthly changes in temperature and humidity. Note that *A. calathoides* generally prefers slightly cooler temperatures and higher humidity than the other three species in the programme; this is further evidence of this species being a relict, adapted for different, more forested conditions than present currently on the islands (Teixeira, pers. comm.). This is reflected in the differences for this species in the charts in Appendix I, which show the seasonality which has led to the highest levels of activity and breeding in this species.

These temperatures are best maintained by heating the room rather than individual enclosures. Using equipment such as heat mats is unsuitable for these species, as it would dry out enclosures far too quickly and desiccate snails. Like temperature, humidity is varied month-to-month to mimic rainfall variation in the Desertas Islands, which is included in the charts in Appendix I. Suitable values can typically be maintained by lightly spraying enclosures 2 times per week in dry season months, and 3 times per week in rainy season months. It is important that the keeper judges whether further spraying is needed by the humidity in the room and level of condensation on the lids and sides of the tanks and adjust spraying amounts accordingly.

2.1.5 Dimensions

D. lyelliana is the largest and most active of the four Desertas Islands species currently in the *ex-situ* programme and thus needs the largest enclosures, or the lowest stocking density. As an example, one style of tanks (see Figs. 24-25) used for this species measure 45cm by 45cm, by 20cm tall at the back.

A tank of these dimensions is suitable for a group of up to 40 adults. A larger colony would need a larger tank, or to be split into smaller groups. These tanks are also now used to house the other species in the programme and can hold up to 80 adult *G. coronula* or *G. grabhami*, or up to 70 adult *A. calathoides*.

Tanks used for smaller groups of *G. grabhami, G. coronula* and *A. calathoides* measure 30 x 30 x 20 cm tall, or 30 x 20 x 30 cm tall (Fig. 34). A tank of these dimensions is suitable for a group of up to 30 adult snails or up to 60 juveniles. Juveniles would need splitting into multiple groups as they grow, the timing of which can be judged by the frequency of tank cleaning required. Once population sizes exceed several hundred snails these tanks can become impractical, however these still make good grow-out tanks for groups of juveniles.

Figure 34. Different tank sizes for *Geomitra* or *Atlantica*: 20x30x30 cm (left) and 30x30x20 cm (right). Both are suitable for housing small groups of adults or as rearing tanks for smaller juveniles (Kieran Richardson).

Initially, petri dishes were used to house hatchling snails of all four species. The petri dishes used to house hatchling snails were 9cm in diameter, with a height of 1.5cm including the lid. The lid has small inserts (>1mm) that keep it lifted to allow for airflow. Petri dishes are good for low numbers of hatchlings of no more than 20 individuals per dish. As populations increase Petri dishes stop being a viable method of housing due to the intensive and time-consuming management required due to the frequency of cleaning needed. For larger numbers of hatchlings, plastic containers with secure lids (see Fig. 35) provide a much more viable housing option. Two sizes have been used: the larger measure 20.5 x 15.5 x 10.7 cm (L x W x H) (Fig. 35). These are suitable to house up to 100 hatchling *A. calathoides*, up to 120 hatchling *G. grabhami* or *G. coronula*, or up to 60 hatchling *D. lyelliana*. These densities will need reducing as the juveniles grow, with a container of the same dimensions being suitable for up to 20 large juvenile *A. calathoides*, *G. grabhami* or *G. coronula*, and 10 large juvenile *D. lyelliana*. Smaller containers can be used, but the number housed in each would need to be reduced accordingly. For example, containers measuring 17.9 x 13 x 8.6 cm (L x W x H) can house numbers approximately half those mentioned for the larger clip boxes above.

Figure 35. Clip boxes used for housing hatchlings, showing mesh fitted into lid for extra ventilation, and setup of furnishings inside. (Imogen Newens-Hill).

In large enough tanks, hatchlings can also be maintained in setups with the adults, with all life stages being housed together and eggs left *in-situ* in the topsoil to develop. This is only advisable once colonies are well-established, have reached a larger size (at least several hundred individuals) and are no longer at risk of extinction from die-offs. This is also useful in terms of reducing keeper workload, as large colonies housed in many small tanks and clip boxes can become very time consuming. Tanks measuring 100 x 40 x 40 cm are now being used to house groups of up to 700 adult *Geomitra* or up to 350 adult *D. lyelliana,* with removable lids made of Foamex® (https://www.cutplasticsheeting.co.uk/product/black-pvc-foamex-board/) with a part of mesh providing ventilation (Fig. 36).

Figure 36. 1 x 0.4 x 0.4m glass vivarium, used to house 700 adult *G. grabhami* (Tamas Papp).

2.2 Feeding

2.2.1 Basic Diet

These snails are generalist herbivores. While apparently relatively unfussy feeders, they do not eat large quantities of food, and the main problem that will be encountered in their feeding is mould growth on excess uneaten food. Groups of 30-80 snails (depending on growth stage and species) are maintained on one slice of pre-peeled sweet potato and one slice of carrot, offered three times a week. These numbers should be scaled proportionally for larger or smaller groups of snails. The vegetables are peeled before being offered to the snails to reduce the risk of parasites or contaminants on the skin or in the soil on the skin. Any uneaten food is removed and replaced with each feed to prevent mould growth. Due to the very small size of hatchlings, risk of drowning is severe, and they are capable of drowning in even tiny amount of water e.g. pools on a lettuce leaf. Therefore, the feeding of leaf vegetables to these snails is not recommended. All food items have supplement powder sprinkled on them and are then lightly sprayed with filtered water. Additionally, liquid food mix can be offered, smeared on a feeding plate. It is important to regularly clean the feeding plates, again to prevent mould growth. See section 2.2.2 below for discussion of both the supplement powder and paste diet. Hatchlings can burrow into food items; therefore it is imperative to double-check food items before discarding.

2.2.2 Special Dietary Requirements

The food items offered should be dusted with 'Bermuda snail powder', a supplement mix developed in-house at the zoo initially for the Bermuda land snails (*Poecilozonites* sp.). The mix consists of spirulina powder, crushed up fish flakes (Fig. 37) and either powdered cuttlebone or pure calcium carbonate, mixed together in a 1:1:1 ratio. Additionally, once a week one food item is smeared with a thin layer of paste diet on one half and calcium paste (made by mixing calcium powder with water to form a yoghurt-like consistency in a 2:1 calcium-to –filtered-water ratio), to provide additional calcium supplementation.

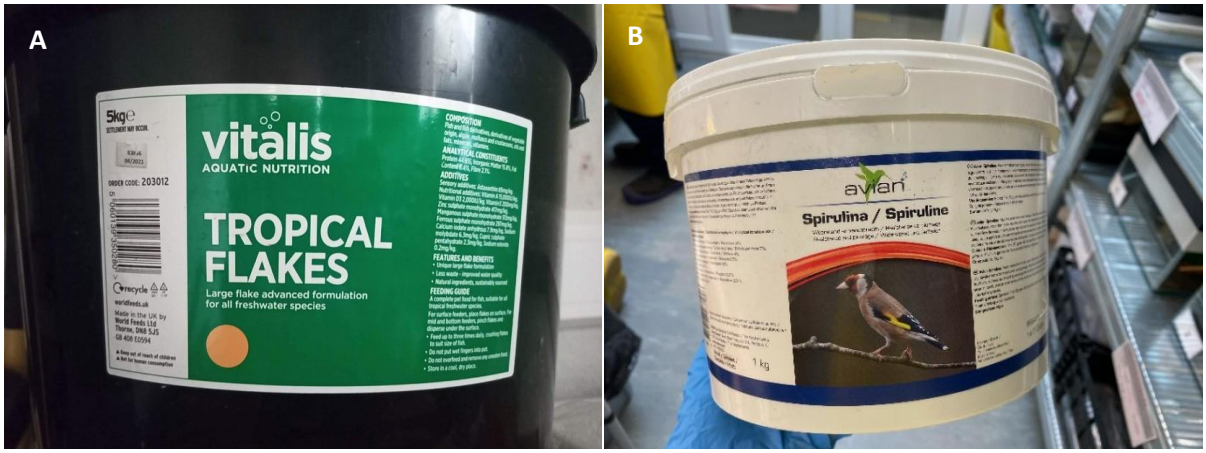

Figure 37A. Fish flake and **B.** spirulina; two principal ingredients of the supplement used for these snails (Katie Kelton).

Another paste diet offered to the snails is the *Partula* paste. It is based on a recipe used for many years in the International Partulid Breeding Programme, and contains 30g of cuttlebone or calcium carbonate, 30g of porridge oats, 60g of grass pellet and 15g of sturgeon pellet. Each ingredient is reduced to a fine powder and the required amount of this dry diet is then mixed with filtered water to form a paste. A 1:3 ratio of *Partula* powder to filtered water is used. If kept dry and in a sealed container, this dry diet mix can be stored for up to six months (Clarke, 2019).

2.2.3 Method of Feeding

Vegetables such as sweet potato and carrot can be sliced (Fig. 38) and placed into the enclosure, with the Bermuda snail powder sprinkled on top (see Figs. 33 and 34 for this in the enclosures). This can then be left in the enclosure for 2-3 days and closely monitored for signs of feeding. Leftover food must then be removed to prevent it going mouldy, and extreme care must be taken to ensure that no hatchlings or small juveniles are discarded along with any leftover food they may be attached to or burrowed into (see section 2.6.2 on how best to remove the very small snails from items within their enclosure).

Figure 38. Slices of sweet potato prepared for feeding to Desertas Snails (Imogen Newens-Hill).

The *Partula* diet can be smeared onto an acrylic food plate as has been used for *Partula* snail species (see Clarke, 2019). This can then be propped up against the side of the enclosure at an angle to allow the snails to climb on it to feed. The angle must be sufficient to avoid risking the food plate falling and crushing any snails; the bottom edge can be dug into the substrate to help stabilise the plate. It is important that the paste is not allowed to touch the soil as this can promote mould growth in the substrate and will require more frequent cleaning of enclosures. Alternatively, acrylic sheets of 5mm thick black Foamex (https://www.cutplasticsheeting.co.uk/product/black-pvc-foamex-board/) cut to 200x100mm have been used successfully as feeding plates.

2.2.4 Water

The only water that should be offered is via spraying; additional water sources are not necessary. Spraying of enclosures can be carried out 2-3 times a week, with the frequency and quantity varied according to the seasonality charts (Appendix I). Make sure to spray the enclosure lightly and only on a very fine mist. Water can also be added via pipetting a small amount directly into the substrate to avoid causing a drowning risk. Enclosures should only be sprayed with water treated with HMA (Heavy metal axe) or filtered through reverse osmosis as snails can be sensitive to some components of untreated tap water.

2.3 Social Structure

2.3.1 Sharing Enclosure with Other Species

It is not recommended to house Desertas land snails with 'clean-up crew' invertebrates such as springtails, due to observations of negative impacts upon their activity and reproductive success (Papp, pers. obs.). Given the small size, incredibly delicate nature, sensitivity to competition from pest invertebrates in their enclosure, and extreme rarity of each of the four species, it is not recommended to house any other species with any of the Desertas land snails.

The limited fauna of the Desertas Islands means that the only species from the same native range kept in EAZA collections is the Deserta Grande wolf spider *Hogna ingens*. It however is not advised to cohabit these spiders with any of the snail species due to differences in the preferred microclimates, with the spiders typically being kept in more arid conditions compared to the snails. Additionally, live food insects offered to the spiders may have the ability to transmit parasites or pathogens onto the snails.

2.4 Breeding

2.4.1 Mating

Both *Geomitra* species and *D. lyelliana* have been observed breeding from September to November, with a pause from December to March, before breeding resumes from April to June. This is associated with the seasonality provided in the ex-situ programme to match conditions on the Desertas, with breeding associated with periods of 18-26°C with 60-80% humidity. Conditions above or below these parameters lead to a reduction in both general activity and breeding.

A. calathoides differs from the other species as their breeding period occurs throughout the winter, from November to March. Levels of breeding activity peak at approximately 14°C and reduce sharply above 18°C (Newens-Hill, pers. comm).

Mating has not been observed in all the species, but anecdotal evidence based on subsequent egg laying suggests an intense spraying following a drying out period can be a trigger of mating. Other observations suggest an increase in egg-laying following substrate changes in the enclosure; this may be related to the soaking of the specimens for cleaning that occurs alongside substrate changes. The duration of gestation, between mating and egg-laying, remains unknown in all four species.

2.4.2 Egg Laying

As discussed in section 1.7.4, the different Desertas species have very different clutch sizes, from 2-3 eggs for *A. calathoides* (although see section 1.7.4 for discussion of *A. calathoides* clutch sizes) to ~55 eggs in *D. lyelliana (*Fig. 39). These are typically laid in a cluster around the edge of the rocks (Fig. 40) or other cover items in the enclosure, near the surface of the substrate, or occasionally scattered across the surface in the case of *D. lyelliana* (Kelton, pers. obs.). Egg clutches can be moved to another container, such as the clip boxes used for hatchlings, to more closely monitor hatching and reduce keeper workload. When the substrate is changed, the old substrate should be kept to one side in a lidded tub and monitored, to allow any unhatched eggs to emerge, as egg clutches can also be difficult to observe with the naked eye. This is especially important in the case of *A. calathoides* due to the potentially long incubation period in this species.

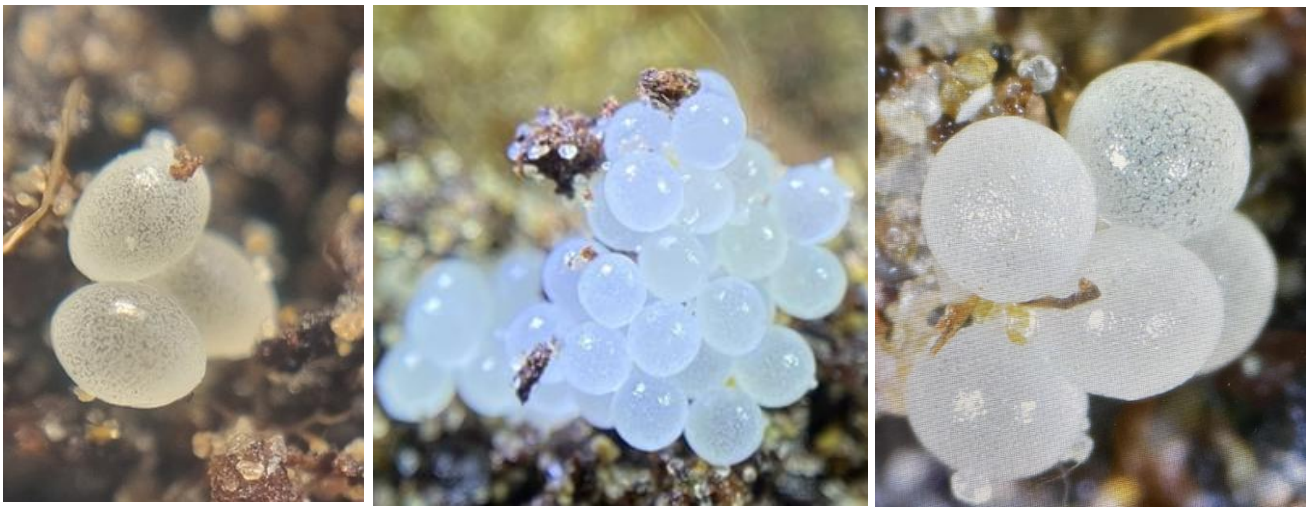

Figure 39. Eggs of (left) *Atlantica calathoides* (Heather Prince), (centre) *Discula lyelliana* (Katie Kelton), and (right) *Geomitra coronula* (Tamas Papp).

Figure 40. Two clutches of *Atlantica calathoides* eggs (circled), laid around the base of a rock that has just been lifted out of the enclosure. Impression of the rock can still be seen. (Heather Prince).

2.4.3 Incubation & hatching

Discula lyelliana: Eggs take around 10-14 days to hatch after being laid. One monitored clutch of *D. lyelliana* eggs were found on 18/11/22, at most 2 days after being laid, and all hatchlings in the clutch emerged exactly 10 days later, on 28/11/22.

Geomitra grabhami*:* Eggs take around 7-10 days to hatch after being laid.

Geomitra coronula: Same as *G. grabhami;* eggs take around 7-10 days to hatch after being laid.

Atlantica calathoides: The incubation time is much longer, with hatchlings still emerging 3-4 months after eggs are laid; one hatchling which emerged on 09/02/23 was from an egg discovered on 06/10/22.

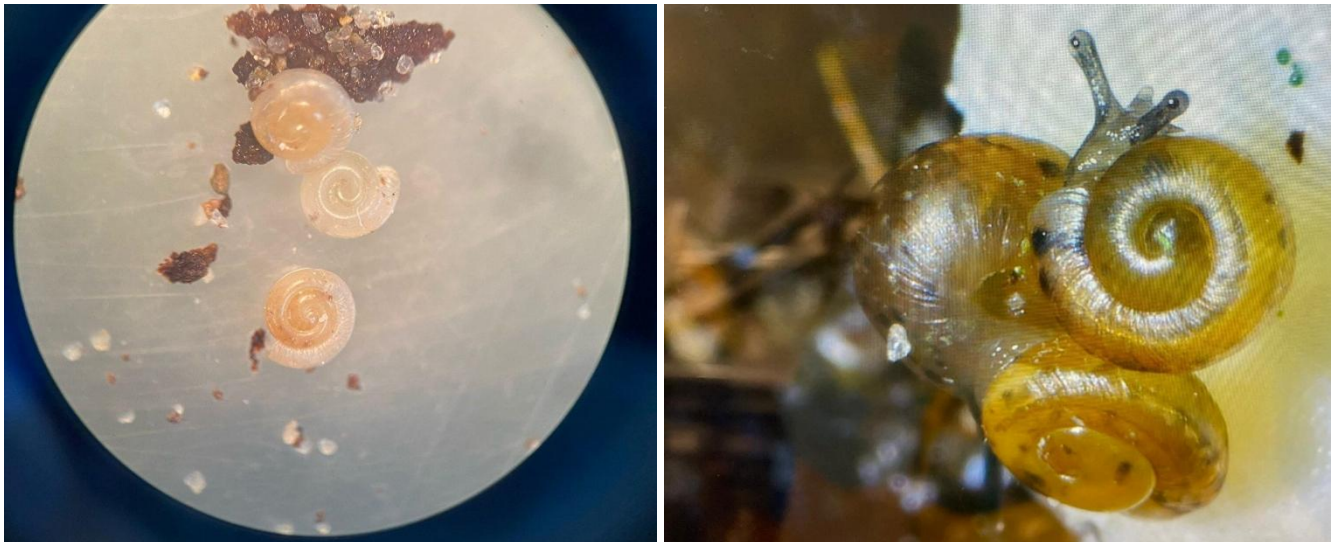

Figure 41. (left) Hatchling *A. calathoides* under a microscope. These are live, retracted hatchlings, not empty shells; the species is extremely pale to the point of near-translucence upon hatching. (right) *D. lyelliana* hatchlings clustered on a piece of cuttlefish bone (K. Kelton).

2.4.4 Development and Care of Young

When hatchlings are found, either in stored tubs of substrate or in adult tanks, they should be transferred to either a clip box or petri dish (See Section 2.1.5 above for discussion of the relative merits and stocking densities of both). This should be labelled with the hatch date (or date found), or the start and end dates of hatchlings added, if added over a period of time. The best technique for moving small hatchlings is by using a fine paint brush (Figs. 42-43), with the tip moistened with O filtered water to ensure the snail sticks to it. Cleanliness in the clip boxes or petri dishes must be closely monitored, as groups will need splitting up into more containers as the hatchlings grow and produce more waste.

Figure 42. Paintbrushes used for handling hatchling Desertas snails (Imogen Newens-Hill).

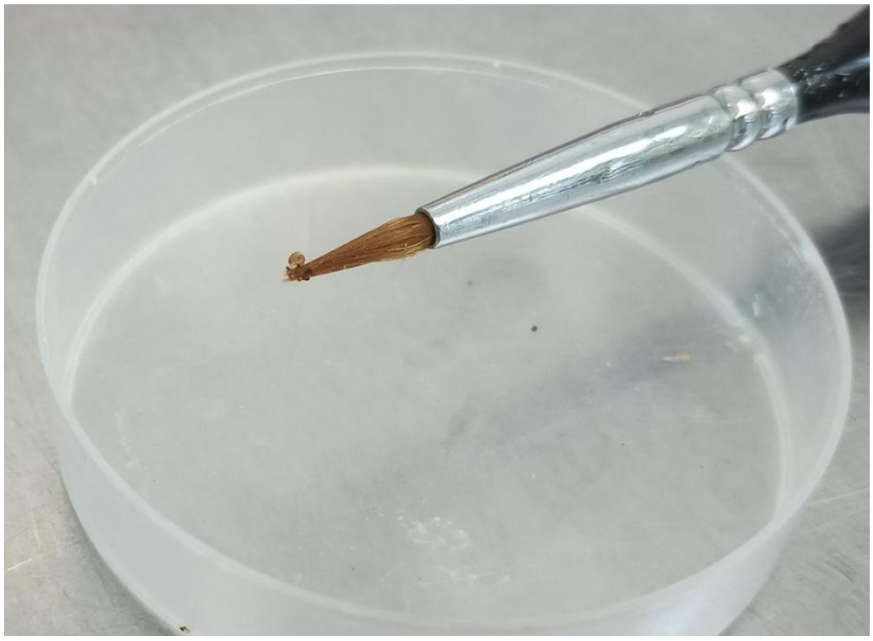

Figure 43. Hatchling *G. grabhami* on the tip of a paintbrush (Tamas Papp).

The very small size of hatchlings and smaller juveniles means that food items should be offered in smaller pieces than for the adults. Great care must be taken to ensure that there are no snails remaining on any food items before they are discarded. If any snails are located on a food item to be discarded, they can be gently removed from it with a paint brush as described above and placed back into their enclosure, or into a separate tub if their enclosure is being fully cleaned out.
In *G. coronula, G. grabhami* and *D. lyelliana*, fully mature adults can be distinguished by the thickened lip around the shell's aperture (see Section 1.7.1). *G. grabhami* start showing shell ridges at the juvenile stage and their shell becomes less flat and larger/higher once they become subadults, in addition to growing in size. As with many invertebrate species, hatchlings and juveniles have a naturally higher mortality rate than adults.

2.4.5 Population Management

Unlike some other snail species, the endemic Desertas land snails all seem quite sensitive to overcrowding, with die-offs rapidly occurring in overcrowded enclosures, due to rapid waste accumulation and cannibalism in overcrowded setups, meaning it is very important to manage population density. Groups of no more than 40 adult *D. lyelliana* and no more than 60 *G. grabhami* and *A. calathoides* are recommended to be housed together in standard-sized enclosures (see section 2.1.5). Juveniles can be housed in higher densities due to lower waste production than the larger adults, but it is important to monitor this closely and split colonies before they grow too large. It also should be noted that the removal of the larger individuals from a group of juveniles seems to accelerate the growth rate of the other individuals, possibly due to reduced competition (Prince, pers. obs.), meaning that groups may need to subsequently be split again sooner than is initially anticipated.

The easiest way of controlling the population size in these snails, to maintain within the carrying capacity of the facilities, is to freeze the clutches of eggs to prevent them from hatching. The eggs can then be disposed of after 48hrs in the freezer. This is a practical and vital part in any long-term breeding programme for these often fast-reproducing molluscs. Euthanasia of the live snails is only to

be carried out as a last resort. Following the seasonality chart (Appendix I) will also limit the time frame of the species' breeding, enabling proper planning of when the peak periods of population growth are.

2.5 Behavioural Enrichment

No specific behavioural enrichment is required beyond provisioning of suitable refugia and food items (see section 2.1.3 and 2.2).

2.6 Handling

2.6.1 Individual Identification and Sexing

In the *ex-situ* breeding program Desertas land snails are managed as groups, as individual level identification is not practical or necessary for the success of the breeding program. Sexing is also a moot point, as the species are all hermaphroditic. VI alpha tags have been used successfully post-release mark-recapture studies in other snail species. These are small (1.2mm x 2.7mm) fluorescent tags which can be superglued to the side of the shell, the methodology of which has been well-developed on other snail species (Flewitt, 2020) and has now been used successfully to tag *G. grabhami*. Each tag has an individual alphanumeric code that enables individual identification of a specimen, with 10,000 different code/ colour combinations (Northwest Marine Technology Inc.).

The ongoing reintroduction programme uses three main marking methods: bee tags (designed for use by beekeepers to mark queen bees), a UV-reflective marker pen, and a regular non-toxic marker pen. Only the *D. lyelliana* are large enough to be marked with the bee tags (Fig. 44), so this species is marked with a bee tag and the UV marker. *Geomitra* spp. are marked with the two marker pens (Fig. 45). All marks should be covered with a layer of clear nail varnish to improve marker longevity. See Appendix II for the exact marking protocol for *Discula* and *Geomitra*. *A. calathoides* are yet to be released as part of the reintroduction programme (as of 2025) but can probably be marked using the same methodology as the *Geomitra* species.

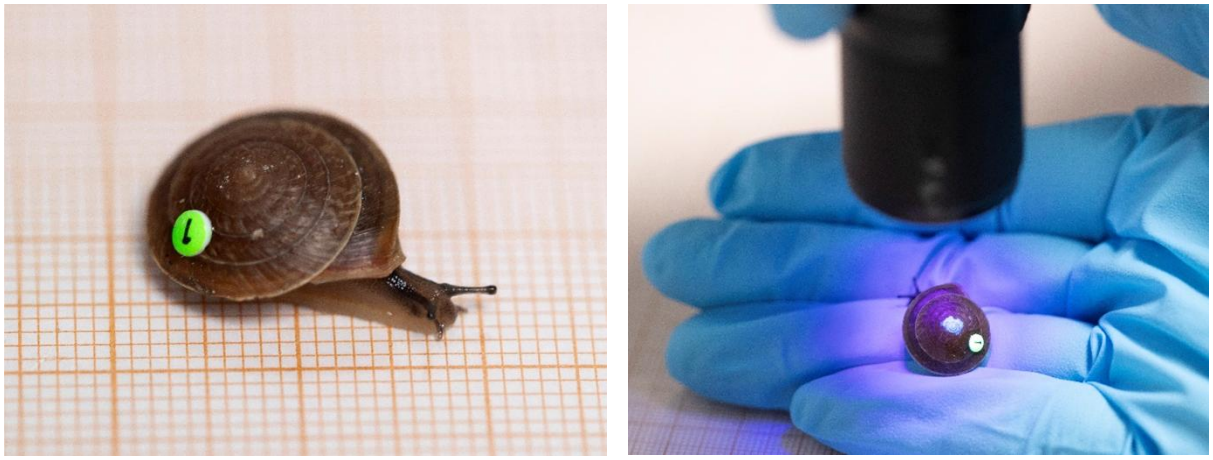

Figure 44. Fluorescent tag glued on a *Discula lyelliana* (left) and shown under a UV light (right) (Mathilde Arnold).

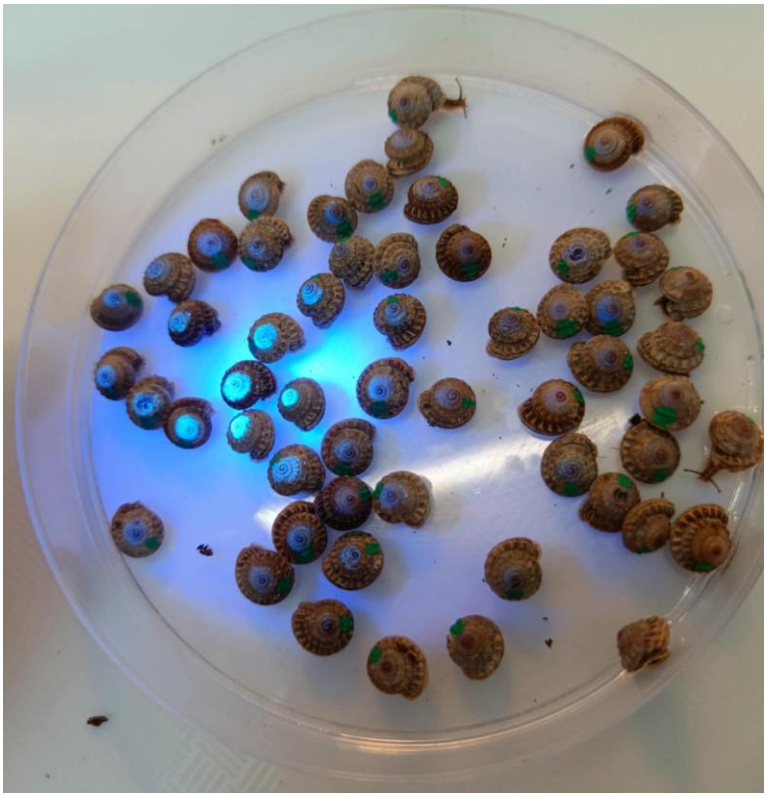

Figure 45. A petri dish of adult *G. coronula* that have been marked ahead of reintroduction, showing the UV reflective marker under a blacklight (Imogen Newens-Hill).

2.6.2 General Handling/ Catching

Adults of all four species can be handled by carefully holding the shell avoiding any contact with the lip of the shell as this is more fragile and this can cause long term shell damage or deformities. It is recommended that non-powdered latex or nitrile gloves are worn to protect both the handler and snail from transfer of pathogens and/or parasites. If adhered to a surface individuals should be removed by very slowly and smoothly pulling on the shell, taking care to avoid sudden jerking movements.

The hatchlings are far too small to move by hand. When hatchlings or young juveniles need to be removed from an enclosure (e.g. when the substrate needs changing, or to separate hatchlings that have emerged in an adult enclosure) the use of a fine-tipped paintbrush is recommended (Fig. 42). The brush tip should be dipped in RO filtered water and then brushed on the back of the hand, so it is only damp, not wet; this moisture improves the ease of moving hatchling snails by allowing them to adhere to the brush. If the hatchlings are clustered on an item of food, piece of cuttlebone or piece of décor, this can be carefully held in plastic tongs, and the small snails then brushed off it into another container. It is important to be aware that, due to the extremely small size of the hatchlings, they are impacted by static electricity: if the brush becomes statically charged, the hatchlings may ping off from the brush if it is brought close to them and be lost.

2.6.3 Transportation

Transporting the snails in between tanks whilst cleaning etc. can be done in any petri dish or small plastic container. When it comes to exporting snails for reintroduction or for longer-distance transport between collections in different countries, the following process can be used; moist paper towels

should be packed into standard Braplast tubs (1.3l; 185x125x75mm) and the snails can then be placed into the tubs. For *D. lyelliana*, up to 30 adults or 50 juveniles can be packed per tub, or up to 50 adults per tub for *A. calathoides* or the *Geomitra* species. These tubs can then be individually labelled and then placed inside a polystyrene box that is then placed inside an IATA-compliant wooden crate (IATA, 2015), or alternatively the insides of the crate itself can be covered by polystyrene (Fig. 46). This is to improve the insulation. Experience has shown that the inclusion of water containers is a great aid in ensuring that desired temperatures are maintained inside the container even if the crate is subjected to unexpectedly high and/or low temperatures at any stage of the journey. Fill the water containers to ¾ of their volume and place in a separated compartment either side of the polystyrene box or separated from the tubs by a polystyrene board. If these water containers have been filled and left in the snail facility overnight prior to packing, they should be at the ideal temperature and tests have shown that they do a good job of maintaining a stable temperature.

Figure 46. A crate used to export Desertas snails for reintroduction. The polystyrene panels either side of the tubs cover the compartments for water containers (Gerardo Garcia).

2.7 Veterinary Considerations for Health and Welfare

Another group of Critically Endangered snails which have been subject to similar *ex-situ* conservation measures as the Desertas snails are the *Partula* species of French Polynesia. Detailed pre-reintroduction health screening protocols have been produced by the Partula EEP and International *Partula* Snail Programme (Clarke, 2019). Many of the features of this protocol are relevant to Desertas species, and so these protocols are here attached as Appendix III.

One potential issue that has been observed is the presence of mites in the enclosures (Fig. 47). Although the mites in question seem to be scavenging/detritivorous rather than truly parasitic, higher mortality is seen in tanks with heavy mite burdens, as well as lower fecundity and a reduction in breeding. The precise identity of the mites involved is yet to be determined (see section 2.8).

Figure 47. Close-up photo of an as-yet-unidentified scavenging mite from a *D. lyelliana* enclosure (Tamas Papp).

Nematodes have also been found in some Desertas snail tanks (Fig. 48). As with the mite outbreaks, these seem to be environmental rather than parasitic, associated with uneaten food and already-deceased snails. Their impact upon the snails remains unclear, as does the specific identity of the worms involved.

Figure 48. Close-up photo of as-yet-unidentified nematodes from a *G. grabhami* enclosure (Tamas Papp).

If mite or nematode numbers are observed to be increasing in a tank, it is important that suitable measures are taken. Affected tanks can be placed in a water bath to prevent pests from spreading to other enclosures, and the substrate should be changed completely to remove all stages of the life cycle. Further barrier management protocols have been trialled, such as double-sided tape or mite paper on the lids or doors of the enclosures, but were not of significant efficacy, as they need changing regularly. Great care must be taken when using mite paper due to the toxins involved and the risk of contaminating snail enclosures or food. Regular substrate changes and removal of uneaten food are the most effective method to prevent the proliferation of mites and nematodes and following a strict service order may prevent them spreading to other enclosures.

Some issues have occurred, particularly in *Geomitra* species, with adults and juveniles feeding on other individuals' shells. This initially starts out as grazing on the periostracum (the living outer layer of the shell) (Fig. 49) but can develop into consuming all layers of the shell, resulting in crevices/holes (Fig. 50). This issue can be resolved by increasing calcium intake (e.g. providing more pieces of cuttlefish bones), increasing available protein and reducing stocking density of the snails.

Figure 49. Examples of periostracum grazing in *Geomitra grabhami.* Intact individual (left), periostracum entirely lost (middle), and individual that has been only partially grazed by conspecifics (right) (Julien Buisson).

Figure 50. Examples of live *Geomitra grabhami* with damaged shells consumed by conspecifics (Julien Buisson).

One individual *G. grabhami* at Chester Zoo developed a strange, elongated growth from the aperture of its shell, impacting its ability to move normally (Fig. 51). This was filed down in order to enable normal movement (Fig. 52), and the overgrowth has not recurred. The reasons for this abnormality are not known but as it has only been observed in one individual of many hundreds of snails, it is not believed to have environmental causes.

Figure 51. *Geomitra grabhami* individual with abnormal growth from aperture of its shell (Heather Prince).

Figure 52. Same *Geomitra grabhami* individual from previous figure with abnormal growth being filed off of its shell (Imogen Newens-Hill).

2.8 Recommended Research

Due to the young age of the *ex-situ* conservation programme, there are a vast number of questions that remain unanswered about the four focal species. Topics of particular interest to the programme going forwards include:

- The wild breeding season for each of the species warrants further study, as do the factors that trigger breeding in the *ex-situ* populations.
- *G. coronula* and *G. grabhami* are extremely similar in external appearance. Full morphological (including genital dissection) and genetic analysis of both taxa should be carried out to either confirm or disprove their status as separate species.
- The varied growth rate of both *Geomitra* species, and any factors that may influence the severe difference between individuals, warrants further investigation.
- Clutch size, incubation period and breeding season in *A. calathoides,* and the impact seasonality systems have upon this, warrant further investigation.
- *A. calathoides'* preference for cooler temperatures is an interesting observation and raises questions about the species' preferred habitat and if the relict population on Deserta Grande is living in suitable conditions for the species; other *Atlantica* species are found in laurel forests in Madeira and the Canary Islands. Investigation into the species' true preferred habitat would be useful to ensure robust populations in the future.
- Marking methodology for *A. calathoides* needs to be trialled ahead of any reintroductions with this species.

- Average longevity and time to maturity of *A. calathoides* and *G. coronula* warrant further investigation.
- Specific identification of the mites and nematodes occasionally impacting *ex-situ* groups would be useful.

Section 3: Acknowledgements, References and Appendices

3.1 Acknowledgements

The authors thank Paul Pearce-Kelly, Dave Clarke and the rest of the invertebrate team at ZSL for developing the snail health screening guidelines attached as an appendix below. Thanks to Adam Button, Mathilde Arnold and Julien Buisson for providing some of the images included here as figures. Thanks also to Tiago de Zoeten and all the team at Mossy Earth and Eric Bairrão Ruivo and all the team at Beauval Nature Association, for supporting *in-situ* conservation efforts. Thanks to Iri Gill, Jay Redbond, Liz Ball, Javier Lopez, Becca McKown, Rebecca Lewis, Laura Naidenov, Heléna Turner and everyone else involved in the Desertas snail project and their export for reintroduction at Chester Zoo. The authors also extend their gratitude to Isamberto Silva, Martinho Pires, Manuel José Jesus, Humberto Silva, Martina Panisi, Robert Cameron, Klaus Groh, Regina Cunha, Roberto Resendes, and all the other IFCN nature wardens and malacologists involved in the *in-situ* conservation efforts. A special thanks to Ana Mafalda Alves from Parque Biológico de Gaia and Luena Marques for their contributions in revising this document.

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3.3 Appendices

Appendix I : Seasonality charts for Desertas species at Chester Zoo

Desertas Island snail

Atlantica calathoides

	Jan	Feb	March	April	May	June
Temperature °C (day/night) ambient)	14-16	15-18	16-18	16-18	16-18	18-20
Temperature °C (soil)	14	14	15	16	17	21
Photoperiod (No. of hours of light)	10	11	12	13	14	14
Humidity %rh (air)	88	91	95	87	92	92
Precipitation	60mm	50mm	50mm	34mm	20mm	13mm

	July	August	Sept	Oct	Nov	Dec
Temperature °C (day/night) ambient)	20-22	21-23	22-24	19-21	17-19	15-17
Temperature °C (soil)	23	23	24	20	16	16
Photoperiod (No. of hours of light)	14	13	12	11	10	10
Humidity %rh (air)	84	86	88	92	92	95
Precipitation	4mm	5mm	36mm	74mm	93mm	84mm

Desertas Island snail

Geomitra grabhami, G. coronula & Discula lyelliana

	Jan	Feb	March	April	May	June
Temperature °C (day/night) ambient)	14-19	14-19	14-19	15-21	16-22	18-24
Temperature °C (soil)	16	16	17	18	19	23
Photoperiod (No. of hours of light)	10	11	12	13	14	14
Humidity %rh (air)	88	91	95	87	84	92
Precipitation	60mm	50mm	50mm	34mm	20mm	13mm

	July	August	Sept	Oct	Nov	Dec
Temperature °C (day/night) ambient)	20-26	21-26	20-26	19-25	17-22	16-20
Temperature °C (soil)	25	25	26	22	18	18
Photoperiod (No. of hours of light)	14	13	12	11	10	10
Humidity %rh (air)	84	86	88	92	92	95
Precipitation	4mm	5mm	36mm	74mm	93mm	84mm

Appendix II: Marking methodology for marking *Discula* and *Geomitra* for reintroductions

Methodology developed by Dinarte Teixeira.

Marking techniques to be used in *Discula lyelliana* and *Geomitra coronula*

1. Discula lyelliana

Bee tag (Example of suitable product: https://www.beeequipment.eu/numbers-for-marking-queens-1-99-various-colours):

1. Clean the shell of all deposits and dirt with a gentle brush. Be careful not to detach the periostracum of the shell (the organic skin on the shell).
2. Use a non-toxic glue solvent-free to attach the tag to the shell (e.g. UHU solvent-free glue).
3. The bee tag should be attached to the shell's penultimate whorl on the mouth aperture's opposite side (see Fig. 1, in green).
4. Lay a coat of chemical-free transparent nail polish on the top of the bee tag to prevent tag erosion when rubbing the rocks. Please ignore this step if you are already using bee tags with a coat of protection on them.

UV Marker (Example of suitable product: https://morgansdirect.co.uk/product/artline-supreme-permanent-marker-uv-pens/):

1. Clean the shell of all deposits and dirt with a gentle brush. Be careful not to detach the periostracum of the shell (the organic skin on the shell).
2. Apply the marker on the protoconch, first and second whorl in a circular form, doubling the coat in the selected area (see Fig. 1, in black).

Fig. 1. Marking areas for *Discula lyelliana*: black (UV marker) and green (bee tag).

2. *Geomitra coronula*

Pen marker (Example of a suitable product: https://www.staedtler.com/uk/en/products/markers/metallic-markers/staedtler-8323-metallic-pen-8323-tb10/):

1. Clean the shell of all deposits and dirt with a gentle brush. Be careful not to detach the periostracum of the shell (the organic skin on the shell).
2. Insert the mark on the top side of the shell, on the penultimate whorl on the mouth aperture's opposite side (see Fig. 2, in green).
3. Lay a coat of chemical-free transparent nail polish to protect the marking area to prevent abrasion.

UV Marker:

1. Clean the shell of all deposits and dirt with a gentle brush. Be careful not to detach the periostracum of the shell (the organic skin on the shell).
2. Apply the marker on the protoconch and the first whorl in a circular form, doubling the coat in the selected area (see Fig. 2., in black).
3. Although indicated in the meeting, we won't mark the specimens on the underside of the shell because of the intense exposure to abrasion.

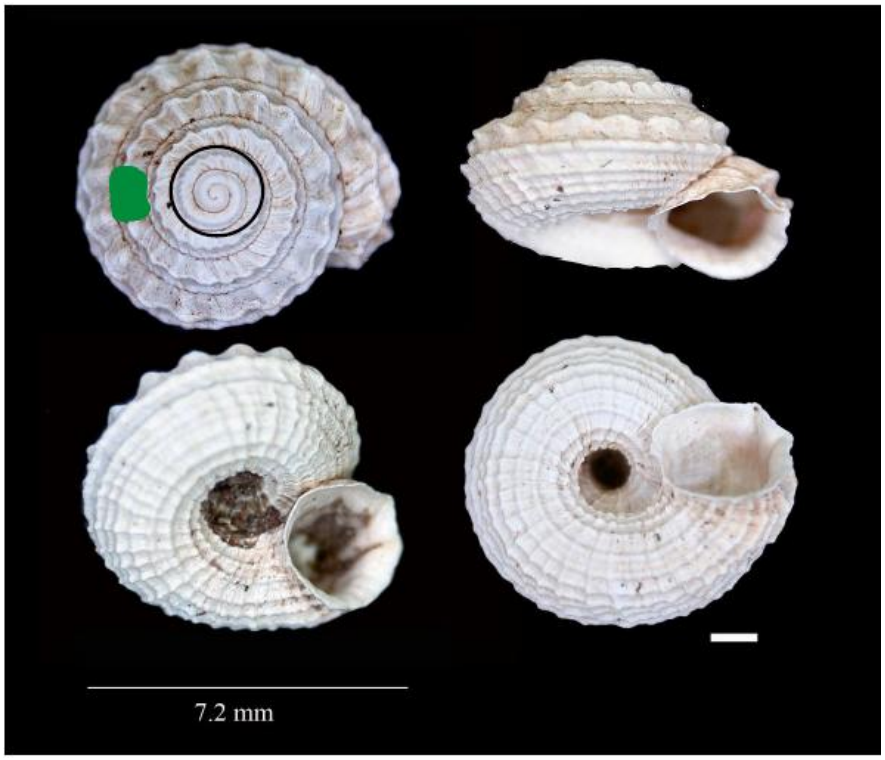

Fig. 2. The marking areas for *Geomitra coronula*: black (UV marker) and green (marker pen).

Appendix III: Pre-reintroduction health screening protocol for *Partula* snails
(From Clarke, 2019)

Authors' note: although *Partula* are ecologically quite different from the Desertas island species covered by this document, the health screening protocol of reintroducing any island endemic terrestrial mollusc should be very similar, and so this is appended here as a reference point on the level of detail and preparation methods required for a pre-release health screening.

Introduction

This health screening protocol is intended to help ensure that all *ex-situ* partulid snail populations have the greatest chance of inclusion in the reintroduction phase of the partulid conservation programme and ensure compliance with the *Partula* reintroduction disease risk analysis (Dalziel et al., 2013) to minimise the risk of infectious and non-infectious diseases compromising reintroduction success. The protocol draws upon and synthesises best health-screening practice employed by the *Partula* programme and other invertebrate programmes.

Background

Intensive health-screening carried out at ZSL (Goodey & Flach, 2015 and 2016) in anticipation of the pre-release export involved monitoring and testing for the presence of potentially significant pathogens and pathogenic lesions. The screening consisted of examination of fresh faeces, individuals found dead, and individuals euthanized, for the presence of parasites and bacteria, plus histopathological examination of snail sections for evidence of microorganisms and pathological processes. Although a variety of parasites and bacteria were observed and cultured, none were considered significant. There was evidence of a suspect microsporidial infection observed commonly in the past and characterised by acid-fast cysts with a diameter of between 4 and 5 microns in the intestines (observed in impression smears of the apex) and *faeces*. Definite microsporidial bodies were identified histologically and were suspected to be *Steinhausia* species, but there was no cell damage, reaction, or inflammation associated with these bodies. Similar bodies were detected in museum-derived, wild-caught individuals, again without evidence of pathogenicity. It was concluded that the health of the snails was good, and that they were free of any pathogens likely to cause disease in them, or to native French Polynesian snails, and other species, after release.

Pre-release Screening

The current screening protocol is based closely on the 2015 and 2016 recommendations, but with three main changes: a) closer monitoring of population numbers with early warning to the veterinarian(s) in charge of any deaths, b) reduced bacteriological culturing from faecal samples; just one faecal bacteriological examination is proposed, and c) snails to be fixed in 10% buffered formalin because of the successful identification of *Cryptosporidium*-like bodies in Partula snails that had been so fixed (Stidworthy & Lopez, personal communication).

Tanks of snails from the resident collection may be monitored and sampled in-situ, but any populations imported from other collections for pre-export screening should be introduced into a separate, bio-secure room and serviced by staff that do not work with the resident snails. If this is not possible, staff dealing with the resident collection should service this first, and then put on laboratory coats and change shoes before working on the quarantined populations. It is acceptable, and makes

final certification of health easier, if tanks from the resident collection intended for export are also moved into this room so that all populations for export are managed and screened together and at the same time.

During the period of quarantine (suggested to be a minimum of one month) the following surveillance should be done:

1. Monitoring of mortality

Each tank population should be checked each morning and all dead snails submitted immediately to the veterinary department. In addition, there should be counts at least weekly of numbers of newborn, juvenile, sub-adult and adult snails. Abnormally high numbers of deaths in a tank should be reported to, and discussed with, the veterinarian in charge and the population withheld from the export, unless the deaths can be proved to be due to an environmental factor (e.g. temperature fluctuation, abnormal humidity, exposure to toxic chemicals on food plates).

2. Faecal screening: A minimum of three faecal samples taken from each tank for parasitological testing, one of which should also undergo bacteriological testing. Faeces should be collected into sterile plastic bijou containers (or similar), labelled with the tank number, species and date, and submitted to the diagnostic laboratory.

3. *Post mortem* examinations: Dead snails should be examined in three circumstances: a) individuals found dead, reasonably fresh and on a day when they could be submitted for immediate examination, b) individuals found dead, but not fresh, or on a day when immediate examination is not possible; these to be fixed in 70% ethanol and then submitted, and c) healthy individuals randomly selected for euthanasia (carried out by the collection's veterinary department and using exposure to an overdose of the volatile anaesthetic drug isoflurane for a minimum of one hour) and submitted for immediate, fresh examination. The aim is to examine approximately 5-10% of the population of adults in each tank, with at least one individual examined fresh after euthanasia from tanks with large enough populations.

As is the case with many other invertebrate species reintroduction programmes, the need to include euthanised specimens in the pre-release health screening process is due to a combination of: a) high prevalence of severe autolysis of dead snails, and b) the need to screening sufficient individuals in order to have confidence of the results of the tests. Unfortunately, despite many years of examining snails there are still no definitive prevalence rates for clinical disease in *Partula* snails due to microbial pathogens. Most are present in low numbers and are detected intermittently, so even when found in populations undergoing unusually high mortality their contribution to the mortality (compared to environmental factors) is unknown.

Snails presented already fixed in ethanol are weighed and measured, but not examined grossly. A proportion may be submitted for histopathology, but the diagnostic value of these individuals is usually very low due to autolytic changes and fixation in ethanol. All others are weighed and measured as previously described (Pearce-Kelly et al., 2007). If the snail does not easily slide from the shell when traction is applied, it is recommended that heavy-duty scissors are used to open the shell to ensure minimal crush and stretching artefacts to the carcass. The extracted body is weighed and examined externally. The body opened with a sterile scalpel blade, an internal ("coelomic") swab is taken for bacteriological culture, and the internal tissues are examined grossly. The tip of the apex is then

removed with the scalpel blade and impression smears of the cut surface made on a microscope slide for parasitological examination (see below). A small piece of foot tissue is removed and frozen as a source of DNA, and finally the remaining carcass, plus the amputated apex, are fixed in 10% buffered formalin, placed in histological cassettes and forwarded to the diagnostic laboratory performing histopathological services. Here at ZSL snails are sent to the Royal Veterinary College (RVC) for processing (including the following stains: haematoxylin and eosin (H&E), Ziehl-Neelsen (ZN), Gram's, Periodic acid-Schiff (PAS) and Luna-Peterson (L-P) specifically for microsporidia (Peterson et al., 2011)) and thence to the International Zoo Veterinary Group (IZVG) for histopathological assessment and reporting.

4. Diagnostic testing

4.1 Parasitology

Faeces are examined by direct microscopy of a wet preparation and microscopy of a dry smear stained with modified Ziehl-Neelsen stain (MZN). Apex smears are also examined microscopically after staining with MZN.

1. Wet preparations are prepared by mixing a small amount of faeces (a bacteriological Nichrome wire loop-full) in two drops of sterile physiological saline solution and examined microscopically for the presence of any parasites or their ova, with particular emphasis on helminths, flagellated protozoa and ciliated protozoa.

2. MZN-stained smears are prepared and examined microscopically under oil immersion for the presence of pink-staining (acid-fast) bacilli (possible *Mycobacterium* species) and cysts (4-5microns diameter; suspect *Cryptosporidium*-like, 1-2microns; suspect microsporidian). Cyst diameter should be measured with an eye-piece graticule that has been calibrated from a micrometer stage slide, or by electronic means.

4.2 Bacteriology

Faecal samples and "coelom" swabs are normally cultured on 5% horse blood agar plates and incubated at 25°c for 48 hours. Plates are examined and, if the culture is mixed, single bacteria colonies of predominant types are sub-cultured onto further plates. The resulting pure cultures are then identified by standard methods: colony morphology, appearance and Gram's staining characteristics, and biochemical reactions (using commercial analytical profile index (API) biochemical test kits). The diagnostic laboratory used for testing should be consulted prior to submission of samples.

4.3 Histopathology

Histopathology reports (external or in-house) should include details of all of the tissues seen in the sections, any cellular changes and the presence of any micro-organisms and interpretation of their significance.

5. Interpretation of results

Results should be recorded in appropriate spreadsheets and reviewed regularly. Examples are given in Appendices 1-4.

It is likely that a large number of micro-organisms will be detected during the screening, but in our experience the majority are of no, or doubtful, significance to the snails' health.

Flagellated protozoa are commonly found in the faeces of *Partula* snails both in captivity and the wild (Cunningham et al., 1996) and are likely to act symbiotically in breaking down cellulose and other plant fibres in the snails' diet. Rhabditid nematodes were also found in the faeces of wild-caught snails, so the presence of nematodes is generally accepted as a normal finding, although they have the capacity to invade snail tissues after death and therefore are potential opportunistic pathogens.

A wide range of bacteria were isolated from the faeces of wild and captive snails by Cunningham et al. (1996). This list included *Flavobacterium breve* that is now *Empedobacter brevis* and was identified in this study, albeit undifferentiated from *Weeksella virosa*. *Myroides* species are also closely related to *Flavobacterium* and one of them, *M. odoratimimus* used to be *F. odoratum*. These, plus *Aeromonas hydrophila*, *Brevundimonas vesicularis* (formerly *Pseudomonas vesicularis*), *Bacillus* species and *Pantoea* species are all common environmental bacteria and therefore likely to form part of the normal flora of plant-eating snails. *Flavobacterium*-related bacterial species were much less commonly predominant in cultures from dead and euthanized snails, as also noted by Cunningham et al. (1996), but *Aeromonas hydrophila/caviae* was predominant on several occasions.

As previously stated, cysts that stain positively in MZN (and ZN) stains are commonly found in faecal and apex smears; most often with a diameter of 4-5microns and assumed to be the *Cryptosporidium*-like protozoa that have recently been identified histologically and by electron microscopy. These have been observed repeatedly in the past, but have often been referred to under different names, including protozoal cysts, protozoal oocysts, protists and microsporidial cysts. They can be found commonly, but intermittently, in captivity (ZSL veterinary records and reports) generally without association with disease or increased mortality. However, increased mortality in populations of *P. gibba* and *P. tohiveana* at ZSL in 2006-8 was linked to an apparent increase in the prevalence of cysts in faeces and apex smears, and also the presence of microsporidial bodies in histological sections of snails testing positive, or from tanks containing positives (Flach et al., 2008). These bodies were similar to the microsporidia described by Cunningham and Daszak (1998), but the *Steinhausia* cysts described in the paper (2 micron diameter) were not identified, and there was no evidence of a cellular, pathological reaction to their presence in the cases seen in 2006-8. In addition, the use of ethanol fixation at the time (and up until this year) was not ideal for sectioning of Partula tissues and the *Cryptosporidium*-like bodies were never identified. In the 2015 health screening it was observed that cysts appeared in the first faecal samples from several tanks, the second faecal sample from a lower number, and were then absent in third and subsequent samples. This suggests that they may be excreted during times of stress, such as transportation, and acclimatisation to a new environment (the quarantine room) and raises the possibility that other stresses causing increased mortality might at the same time lead to increased shedding and therefore detection of the cysts. Gouveia (2011) investigated many factors that affected the well-being of captive *Partula* species and identified different species sensitivities to a number of environmental factors, especially temperature, humidity and diet.

Microsporidial bodies were frequently seen in histological sections, mainly in digestive glands, of snails during the 2015 and 2016 health screening, but with no associated cellular lesions. Interestingly, and tellingly, similar microsporidial bodies were found in wild caught, museum-archived individuals of two of the three species screened for export in that year (*P. hyalina* and P*. nodosa*); again with no evidence of any pathological reaction associated with them. Microsporidia have been recorded in the literature to infect all vertebrates and most invertebrates (Keeling and Fast, 2002), and Weiser (1976) even estimates there to be microsporidia in every living invertebrate. Therefore, the vast majority of infections are sub-clinical, but because of the occurrence of microsporidia belonging to the genus *Steinhausia* in some of the last individuals of *Partula turgida* that died at London Zoo in 1996 (Cunningham and Daszak, 1998) they have always been considered pathogenic in *Partula* and potential causes of death. The histopathological evaluation of fresh snails, and lack of cellular response to any microsporidial bodies seen, is the best indicator that the source population is unaffected by them.

6. Certification

The veterinarian in charge will have to be confident that he/she can sign the health certificate issued by the exporting country's official animal-health service that, in turn, will be based on the requirements of the importing country (French Polynesia). Discussions should be held at an early stage to ensure that extra testing may be added, if requested, and additional requirements met (e.g. use of sterile substrate prior to and during transportation).

Follow-up

It would be helpful if the results of health screening be shared with all collections holding *Partula* species, so that we can increase our knowledge of normal and potentially significant micro-organisms. Also, details of mortality during transportation, during post import quarantine and acclimatisation, and in the post release monitoring period, should be recorded and shared. Whenever possible, any freshly dead snails found during this period should be fixed in 10% buffered formalin and examined histopathologically, (postage back to Europe for examination by the same histopathologist(s) that did the pre-export screening is preferable).

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