APPENDIX ES-3

STUDIES ON HAWAIIAN TREE SNAILS

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Current status of captive endangered tree snail populations:
As of our latest lab census (April 2016) we are caring for 504 Endangered Hawaiian tree snails (up from 426 last year at this time), the majority of which, 461, are members of the genus *Achatinella*, from Oahu. The remaining two species in the lab are members of the genus *Partulina*, with 43 individuals (up from 27 last year at this time), from the island of Lanai. The snails are housed in 15 large cages (35 x 20 x 20 cm, total volume of 14,000 cm^3 each) and 6 small cages (20 x 13 x 10 cm, 2,600 cm^3 volume) (large cages shown in Figure 1). Populations exceeding 20 individuals are kept in large cages while for smaller populations (<20 snails), small cages are sufficient. The snail cages are housed in 3 environmental chambers (see Figure 1). Each week we inoculate and grow up about 50 potato dextrose agar plates of tree fungus dietary supplement (see **Fungus Culture**). We currently have a staff of three technicians and one lab manager who are directly involved with care and maintenance of the captive tree snails.

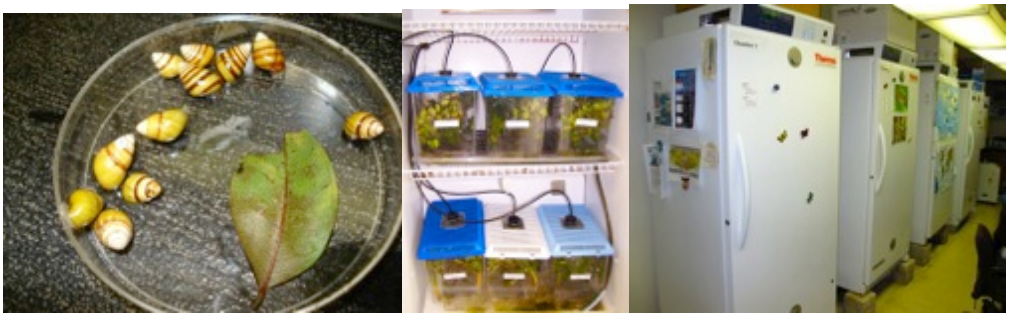

Figure 1. Captive breeding and care entails scheduled daily cage watering, changing, cleaning and documentation of mortality, births, and population status. Cages are changed by exchanging native foliage collected in the field and laboratory cultured leaf fungus, and cleaning and sterilizing the interior and the lid.

General Daily Tasks
Incubators are checked and inspected daily, first in the morning and at the end of each work day, at a minimum. Priority system checks include visual examination of rubber sprinkler stopper placement. Stoppers are sealed, and in place, temperature panels are

checked throughout each day. Note that operating temperatures have been corrected via Hobo® data loggers and analog thermometers, such that panels do not always reflect target temperatures. Thermal profile printouts, are affixed to upper left side of each chamber door. Internal drainage systems should also be checked daily for leaks, overflows, and clogged nozzles or drains.

Environmental Chambers - We currently use three diurnal incubators, also referred to as environmental chambers, for snail propagation in the Hawaiian Tree Snail Conservation facility at the University of Hawaii, Manoa. The units currently in operation, are manufactured by Precision Thermo Scientific 818 (Model # 3751), two are newer Thermo Scientific (Model # 3751), and one VWR Sheldon Manufacturing (Model # 2015) (Figure 2). All of the environmental chambers run on 120 volts, and are equipped with electronic programmable temperature and photoperiod controls, as well as alarms in the event that the temperatures exceed preset high or low set-point limits. Light bulbs used in each chamber are full spectrum, 40 watt, 48 inch fluorescent bulbs, producing between 1,980 and 3,300 lumens. Chambers require regular maintenance, including periodic light bulb and drain tube replacement, rust removal and repainting, fuse replacement and temperature calibration.

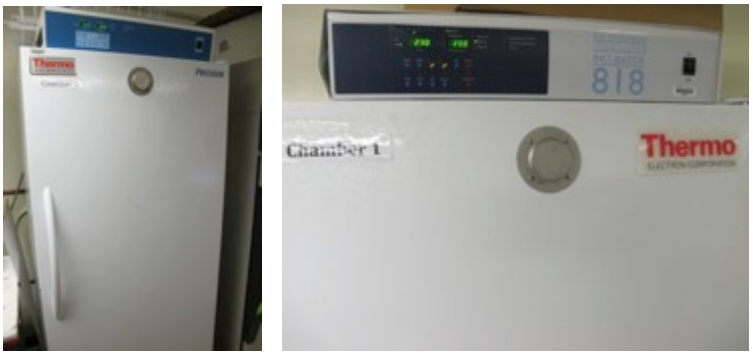

Figure 2. Environmental chambers, showing Thermo model and control panel.

Temperature - For snails collected from to upper-elevation habitat (670 + m.), chamber temperature is maintained at 20° C during the 12 hr day (light) cycle and 16° C during the 12 hr night (dark) cycle (*Achatinella lila, A. bulimoides, A. livida, A. fuscobasis, A. decipiens*). For the snails collected from slightly lower elevation habitat, day temperature is set slightly warmer, to 21° C, and at night is 18° C (*A. mustelina, A. fulgens, A. apexfulva, Partulina variabilis, P. semicarinata*). We currently have Hobo® data loggers (on loan from SEPP). We are keeping these in all active chambers, and we currently monitor temperature profiles, to ensure that actual operating temperatures are within range and that chambers are holding consistent temperature profiles.

By monitoring chamber temperatures we have found that each internal actual operating temperature differs slightly from the control panel setting and display, by differing

amounts, such that temperatures shown on panels vary slightly among chambers. We have incrementally adjusted each chamber thermal control, so that internal temperatures are now set correctly to thermal targets. Conversions are shown on printouts placed on the left side of each chamber door. We have two chambers set to actual internal operating temperatures of higher elevation habitat, 20°C day and 16° C night, and one chamber as shown above, at the lower elevation temperature profile, 21° C day and 18° C night.

Cage Changing & Cleaning Procedure

We have developed a stepwise, standardized procedure for cleaning individual snail cages. Strict adherence to these steps is essential to the maintenance of populations of tree snails. Changing schedule is: each week for small cages, every two weeks for large cages. We keep track of fungus consumption at each changing cycle, and the general rule of thumb is between 2 and 4 plates (or discs) for large cages and one plate or less for small. We carefully document and measure new births, as well as any mortality during each cycle.

Fungus Culture

Cultured fungus is provided as a supplement for captive tree snail dietary requirements. We maintain a line of fungus that is fed to all snails. The Potato Dextrose Agar (PDA)(Difco or Cole Parmer) medium on which the fungus grows is supplemented with calcium carbonate to help with shell maintenance and growth. Fungus culture is done in several steps, the first is preparing and sterilizing the PDA, then pouring the PDA plates (Figure 3), and finally is inoculating the cured PDA plates. Currently we prepare 45-50 Petri plates per week.

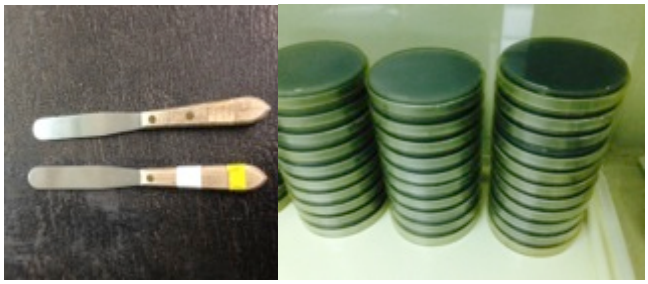

Figure 3. Image shows fungus spatulas (left) and plates of cultured fungus (right).

Leaf and Branch Collection

Once per week, plant material is collected for snail cages by hiking on designated trails and clipping small branches from native host plants. In the field, personnel wear general hiking apparel including closed-toed hiking or running shoes, sun protection, rain gear, and carry bottled water, sun screen and at least one cell phone. In addition, clippers and large trash bags are required. Hawaiian tree snails typically prefer host trees with glabrous leaves (shiny, smooth leaves), including Ohia lehua (*Metrosideros polymorpha*) as well as other native species such as Kopiko (*Psychotria grandilora*), Kawa'u (*Ilex*

anomala), Olopua (*Nestigis sandwicensis*), Pāpala (*Pisonia umbellifera*), Akiahala (*Broussaisia arguta*), Lama (*Diospyros sandwichensis*), and Alani (*Melicope* sp.). These tree species are the main focus of leaf collections for tree snail maintenance. Branches are cut and collected in the field using hand-held clippers. Branches are maintained as intact as possible, to maximize the time that they remain fresh. *Freycinetia arborea* (I'e i'e) is another host plant favored by tree snails. I'e i'e leaves have an unusual structure, and are long and thin, and can be acquired in the field by pulling them away from the lower portion of the cluster along the stem at the base, 3-5 leaves at a time, rather than clipping. Thanks to the recent work done with our collaborators in the Botany Department, we now know that at a given locality, the microbial community tends to be the same on native and non-native tree surfaces. This has allowed us to slightly expand the scope of host plant leaf collecting to now include several nonnative broad leaved tree species, that we harvest from mid-elevation forests along with the usual native host plants, Octopus tree (*Schefflera actinophylla)* and Ti plant (*Cordyline fruticosa*). Short branches with well-developed foliage, of approximately 30 cm are generally preferred for clippings. Leaves tend to remain fresher, longer this way, as opposed to cutting shorter twigs. Generally, if given an option, we do not clip branches with flowers, fruit, seeds or other reproductive structures. Also since tree snails feed on the microbial phyllosphere (leaf and branch surface fungal community), which is not as likely to be well-developed on immature plants, therefore we do not collect juvenile or undeveloped leaves, buds or branches. Leaves collected in the field are doused with water in the collection bags, maintained in shade when possible, and kept wet in the field.

In the lab, all branches and leaves are sprayed with water and placed at 4º C in closed plastic trash bags. Plant material can continue to be used for 5 to 7 days following collection and this way leaves will stay fresh within snail cages until the next scheduled cleaning.

Publications (accepted within the past 18 months, with assistance of OANRP support)

Van Kleeck, M.J. & **B.S. Holland**. Chemical control of the invasive Jackson's chameleon. *International Journal of Pest Management* (in revision)

O'Rorke, R., **B.S. Holland**, G.M. Cobian, K. Gaughen & A.S. Amend. (2016) Enhancing captive breeding of endangered species by determining dietary preferences. *Biological Conservation* (in press)

Holland, B.S., L.M. Chiaverano & C.K. Howard. (2016) Diminished fitness in an endemic Hawaiian snail in nonnative host plants. *Ethology, Ecology and Evolution* (in press)

Van Kleeck, M.J., L.M. Chiaverano & **B.S. Holland.** (2015) Prey-associated head-size variation in an invasive lizard in the Hawaiian Islands. *Biological Journal of the Linnean Society* 116(3):626-636.

O'Rorke R., G.M. Cobian, **B.S. Holland**, M.R. Price, & A.S. Amend. (2015) Dining local: the diet of a snail that grazes microbial communities is geographically structured, *Environmental Microbiology* 17(5):1753-1764.