JZAR www.jzar.org JOURNAL OF ZOO AND AQUARIUM RESEARCH

EAZA

JZAR Evidence-based practice

OPEN ACCESS

Evidence-based practice

# Tagging and location preferences to inform post-release monitoring of the Greater Bermuda land snail *Poecilozonites bermudensis*

**Amber Flewitt[1,2], Leah J. Williams[1], Richard Preziosi[3] and Gerardo Garcia[1]**

*[1]North of England Zoological Society (Chester Zoo), Upton-by-Chester, CH2 1LH, UK*
*[2]Manchester Metropolitan University, All Saints, Manchester, M15 6BH, UK*
*[3]School of Biological and Marine Sciences, The University of Plymouth, UK*

*Correspondence: Leah J. Williams, email; l.williams@chesterzoo.org*

**Keywords:** captive breeding, mollusc, reintroduction, zoo

**Article history:**
Received: 22 Feb 2023
Accepted: 12 Jun 2023
Published online: 31 Jul 2023

**Abstract**

The Greater Bermuda land snail *Poecilozonites bermudensis* was once thought to be extinct in the wild, however, recent captive population increases have allowed reintroductions to take place. Post-release monitoring of reintroduced individuals is a fundamental component of the reintroduction process and requires individuals or groups to be identifiable. In this study, a novel method of identification through tagging (using Alpha fluorescent tags glued to sanded and non-sanded shells) was assessed in a captive population (n=400) at Chester Zoo. Tag loss rate and snail survival was monitored over six months, and the location preferences of the snails within their tanks was also recorded under different conditions (day/night, dry/wet) in order to inform survey methods in their native habitat. The tagging method is recommended based on a 0% tag loss rate over the study period, although sanding was unnecessary and had a negative effect on survival. Environmental conditions should be taken into account when planning post-release monitoring surveys. Under dry daytime conditions, more snails were found in higher areas, whilst snails were more likely to be found in both high and rocky areas during wet daytime conditions. During darkness, most snails were found on rocks and in the leaf litter. A consistent low number of snails were found buried within the soil. These results will inform the post-release monitoring of this species, enabling more accurate population and survival estimates to be made, and could act as a model for other reintroduction projects for land snail species.

## Background

The Greater Bermuda land snail *Poecilozonites bermudensis*, is endemic to Bermuda (Gould 1969), and was believed to have become extinct by the 1990s (Outerbridge and Sarkis 2018). However, a small remnant population was discovered in 2014 in an urban alleyway (Outerbridge 2014), and all known specimens were collected and taken into captivity for safeguarding and conservation purposes. The species reproduced well in captivity which facilitated an initial reintroduction in 2017 (Outerbridge and Sarkis 2018; Ovaska 2018). Some of the released snails were marked with either small fluorescent tags, Passive Integrated Transponder (PIT) tags (glued to the shell) or nail varnish, to allow populations to be monitored via mark-recapture surveys. However, many of these tags were lost over the duration of one year of surveys (Ovaska 2018).

Many mark-recapture models have a crucial assumption that tags are not lost from individuals. This assumption is often violated, which can cause severe biases in survival and population estimates (Arnason and Mills 1981; Cowen and Schwars 2006; McDonald et al. 2003; Seber and Felton 1981). To account for this, the rate of tag loss in a population can be assessed, by either double tagging (Alisauskas and Lindberg 2002; Nichols and Hines 1993; Robson and Regier 1966; Seber 1982), or the use of other field methodologies (McDonald et al. 2003). The time required for tag loss to occur has been shown to significantly affect estimates of population size (McDonald et al. 2003), so to obtain accurate estimates, knowledge of tag loss rate is crucial. Only the tags or marks with the highest retention rates should be used, however, research into such factors can often be logistically or financially restrictive (McDonald et al. 2003).

Mark-recapture methods are commonly used to study snails. Various techniques have been used such as drilling holes, nail polish or pencil marks. However, in many of these studies either the marking technique or tag loss rate are not reported (Bertness et al. 1981). Only 21% of such studies mention the issue of mark loss and of those studies only a couple accounted for mark loss during the analysis (Henry and Jarne 2007). The method found to have the lowest tag loss rate (Henry and Jarne 2007) was gluing small plastic tags to the shell (Chen and Soong 2002) with cyanoacrylate glue (Pyron and Covish 2003) or using epoxy resin (Kraeuter et al. 1989; McShane and Smith 1992). The crucial aspects of a mark-recapture study are knowing the impact on the individual (marking trauma), and mark persistence and detectability (Severns 2009; Williams et al. 2002). Detectability has been addressed in very few studies focusing on land snails (Bennetts et al. 1999; Martin et al. 2007). In a study which set out to address this problem for the first time, up to 50% of land snail species were found to have an exceptionally low detection rate (Albano et al. 2015). When searching both the leaf litter and the soil, rather than just under refuges, a greater proportion of snails were detected. If the detection rate was not known, site occupation estimates were found to be between 15-85% lower than the actual value (Albano et al. 2015). Little is known about *P. bermudensis* habitat preference due to its limited distribution; monitoring the species in a captive setting could provide important information on activity and microhabitat selection, which in turn can inform survey efforts following reintroduction. The species is reported to be predominantly active during rainfall, and periods of darkness or subdued lighting (Ovaska 2017; Pearce-Kelly and Walker 2006). Individuals of *P. bermudensis* were historically reported upon weathered rocks in private gardens (Gould 1969), but more recent surveys have often found them in association with the leaf litter of bay grape *Coccoloba uvifera*, and the endemic Bermuda palmetto *Sabal bermudana* (Lines 2002). Additional observations of *P. bermudensis* on Port's Island have shown that moist microhabitats under rocks and decaying logs are also important (Outerbridge and Sarkis 2018). Under these conditions a marking technique was required that was at least semi-permanent, minimized trauma to the individual, and that could be efficiently applied under high relative humidity and occasional high temperatures.

In this study, the aim was to determine the tag loss rate of gluing standard VI Alpha tags™ using Ethyl Cyanoacrylate adhesive, which was chosen over epoxy resin due to ease of use in the field and affordability. Testing was also conducted to determine whether lightly sanding the snail shells would improve tag retention. By sanding, the aim was to increase the roughness of the shell (Huber et al. 2007), increase the surface area between the shell, the adhesive, and the tag (Marshall et al. 2010, Song et al. 2016) and consequently reduce the risk of tag loss.

The location preferences of the captive group of snails within their tanks was assessed in order to improve detectability in the post-reintroduction surveys. It is hoped that the findings from this study will enable more accurate survival and population estimates to be made, to determine the success of the reintroduction programme, and act as a model for further reintroduction and monitoring projects for other land snail species.

## Action

### *Tag Retention*

Standard Alpha fluorescent tags (1.2mm x 2.7mm; Northwest Marine TechnologyTM, Figure 1a) were used for the study and were glued to the shell of the snail using Gorilla glue™. The tags were glued to the same location on each shell (Figure 1b). Gluing took place two weeks before data collection occurred. Each snail shell was first wiped with a paper towel to remove dirt and

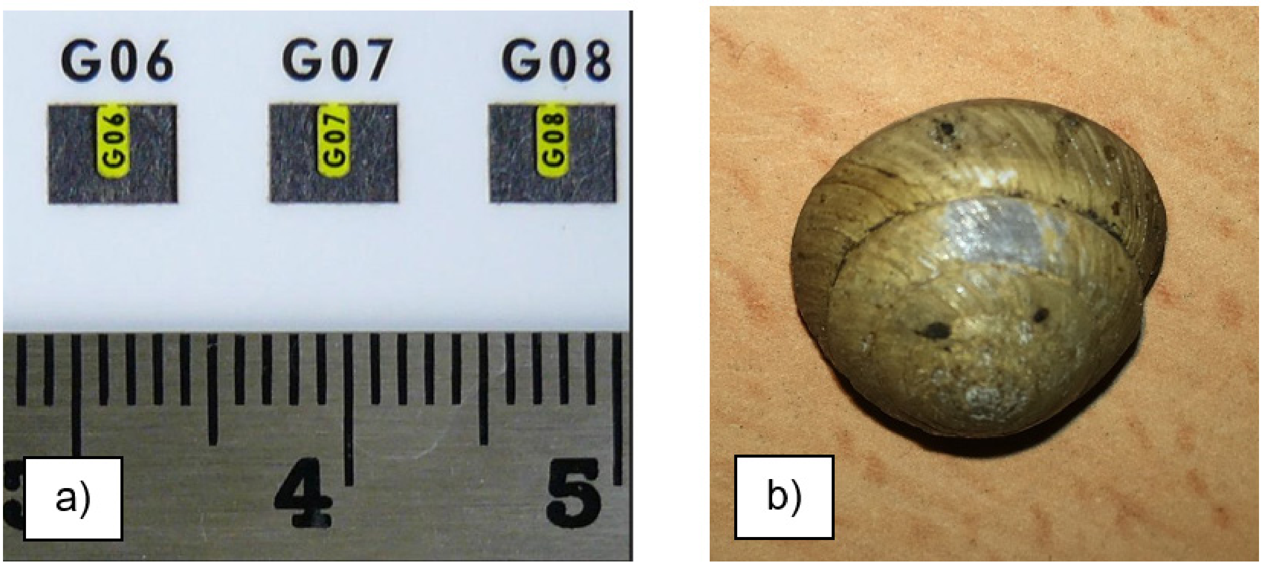


**Figure 1.** (a) Individually identifiable Alpha fluorescent tags used for marking individual *Poecilozonites bermudensis* snails (b) A *P. bermudensis* shell that has been lightly sanded (the grey part) prior to gluing a tag on. All tags were glued to this location on each snail's shell.

**Table 1.** Definition of snail locations within tanks of *Poecilozonites bermudensis.*

| Location | Definition |
|---|---|
| Soil | Completely buried under the substrate |
| Litter | 0-3cm above substrate level |
| Rock | On or under the rocks |
| High | >3cm above substrate level |

moisture. The "sanded" group had a small part of their shell lightly sanded before gluing, using a titanium/ diamond needle file. This was done lightly by hand for approximately 10 seconds, until the first periostracum layer was removed. The sanded area was roughly twice the size of the Alpha tag. Gluing was only carried out once the snail had retreated into its shell. A small drop of glue was applied to the shell at the tagging location, and the tag was then pressed into the glue spot using a cocktail stick; ensuring the glue covered the surface of the tag. Once the tag was secure, the snail was placed in a small individual plastic container (2x2cm) with air holes, to allow the glue to dry completely without the risk of other snails crawling on it. After 3 hours (when the glue was completely dry) the snails were placed into their designated tank. Beginning two weeks post-gluing, the location of each snail within its tank was recorded every 14 days and its tag visually inspected. It was noted whether the tag had been lost or retained.

***Location preference***

During each data collection event, the location of each snail within the tank was recorded (Table 1). The tanks contained around 4 inches of substrate (75% coir, 25% sand), some freshly cut palm fronds, rock structures, leaf litter (palm leaves), and cuttlefish bone as a source of calcium. Data were collected under four differing conditions to replicate potential post-release monitoring surveys (Table 2). To ensure no ambient light seeped into the tank during "night" collection events, a blackout material was placed over the tanks at approximately 1630 before data collection and removed immediately prior to data collection, to enable the snails to be located as though it were night. Due to data collection happening first thing in the morning, this would occasionally mean that the day:night cycle length of the tank was increased by up to two hours. If a snail died during the study it was removed from the tank as soon as possible, and data collection carried on with the reduced number of specimens per tank.

***Husbandry***

Five hundred adult snails (>15mm) were selected randomly from the group housed at Chester Zoo. These snails were then randomly allocated to either the sanded (n=200) group, the non-sanded (n=200) group, or the control group (n=100). The snails were kept in Biopod AquaTM tanks (LWH 65x38x53cm) for the duration of the study, with 100 snails being placed in each. Each tank had one SkyLight (680 lumens, 6000k) placed above it in one corner. The lights were on a timer and were on for 12 hours per day (0800-2000). Outside of these hours, snails were kept in complete darkness as the room they were housed in had no windows. The temperature of the tanks was maintained between 18-25°C at all times during the study. The snails husbandry routine was consistent throughout the study. Feed was provided three times per week and consisted of romaine lettuce, sweet potato, and carrot, sprinkled with a powder containing spirulina, cuttlefish scrapings, and fish flakes (1:1:1). At feeding times, the tanks were also sprayed with reverse-osmosis water for roughly ten seconds. Aside from data collection periods, no other handling or disturbance of the snails occurred. Data collection occurred once every 14 days beginning on 5 February 2019 until 30 July 2019.

***Data analysis***

The survival of the snails was calculated by investigating the number of snails recorded at each data collection event. These were converted into percentage per group due to the differing numbers of animals at the start of the study for the two groups (tagged = 400, control = 100). A general linear model (GLM, family=Gaussian) was used to test for any interaction between the days since data collection began and whether the snails had a tag or were tagged and sanded followed by a post-hoc ANOVA. To test for differences in the location preferences of the snails dependent on time of day (day/night), condition (wet/dry- depending on time of last spraying) and whether the shells had been sanded or not, chi-square tests were used. All Data analysis was conducted in R Studio Version 4.2.2.

## Consequences

***Tag retention***

There was no detrimental effect on the survival of snails who had a tag glued on compared to the control group. Indeed, the tagged snails lived on average, longer than the un-tagged snails (GLM Tagged*Day interaction, $F=10.3$, $df=1$, $P<0.005$, Figure 2a). No analysis was carried out to calculate a tag loss rate, due to a 100% retention of the tags during the study period. Although data were only formally collected over the course of 6 months, the tags were still observed to be attached to the snail's shells over two

**Table 2.** The four tank conditions for *Poecilozonites bermudensis*.

| Condition variables | Description |
|---|---|
| Day | Approximately 2 hrs after lights above tank switched on in morning |
| Night | After tanks had been in complete darkness for ~8 hrs |
| Dry | No spraying in tank for at least 24 hrs prior |
| Wet | Tanks sprayed for ~20 seconds one hr prior to data collection |

years after they were originally glued on (pers. obs.), suggesting that this is a sound and effective method of tagging land snails.

As no tags were lost in either the sanded or the un-sanded group it appears that sanding the shell (which carries a small risk of breakage) is unnecessary. Sanding did have a significant effect on survival, with those individuals whose shells were sanded showing a much higher probability of mortality (GLM Sanded F=360.4, df=1, P<0.001; Sanded*Day interaction F=67.7, df=1, P<0.001, Figure 2b). A principle requirement for strong adhesive bonds to occur is a clean surface (Marshall et al. 2010), and therefore the cleaning of the tagging location prior to the use of an adhesive is still recommended.

***Location preference***

When investigating the location preferences of the snails, the control tank was found to have a significantly different distribution to the other four tanks ($X^2$=184.7, df=3, P<0.001); there was a consistently significantly lower proportion of snails located on the rock structures of the control tank (7%) compared to all other tanks (30%). Due to resource limitations, the control tank contained a different type of rock which was smoother than in the other four tanks (highly textured porous limestone rocks), as this component was mainly set up to compare survival across tagged and untagged snails. In line with a previous study (Solymos et al. 2009), land snails appear to not favour smooth rock surfaces as they offer little opportunity for shelter. It seems likely that the tagging of the snails did not affect their behaviour during this study but rather they were affected by the environmental conditions and microhabitats presented to them, however, rock type and tagging are confounded in our data. For the purposes of further data analysis of the location preferences of the snails, the control tank was excluded.

The condition (dry/wet, $X^2$=43.28, df=3, P<0.005) and the time (day/night, $X^2$=175.46, df= 3, P<0.005) were both found to be important predictors of the snails location. If surveying for *P. bermudensis* during the day with no rainfall, the majority of snails are likely to be found at a higher level than the ground, such as up the sides of the tank or a tree, and few will be found in the leaf litter (Figure 3). This links to the species biology, *P. bermudensis* cannot seal the aperture of its shell as some other land snail species can, making it prone to desiccation during hot and dry weather (Outerbridge and Sarkis 2018). Instead, the species aestivates during unfavourable conditions (Copeland 2018). If data collection occurs during a dry night, more snails are found on rock structures and within the leaf litter, and fewer are high up (Table 3, Figure 3). Rain at night causes a slight increase in snail numbers from the leaf litter to the rock structures, and rain in the daytime causes more snails to be found on the rocks than higher up (Table 3, Figure 3). The number of snails buried in the soil is relatively equal under all conditions, at approximately 6%; this aligns with wild observations where snails have been documented using refuges such as rocks and leaf litter to hide under, however, wild specimens have not been recorded burrowing into the soil. Sanding had no impact on location preference ($X^2$=4.41, df=3, P>0.05).

The results of this study demonstrate that for post-release monitoring of *P. bermudensis*, the use of Alpha fluorescent tags for marking individual snails is recommended. These should be attached using an Ethyl Cyanoacrylate adhesive following wiping the shell to remove dirt or moisture. Sanding the shell is not necessary to aid tag adhesion, and indeed sanding had a negative effect on survival, so should be avoided. Environmental conditions should be considered when planning post-release monitoring surveys. Following dry weather, daytime surveys should focus on higher areas, whilst daytime surveys following wet weather snails are likely to be found in both high and rocky areas. During night surveys, effort should focus on rocky areas and in leaf litter.

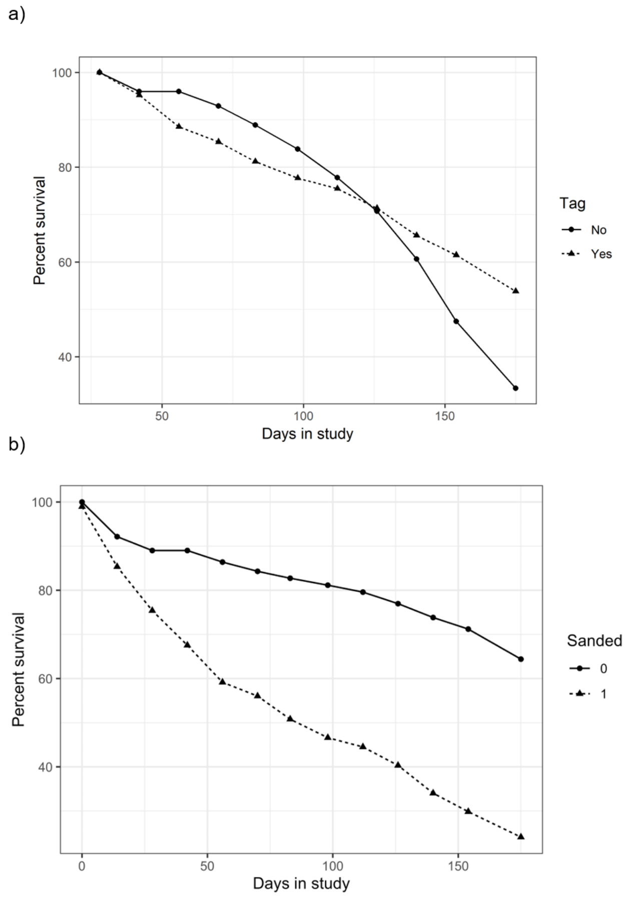


**Figure 2.** (a) The survival rate of tagged and non-tagged *P. bermudensis* over the course of the study period, (b) The survival rate of sanded and non-sanded *P. bermudensis* over the course of the study period.

These findings should prevent errors in the calculation of both survival and population estimates during the in-situ studies of recently reintroduced populations of *P. bermudensis*. Being able to attain accurate population estimates will prove vital in choosing the most effective management strategies for the conservation of this species, and it is hoped that other similar species can benefit from the results of this project and be the subject of further reintroduction success.

## Acknowledgements

With thanks to the Ectotherm team at Chester Zoo who assisted in the set up and husbandry of the snails, and the researchers in Bermuda who inspired the project.

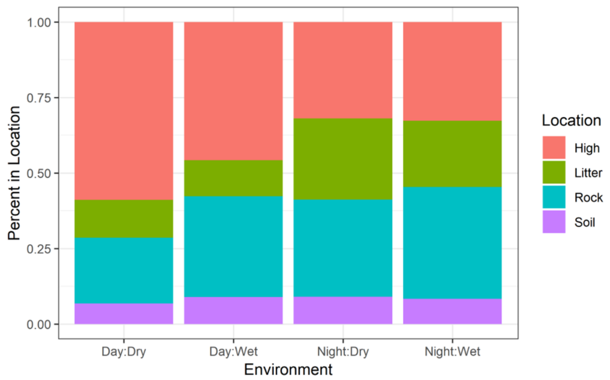


**Figure 3.** The percentage of *P. bermudensis* found in different locations within their tanks under four different environmental conditions.

## References


Albano P.G., Strazzari G., D'Occhio P. Succetti, F. (2015) Field estimates of detectability and site occupancy show that northern Italy forest molluscs are spatially rare and poorly detectable. *Italian Journal of Zoology* 82(4): 592–608. https://doi.org/10.1080/11250003.2015.1040084

Arnason A.N., Mills K.H. (1981) Bias and loss of precision due to tag loss in Jolly-Seber estimates for mark–recapture experiments. *Canadian Journal of Fisheries and Aquatic Sciences* 38(9): 1077–1095. https://doi.org/10.1139/f81-148

Barker G.M. (2004) *Natural enemies of terrestrial molluscs*. CABI.

Bertness M.D., Garrity S.D. Levings S.C. (1981) Predation pressure and gastropod foraging: a tropical-temperate comparison. *Evolution* 35(5): 995–1007. https://doi.org/10.2307/2407870

Cameron R.A.D., Pokryszko B.M. (2005) Estimating the species richness and composition of land mollusc communities: Problems, consequences and practical advice. *Journal of Conchology* 38(5): 529–548. https://doi.org/10.1111/j.1541-0420.2006.00523.x

Chen M.H., Soong K. (2002) Estimation of age in the sex-changing, coral-inhabiting snail *Coralliophila violacea* from the growth striae on opercula and a mark–recapture experiment. *Marine Biology* 140(2): 337–342. https://doi.org/10.1007/s002270100700

Copeland A. (2018) Determining the habitat preferences of the Bermuda Land Snail (*Poecilozonites bermudensis*) on Port's Island, Bermuda. [unpublished report] University of Oxford.

Cowen L., Schwarz C.J. (2006) The Jolly–Seber model with tag loss. *Biometrics* 62(3): 699–705. https://doi.org/10.1111/j.1541-0420.2006.00523.x

Gould S.J. (1969) An evolutionary microcosm: Pleistocene and recent history of the land snail P. (*Poecilozonites*) in Bermuda. *Bulletin of the Museum of Comparative Zoology* 138(7): 407–532. https://doi.org/10.2307/2412347

Henry P.Y., Jarne P. (2007) Marking hard-shelled gastropods: tag loss, impact on life-history traits, and perspectives in biology. *Invertebrate Biology* 126(2): 138–153. https://doi.org/10.1111/j.1744-7410.2007.00084.x

Huber G., Gorb S.N., Hosoda N., Spolenak R., Arzt E. (2007) Influence of surface roughness on gecko adhesion. *Acta Biomaterialia* 3(4): 607–610. https://doi.org/10.1016/j.actbio.2007.01.007

Kraeuter J.N., Castagna M., Bisker R. (1989) Growth rate estimates for *Busycon carica* (Gmelin, 1791) in Virginia. *Journal of Shellfish Research* 8(1): 219–225.

Lines A. (2002) Survival and distribution of Bermuda's native and endemic terrestrial snails. BBP Student Project Report. Bermuda Aquarium Museum and Zoo. BAMZ#1184.

Marshall S.J., Bayne S.C., Baier R., Tomsia A.P., Marshall G.W. (2010) A review of adhesion science. *Dental Materials* 26(2): 11–16. https://doi.org/10.1016/j.dental.2009.11.157

Martin J., Kitchens W.M., Hines J.E. (2007) Importance of well-designed monitoring programs for the conservation of endangered species: case study of the snail kite. *Conservation Biology* 21(2): 472–481. https://doi.org/10.1111/j.1523-1739.2006.00613.x

McDonald T.L., Amstrup S.C., Manly B.F. (2003) Tag loss can bias Jolly-Seber capture-recapture estimates. *Wildlife Society Bulletin* 31(3): 814–822. https://www.jstor.org/stable/3784604

McShane P.E., Smith, M.G. (1992) Shell growth checks are unreliable indicators of age of the abalone *Haliotis rubra* (Mollusca: Gastropoda). *Marine and Freshwater Research* 43(5): 1215–1219. https://doi.org/10.1071/MF9921215

Outerbridge M., Sarkis S. (2018) *Recovery plan for the endemic land snails of Bermuda,* Poecilozonites bermudensis *and* Poecilozonites circumfirmatus*.* Department of Environment and Natural Resources, Government of Bermuda. [online]. https://static1.squarespace.com/static/501134e9c4aa430673203999/t/5b59e5c4758d468420b61b51/1532618184222/Bermuda+land+snails+recovery+plan+.pdf

Outerbridge M.E. (2014) *The Greater Bermuda Land Snail* = Poecilozonites bermudensis*–a Lazarus species discovered in the center of an urban environment.* Department of Environment and Natural Resources, Government of Bermuda. [online]. https://static1.squarespace.com/static/501134e9c4aa430673203999/t/5a1db4ec085229dccc243f2d/1511896308235/Rediscovery+of+the+greater+Bermuda+land+snail.pdf

Ovaska K. (2017) *Introduction and monitoring of* Poecilozonites *on Nonsuch Island, Bermuda*. Report prepared by Biolinx Environmental Research Ltd. for the Department of Environment and Natural Resources, Government of Bermuda. 41.

Ovaska K. (2018) *Reintroduction of the endemic Bermuda Snail (*Poecilozonites bermudensis*) to Nonsuch Island – results of February 2018 surveys.* Preliminary report, prepared by Biolinx Environmental Research Ltd. for the Department of Environment and Natural Resources, Government of Bermuda. 5

Pearce-Kelly P., Walker, C. (2006) Progress report on the conservation breeding programme for the Bermudian land snail (*Poecilozonites circumfirmatus). Zoological Society of London Report* 7.

Pyron M., Covich A.P. (2003) Migration patterns, densities, and growth of *Neritina punctulata* snails in Rio Espiritu Santo and Rio Mameyes, northeastern Puerto Rico. *Caribbean Journal of Science* 39(3): 338–347.

Seber G.A.F., Felton R. (1981) Tag loss and the Petersen mark-recapture experiment. *Biometrika* 68(1): 211–219. https://doi.org/10.1093/biomet/68.1.211

Servens P.M. (2009) A conspicuous, water-resistant ink for marking terrestrial snails. *Journal of Molluscan Studies* 75: 93–94. https://doi.org/10.1093/mollus/eyn040

Song Y., Dai Z., Wang Z., Ji A., Gorb S.N. (2016) The synergy between the insect-inspired claws and adhesive pads increases the attachment ability on various rough surfaces. *Scientific Reports* 6(1): 1–9. https://doi.org/10.1038/srep26219

Sogutlu C. (2017) Determination of the effect of surface roughness on the bonding strength of wooden materials. *BioResources* 12(1): 1417–1429.

Sólymos P., Farkas R., Kemencei Z., Páll-Gergely B., Vilisics F., Nagy A., Kisfali M., Hornung E. (2009) Micro-habitat scale survey of land snails in dolines of the Alsó-hegy, Aggtelek National Park, Hungary. *Mollusca* 27(2): 167–171.

Treble R.J., Day R.W., Quinn II T.J. (1993) Detection and effects on mortality estimates of changes in tag loss. *Canadian Journal of Fisheries and Aquatic Sciences* 50(7): 1435–1441.