

Husbandry Manual for the Campbell's Keeled Glass Snail (*Advena campbellii*, Gastropoda: Pulmonata: Microcystidae) and *Mathewsoconcha suteri* (Gastropoda: Pulmonata: Microcystidae)

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Top: *Advena campbellii*, (photo: T. Williams Clow) bottom: *Mathewsoconcha suteri* (Photo: A. Daly)

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Preface

The information provided in this document has been acquired and compiled in the context of an ex-situ conservation program. Neither species discussed in this document has been displayed to the public, and the chief aim of husbandry has been to maintain insurance populations of both species with the longer-term aim of breeding snails for release on Norfolk Island. The approach recommended in this manual is therefore a product of that context, and as such may not be appropriate for a husbandry program with different aims, even for a closely related species.

This husbandry manual has been compiled using the guidelines for terrestrial vertebrate husbandry manuals suggested by Jackson (2003). To the authors knowledge, no such guidelines have been suggested for the husbandry manuals of invertebrate species. To produce the most useful document possible, the authors have altered the format and content suggested by Jackson (2003) where they thought appropriate.

Both species of snail were imported to Taronga Zoo, Sydney, and are kept under all relevant permissions from the Department of Agriculture, Water and the Environment and under the Environment Protection and Biodiversity Act 1999.

All photos in this manual were taken by A. Daly, except where otherwise acknowledged.

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1. Introduction

Campbell's keeled glass snail (*Advena campbellii*) and Suter's striped glass snail (*Mathewsoconcha suteri*) are microcystid snails endemic to Norfolk Island. Norfolk Island has a rich collection of endemic flora and fauna. This includes land snails, with 70 recorded species on the island (Hyman, 2020a). Terrestrial snails on oceanic islands are particularly vulnerable to extinction (Régnier et al., 2009), and the snail fauna of Norfolk Island are no exception - both *A. campbellii* and *M. suteri* are currently listed as Critically Endangered under the Environmental Protection and Biodiversity Conservation Act 1999 (Threatened Species Scientific Committee [TSSC], 2009a, TSSC, 2009b). *A. campbellii* is listed as Extinct by the IUCN Red List (Mollusc Specialist Group, 1996). A single living population of *A. campbellii* was rediscovered in 2020 (Hyman, 2020b) and *M. suteri* is found in a single location within a reserve on the island.

Pressures on populations of both snails include predation by introduced predators (Polynesian and black rats, and chickens) and habitat destruction and degradation (TSSC, 2009a, TSSC 2009b). Climate change is also suspected to be negatively impacting both species, though how climate change is affecting land snail populations has been poorly studied in Australia (Parkyn and Newell, 2013).

Scientists from the Australian Museum have been monitoring and researching the snail fauna on Norfolk and nearby islands since March 2020. Following the rediscovery of *A. campbellii*, and with ongoing concerns about the future of *A. campbellii* and *M. suteri*, the Australian Museum approached Taronga Conservation Society Australia about establishing a captive breeding program. The program could serve multiple purposes. Firstly, as an insurance population in the case of a catastrophic event on Norfolk Island, and secondly as a source of snails for potential reintroductions and range expansions.

Neither *A. campbellii* nor *M. suteri* have been held in a zoo collection prior to this program. However, there are existing and long-running captive-breeding programs for other endangered terrestrial snails, including the Hawaiian Snail Extinction Prevention Program (SEPP) and the European Association of Zoos and Aquaria's (EAZA) *Partula* sp. breeding program.

The first founder individuals of *A. campbellii* and *M. suteri* were imported from Norfolk Island to Taronga Zoo, Sydney, in May 2021. The following husbandry manual outlines what we have learned about the captive management of both species since that time.

2. Taxonomy

Advena campbellii and *Mathewsoconcha suteri* were traditionally placed in family Helicarionidae (e.g., Baker, 1941; Smith, 1992). However, taxonomic revisions by Hyman (2005) and Hyman *et al.* (submitted) show that these species belong to family Microcystidae. Microcystidae is distributed throughout the Pacific and into southeast Asia and Madagascar (Baker, 1941). Apart from a few cosmopolitan species, no microcystids are known from Australia, New Zealand or New Caledonia (Baker, 1941), indicating that the origins of the Norfolk Island Microcystidae must lie in the islands of the Pacific.

Advena campbellii contains two subspecies: *Advena campbellii campbellii* and *Advena campbellii nepeanensis*. The latter is only known from Nepean Island, which was completely cleared by 1840, causing the extirpation of all its land snails. Consequently, only subspecies *Advena campbellii campbellii* remains extant. For simplicity, we refer simply to *Advena campbellii* throughout this manual.

Mathewsoconcha as currently formulated contains three species: *M. suteri*, *M. grayi* and *M. phillipii* (Hyman *et al.*, submitted). All three species are listed as Critically Endangered under the Environmental Protection and Biodiversity Conservation Act 1999. *Mathewsoconcha suteri* is only known from Norfolk Island; *M. grayi* and *M. phillipii* were last recorded from Phillip Island.

Class: Gastropoda

Order: Stylommatophora

Family: Microcystidae

Genus: *Advena*

Species: *Advena campbellii* (Gray, 1834)

Synonyms: *Advena campbellii charon*

Other common names: Campbell's keeled glass snail

Class: Gastropoda

Order: Stylommatophora

Family: Microcystidae

Genus: *Mathewsoconcha*

Species: *Mathewsoconcha suteri* (Sykes, 1900)

Synonyms: *Mathewsoconcha belli*, *Mathewsoconcha albocincta*, *Belloconcha compacta*

Other common names: Suter's striped glass snail

3. Natural History

Both *Advena campbellii* and *Mathewsoconcha suteri* are terrestrial snails endemic to Norfolk Island (Director of National Parks, 2010). They are both hermaphroditic and are presumed biofilm feeders. There is little known about their ecology and life history and much of the information provided in this manual has been ascertained by observing the breeding populations.

3.1 Morphometrics

Table 1 – Morphometrics of two species of Norfolk Island land snail

	<i>A. campbellii</i>	<i>M. suteri</i>
Shell diameter	15-22 mm	11-14 mm
Shell height	10.5-18 mm	7.5-9.5 mm
Weight	0.71-1.76 g	0.25-0.58 g
Lifespan	10-13 months	At least 3 months post maturity
Distinguishing features	Shell conical with a raised spire. Whorl profile flattened above and rounded below an angulate periphery. Umbilicus closed, outer lip smooth and simple. Colour fawn above periphery with a chocolate brown to black base. The largest of the native Norfolk Island land snails (Director of National Parks, 2010)	Shell depressedly conical, spire and apex slightly raised. Whorl profile rounded. Colour orange-brown to fawn with a narrow white peripheral band (Hyman <i>et al.</i> , submitted)

3.2 Habitat and distribution

Norfolk Island has a sub-tropical climate, with small seasonal variation in temperature and rainfall, and high relative humidity (Bureau of Meteorology). Across a year, the minimum daily temperature ranges from 11.0°C to 23.4°C, the daily maximum temperature ranges from 15.8°C to 26.5°C. An average of 1,320 mm of rain falls each year, peaking in winter (Norfolk Island Recovery Plan, 2010). Prior to European settlement, the island was covered in subtropical rainforest, now remaining only in the National Park and other isolated fragments. The flora is diverse, with approximately 180 native species, of which 25% are endemic (Director of National Park, 2010).

3.2.1 *Advena campbellii*

Presumed extinct for a number of years, *A. campbellii* is (at the time of writing) found only in three valleys within the National Park. The vegetation is native subtropical rainforest, with a notable layer of leaf litter and fallen vegetation including fallen palm fronds. Observations of *A. campbellii* populations over the last decade suggest that populations may expand and contract up the valley walls, possibly in response to variable rainfall (I Hyman, pers. comm.). While currently restricted to the National Park, evidence has been found which suggests a greater historical range (Neuweger *et al.*, 2001).

On-island observations found that adult *A. campbellii* were primarily found in the folds of fallen and partially decomposed frond bases, where they were sheltered from direct sunlight and presumably experienced a cooler and more humid microclimate (data logger from inside the fronds sometimes read 100% relative humidity). Conversely, neonates were found on the leaflets or rachises of the same fallen fronds, far more exposed to direct sunlight and ambient conditions and often aestivating or otherwise inactive.

3.2.2 *Mathewsoconcha suteri*

M. suteri is restricted to a single population within the 100 Acres Reserve, occurring on the slopes either side of a walking trail leading to Rocky Point, on the south-west corner of the island. *M. suteri* is far more prevalent on the southern slope, but specimens have also been found on the north-eastern slope (pers. obs.). The canopy in this area is comprised mostly of Norfolk Island pine (*Araucaria heterophylla*) and white oak (*Lagunaria patersonia*) with little to no understory or groundcover. *M. suteri* is found within or underneath fallen branches/logs/deadfall of Norfolk Island pine. As with *A. campbellii*, *M. suteri* likely had a broader distribution in the past (Neuweger et al., 2001).

3.3 Conservation status

A. campbellii

IUCN – Extinct

EPBC Act – Critically Endangered

M. suteri

IUCN – Endangered (listed as *Mathewsoconcha belli*)

EPBC Act – Critically Endangered

3.4 Wild diet

Unknown. Both species are presumed biofilm feeders. *A. campbellii* has been observed leaving potential scrape marks on fallen leaves (brush bloodwood, *Baloghia inophylla*) and palm fronds, and fresh faeces observed in the field were bright green, suggesting consumption of leaves and other vegetation (T. Williams Clow, pers. comm.)

3.5 Longevity

3.5.1 General

Longevity of both species in the wild is unknown. Tracking of individual *A. campbellii* in a zoo setting suggests a maximum captive life span of 13 months, with an average life span between 10 and 12 months. The longest surviving *M. suteri* founder died approximately three months after collection, suggesting that they will survive at least that long after maturation. The longest recorded lifespan for *M. suteri* born in captivity is approximately 5 months.

It is likely that wild longevity is greater, due to captive snails spending less time in aestivation than wild individuals – this has been observed for Hawaiian snails (D. Sischo, pers. comm.)

3.5.2 Determining age in adults

Growth is indeterminate in microcystid snails, so the snails continue to grow their entire adult life. In general, both species are assumed to have reached maturity at 4.5 to 5 whorls (Hyman et al., submitted). However, determining whorl count is difficult without a microscope, so we prefer to use shell diameter as a gauge to minimise disturbance to the snails. *A. campbellii* is assumed to have reached maturity once its shell diameter is 15 mm or more (approximately 5 whorls)(Hyman et al., submitted), at approximately five or six months of age – this is the smallest size at which birthing has been observed (unpublished data). *M. suteri* is assumed to have reached adulthood at 10 mm shell diameter (approximately 4.5 whorls) (Hyman et al., submitted) – the age is unknown. It is difficult to determine age in adult snails beyond this point. Rate of growth is likely determined by several environmental variables (Barker, 2001). The development of growth charts may nonetheless assist in determining age; this has not been undertaken to date due to the priority to minimise disturbance and stress.

4. Housing Requirements

4.1 Quarantine

Due to biosecurity requirements, all Norfolk Island snails including *Advena campbellii* and *Mathewsoconcha suteri* need to be housed in a facility that has undergone Approved Arrangement Accreditation by the Department of Agriculture, Water and the Environment (DAWE). All staff who need to work unsupervised in these areas are required to become Approved Arrangement accredited persons, by undergoing DAWE training and certification.

4.2 Enclosures

Snails are housed in small groups (3-5 adults) in Pen Pals plastic terrariums. All four available sizes have been used, depending on the number of snails, but sizes medium (30cm (L) x 18cm (W) x 18cm (H)), large (36cm x 21cm x 21cm) and extra-large (40cm x 23cm x 28cm) are most commonly used. Each terrarium should have a collar of black tape on the outside, at the base, between 4 to 5 cm high to reduce light intensity on the bottom of the terrarium. Four layers of brown, and bleach and peroxide free paper towel are cut to size and layered over the bottom of the tank. This paper is misted with filtered water until it is damp to touch. Each medium terrarium should have one food plate placed in a corner, flat on the bottom at one end of the terrarium, and one calcium plate also flat at the other end of the terrarium (Figure 1). In a large or extra-large tank, two food and two calcium plates are placed in the terrarium (Figure 2). Non-PVC cling wrap (a permeable plastic wrap) is placed over the top of the terrarium before the lid is placed on top.

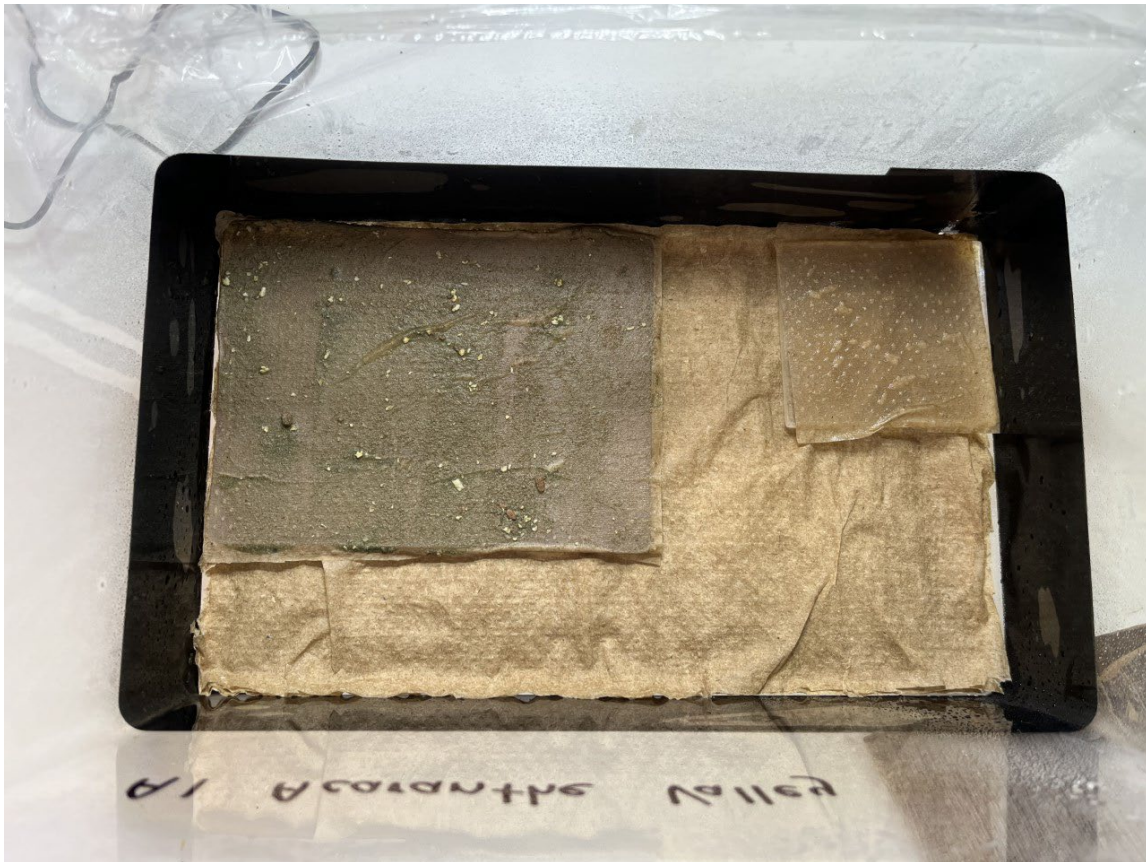


Figure 1 - Positioning of a food plate (left) and calcium plate (right) within a terrarium



Figure 2 - Positioning of food plates (back left and back right) and calcium plates (front left and front right) in a medium or larger terrarium

4.3 Density

Maintaining the right density of snails is important for overall survivorship, and for development of immature snails (D. Sischo, pers. comm.). Neonate snails in particular appear to need to follow adult slime trails to find food (D. Sischo, pers. comm.) and in our program seem to be more active in the presence of juvenile or adult snails.

Wherever possible, we aim to maintain adults in groups of three or more. Similarly, neonates are ideally housed with adults present, even if it is only one. Overall, higher density is preferable to lower, though the need for high density must be balanced against a low-intervention approach to husbandry (e.g. frequency of necessary cleaning). Given the dynamic nature of a breeding population and the many possible combinations of numbers of adults, juveniles and neonates, we do not currently have set “rules” for density.

4.4 Enclosure furnishings

4.4.1 *A. campbellii*

Each tank should have a palm frond base to act as a hide, and one or more palm rachises with leaflets. The frond base should lie flat on the base of the terrarium. The rachis or rachises should have the thicker end placed in a corner of the terrarium and be leaned diagonally across the tank, or in any way such that it sits securely and will not fall. The leaflets can be trimmed to help the rachis fit, but keepers should be mindful that there are enough leaflets

and sufficient surface area that the snails can use the leaflets. All plant material should be sterilised (see husbandry section).

4.4.2 *M. suteri*

Each tank should have a “hide” or “roll” of brown butcher’s paper folded and twisted into shapes to provide cover and structure for the snails. It is very likely this type of furnishing will be replaced in future collections of *M. suteri*, to more closely mimic the physical structure of their natural habitat.

4.5 Temperature and humidity

The facility at Taronga Zoo Sydney is maintained at temperatures between 16 – 25 °C using two reverse-cycle air conditioners. Having two air conditioners provides a fail-safe system in the case of one malfunctioning.

Humidity is monitored within a tank using a Hobo datalogger. Relative humidity is maintained between 60-70% pending more information from Norfolk Island.

4.6 Photoperiod

Snails are maintained on the natural day/night cycle of Sydney, Australia. While factors such as day length and light intensity may differ slightly from those on Norfolk Island, we believe that broadly it is an adequate match. Snail terrariums are protected from over-exposure to direct sun light, as both species occur in relatively well shaded areas. Having a distinct day/night cycle is important for promoting natural behaviours and welfare (D. Sischo, pers. comm.)

4.7 Positioning of enclosures

Terrariums are placed on shelving (Figure 3). Terrariums holding different species should not occupy the same shelf, to minimise the chance of cross contamination. Similarly, where space allows, terrariums holding snails of the same species but from different populations should also be placed on individual shelves. Shade cloth attached to the shelves, or the roller blinds attached to the facility windows are used to block direct sunlight where necessary.

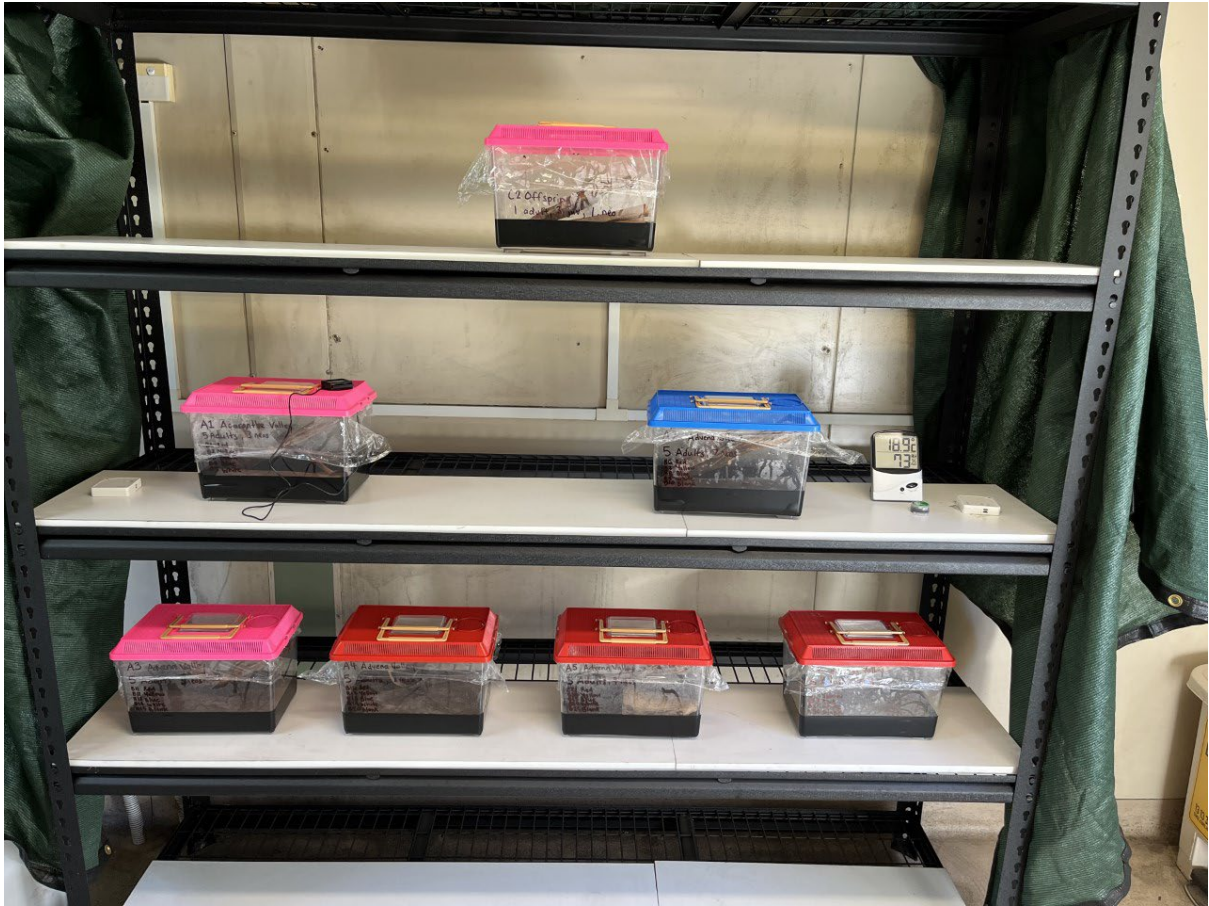


Figure 3 - Terrariums holding Advena campbellii snails in quarantine facility at Taronga Zoo, Sydney

5. General Husbandry

Norfolk Island snails have proven susceptible to acute stress and have had notable mortality events after significant interference such as tank cleans. As such, day-to-day husbandry is minimal, seeking to minimise disturbance to the snails.

5.1 Hygiene and cleaning

At the start of husbandry, the work area should be wiped down with 70% ethanol. Gloves should be worn on hands, which should then be washed with an antiseptic skin cleanser and filtered water. After husbandry in each terrarium, the work area should be wiped down with ethanol and gloves should be washed as above, or replaced, before starting husbandry with the next terrarium.

Day-to-day cleaning of terrariums is minimal. Heavily soiled tanks may have one wall wiped down with paper towel, being careful not to disturb any snails in the process. Different walls may be wiped down over successive days, ensuring that there isn't excessive disturbance on any one day.

Food and calcium plates are changed twice weekly, on Mondays and Thursdays, or more frequently if necessary e.g. heavy mould or mite load on food plate.

A full tank change for every tank occurs every 5 to 6 weeks, depending on how soiled the terrarium becomes. Ideally, this will co-occur with a feed day. A clean terrarium is set up from scratch (as described Section 4. Housing requirements) with fresh food and calcium plates.

Hides and fronds should be replaced every 5 or 6 weeks. This should be separate from full terrarium cleans to avoid overly stressing the snails by completely changing their environment.

Great care should be taken when moving or handling snails during any form of cleaning. Please refer to handling instructions in Section 7. Handling and Transport.

All waste material should be disposed of within the quarantine bin. The rubbish bag should be changed at a minimum after every feed day. Rubbish is double bagged as per quarantine requirements, and taken to Taronga Wildlife Hospital (TWH) to be placed in their quarantine bins. This should occur at least weekly, and ideally whenever rubbish bins are changed.

An example daily routine is present in Appendices 15.1.

5.2 Record keeping

Because of the difficulty in tracking individual snails, particularly when there are large numbers of snails, photos are taken of the terrariums each day, showing the positions of as many snails as possible, and shared in a group chat with all the snail husbandry team. This allows easier comparison of the snails' positions day-to-day.

The population composition of each terrarium should be recorded on the outside of each terrarium (e.g. 3 adults, 2 neonates), as well as the identity of any founder snails. This should be updated immediately following any changes. Other routine record keeping is outlined in Table 2.

Table 2 – examples of record keeping requirements for Norfolk Island snails

Daily	Weekly	Incidentally
Room temperatures	Food or calcium plate changes	Births
Relative humidity	Movement of rubbish to TWH	Deaths
Position of adults		Movement of snails to TWH
Position of any other conspicuous snail e.g. on cling film		Change in size class
Signs of life, or their absence – movement, eyestalks out, responsiveness to misting		Animal counts
Incidence of aestivation		Incidence of pests
Notable behaviours, health concerns		Pest control activities
Any cleaning activities		Diet changes
		Terrarium changes
		Furnishing changes

5.3 Methods of identification

Individual snails are difficult to identify by the naked eye alone. There are a variety of methods of marking snails for identification, such as metallic pen ink (Severns, 2009), nail varnish (S. Aberdeen, pers. comm.) and water-based enamel paint (Clarke, 2019). Each technique has advantages and disadvantages. Both *A. campbellii* and *M. suteri* are terrestrial species that likely have their shells rub incidentally against conspecifics and environmental features, and therefore need a method that is resistant to wear. We decided the least intrusive method with the highest probable longevity was to use water-based enamel paint (Clarke, 2019, S. Aberdeen, pers. comm.). We have used oil-based enamel paint in the past, but aversive behaviour in the form of “flinching” has been observed during application. Since this same behaviour has not been observed applying water-based enamel, we theorise that the snails may have reacted to the oil fumes.

Snails are marked by placing a small dot water-based enamel paint within a whorl (i.e., not on or overlapping a suture) on the shell with a fine-tipped paintbrush (Figure 4). For adult founders, each individual within a tank will be marked with a unique colour. From May 2022 after our second import of founders, we assigned a colour to each month, and at the end of each month all unmarked neonates are marked with that colour. While this doesn't allow individual identification it does allow rough estimation of age.

5.4 Routine data collection

Most husbandry record keeping will collect the necessary data for monitoring of the program and improving life history knowledge. Daily husbandry records, such as dates of birth or death can be used to extrapolate life history parameters such as longevity, birth rate etc. Additional data collection is necessary upon the death of a snail (Section 8.6).

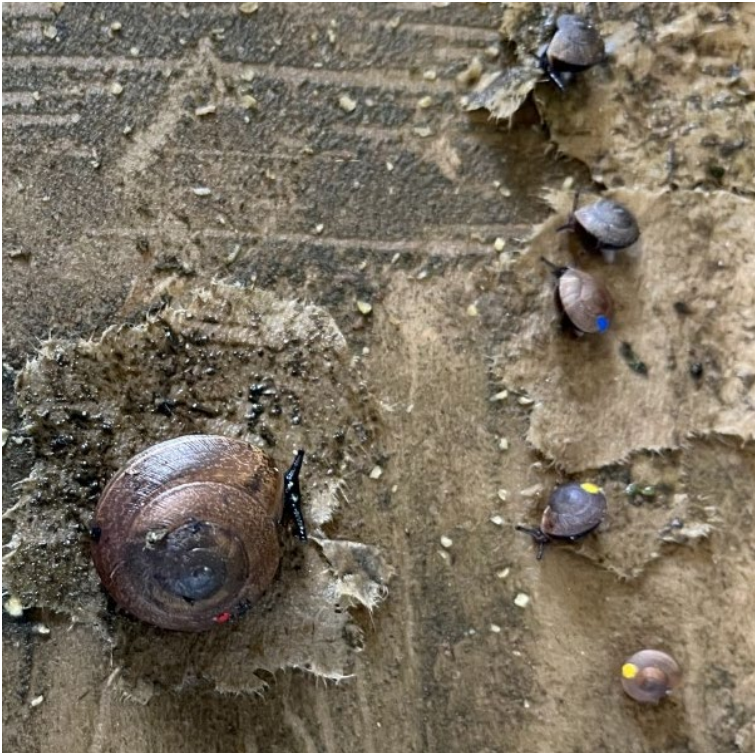


Figure 4 - *Adena campbellii* snails showing marks with water based enamel paint. An adult (left) shows an individual red paint mark while neonates left show age marks, blue and yellow.

5.5 Sterilising organic material

All organic material should be sterilised before being placed in terrariums with snails. Norfolk Island snails may be naïve to diseases common to Australian mainland snails, and other pathogens may be present on organic material. The following method to sterilise organic material is adopted from the Hawaiian Snail Extinction Prevention Program (SEPP) (D. Sischo, pers. comm.)

- source fronds onsite
- wash all dirt, debris etc from the fronds with tap water
- cut hides and rachises to convenient size
- store in rubbish bag in fridge until desired level of decomposition has been achieved
- freeze organic material until needed
- microwave all material for four minutes before adding to terrarium

5.6 Washing terrariums and enclosure furnishings, and other materials that have contacted snails

All dishes and utensils used in the snail terrariums (including terrariums) should be cleaned following the method below

1. Scrub underneath hot water, removing any physical and visible mess. Dishes should look clean to the naked eye. Leave on dish mat to dry overnight.
2. All surfaces of dishes should be sprayed with 70% ethanol. Spraying should be light, but cover all surfaces of the dish/utensil. Dishes are left to air dry overnight.

3. Dishes/utensils should be rinsed thoroughly with filtered water to remove any ethanol residue. Leave to air dry overnight, after which dishes/utensils can be put away or used again.

6. Feeding requirements

Both *A. campbellii* and *M. suteri* are suspected biofilm feeders and/or detritivores, though *A. campbellii* has been observed eating leaf material (pers. obs.).

6.1 Captive diet

Both species of snail are offered a custom diet developed at Taronga Zoo Sydney by the zoo nutritionist, taking diets offered to *Partula* sp. snails from various institutions as a starting point. The diet was adjusted based on the results of feed trials with *Hadra* sp. before importation of Norfolk Island snails, and feedback from the husbandry team after the Norfolk Island snails arrived.

The primary ingredients of the diet are oatmeal and nettle leaf. The diet is prepared as a powder that is stored in a refrigerator in the snail facility. The food is made up at a ratio of 1 teaspoon of dry powder to 5 ml filtered water, forming a thin paste. A list of ingredients is available in Appendices 13.2.

6.2 Presentation of food

Brown paper towel is cut to size to match a Perspex plate (11.1 cm x 14.6 cm) and sprayed with filtered water to damp it down. A thin layer of food paste is then spread evenly over the paper using a spatula. Food plates with a thin layer of food paste have a lower incidence of mould than those with thicker layers of food paste (pers. obs.).

6.3 Supplements

While calcium is present in the diet, additional calcium is provided in syrup form as calcium glubionate and calcium lactobionate, at a concentration of 22 mg/mL.

A square of brown paper towel is cut to fit a Perspex square (6.5 cm x 7 cm). The paper is sprayed with filtered water and then 9 drops of supplement from a 1 ml syringe are placed on the paper towel (approximately 0.3 ml).

While snails are frequently seen on the food plate, they are very rarely seen on the calcium plates (pers. obs). This may be in part due to the respective locations and sizes of the plates, but snails are also seen less often on the calcium plate than on the walls of the terrariums.

7. Handling and transport

7.1 Timing of capture and handling

As any handling of snails will occur within the temperature-controlled snail facility, there is no need to avoid handling snails at certain times of day. However, all handling should be kept as brief as possible and only undertaken when absolutely necessary.

7.2 Capture and restraint techniques

Snails should be handled as little as possible, and all forms of unnecessary disturbance avoided. If a snail needs to be moved for husbandry purposes:

1. If snail is retracted in shell, and not sealed to a surface, it may be handled with gloved hands or blunt nosed forceps.
2. If snail is actively moving on or sealed to a substrate e.g. paper towel substrate, food plate or palm rachis, the substrate itself can be moved rather than the snail. If the whole piece of substrate cannot be moved (e.g. tank change), a small piece of that substrate should be cut around the snail, taking due care not to injure or disturb the snail, and the whole piece transferred to the new terrarium etc. The weight of the snail should be directly supported from underneath.
3. If snail is moving along the terrarium, place small pieces of damp paper towel around the snail, and when it moves on to a piece of paper, it can be moved on that piece of paper.
4. If snail is sealed to terrarium, spray lightly with filtered water. This should disturb the seal and may promote movement, in which case method 3 can be used. If after a reasonable amount of time the snail has not moved, blunt nosed forceps can be used to dislodge the snail from the surface.
5. Only in the last instance should an actively moving snail be removed directly from a surface or substrate. If this is necessary, use blunt-nosed forceps to move the snail.

Snails can be held in the hand directly where necessary, such as for close examination. However, many keepers feel more comfortable using forceps, especially for the smaller *M. suteri* or for neonates. Placing snails in a petri dish for close observation is safer and therefore preferable to hands and is recommended wherever possible.

7.3 Weighing and examination

Live snails are not routinely weighed to minimise stress. If an opportunity arises, they may be weighed by placing in a petri dish on scales.

If a snail needs to be examined, it should be placed in a petri dish on top of a piece of damp paper towel. Use of microscope or magnifying glass is recommended. If needed, the position of the snail may be manipulated by blunt nosed forceps or gloved hands.

For both weighing and examination the handling method described in 7.2 should be followed as closely as possible.

7.4 Transport requirements

7.4.1 First import

The first import of Norfolk Island snails to Taronga Zoo occurred in May 2021. The aim was to collect 15 snails of each species but given their scarcity on a previous field trip – particularly *M. suteri* – it wasn't guaranteed that 15 snails would be found (B. Finlayson, pers comm). Therefore, snails were collected opportunistically. Each snail was weighed on collection, and then kept individually until the night before transportation in a plastic takeaway container with perforated lids and a 5 cm layer of leaf litter/substrate collected with the snail. Each snail was given a unique code or number, this was recorded on the lid of the container. The containers were misted with water twice over approximately one week.

The night before transport back to Sydney, the following variables were measured for each snail

- weight
- shell diameter and height
- number of whorls

At that point we followed the EAZA guidelines for *Partula* transport (Clarke, 2019). Adult snails were individually wrapped in brown paper towel, which was gently folded over, and then placed in a cardboard (toilet paper) roll with the ends folded over. The snail's identification number/code was written on the toilet paper roll. Some of the snails had given birth over the week, in this case the neonates were packed with their parent. The rolls were then placed in a plastic storage container, which was then set in a wooden transport box with Styrofoam lining. Around the plastic container, half-filled water bladders were used as temperature buffers. The box was then latched close and secured with ratchet straps.

It was noted that between collection and packing for transport that many of the snails lost weight, presumed to be water loss. Snails again lost weight during their time in transport storage (approx. 36 hours). One adult snail died in transit

7.4.2 Second import

Our second import of snails was for 30 *A. campbellii* adults only.

Given the weight loss and mortality event in the first import, and with increased confidence that 30 *A. campbellii* founder snails could be found relatively quickly, we decided that snails would be collected the afternoon before or morning of transport and placed straight in to transport conditions. Snails were still individually wrapped in unbleached paper towel, placed in toilet paper rolls and then stacked within a plastic storage container. In this case, that storage container was placed in an esky rather than the transport box.

There was no mortality event in transit, and survivorship over the first 5 weeks suggests that the second group of founders experienced less stress during the collection and transportation process (80% survivorship in first group of founders, compared to approximately 87% survivorship in second group of founders – unpublished data).

7.4.3 Water and food

It is not necessary to provide water or food for either species during transport. The packing method should encourage snails to enter aestivation. If the snails fail to aestivate, the paper towel can be consumed without harm to the animal.

7.4.4 Timing of transportation

Minimising the time between collection and release into terrariums is the most important variable. However, potential heat stress can be avoided by collecting, processing and packing of snails in cooler parts of the day e.g. morning, evening.

7.4.5 Release from box

Ideally, snails should be weighed again on arrival, given a health check and marked for identification. Snails are placed in pre-prepared terrariums, and the ID of each adult founder should be recorded on its terrarium (see 5.2).

8. Health Requirements

8.1 Daily health checks

It is not necessary to sight or health check each individual daily. However, photos and written records should be compared each day to ascertain, where possible, that snails are moving or showing other signs of life.

Keepers should place any snail suspected of being unwell, or any snail that has not obviously moved in more than 24 hours, on a petri dish lined with damp paper towel. The snail can then be observed under a magnifying glass. The petri dish should be placed back in the terrarium and the snail's behaviour/movement carefully observed over the following days.

8.2 Detailed physical examination

8.2.1 Chemical restraint

N/A. Application of chemicals is likely to result in illness or even death for the snail.

8.2.2 Physical examination

Any snail requiring detailed examination should be placed in a petri dish with damp paper towel, and examined under a magnifying lens and light. The shell should be examined for damage or wear. The state of the snail should be determined – is it retracted in its shell? Is the foot extended? Are both eyestalks present, and without damage? The presence or absence of external parasites or commensals (such as mites and/or nematodes) should be determined – a light spray with filtered water may make nematodes easier to spot. Nematodes, especially when present in low numbers, often look like water glistening or a spot of reflected light. However, the light will move even if the snail is being held still.

8.3 Routine treatments

There are no routine treatments currently used for either species.

8.4 Known health problems

8.4.1 Prolapse

The prolapse of organs through the mouth parts is a common health problem in snails and is relatively frequently seen in pet snails e.g. giant African snails (Pellet and Bushell, 2015, Pellet, O'Brien and Kennedy, 2020). Mouth part prolapse was observed in *A. campbellii* after the first collection of founders (Figure 5). Prolapse is generally considered an indicator of poor overall snail health. However, after changing our feeding method such that the food paste was smeared on to damp paper towel on top of a Perspex plate (rather than directly on to the plate) prolapse has not been observed in any snail of either species.

8.4.2 Strongyloid nematodes

On several occasions, deceased snails were observed to have high numbers of strongyloid nematode worms present. Occasionally live snails have been observed to have one or two worms on them, generally these snails had been observed to be unwell and have died shortly thereafter. Histopathology analyses of deceased snails have mostly shown no tissue reaction to the presence of the nematodes. Examination of a sample wild *A. campbellii* adult brought



Figure 5 - An adult *Advena campbellii* with mouth part prolapse (Photo: Brett Finlayson)

back from Norfolk Island confirmed the presence of nematodes in wild snails. The presence of nematodes did not appear to be causing ill health and it is likely the nematodes are commensal with live snails, proliferating after the snail becomes unwell or dies.

8.4.3 Mites

Mites have been seen in the terrariums of *A. campbellii* on several occasions. Samples were sent away for identification and the mites were identified as *Tyrophagus putrescentiae*, a species with global distribution that feeds on mould. There is no indication that *T. putrescentiae* has caused any health problems in *A. campbellii*.

8.4.4 Trauma

Trauma or damage to the shell can occur from falls or other impacts and may or may not cause loss of haemolymph, but can lead to desiccation of exposed tissue. Perforation of soft body and loss of haemolymph will generally result in death soon after (Pellet and Bushell, 2015).

8.4.5 Aestivation

Snails may enter aestivation in response to unsuitable environmental conditions (Pellett and Bushell, 2015). Aestivation is a normal behaviour and snails can take in some amount of water through their shells, however, extended aestivation may result in dehydration or malnutrition (Pellett and Bushell, 2015, Schweizer, Triebkorn and Köhler, 2019). Younger, smaller snails are more prone to both dehydration and malnutrition. Manual disruption of the

mucous membrane can also cause damage to the snails, but they can be encouraged to leave aestivation by spraying water across the membrane (Pellet and Bushell, 2015, Sischo, D., pers. comm.)

8.4.6 Calcium deficiency

Calcium deficiency results from the lack of appropriate calcium in diet and can cause shell to become brittle, and increase the incidence of shell fractures.

8.4.7 Shell predation

In the absence of other appropriate sources of calcium, some snail species will predate the shells of other living or deceased snails (pers. comm., D. Sischo). There have been possible incidences of this in *A. campbellii* – a juvenile snail was observed spending a prolonged amount of time on the shell of an adult founder, and the next day the adult snail was found with a large hole in its shell.

8.4.8 Senescence and signs of old age

Some common signs of old age in snails include separation of mantle from body, thinning of periostracum (the thin waterproof outer layer of shell), and a general loss of condition (Pellet, O'Brien and Kennedy, 2020). A snail that has mantle that has become separate from the body should be housed alone in a shallow tank, with restricted ability to climb. In some cases, the mantle will rejoin the body, though often the problem never resolves and the snail will die (Pellet, O'Brien and Kennedy, 2020).

8.5 Problems determining ill health

Snails present a unique challenge in determining ill health and cause of death. It is not unusual for healthy snails to remain still for several days before resuming activity even without entering aestivation, but a lack of movement can also be an indicator of ill-health. In neonates, death can be confirmed by candling the shell (i.e., shining a bright light through the shell) and observing if the soft tissue has dried up. This is more difficult in adults as they can retract well within their shell (Clarke, D. 2019). Given the Critically Endangered status of both species, snails are given every chance to show they are alive and by the time death is conclusively established very little suitable tissue remains for pathology.

8.6 Deceased snails

Veterinarians should be contacted whenever a deceased snail is found. If vets request the sample, it should be transported fresh to the hospital following established biosecurity transport protocols. If vets do not request a fresh sample, the deceased snail should be placed in a sample jar with formalin or 70% ethanol, following vet instruction. Generally, snails should be placed in individual jars, however multiple neonates of a similar age or size can be stored in a single jar. Before immersion in preservative, the following information should be recorded on the jar and in digital records

1. species of snail
2. number of snails in sample jar and age class (e.g. 1 x neonate)
3. enclosure from which the snail(s) came
4. weight and approximate shell diameter for each snail

5. where available, the identification or any identifiers of the snail (e.g. blue marked, unmarked, adult B20)
6. approximate date of birth
7. date of death
8. preservative used

8.7 Quarantine requirements

Norfolk Island snails are kept under strict quarantine conditions under the Biosecurity Act 2015. The facility has Approved Arrangement (AA) certification, and all staff working in the facility must complete Department of Agriculture Water and the Environment (DAWE) Approved Arrangements for Accredited Persons and Biosecurity Awareness certification, or be working under the direct supervision of a certified person.

All waste disposal and movement of material in and out of the Approved Arrangement facility needs to be compliant with AA regulations. A copy of Taronga's standard operating procedure for collection and disposal of biosecurity waste is available in the appendix 15.3.

Additionally, terrariums holding different species, and terrariums holding individuals of the same species but distinct populations, should be kept physically distant within the facility. No materials should be transferred between terrariums.

9. Behaviour

9.1 Activity

Field and captive observations suggest that snails are most active overnight. Generally, this is when conditions would be most favourable for snail to be active i.e. lower temperatures and higher humidity (Schweizer, Tribskorn and Köhler, 2019). In captivity, snails are often observed on one day to be aestivating, retracted into their shell or otherwise inactive, and in a similar the state the following day, only in a different location within the terrarium. On the island, snails have to be searched for within leaf litter during the day, at night they are easily observed by torchlight as they move on and within the substrate.

If environmental conditions such as temperature or humidity are sub-optimal, snails may enter a state of dormancy known as aestivation (Pellett and Bushell, 2015). In aestivation snails will form a mucous barrier across the shell aperture and attach themselves to a solid surface. The mucous membrane is dissolved by water, often prompting the snails to become active (D. Sischo, pers. comm.).

9.2 Social behaviour

Neither species appear to actively seek company, but they appear to coexist in high density. Adult snails have been observed in close proximity both in the wild (T. Williams Clow, pers. comm.) and in captivity, with 4 or 5 adults seen occupying the same palm base (pers. obs). Neonates also appear to aestivate and feed in close proximity. Snails will climb over one another and active snails may bump or dislodge aestivating snails, seemingly without obvious detrimental result.

9.2.1 Possible shell predation

On one occasion a juvenile snail was observed spending an extended period of time on an adult snail (i.e., did not appear to be climbing over the adult), and the adult was found with missing shell fragments the next morning. This may be a case of shell predation, but this cannot be confirmed and no similar incidents have been observed in this program.

9.3 Reproductive behaviour

Behaviours associated with reproduction in both species are unknown. In a closely related species from Norfolk Island, snails appear to exchange gametes by intertwining everted penises, and this coupling behaviour has only been observed after rain (I. Hyman, pers. comm.)

9.4 Behavioural problems

Neither *A. campbellii* nor *M. suteri* have been observed displaying stereotypical behaviour. As primarily nocturnal species being managed in a low intervention approach, extensive behavioural observation has not occurred for either species, and so baselines for activity budgets, or normal versus abnormal behaviours have not been well established.

9.5 Signs of stress

Signs of stress in Norfolk Island snails are not well established. Increased birth rate is likely to be indicative of acute stress in both species (D. Sischo, pers. comm.). While a normal

behaviour, extended or overly frequent aestivation may be a sign that environmental parameters are sub-optimal.

9.6 Introductions and removals

It is recommended, and our current practice, that snails from distinct populations are not introduced to one another, so that potential pathogens present in snails from one population are not introduced to those of another population.

There have not been any observations of behavioural issues between snails after introductions or removals of snails. Generally, introductions and/or removals only occur to maintain favourable densities and population age structures within terrariums e.g. ensuring all neonates are in a terrarium with an adult.

9.7 Intraspecific compatibility

Both *A. campbellii* and *M. suteri* are hermaphroditic, and the species are not likely to have compatibility problems that occur in some other animal species with regard to sex. There has been no observation of health or behavioural problems in either species that might be associated with housing different size classes together.

9.8 Interspecific compatibility

Interspecific compatibility has not been investigated. Due to quarantine requirements and the conservation focus of the program at Taronga Zoo, no other species will be introduced to terrariums housing Norfolk Island land snails.

9.9 Suitability to captivity

The paucity of life history and ecology knowledge for both species has posed a significant challenge to successful captive husbandry. This same lack of baseline knowledge (e.g. longevity) also means that evaluating husbandry approaches is also difficult, as there are few to no known parameters with which to compare.

Snails can have very particular environmental requirements that are specific to individual species. The conservation focus of the Taronga program, in addition to small wild population sizes, has meant that experimental evaluation of different conditions or husbandry practices is inappropriate.

10. Reproduction

10.1 Mating system

Both *A. campbellii* and *M. suteri* are simultaneous hermaphrodites. Most terrestrial pulmonates breed by cross-fertilisation, though some species are capable of self-fertilisation (Heller, 2001) – it is not known if *A. campbellii* or *M. suteri* can self-fertilise.

10.2 Ease of breeding

Both species appear to readily reproduce in captivity – founders of both species began birthing soon after importation. It is worth noting that stress can increase birthing rate in other terrestrial snails (D. Sischo, pers. comm.) and therefore poor husbandry or unsuitable environmental conditions may result in frequent birthing. There have been three generations of *A. campbellii* born at Taronga Zoo, indicating that mating has occurred in two successive generations in captive conditions.

10.3 Reproductive condition

Both species appear to be reproductive immediately upon reaching adult size. Observations suggest that larger *A. campbellii* give birth to a greater number of neonates, and those neonates have greater survivorship than the neonates birthed by smaller adults (unpublished data). Further investigation is required.

10.4 Age at first and last breeding

A. campbellii can breed from approximately six months old (i.e., upon reaching adult size – approximately 15 mm shell diameter) through to death.

M. suteri – unknown, though possibly similar to *A. campbellii*.

10.5 Environmental requirements for breeding

Neonate snails are often found high within terrariums, even on the clingfilm. An adult *A. campbellii* was recorded giving birth halfway up a terrarium wall, and the neonate snail immediately began climbing higher. It is hypothesised that *A. campbellii* may like to birth in higher locations, and so the provision of palm rachises and leaflets is designed to accommodate this (as well as provide additional structure for neonates).

10.6 Breeding diet

Diet as described in Section 6.1 has proven sufficient for successful breeding (see also Appendix 13.2)

10.7 Gestation period and birth rate

Dissection of the reproductive systems reveals that adults of both species often have several unborn snails present, becoming progressively less developed further from the genital pore.

The birth rate of both species in the wild is unknown, and a limited amount of information has been garnered by observing singly-held adult *A. campbellii* (Table 3). However, birth rate does appear to be flexible and potentially in response to environmental stimuli – disturbance can increase the birth rate of snails (Dave Sischo, pers. comm.) and increased birth rate seems

to correlate with premature births. Premature neonates are born smaller, and their shells often appear thinner (more translucent) than those of other neonates.

Table 3 – Reproductive parameters for Norfolk Island land snail species *A. campbellii* and *M. suteri*

	<i>A. campbellii</i>	<i>M. suteri</i>
Birth interval	7-10 days	Unknown
Size at birth (shell diameter)	~ 4 mm	~ 2 mm
Time to maturity	~ 6 months	Unknown
Size at maturity	≥ 15 mm	≥ 10 mm

One adult snail born at Taronga Zoo (“William”) gave birth to 13 neonates over 82 days. Birth intervals ranged between 3 and 14 days, with an average birth interval of 6.8 days. The first confirmed birth by this individual occurred at approximately 6 months of age, though it is possible the snail had been giving birth prior to this because it was housed with other adults. Another adult born at Taronga Zoo (“Big Red”) gave birth to only 5 neonates over a 21-day period – this was the entirety of its reproductive output. Interestingly, “William” lived to 10 months of age, whereas “Big Red” lived to approximately thirteen months old, indicating a possibly link between reproductive output and lifespan.

10.8 Litter or clutch size

Both species give live birth to a single neonate snail at a time.

10.9 Independence of neonates

Neither species is known to provide parental care. Neonates are born fully formed, and capable of locomotion, feeding and all other functions necessary for survival. In other species of snail, neonates rely on the slime trails of adult snails to find food (D. Sischo, pers. comm.) and *A. campbellii* neonates housed without adults or juveniles were less active than neonates housed with older snails. It is therefore recommended that neonate snails are housed with adult conspecifics wherever possible until they reach juvenile status.

10.10 Growth and development

We have divided the life cycle of *A. campbellii* in to three life stages or size classes – neonate, juvenile and adult. Other species of snail, such as *Partula* sp., have a sub-adult life stage (Clarke, 2019). This stage is defined as snails who have reached adult size but are lacking the thickened shell lip that indicates maturity. As *A. campbellii* lack an obvious physical indicator of maturity, we have not included a sub-adult stage.

The growth and development of *M. suteri* has been less extensively studied, and therefore life stages are yet to be defined.

Table 4 – Size classes for Norfolk Island land snails *A. campbellii* and *M. suteri* based on measurement of shell diameter

	<i>A. campbellii</i>	<i>M. suteri</i>
Size at birth	~ 4 mm	~ 2 mm
Neonate	< 6 mm	Undefined
Juvenile*	6 mm – 15 mm	Undefined

Adult	≥ 15 mm	≥ 10 mm
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* 6 mm shell diameter was chosen at the point at which to distinguish based on increased survivorship when snails reached that size.

11. Acknowledgements

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13. Appendices

13.1 Example daily routine

1. Sign in to quarantine area on sheet outside the snail facility. Remove shoes.
2. Step in to pair of crocs as you enter room. Turn on lights and tablet/laptop, put on labcoat
3. Read previous day's notes in physical and digital diaries.
4. Record room temperatures.
5. Spray work area with 70% ethanol and wipe down with paper towel. Put on gloves and wash with filtered water and antiseptic cleaner.
6. Remove a terrarium from the shelves. Record tank humidity data. Count and health check snails. Record position and behaviour of snails in diary, and take photos for reference. Mist with filtered water.
7. Food and calcium plates changed Monday and Thursday. Full tank cleans/changes every 5 or 6 weeks
8. Place snails suspected of being unwell in petri dish with damp brown paper towel. Dead snails to be placed in 70% ethanol and/or formalin depending on vet team preferences
9. Place terrarium back on shelf. Wipe down work area with ethanol and wash gloves between tanks to prevent cross-contamination.
10. Repeat steps 3-6 for each terrarium.
11. Complete any dishwashing as necessary.
12. Change bin liner as necessary
13. Turn lights off and tablet/laptop. Remove lab coat. Step out of crocs as you exit the facility. Take rubbish to TWH as necessary, obtaining permission first.

13.2 Captive diet

Ingredient	%	1 Batch (g)	Notes
Calcium carbonate	37.2	140	
Oatmeal	24.4	92	Rolled oats
Nettle leaf	24.4	92	
Hikari Massivore or Koi	12.8	48	
Wombaroo Big Carnivore Supplement	1.2	4 (2/3 tsp)	
Snow E Vitamin E Powder (100IU/g) *Or White E	0.1	0.4 *(0.6g = 1/8 tsp)	Higher concentration than formulas from other zoos (ie. Disney). *If using White E product 1 batch requires 0.6g (1/8 tsp)
TOTAL	100	376.4	

INSTRUCTIONS

- 1) All ingredients should be ground individually in the Coffee Grinder prior to being weighed out.
- 2) Weigh out Vitamin E Powder on the lab scale in Michelle's office.
- 3) Mix ground ingredients together in a large bowl until a homogenous mixture is formed.
- 4) Package in a tupperware container and set expiration date for 3 months.
- 5) Send to B2B to store in fridge (needs to be in dark, cool area).

KEEPER PREP


Mix 1 tsp dry snail diet with approximately 5 ml filtered water to form a paste.

15.3 Taronga Zoo biosecurity waste collection standard operating procedure

Taronga Conservation Society Australia

Standard Operating Procedure

Taronga Wildlife Hospital



Biosecurity Waste collection and disposal for Christmas Island reptile Rooms Norfolk Island snail room

Purpose

This Standard Operating Procedure describes the steps to be followed to ensure correct disposal of biosecurity waste from the two Christmas Island reptile located at Taronga Wildlife Hospital and the Live Breeding Unit. The reptiles (Christmas Is. Blue-tailed skinks and Lister's Geckos) are held in permanent quarantine under the direction of the Department of Agriculture, Water and the Environment (DAWE). The purpose of this SOP is to ensure compliance with permanent quarantine requirements.

Procedure

- Waste from the Christmas Is. rooms and Norfolk Is. snail room must always be placed in bins clearly labelled as 'Biosecurity Waste'.
- A 1 x 120L locked SUEZ Biosecurity Waste bin is to be stored within the TWH Quarantine area upon delivery.
- The Biosecurity Waste bins in the Christmas Is. rooms and Norfolk Is. snail room are to be labelled as 'Biosecurity Waste' (labels provided by SUEZ).
- Keepers are to place all Christmas Is. room and Norfolk Is. snail room waste into the designated Biosecurity Waste bins in each of the quarantine rooms.
- Once the bins are full, keepers are to double bag waste and notify TWH nurses who will unlock the 120L Biosecurity Waste bin for the waste to be placed in.
- The 120L bin will then be locked and remain in TWH Quarantine.
- When the 120L Biosecurity Waste bin is almost full, TWH nurses are to contact Suez to organise collection and replacement of the Biosecurity Waste bin stating specifically that it is Biosecurity Waste bin. Contact Scott Bayliss 0402 228 536 or Michelle Runge 0411 147 021 to arrange this.
- A key for the padlock of the 120L Biosecurity Waste bin is in the nurse's office locked key box.
- The Transport provider for Biosecurity Waste is SUEZ (210-205 Newton Rd, Wetherill Park, 2164). Approved Arrangement number N2553. The driver assigned for the collection will be trained and approved for Biosecurity Waste. The vehicle will be an approved Biosecurity licenced vehicle fitted with appropriate spill kit including Virkon.
- The Approved Arrangement where the Biosecurity Waste will be delivered and incinerated is SteriHealth, (2-16 Wiblen Street, Silverwater, NSW 2128). Approved Arrangement number N0183.

13.4 Products used for snail husbandry at Taronga Zoo, Sydney

13.4.1 Terrariums

Pen Pals terrariums, sizes medium, large and extra-large

(<https://www.petcircle.com.au/search?searchTerm=Pen%20Pals>, accessed 12/04/2022).

13.4.2 Calcium supplement

Troy Calcium Supplement for Cats and Dogs (<https://troylab.com.au/product/troy-calcium-syrup-250ml/>, accessed 24/10/2022)

13.4.3 Forceps

Australian Entomological Supplies – Feather Light Forceps (Blunt)

(<https://www.entosupplies.com.au/equipment/laboratory/forceps-tweezers/feather-light-forceps/>, accessed 24/10/2022)

13.4.4 Marking paint

White Knight Splashes water based enamel

(<https://www.whiteknightpaints.com.au/products/craft-and-decorative/white-knight-splashes/>, accessed 24/10/2022)

13.4.5 Cling film

Biogone Landfill-biodegradable, caterer's pack cling wrap 450 mm x 600 mm

(<https://www.biogone.com.au/product/caterers-pack-cling-wrap-biodegradable/>, accessed 24/10/2022)