DEVELOPING CAPTIVE *EX SITU* POPULATIONS OF THE ENDANGERED CHITTENANGO OVATE AMBER SNAIL *Novisuccinea chittenangoensis* (GASTROPODA: PULMONATA: SUCCINEIDAE) FOR POPULATION AUGMENTATION IN NEW YORK STATE

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ABSTRACT

Nearly half of all animal extinctions recorded since 1500 A.D. have been molluscs, and most of these are nonmarine molluscs. Few land snail species have been rigorously assessed with respect to conservation status, but over 1200 land snails are listed as endangered or threatened. Land snails are important in nutrient cycling, and thus land snail species' decline and extinction could have dramatic impacts on forest ecosystems. Captive breeding is an important tool for saving some of these land snail species from extinction, but it can also help us better understand understudied invertebrates, particularly life histories. The endangered Chittenango ovate amber snail *Novisuccinea chittenangoensis* (Pilsbry, 1908) (Mollusca: Gastropoda: Pulmonata: Succineidae) (COAS) is known from only one locality: the spray zone of one side of the main waterfall at central New York State's Chittenango Falls State Park (Cazenovia, New York, USA). The main goal of my conservation research is to contribute to COAS's removal from the United States Endangered Species List by successfully rearing COAS in captivity for augmentation of the wild population. I propose to: (1) examine the diversity and distribution of Succineidae within New York State (NYS) (2) determine the optimal captive diet for COAS, which will be measured using comparisons of fecundity, food preference, and growth rate; and (3) measure habitat parameters including abundance of detritus or decaying plant matter for differences on the side of Chittenango Falls where COAS do not live.

INTRODUCTION

Living among limestone rocks in the spray zone at the base of a waterfall is a small terrestrial snail. *Novisuccinea chittenangoensis* (Pilsbry, 1908) or the Chittenango ovate amber snail (COAS) is endemic to only one location in Upstate New York. COAS (Mollusca: Gastropoda: Pulmonata: Succineidae) is federally-listed as threatened and protected by the United States Fish and Wildlife Service (USFWS). According to the New York State Department of Environmental Conservation (NYSDEC), COAS is classified as the most endangered species in New York State. USFWS will keep the current listing until a status review is completed in which the federal listing may be changed to endangered (USFWS, 2006). The entire COAS population is limited to one side of a waterfall at Chittenango Falls State Park in Madison County (central New York State (NYS). It is the only known population, and the historic geographical distribution has been presumed to be fairly restricted, but may have been larger than its current range. COAS's historical geographic range is poorly understood because definitive and distinctive shell characters are currently lacking, which complicates comparisons with recent and subfossil shells (Molloy, 1995).

COAS threats: In 2006 the natural disturbance of a rockslide destroyed a large portion of the COAS habitat, resulting in a substantial population decrease. USFWS performs mark recapture surveys every other year to estimate and monitor growth rate and abundance of the COAS population. The 2009 population estimate of 339.2 (± 52.85) was 56.7% lower than the pre-rock slide estimate of 784.2 (±38.10) in 2005 (USFWS, 2012). Chittenango Falls is geologically dominated by shale, which is known for its non-cohesive properties resulting in a higher risk of rockslides especially during times of heavy precipitation because this type of clay shale may absorb water more easily and swell (Keller, 2011). These rockslides exhibit a long term risk to the COAS population since their habitat will always be under threat from recurring rockslides. Climate change, which may lead to unpredictability or increased severity of weather patterns, may also impact the COAS population negatively. For example, increases in drought duration may cause a decrease in water flow, which would reduce mist zones and increase the potential for COAS desiccation. Inversely, an increase in precipitation may cause an overflow of water, washing away COAS eggs and adults downstream to an unsuitable habitat. An increase in water may also drown hatchlings or adults. Water pollution remains a concern: although raw sewage is no longer being flushed into Chittenango Creek, herbicides and pesticides from agriculture still flow into the aquifer. The town of Cazenovia now treats the water, but it is chlorinated from May to October (Molloy, 1995).

Aside from abiotic threats, there are a number of other land snail and slug species present in the surrounding area of the falls, including a succineid land snail species similar in appearance and size to COAS currently referred to as "species B" (sp. B). COAS and sp. B may compete for prime egg laying locations which could cause stress to the COAS population (Campbell, 2010). It is thought that this introduced species is an invasive snail brought to the United States from Europe (Molloy, 1995). Evidence from mitochondrial DNA (mtDNA) sequences demonstrates that COAS is distinct from sp. B. There is no evidence of past hybridization between COAS and sp. B (King *et al.*, 2011). Potential biotic threats to COAS include sp. B and encroachment of invasive plants such as pale swallow-wort (*Cynanchum rossicum*) as well as human trampling of COAS habitat crushing snails underfoot. The overlap of sp. B and COAS feeding on similar vegetation is present, but limited which may help in co-existence. Density of sp. B in a competition experiment was far greater than that of the natural density of sp. B in COAS habitat. It is therefore likely that in the 30 plus years they have lived together, density of sp. B has stabilized at a level lower than numbers that would affect COAS (Campbell, 2010). Predation by sciomyzid fly larvae, beetles, salamanders, birds and small mammals may be reducing COAS numbers, but research is lacking on these potential factors.

<u>Species-centered management and ecosystem management approaches</u>: A collaborative effort between the Rosamond Gifford Zoo (Syracuse, NY), Seneca Park Zoo (Rochester, NY), USFWS, NYSDEC, New York State Office of Parks Recreation and Historic Preservation (NYSOPRHP) and the State University of New York's College of Environmental Science and Forestry, was convened to help ensure persistence of the COAS population, as mandated by the species' conservation status. The USFWS is required to enforce the Endangered Species Act (ESA) and therefore to provide and follow a Species Recovery Plan for COAS by law to recover population size and stability. The ESA focuses on protecting individual species, and the ecosystem that they live in, which may contain other species under threat. On page 1 of the Endangered Species Act of 1973 Section 2(b) states: "The purpose of this Act are to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved." COAS inhabits a very small area and thus is a good candidate for a focused conservation effort (i.e. captive breeding for translocation or augmentation of the wild population). An endangered fern species, American hart's-tongue fern (*Asplenium scolpendrium var. americanum*) lives in the park just outside of COAS habitat. Therefore, habitat protection of the entire area would benefit other species as well. NYSOPRHP has made efforts to lower human activity within COAS habitat and the surrounding area. Fencing was installed to prevent people from entering COAS habitat. This has limited trampling of COAS habitat, which is favorable for all species within the area protected. However, during my recent work at the falls I witnessed groups of people in the restricted area. Protection and monitoring of entire habitats, not just one species would be necessary for prevention of declines in overall abundance of invertebrates. It would be far less costly and more effective to designate large protected areas for all species within those areas, particularly when the ecology (e.g. food preferences) of the target invertebrate species are poorly known (as is true in many if not most cases)..

Although there are multiple methods of protecting endangered species, such as habitat protection, limiting human access, and *in situ* management of species; captive breeding in some cases (e.g. small isolated populations under threat) may be the best way to ensure the population does not go extinct. For example, when the effective population size is beginning to drop below a genetically viable size, species management through captive breeding may increase the chance of species survival. Successful conservation efforts with tropical tree snail species have provided templates for husbandry methods (e.g. Pacific-endemics Partulidae and Achatinellinae: Tonge & Bloxam, 1991). Because of introduced predatory snails, rodents, over-collecting and habitat destruction, these endemic tree snail species have been decimated (Clarke *et al*., 1984; Cowie and Cook, 2001; Gould, 1991; Hadfield *et al*., 2004; Murray *et al*., 1998). Captive propagation efforts have maintained some tree snail species that otherwise would have gone extinct (Coote *et al.*, 2004; Hadfield *et al.*, 2004; Tonge & Bloxam, 1991). The USFWS recovery plan for COAS states if at least 2 healthy captive colonies are established successfully, then population augmentations and buffers against extinction will potentially lead to stabilization of the population and delisting of the species (USFWS, 2006). The short term objectives are to stabilize the COAS population in captivity as well as in the wild, and the overall goal of the recovery plan is to achieve a long-term sustained population(s) in the wild (USFWS, 2006).

COAS is a temperate species and thus may need a different set of conditions for captive breeding (e.g. temperature and diet) than conditions required for tropical snail species. COAS also have a short lifespan of about 2.5 years and high reproductive rates whereas partulid and achatinelline tree snail species live for 8+ years and have a lower fecundity (Murray *et al*., 1988; Hadfield and Mountain 1980). Captive breeding of COAS under the right conditions could produce high numbers of individuals over a short period of time. If genetic diversity is maintained, and diet is matched with their natural diet, breeding individuals, followed by raising and releasing them into the wild at a larger size may help decrease the threat of extinction. Mature COAS individuals readily copulated in captivity with hatchlings often numbering into the hundreds (J. Brown, personal communication, 2013). With proper care, a large colony could be kept and split into multiple colonies for back up populations. But having too many individuals may become a problem if there is no wild habitat to receive translocated captive bred COAS. Managers of the colony would either have to increase time and resources into care of a larger colony which can be costly, train other facilities to take on the care of "surplus" individuals, or euthanize some of the snails. The number of founders collected for a captive population may determine the success or failure of a long term sustainable population. Only 6 founders were used in one past attempt at an *ex situ* captive population of COAS in 1995. The population failed due to a die off of the snails at around their 7th generation. The last remaining snail died 11 January 2003. (J. Brown, personal communication, 3 May 2013) It is thought that having such a small founder population contributed to the failure of establishing a long term captive population.

Avoiding inbreeding and adaptations to captive conditions is crucial in a successful breeding program since the ultimate goal is to continue populations in the wild. Zoos ensure this though Species Survival Plans, which also exist for invertebrates. Some examples include: The Oregon silverspot butterfly at the Woodland Park Zoo in Seattle, Washington, the American burying beetle at the Roger Williams Park Zoo in Providence, RI, and the Karner Blue Butterfly at the Toledo Zoo in Ohio. Starting captive bred populations with more founders will add value to conservation biology (i.e. increase genetic diversity/stability of the captive population) in a zoological setting (Barker, 2007). Relatedness between founder individuals also plays a role for captive populations, however Ledberg and Firmin (2008) claim that species with smaller geographic ranges may be less affected by inbreeding depression due to adaptations to living in small isolated populations. For example, a plant study in western Montana, USA on *Arabis fecunda*, which is a perennial herb that only lives on calcareous soil outcrops demonstrated the plants ability to adapt to a microclimate despite little genetic diversity with the use of common garden experiments (McKay *et al*., 2001).

The overall goal of this research is to determine how many other succineid species are known from only one location, establish at least 2 healthy captive COAS colonies for augmentation of the existing population as well as investigate habitat suitability for COAS by (1) conducting a distribution analysis of *Succinea* species in NYS (2) deciphering optimal diet for captive propagation as a means of keeping a successful long term backup populations of COAS for augmentation and future research, and (3) measuring habitat parameters for differences on the side of Chittenango Falls where COAS do not live, to determine habitat suitability for COAS.

METHODS

Objective 1: Examine the diversity and distribution of Succineidae within the North East United States: From museum collections on succineid snail species, data will be used to make comparisons between small geographic distributions and widespread population distributions. Possible translocation sites for COAS can be identified by locating past and present distributions of succineid species. The following questions can be addressed: Are succineid snails common in NY? Are there other populations that only lived or live in isolated habitats (e.g. spring snails)? Is there a decline in succineid species from past to present populations? I expect to find a decrease in numbers of different species because of the introduction of invasive snails possibly outcompeting native snails for resources. However, if there is no change, the invasive snails may just co-exist with other Succineidae. Another possible explanation would be that there are so many isolated populations that sp. B has simply not spread to those locations yet.

Objective 2: Determine the optimal captive diet for COAS, which will be measured using comparisons of fecundity, food preference, growth rate and health of the snail: Ten COAS adults measuring at least 13mm (suspected shell length for reproductive onset: Molloy, 1995), will be brought into captivity to breed and fed vegetation from their habitat as well as wild-collected detritus and leaf litter. Adults will be released back to their habitat and hatchlings will be raised on three different diets. New adult individuals will be brought in occasionally to keep genetic diversity within the captive population. To test conditions before COAS are held in captivity, sp. B snails were collected from Chittenango Falls state park on 14 September 2013. 217 snails were housed in 6 plastic containers within an incubator at the Rosamond Gifford Zoo. These snails are being used to refine settings such as temperature, humidity, enclosure model and diet for eventually keeping COAS. Each of the 6 enclosures had about 35 snails in them. Two brown paper towels are saturated with water from the falls and placed on the bottom of each container as a substrate. A food preference on sp. B was conducted as a pilot study to refine conditions for COAS. Romaine lettuce/ fish flakes and calcium carbonate/bone meal powder and wheat grass powder along with various food items such as sweet potato, grapes, kale, swiss chard, kale, and others were used for the first months to determine preferences and leaf litter was used along with the previous diet for the remaining months. The partulid diet was also tested for preference. The partulid diet consists of: 6 teaspoons of ground nettle leaf, 2 teaspoons of oat flour, 1 teaspoon of wheat grass powder, 2 teaspoons of calcium carbonate, and 1 teaspoon of ground trout chow. These dry ingredients were mixed with water to form a paste and spread on the walls of the container for snails to feed upon.

The diet requirements of COAS are still poorly known. Do COAS eat mostly fungus growing on the vegetation and/or rocks? Or are they simply eating vegetation? By setting up an experiment with inoculated petri dishes using sterile techniques with the fungus that is found on wild watercress and other vegetation or detritus that COAS live on, we can study COAS food preference. COAS was more often found on detritus or leaf litter whereas sp. B were more likely to be found on live vegetation (Campbell *et al*., 2010), but is it the leaves or the fungus they are ingesting? A simple percentage measurement shows how much fungus has been eaten in a given amount of time. The set up for enclosures of COAS will be similar to the previous captive attempt; however diet will be modified to test if one over the other yields higher fecundity and health. All enclosures will be kept in an incubator with a 12 hour light cycle using full spectrum plant/aquarium lights. The temperature will be kept at 15.5 degrees Celsius (60 degrees Fahrenheit).

For the design of the experiment (i.e. measured variables), 3 enclosures will be used. One container will have romaine lettuce sprinkled with fish flake food, calcium carbonate powder or cuttlebone and spirulina powder as a control. This diet was used for the first captive population. Flat shale rocks and brown paper towel will be used as substrate. One container will have leaf litter (mostly sugar maple (*Acer saccharum*), cherry (*Prunus serotina*) and English walnut (*Juglans regia***)** **(**since these were preferred by sp. B) and the control diet. One container will have fungus pies or soil from COAS habitat, leaf litter and the control diet in the enclosure to test micro flora preference. All other factors will remain unchanged (humidity, light cycle, temperature). Differences in success will be assessed using fecundity (number of hatchlings that survive to reproduce), growth rate, and survival rate. Growth rate data will be collected by taking measurements of shell length from all individuals with digital calipers in millimeters every 2 weeks. Mean growth rates will be compared for each type of diet given. The same method used by USFWS for mark-recapture surveys will be used to mark the snails. Honey bee tags with numbers are glued to COAS shells to keep track of individuals over 9mm in length, which is effective, non-invasive and non-lethal.

Small sample size may be an issue due to dealing with an endangered species. We cannot take many snails from the habitat because of the possibility of decreasing genetic diversity in the wild population, and USFWS's mandate to protect these snails from further decline. We will only be able to observe the number of hatchlings that survive and reproduce in captivity and their food preferences. This will be carried out over 2 years. Keeping the temperature set to 15º Celsius will produce continuous egg laying of captive COAS (Joe Brown, personal communication). To study which diet results in the highest fecundity, this method will be suitable in the short term. Long term, producing and raising hundreds of hatchlings is not feasible due to the number of hours needed to care for them. A more practical approach would be to work with 4-6 egg masses and raise those hatchlings to a certain shell length and release them back to the wild population habitat as an augmentation study. This would be a "head start" for small hatchlings if successfully reared in captivity. Hatchlings will be counted and measured every 2 weeks; growth rate and fecundity will then be compared to the first captive population's fecundity. Extracting data on growth rates and maximum shell lengths from mark recapture surveys will provide a control for any needed adjustments in captive conditions. All data will be analyzed using Minitab (Pearson Education, Inc. Boston, Massachusetts) to chart average growth rates and fecundity between the three different food choices using one-way analysis of variance (ANOVA).

<u>Objective 3: Test parameters on the other side of the falls where COAS do not live</u>: Hypothesis: COAS cannot survive on the west side of the falls because of the amount of time the area is in

the shade. This may create lower temperatures that are problematic for COAS survival. To test this hypothesis, using HOBO data loggers, temperature and humidity on the west side of the falls as well as the east side where the COAS population resides will be measured. Logging the information for at least 2 field seasons from April to May covers the COAS breeding and egg laying months as well as the winter temperatures. Comparing all parameters using one-way ANOVA for statistical analysis will determine if there are differences between habitat parameters on either side of the falls. I can also look into vegetation differences. If there are no significant differences, then the west side of the falls may be considered a suitable translocation site to establish a second COAS population at in the future.

CONCLUSIONS

In conclusion, my work will include: (1) captive-bred live snails that will supplement the current COAS population and thus enhance persistence of the species in NYS; (2) management recommendations for future captive breeding of COAS and for endangered temperate land snails generally, particularly those that are geographically restricted (e.g. Is a long term captive population feasible? Will supplementing the wild population increase numbers?) and (3) recommendations for future reintroductions of COAS and other temperate land snails. This research will contribute to slowing the loss of biodiversity, increase the body of knowledge about understudied species, and provide a template for conservation strategies of other temperate land snail species.

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