Ann Holmes, ECL290: Grand Canyon Seminar, Spring 2018

Conservation of the Kanab ambersnail: Snail ecology, species boundaries, and adaptive management in the Grand Canyon

Charles Darwin knew the value of studying invertebrates. In addition to his most famous evolutionary studies of Galapagos finches, Darwin also spent much of his career researching lowly barnacles and earthworms. Invertebrates, still often described as "lower organisms," are scientifically undervalued. However, invertebrates play important ecological roles and should be considered in conservation efforts. Like Darwin's barnacles and worms, snails are not, at first glance, charismatic species. However, snails consume detritus and plants, concentrate calcium to pass up the food chain, and provide food for predators such as birds, fish, and amphibians. Many of these predators are declining or at risk of extinction.

Snails living in aquatic habitats are also at risk of extinction due to climate change, pollution, and loss of freshwater habitat. Close association with water and limited mobility puts aquatic snails and other mollsucs on the front line of anthropogenic impacts to their habitat. In fact, the number of mollusc extinctions caused by humans exceeds those of birds and mammals combined (Ponder et al. 1995). An estimated 450 species of freshwater snails live in the Western US (Hurt 2004). In Utah, an intensive survey of terrestrial and aquatic snails found several species in peril (Clarke 1991). The results recommended four snails be listed as endangered and threatened; several other snails were not detected and were presumed extirpated from Utah. One of the snails recommended for listing as endangered, the Kanab ambersnail (*Oxyloma haydeni kanabensis)*, was found at a single site (Three Lakes) that was slated for imminent development. The threat of habitat destruction created an urgency for conservation action. Subsequent surveys found the Kanab ambersnail at only one additional site, Vasey's Paradise in the Grand Canyon. Population stability at these sites is poorly understood; the Kanab ambersnail has highly variable population sizes, which makes it hard to accurately census (Sorensen and Kubly, 1997; Gloss et al., 2005; USFWS 2011). The Kanab ambersnail was federally listed endangered in 1992 (USFWS, 1991; USFWS, 1992).

Kanab ambersnail. Image from Arizona Game and Fish.

The Kanab ambersnail is faced with a complex set of challenges for conservation and management. The challenges include: 1. a restricted range and narrow set of habitat requirements; 2. lack of clarity of species boundaries; and 3. conflict between endangered species management and ecosystem adaptive management. Although the combination of these challenges complicates conservation efforts of the species, no single challenge is unique to Kanab ambersnails. Further analysis of these issues can inform conservation and management of endangered species generally, perhaps improving conservation outcomes for both Kanab ambersnails and other species.

Biology and Ecology of the Kanab ambersnail

The Kanab ambersnail is a tiny, air-breathing snail that inhabits spring-fed wetlands. The snail's shell is approximately 1 cm long and amber in color, as indicated by the common name. Related species of ambersnail are difficult to distinguish from each other and show only subtle morphological differences. The Kanab ambersnail was originally distinguished from other *Oxyloma haydeni* subspecies by a slender shell spire and smaller shell opening (Pilsbry 1948; Spamer and Bogan 1993). However, subsequent genetic analysis indicated that shell morphology alone is not a reliable way to distinguish *Oxyloma* species from each other (Culver et al. 2007). Shell morphology may be a plastic character that is either highly variable between individuals or reflects environmental conditions rather than species affiliation. Additional morphological features such as the anatomy of genitalia, proportion and arrangement of organs, and pigmentation of tissue, as well as habitat assessment should be considered when distinguishing *Oxyloma* species (USFWS, 2011). The habitat of the Utah population at Three Lakes is poorly known because this site is located on private land and thus more difficult for scientists to access. In the Grand Canyon, the Kanab ambersnail is closely associated with native crimson monkeyflower, *Mimulus cardinalis* (USFWS 2011).

Vasey's Paradise. Image by Andrew Gulliford.

The range of the Kanab ambersnail is very limited, even in comparison with other aquatic snails. Loss and modification of wetland habitat is a major threat to Kanab ambersnails (USFWS, 2011) and other species that depend on springs. Outflow from springs is sensitive to both low precipitation and groundwater pumping, and changes in flow can impact snail populations (Myers & Resh, 1999). To expend the number of populations, Kanab ambersnails were translocated from Vasey's Paradise to three other Grand Canyon sites in 1998; Kanab ambersnails successfully established at only one of the sites, Upper Elves Chasm (Sorenson, 2005). This site is significantly removed from the bank of the Colorado River. Populations of both *O. h. haydeni* and *O. h. kanabensis* have also been described from Alberta, Canada based on morphology (Harris and Hubricht, 1982). It is hypothesized that ambersnails are dispersed by birds; indeed, a small proportion of consumed snails can survive passage through birds' digestive tracts (Green & Figuerola, 2005; Wada et al., 2011). Genetic identification of specimens from Alberta do not always agree with the morphological identification (Stevens et al., 2000; Culver et al. 2013). If these Albert snails are indeed Kanab ambersnails, it is unclear why no populations have been identified between the US Southwest and Alberta. It is possible that such Kanab ambersnail populations do exist along the flyway but have yet to be discovered, or that historical populations along the flyway are now extirpated.

Conservation and species boundaries of Kanab ambersnails

In order to justify endangered species protections, it is important to demonstrate that the Kanab ambersnail is distinct from the closely related and more abundant Niobrara ambersnail (*O. h. haydeni*). Unlike vertebrates, invertebrates cannot be listed for protection as distinct population segments. The Kanab ambersnail was originally listed under the Endangered Species Act based on morphological features (USFWS, 1991; USFWS, 1992). While morphological features can be used to identify populations for conservation, morphological identification alone can be unreliable. First, some species exhibit significant phenotypic variability such that identifying and differentiating individuals from the species of interest is challenging or impossible (Meier et al., 2006). Second, related species can show little to no morphological variation. Cryptic species are a frequently unrecognized aspect of biodiversity (Fišer et al., 2017). DNA sequencing has provided an important window into previously unrecognized aspects of biodiversity (Bickford et al., 2007). Cryptic differences in invertebrates such as mosquitoes (Endersby et al., 2013) and copepods (Goetze 2003) has ecological implications. Finally, morphological and genetically similar individuals rarely exist in homogenous populations. Some individuals or populations may have unique characteristics that simply do not exist throughout the population. Thus, genetic data is crucial for listing distinct population segments of vertebrates for protection. For example, in Pacific salmon, run migration timing is a unique phenotype that deserves conservation protection because it appears to be the result of a rare evolutionary event (Prince et al., 2017). The appropriate metric for conservation incorporates the evolutionary significance of variants in populations or species (Moritz, 1994).

Conservation increasingly incorporates genetic data as a means to define the appropriate "unit" to conserve (e.g. a population, regional populations, or species and subspecies). Genetic methods have been used to compare ambersnail populations in order to understand species boundaries and the appropriate conservation unit for the species. It is important to note that genetic methods incorporate a wide range of approaches and have developed significantly over nearly three decades. Early methods such as amplified fragment length polymorphism (AFLP) were used to compare a very limited number of genetic differences between individuals or populations. These types of analyses are useful, but often do not tell the whole story. Mitochondrial sequences and microsatellite markers are more powerful ways to determine conservation units. However, these methods can also have weaknesses such as the inability to distinguish species or populations. State of the art methods such as restriction site-associated DNA sequencing (RAD-seq) are better able to compare thousands or hundreds of thousands of genetic differences, this confers orders of magnitude more power to genetic analysis. However, RAD-seq is more expensive and has certain requirements such as the availability of genome data for mapping genetic differences.

Genetic methods have been used to evaluate the Kanab ambersnail populations to determine metrics relevant to conservation status. AFLP and mitochondrial markers indicated that both populations of the Kanab ambersnail were distinct from each other and from two populations of Niobrara ambersnails in the Grand Canyon (Miller et al., 2000; Stevens et al. 2000). The genetic distances observed in this study appear to support the genetic distinctiveness of the populations from each other when compared with similar analyses in other land snails (Jarne, 1995; Miller et al. 2000). However, the results indicated that the two US southwest Kanab ambersnail populations were not each other's closest relative (Miller et al., 2000). It is unclear whether protected status has been incorrectly applied to the Kanab ambersnail (because it is not distinct from the Niobrara ambersnails), or if all populations in the Grand Canyon are distinct from other *Oxyloma* and should be given protected status.

Additional genetic analysis did not support the differentiation of *O. h. kanabensis* from *O. h. haydeni* (Culver et al., 2013), but the study had some flaws. The same mitochondrial markers used in previous analysis were used in a new analysis, along with nuclear markers and one microsatellite marker (Culver et al., 2013). Like the previous study, mitochondrial markers showed evidence of population structure between snails at different sites, and evidence that the Vasey's Paradise population was more different genetically. This result supports the designation of the Vasey's Paradise as an endangered subspecies or, at the very least, a population that may harbor important genetic diversity. Genetic diversity has been identified as a key conservation priority (although distinct population segments are not currently protected for invertebrates). Phylogenetic relationships of ambersnails observed using nuclear markers were not consistent with relationships observed using mitochondrial markers (Culver et al., 2013). The nuclear markers analyzed indicated gene flow between populations, which does not support the designation of the Vasey's Paradise as an endangered subspecies (Culver et al., 2013). However, the most commonly used nuclear markers for genetic comparison of closely related populations (microsatellites) were not used effectively in this study; only one microsatellite marker could be developed. Usually at least 6-10 microsatellite markers are used for this type of analysis. For example, genetic analysis of the flat-spired three-tooth land snail (*Triodopsis platysayoides*) developed ten microsatellite markers to analyze population structure and glean demographic insights relevant to conservation (King et al., 2015). The inability to develop multiple microsatellite markers for the Kanab ambersnail could indicate genetic differences relevant to conservation. Moreover, the study authors admit that sample degradation (which can make DNA sequencing more difficult or impossible) may have been a confounding factor for genetic analysis.

Overall, the genetic data on Kanab ambersnails is conflicting and incomplete. Although multiple loci were analyzed, many of the approaches are outdated and analyses have been completed lacking important data. Despite the confusing results from these genetic data, analyses do pair genetic analysis with a morphological comparison (Culver et al., 2013). The results strongly indicate that morphology is not a reliable method for identifying *Oxyloma* (Culver et al., 2013). Given the unreliability of morphological characters and the conflicting genetic results and interpretations, the Kanab ambersnail remains a listed species. More in-depth genetic studies (e.g. RAD-seq) are likely to provide useful and needed genetic data for management of *Oxyloma* in Arizona and Utah. RAD-seq has yielded surprising information on fine-scale genetic structure that has been crucial for proper management of other at-risk species such as Atlantic cod (*Gadus morhua*; Therkildsen et al., 2013). A better understanding of genetic structure in *Oxyloma* is especially crucial for the Vasey's Paradise population, which faces particular risks due to management of flow in the Colorado River.

Conservation of Kanab ambersnails and high-flow events

Kanab ambersnails at Vasey's Paradise inhabit a region that is extensively managed for human use. Vasey's Paradise is directly along the bank of the Colorado River, an ecosystem which was historically subject to high hydrological variability. At Vasey's Paradise, non-native watercress (*Nasturtium officinale*) and native monkeyflower constitute the snails' riparian habitat. The impact of watercress on the Kanab ambersnail at Vasey's Paradise is unclear; while non-native plants are usually undesirable, a single study actually indicates improved growth and fecundity of Kanab ambersnails raised on watercress compared with snails raised on native crimson monkeyflower (Nelson, 2001). Although the role of host plant quality is poorly understood, hydrological modification after dam closure has increased vegetation cover available for Kanab ambersnail habitat by approximately 40% (KAIMG 1997). Historical photographs indicate that prior to 1963 flooding routinely scoured the lower limit of ambersnail habitat (Meretsky et al., 2000).

Although hydrological modification increased snail habitat at Vasey's Paradise, dam closure is had an overwhelmingly negative impact on the Colorado River ecosystem as a whole. With some exceptions, the river has not flowed through to its original endpoint in the Gulf of California since dam closure. Dammed rivers can no longer serve important functions such as flushing of sediments, dilution of polluted water, and control of salinity intrusion on natural and agricultural landscapes (Molle et al., 2010). In dammed ecosystems, sedimentary processes are greatly altered, degrading river features used by fish and for recreation. Deliberate high-flow events are one solution to address problems caused by dams (Melis et al., 2015). High-flow events are proposed to improve ecosystem health by scouring banks, mobilizing sediments, and deepening channel areas used by native fishes (Meretsky et al., 2010). However, high-flow experiments must balance potential benefits to whole ecosystem health with potential risks to endangered species such as Kanab ambersnails and their habitats. High-flow experiments can require careful balancing of these two priorities.

The 1996 high-flow experiment, conducted in an adaptive management context, was the first experiment of its type conducted in the Grand Canyon. At that time the Kanab ambersnail population at Upper Elves Canyon had not yet been established; Vasey's Paradise adjacent to the Colorado River was one of only two known populations of Kanab ambersnail. The high-flow experiment carried a risk of a severely negative impact on the Kanab ambersnail (by washing snails downriver) and its habitat (by removal of vegetation; Meretsky et al., 2000). Full replication and control of the high-flow experiment, as is expected in conventional scientific approaches, was not possible (Walters and Green, 1997). Though conventional science provides a suboptimal framework for addressing uncertainty is management scenarios, adaptive management provides a better framework. Endangered Species Act protections required management to mitigate negative impacts on Kanab ambersnails. However, given the experimental nature of the flood, important metrics such as the maximum water levels during the flood were unknown. Managers developed a plan to ensure that enough snails were removed from areas likely to be scoured away without unnecessarily disturbing habitat that was unlikely to be damaged in the flood. Based on observations of high snail abundance from biologists in the field, USFWS provided last minute adjustments their previous directive in order to preserve more habitat (Meretsky et al., 2000). The 1996 flood did in fact damage some lower areas of the ambersnail habitat, which took about 2.5 years to recover (Stevens et al., 1997). For a subsequent high-flow experiment in 2004, Arizona Fish and Game Department removed 25-40% of the vegetative habitat prior to flooding and replaced the vegetation after the flood water subsided; this approach resulted in a quicker habitat recovery time of just 6 months (USFWS 2011). Management of Kanab ambersnails at Vasey's Paradise can be seen as an adaptive management success story: agency biologists accurately assessed and mitigated risks to an endangered species while supporting positive ecosystem outcomes. A healthy population of Kanab ambersnails persists at Vasey's Paradise (Meretsky et al., 2000).

Future Directions

Continuing protection of Kanab ambersnails presents a complex set of challenges. At a basic level, species boundaries and genetic diversity in *Oxyloma* is poorly understood. In fact, biodiversity of snails in the Western US (Hershler 1998) and invertebrates generally (Dunn et al. 2008) is also poorly understood. Agency managers have taken a conservative approach by retaining endangered species protections for Kanab ambersnails in the face of conflicting genetic analyses. Newer and more powerful genetic approaches may help to resolve species boundaries in *Oxyloma*, but given the limited number of populations, the Kanab ambersnail and genetically related snails do appear to be at risk of extinction. In terms of habitat, spring-fed wetlands continue to be at risk due to heavy exploitation of groundwater in the US Southwest (Falkenmark and Molden, 2008). On a more positive note, many of the lessons learned from adaptive management in the Grand Canyon can be applied to other species. Kanab ambersnails in the Grand Canyon present an excellent case study to develop adaptive approaches that benefit species and ecosystems. However, ecologically important species will continue to face extinction unless management broaden to include improving conservation outcomes for more invertebrates.

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