Abundance, Distribution, and Habitat Use of the Grand Wash springsnail (*Pyrgulopsis bacchus*), Grand Canyon-Parashant National Monument, Arizona

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By

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and

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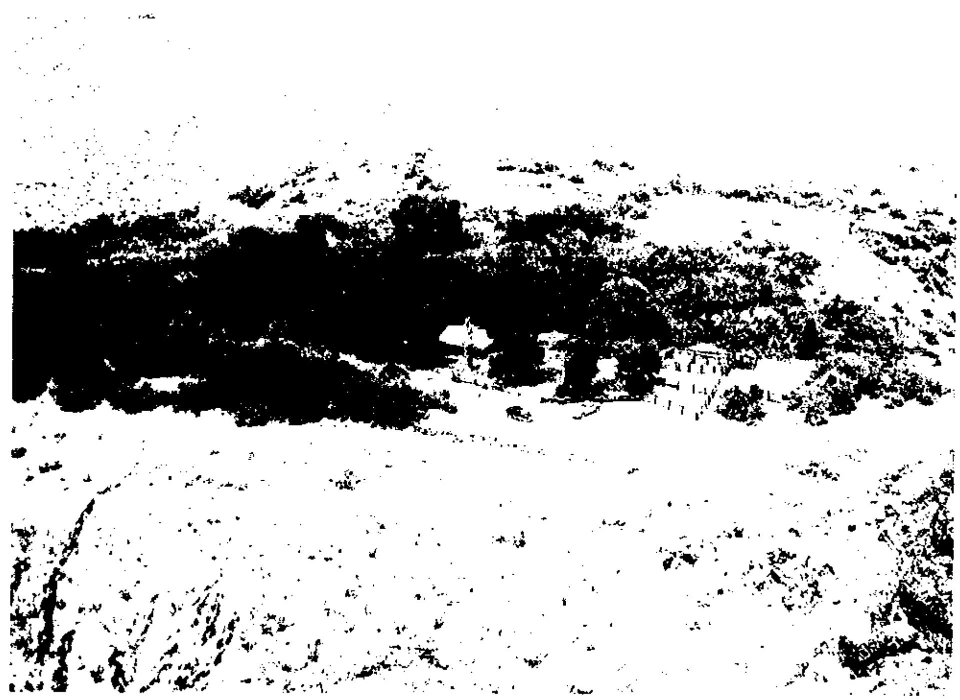

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

The Grand Wash springsnail (*Pyrgulopsis bacchus*) is a crenobiontic species (ergo obligatory spring-dwelling) that is endemic to springs in the Grand Wash system, Mojave County, Arizona. The only known population on Federal land occurs at Tassi Springs, which is an historic homestead and a province of three springs. This report summarizes studies that were conducted during May 2004 to quantify the distribution, abundance, and habitat preference of the Grand Wash springsnail, and to recommend management for its conservation.

Tassi Springs lies at approximately 500 m elevation in the Mojave Desert and consists springs, several buildings, and a system of dry ditches that historically delivered spring water to croplands. Each spring has been altered from its natural condition; one has been captured in spring box, one in pipe, and the other excavated by heavy equipment and diverted into a new channel. Not only have the springs been altered, but the path of historic spring brooks is now in conflict with homestead buildings. As a result, spring brooks do not follow natural pathways. In spite of these alterations, two of the springs support approximately 200 m of isolated, and comparatively large, hypocrenal and wash habitat in the desert landscape.

During the May 2004 surveys, the Grand Wash springsnail was extant in only the excavated spring where it was limited to the upper 65 m of the upper spring brook (the other springs have been so thoroughly altered that historic springsnail occupation is uncertain). It occupied approximately 70 m^2 of habitat. Mean density within 25 cm^2 quadrats ($n = 85$) was 29.0 individuals (range 0-160), and greatest density occurred in the reach of spring brook from 10 m to 40 m from the spring source. It was the most abundant member of the aquatic macroinvertebrate community in this habitat, and it preferred habitat that was shaded by riparian vegetation or instream cover, and aquatic habitats that were deep (4 cm – 10 cm), slow ($<$ 30 cm/sec), and warm ($\sim$ 24°C). It also preferred watercress (*Rorippa* sp.) and submerged roots of the riparian vegetation (e.g., willow).

There was no preference for substrate size, but it avoided cold and warmer waters and habitats with *Potomoeton* sp. (an aquatic plant).

Consistent with other persistent arid land springs, this spring is also occupied by a variety of additional, often rare, aquatic species, including toad bugs (*Nerthra* sp.), riffle beetles (*Microcylloepus* sp.), true bugs (*Ambrysus* sp.), speckled dace (*Rhinichthys osculus* ssp.), and red-spotted (*Bufo punctatus*) and southwestern toads (*Bufo microscaphus*). Presence of this suite of aquatic species indicates that Tassi Springs has been a persistent aquatic habitat and a refuge for a long time (probably > 10,000 years). Because of their persistence, these springs also support dense riparian vegetation and a wide variety of terrestrial animals.

Management recommendations include maintaining upper Tassi Spring and the spring brook created by discharge from all springs in their existing condition, decrease arrow week (*Pluchea* sp.) to allow reestablishment of willows in the riparian zone, conduct surveys to identify other distinctive crenobionitic species, and install gauges to monitor spring discharge.

Qualitative and quantitative monitoring programs to assess changes in the aquatic environment and the abundance and distribution of *P. bacchus* are described.

INTRODUCTION

The Grand Wash springsnail (*Pyrgulopsis bacchus)* was described in 1988 from several springs in Grand Wash, Mohave County, Arizona (Hershler and Landye 1988). It is endemic to springs in this area, which occur on private lands, and lands administered by the U.S. National Park Service (NPS) and U.S. Bureau of Land Management. The only known locality on Federal land is Tassi Springs, which is an important historical site for homesteading, ranching and agriculture. No studies have examined the demography, habitat use, distribution, or environmental factors affecting the abundance and distribution of the Grand Wash springsnail. Ecological information is needed to guide management through the challenges of protecting the historical and ecological values of Tassi Springs. This study assessed *P. bacchus* demography by examining its habitat use, distribution, and abundance at Tassi Springs. The specific questions addressed were: 1) What is the distribution and abundance of *P. bacchus*? 2) What habitat does *P. bacchus* prefer? and 3) What recommendations are necessary to restore springs and preserve *P. bacchus* populations while maintaining the historic landscape at Tassi Springs?

GENERAL SPRINGSNAIL ECOLOGY

The family Hydrobiidae has a worldwide distribution that is represented in North America by approximately 285 taxa in 35 genera (these numbers change frequently because additional species are being described at regular intervals). There are several genera in the western U.S. Species in the genus *Pyrgulopsis* are the most widespread with approximately 130 species (Hershler 1998, Hershler and Sada 2002). *Tryonia* is the next most common with 22 extant species in North America (Hershler 2001), which is followed by the genus *Fluminicola* with 6 North American species (Hershler and Frest 1996, Hershler 1999). *Eremopygrus* is a monotypic genus endemic to Nevada (Hershler 1999), and *Ipnobius* is a monotypic genus endemic to Death Valley National Park (Hershler 2001).

Current taxonomy is based largely on penial morphology that can be examined only by microscope after rather tedious methods of relaxation and preservation have prepared the material. There are no keys to assist with identification, but existing literature can be used to identify species by those familiar with terminology. Recent genetic studies have shown that most described species are valid taxa, but that morphologically-based taxonomy is usually conservative and that further genetic analysis is likely to show that populations of some currently recognized species maybe described as new species in the future. Hamlin (1996) found fixed allelic differences among populations of *P. wongi* in eastern California and western Nevada and Hershler et al. (1999) found similar differences among populations of *T. variegata* in southern Nevada and southeastern California. Genetic analysis of *P. micrococcus* populations also revealed greater diversity than was suggested by morphological analyses (Liu et al. 2003). These results are contrary to genetic analyses of *Pyrgulopsis* in the Snake River system and northern Great Basin that resulted in synonomy of several species (Hershler and Liu 2004). A genetic study of 50 *Pyrgulopsis* spp. has been initiated, which should be completed during 2005.

The distribution of most springsnails is limited; a number are known only from single valleys, others from several valleys, and some species appear to be widespread. It is uncommon for a habitat to be occupied by more than one species, and less common for a spring to be occupied by more than a single species in a genus. Only *P. kolobensis* and *P. gibba* are comparatively widespread in the western U.S. *P. kolobensis* is known from habitats along the Virgin River, northward along the east side of the Great Basin to the Snake River, then westward across the Snake River Plain into central Nevada. This taxon includes a number of different morphological types and it will probably be separated into a number of different species when genetic studies have been conducted. *P. gibba* occurs along the western Great Basin from the Walker River system north into southeastern Oregon and east to central Nevada (Hershler 1998).

It is difficult to determine the number of springs in the southwestern U.S., but it is reasonable to estimate that there are more than 100,000. Springsnails have been collected from approximately 800 habitats. Most surveys have been limited to habitats lower than 2,000 m elevation (springsnails are rarely found above this elevation) and most surveys were made to collect material for taxonomic and biogeographic studies. Although these surveys provide some status information, it is unlikely that all populations of most species have been located, and new species continue to be found at previously unvisited sites. Sampling effort was limited during these surveys because funding was inadequate to visit all potential habitats, springsnail diversity that is known today was not anticipated, and taxonomic diversity is not high in most basins, which indicates that it is unnecessary to locate all populations to determine the taxonomic diversity of most basins. It would be surprising if new populations of most species are not found with additional surveys. It is important to realize the infantile status of springsnail taxonomy and that new techniques and future studies will increase knowledge of diversity and distribution. It has taken Great Basin fish taxonomy more than 70 years to reach the current level of understanding about genetic diversity; clearly it will be years before this level of knowledge can be achieved with springsnails.

Much of the knowledge about springsnail ecology comes from observations made during taxonomic and biogeographic surveys, but recent work by Mlandenka (1992), Sada and Herbst (1999, 2001, 2004) and Sada (2001, 2000) quantitatively describe habitat use and environmental factors affecting community structure of several species. Springsnails are small (usually < 5mm high) and they are generally limited to spring sources and a short length of spring brook. Few species occupy large rivers (e.g., *Pyrgulopsis archimedis* and *P. robusta* from the Klamath and Snake Rivers, respectively) and lakes (e.g., the now extinct *Pyrgulopsis nevadensis* that occurred in Pyramid and Walker Lakes). They feed on algae gleaned from substrate and aquatic vegetation, and they occupy habitats with good water quality. Diatoms may be their preferred food, but food ingested by *P. bruneauensis* was proportional to the availability of algae in its habitat (Mladenka 1992). Hydrobiids occupy a number of different substrates and it appears that most species prefer either sand, gravel, or cobble. They also occur on aquatic vegetation, and they usually occupy slow to moderate (> 0 cm/sec and < 40 cm/sec) currents. Water temperature of their habitats ranges from 3°C to 36°C and discharge rates of springs they occupy ranges from < 0.0001 m^3/sec to 0.28m^3/sec. *Pyrgulopsis* spp. and *Fluminicola* spp. generally occur in colder water (although several *Pyrgulopsis* spp. occur in thermal springs, e.g., Ash Meadows, Soldier Meadow, and Fly Ranch Geyser, NV, and Bruneau Hot Springs, ID) and *Tryonia* spp. always occur in thermal habitats. Populations may be large (> 10,000/m^2, and habitats may be several hundred meters long) and small populations (< 1,000 individuals) are uncommon, even though habitat size may be limited. Longevity is probably about one year. Reproduction may occur throughout the year, but peak periods are in the late winter, spring, and late summer. Mladenka (1992) and Sada (2001) observed several cohorts in monthly and seasonal samples of *P. bruneauensis* and Badwater snail (*Assiminea infima*, a close relative to springsnails) populations, respectively. In warm habitats preferred by *P. bruneauensis,* Mladenka (1992) found that sexual maturity was reached in approximately two months, and maximum size was reached in four months. Sex ratio in this species was 1:1. Nothing is known about reproductive habitat requirements.

Springsnails are not cosmopolitan inhabitants of their habitats, and it appears that many species occupy different microhabitats. In thermal springs of the Muddy River, Clark County, Nevada (inhabited by three species of springsnails), *P. carinifera* preferred shallow habitats with moderate current velocities between 30 cm/sec to 40 cm/sec, and avoided current velocities > 50 cm/sec. It showed no preference for substrate type. *P. avernalis* preferred deeper water with swift current (> 50 cm/sec) and gravel substrate. *T. clathrata* was less abundant than either *Pyrgulopsis* spp., and it preferred shallow habitats with low current velocity (< 10 cm/sec). It strongly avoided deeper habitats and current velocities > 10 cm/sec, while selecting habitats without gravel or cobble substrates. It slightly and strongly preferred fines and sand substrates, respectively. It also preferred habitats with algae and CPOM. In SoldiersMeadow, Humboldt County, *P. notidicola* occurs only near the source of thermal springs in semi-aquatic habitat that is within 1 cm of water and in the splash zone at the base of riparian grasses and emergent rocks, and *P. limaria* occurs in downstream habitats where comparatively swift water flows over cobble substrate. In thermal springs along the east side of Death Valley, Sada and Herbst (2004) found that *Ipnobius robustus* was scarce near spring sources and most abundant from 50 m – 150 m downstream. It preferred warm water (approximately 34°C), and moderate current velocity and water depth. It avoided slow and fast currents (< 5 cm/sec and >35 cm/sec), low and high water temperatures (<32°C and > 40°C), shallow and deep water (< 4 cm and > 14 cm), and open riparian cover (< 20% and > 80%).

Temporal variation in Badwater snail abundance at springs on the Death Valley floor was not statistically significant, however, spatial variation was high between Badwater and Cottonball Marsh habitats (Sada 2001). In four Muddy River springs, Sada (2000) found temporal variation was significant for populations of *P. carinifera* and *P. avernalis,* and *T. clathrata*. Temporal variation in *P. owensensis* was not statistically significant in a single cold spring along the east side of the White Mountains, Mono County, California (Sada and Russi fieldnotes). Temporal variation was also minimal in *I. robustus* populations occupying thermal springs in the Funeral Mountains of Death Valley National Park (Sada and Herbst 2004).

Mladenka (1992) reported that *P. bruneauensis* abundance was influenced by food availability, predators on juvenile snails (by guppies, *Libestes reticulatus*, and aquatic insects, Zygoptera), and water temperature. Sada and Herbst (1999, 2004) found that macroinvertebrate communities dominated by hydrobiids were most strongly influenced by water temperature, water velocity, substrate, distance from the spring source, water depth, conductivity, and riparian cover. Their work in several Death Valley thermal springs also indicated that different environmental factors affect communities in each spring.

Cultural factors affecting spring condition also affect springsnail abundance and distribution. Mladenka (1992) reported decreased *P. bruneauensis* abundance from livestock trampling. Sada and Nachlinger (1998) reported extirpation of springsnail populations by diversions, and Sada et al. (2000) speculated that springsnail populations on Ruby Lake National Wildlife Refuge, Elko County, Nevada, had been extirpated by dredging. Hershler and Sada (1987) reported that springsnails were lost when Jackrabbit Spring was dried by ground water pumping, Landye (1981) found that ground water pumping caused extinction of several species in northern Mexico, and excessive ground water extraction is affecting *P. bruneauensis* (Mladenka 1992). Recent surveys indicate that extinction of *P. coloradensis* (known only from Blue Point Spring, Lake Mead National Recreation Area) may have been caused by introduction of the red-rimmed thiara (*Mealnoides tuberculata*) (Sada field notes). Sada and Vinyard (2002) reported that extinction of *P. ruinosa* occurred after springs were impounded on the McNett Ranch in Fish Lake Valley, Esmeralda County, Nevada. Sada (field notes) reported extirpation of a *P.*

hubbsi population from Hiko Spring, Lincoln County, Nevada, following development of the spring source to maximize diversion potential.

TASSI SPRING SITE DESCRIPTION

Tassi Springs occur on Tassi Ranch in Section 13,T 33N, R16W, Mojave County, Arizona. They lie approximately 15 km upstream from Lake Mead along a wash that is tributary to Grand Wash. Three springs, a homestead with a house, several out-buildings that cover approximately 0.5 hectare (Figure 1), and several hectares of pasture and agriculture land comprise Tassi Ranch. Much of the area has been modified since the early 1900's by agricultural uses that cleared land and diverted spring water through a series of ditches to support alfalfa and forage crops related to cattle production. These ditches were maintained through the 1980s for ranching and they are part of the historical Tassi Ranch.

All of the springs on Tassi Ranch have been modified from natural conditions when they flowed to the southeast, off of a hillside, and onto the wash floor. The highest, and largest, spring (and the only spring occupied by *P. bacchus*; No. 1 on Figure 1) has been excavated and diverted southward from its historic path in a naturalized ditch. The ditch extends for approximately 50 m where the channel bends sharply and cascades down a steep slope onto the wash. The existing ditch may have supplied water to historical ditches that delivered water to agriculture lands that lie above the wash and northeast and southwest of the upper spring (No. 9, Figure 1). All of these routes differ substantially from the historic, undiverted spring brook that followed the fall-line into the wash. The middle elevation spring is small and has been modified by a springbox (No. 2, Figure 1). There is no discernable discharge or spring brook at this spring, but its water supports obligatory wetland plants over approximately 30 m^2. It is not possible to discern the historic, natural condition of this spring. The lowest spring (No. 3, Figure 1) has no natural features, and its location is indicated only by a rubber pipe (approximately 3 inches in diameter) emerging from the ground. All of its discharge is captured in this pipe and delivered to a metal trough (No. 6, Figure 1) that is located approximately 40 m from where the pipe emerges from the ground. Similar to the middle spring, it is not possible to determine the natural

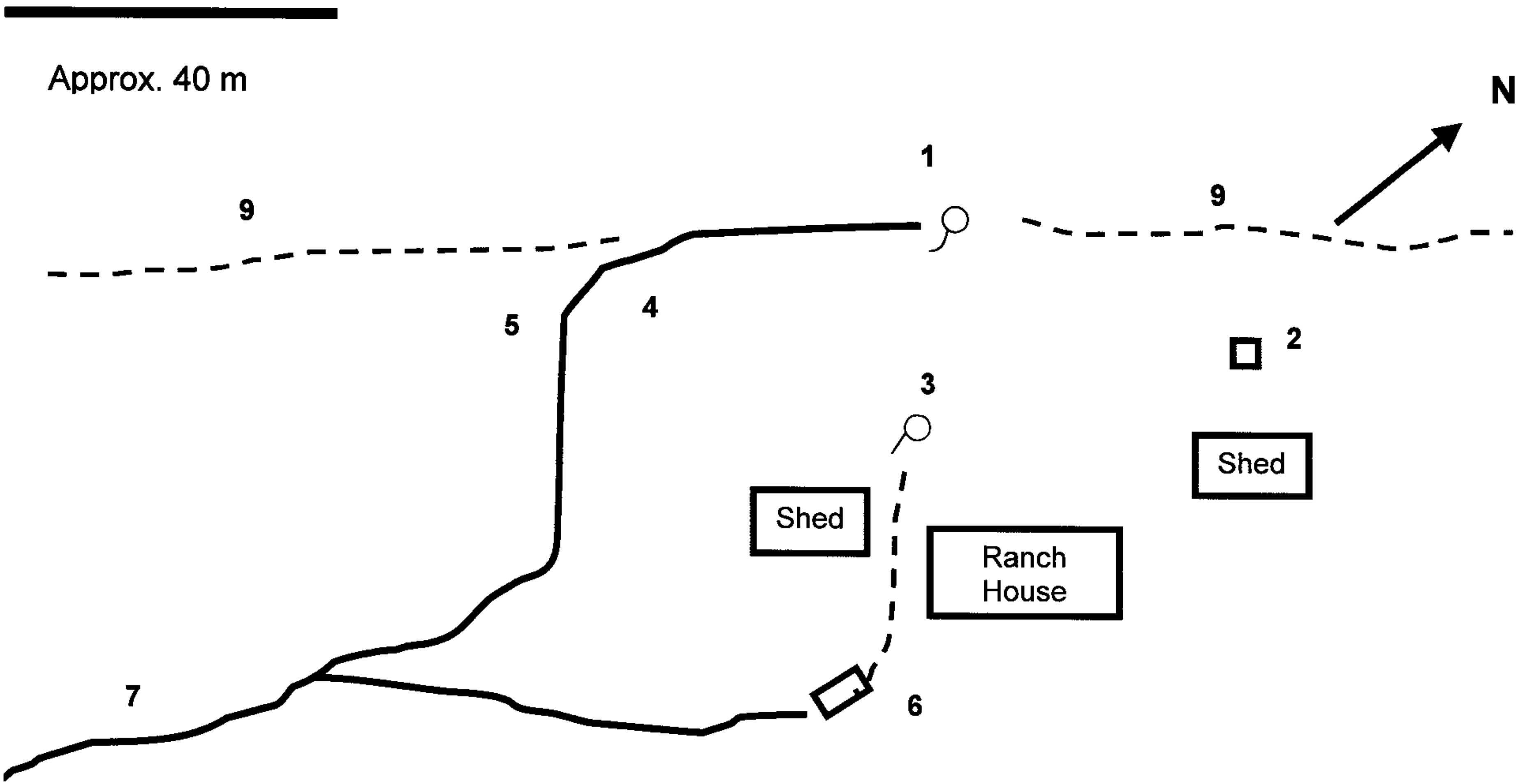

Figure 1. Schematic representation of the location of ranch buildings, spring sources, spring brooks, historic ditches, and the downstream distributional limits of important aquatic species at Tassi Springs, Mojave County, Arizona. Dashed blue line shows the pipeline route and the probable historic path of the lowest spring's spring brook; dash orange lines indicate historic diversion ditches. Numbers represent important locations on property. See Table 1for description and UTM coordinates of important sites.

Table 1. UTM coordinates (Zone 12) of important points shown on Figure 1 that represent the distribution of important aquatic species and important features of Tassi Springs, Mojave County, Arizona.

Number	Feature	Northing	Easting	PDOP
1	Source, Upper Tassi Spg.	4016471	234237	4.16
2	Middle Spring Springbox	4016443	234276	3.75
3	Where Pipe Capturing Lower Tassi Spg. Enters Ground	4016442	234238	4.42
4	Point Where Upper Tassi Spg. Turns & Begins Down Cascade	4016451	234179	3.77
5	Lowest Point *P. bacchus* Observed	4016441	234181	3.89
6	Location of Trough	4016415	234232	3.56
7	Lowest Point *R. osculus* Observed	4016314	234085	3.76
8	Terminus of Water in Wash Spring Brook	4016149	233872	3.17
9	Location of Historic Ditches that Diverted Water to Agriculture lands			

condition of the lower spring. After leaving the trough, its water flows onto the wash and combines with upper spring water to flow approximately 150 m before percolating into the wash and drying (No. 8, Figure 1). Springsnails presently occur only in approximately 65 m of the upper spring (No. 5, Figure 1). They do not occupy the middle and lower springs, and there is no evidence that these springs were occupied by springsnails. The substantial discharge from the lower spring and its possible confluence with the upper spring suggests that it was historically occupied by springsnails. The small size of the middle spring suggests that it was not a persistent aquatic habitat and the historic occupation by springsnails was less likely. Aquatic communities in the two lower springs are depauperate and typical of springs that are ephemeral or highly disturbed by cultural activities (Sada et al. 2005).

The upper and middle springs have naturalized from past disturbance and the currently support functioning riparian and aquatic communities. Upper reaches of the highest spring are densely covered by arrow weed (*Pluchea* sp.) and willow (*Salix* sp.). In the wash, these species are replaced by dense mesquite (*Prosopis velutina* and *Prosopis glandulosa*) and salt cedar (*Tamarix* sp.). Several large cottonwood trees (*Populus fremontii*) are near the homestead and along the wash. The lower spring also supports these community types, but only downstream from the trough.

METHODS

Aquatic Communities

A 12″ D-frame net was used to qualitatively sample (ergo 'grab sample') the aquatic community near the source of the upper spring and determine the potential presence of other rare species. The sample was a composite taken from all types of aquatic habitat (e.g., center of channel, backwater, open and densely covered by vegetation, etc.). All vertebrates were released and only macroinvertebrates were preserved. Samples were preserved in 90 percent ethyl alcohol and returned to the laboratory for sorting, enumeration, and identification.

Habitat and Demography

Distribution, habitat, and population studies were conducted in early May 2004 and followed similar methods described in Sada (2000). All aquatic habitats on the homestead were qualitatively sampled to assess springsnail distribution. Quantitative sampling was limited to the upper spring (which supported the only springsnail population) and the wash. This sampling occurred where vegetation density was sufficiently low and did not prevent access to the spring brook. Samples were taken along transects spanning the spring brook and oriented perpendicular to the flow of water. Transects were placed every 2.5 m along the spring brook that supported springsnails and where riparian vegetation was sufficiently open to allow access to the aquatic environment. Transects were placed every 20 m along the spring brook in the wash, which did not support springsnails. A total of 23 transects were sampled ranging from the spring source to 162 m downstream. Habitat measurements and population estimates were made within 25 cm^2 (5 cm X 5 cm) quadrats that were placed at 5 evenly-spaced points along each transect, yielding a total of 114 habitat and population sample points along the spring brook (one sample at 50 m from the spring source was eliminated because the population estimate was questionable and possibly inaccurate).

Springsnail density in each 25 cm^2 quadrat was estimated using a modified surber sampler to collect snails and temporarily remove them from the spring brook. Estimates were made using depletion techniques (White et al. 1972); most estimates were made from three depletions. Two species of mollusks occurred along the spring brook: *Physa* sp. and *P. bacchus*. Mollusks were identified to species, counted, and returned immediately to the spring brook. A maximum-likelihood analysis was used to estimate abundance with Microfish (Van Deventer and Platts 1985).

Habitat data were collected to quantify characteristics of channel morphology and characteristics of habitats within the wetted width. Table 2a shows parameters measured to describe channel morphology, and Table 2b shows parameters measured to describe habitat characteristics within the wetted width. Presence/absence of substrate types, algae, and submerged vegetation were recorded. Substrate types included course particulate organic matter (CPOM), fines, sands, gravel, or cobble (using a Wentworth particle scale analysis, which classifies materials as: Fines (<1 mm), Sand (1 mm - 5 mm), Gravel (>5 mm - 80 mm), Cobble (>80 mm - 300 mm), Boulder (>300 mm), or bedrock. Size was defined as the minimum particle size of substrate as measured on a two-dimensional axis, as would pass through a substrate sieve. Submerged vegetation and algal types were recorded as *Potomogeton* sp., watercress (*Rorippa nasturtium-aqaticum*), *Chara* sp., riparian vegetation roots, blue-green algae (cyanobacteria), and filamentous green algae. Percent of the substrate that was shaded (either by riparian or instream vegetation) was also recorded. Mean water column velocity and water depth were measured at the center of each quadrat. Water temperature (measured using a YSI Model 30 hand-held salinity, conductivity, temperature meter) and wetted width were measured at each transect. Current velocity was measured using a Marsh-McBirney Model 2000 current meter.

Data Analysis

Habitat preference and niche breadth were calculated for 16 measured and categorical habitat parameters. Preference was calculated using the formula of Jacobs (1974): $D = r - p / r + p - 2rp$; where p is the proportion of the resource available in the habitat and r is the proportion of the resource utilized by the species (Manly et al. 1993). Habitat availability was determined using data collected within quadrats (N = 114 for each variable, see Table 3), and habitat preference calculations were made by weighting resource use in accordance with springsnail abundance within quadrats. Preference was categorized as moderate (between 0.25 and 0.5) or strong (> 0.5), or strongly (< -0.5) or weakly avoided (between -0.25 and -0.5).

Table 2. Habitat parameters measured (units shown in parentheses) in the wash and upper spring at Tassi Springs: **a**—variables measured at transects placed along the spring brook continuum, and **b**—at five, evenly-spaced points across the wetted width of each transect.

a. Parameters Measured At Transects	**b. Parameters Measured At 5 Equally-Spaced Points Across Transects**
Wetted Width (cm)	Water Depth (cm)
Water Temperature (°C)	Mean Water Column Velocity (cm/sec)
	Riparian Cover (%)
	Substrate Category (Presence/Absence)
	Vegetation Presence/Absence
	Vegetation Type (roots, algae, etc., Presence Absence)
	CPOM Presence/Absence

RESULTS

Habitat

During the May 2004 surveys, Tassi Springs supported approximately 200 m of aquatic habitat. The length of habitat varied seasonally and daily in response to air temperature and evapotranspiration rates. During this study, the daily variation exceeded 30 m, with greatest lengths observed during early morning and the spring brook was shortest during mid-afternoon. Environmental characteristics of the aquatic habitat along the spring brook are summarized in Tables 3 and 4. Discharge from upper Tassi Spring (as calculated from measurements taken within each quadrat, N = 74) was 0.00399 m^2/sec, and in the wash (with combined discharge from upper and lower springs) it was 0.00495 m^2/sec (N = 40).

Table 3. Mean and range of wetted width, current velocity, water depth, cover, and water temperature recorded during quantitative springsnail and habitat surveys at Tassi Springs, May 2004.

Parameter	Mean	Range
Wetted Width (cm)	108.5	50 – 270
Mean Water Column Velocity (cm/sec)	12.7	0 – 75
Water Depth (cm)	3.4	1 – 10
Cover (%)	49.2	0 – 100
Temperature (°C)	24.3	23.4 – 25.2

Table 4. Proportion of quadrats where environmental parameters were recorded as present during quantitative springsnail and habitat surveys at Tassi Springs, May 2004.

Parameter	Percent Occurrence
Substrate	
Fines	0.0
Sand	0.42
Gravel	0.63
Cobble	0.0
CPOM	0.27
Submerged Vegetation	
Green Algae	0.18
Blue-Green Algae	0.0
Chara sp.	0.0
Potomogeton sp.	0.13
Roots	0.10
Watercress	0.43

Aquatic Communities

A total of 14 taxa were collected by grab sample near the source of upper Tassi Spring (Table 5). *P. bacchus* was the most abundant organism in the community, which included several rare species in addition to other potential crenobiontic macroinvertebrates. The presence of *Microcylloepus* sp. and *Ambrysus* sp. is particularly interesting because these genera include crenobiontic species in arid land springs of the western U.S. (e.g., La Rivers 1953, Shepherd 1992, Schmude 1999, Polehmus and Polhemus 2002). Identification of these specimens will be verified by scientists that are experts with these groups. *Nerthra* sp. is another uncommon group of semi-aquatic heteropterans with limited distribution. The occurrence of speckled dace is also notable because of its virtual absence from historical habitat in the lower Colorado River.

Table 5. Taxonomic list of macroinvertebrates collected during grab sample near the source of upper Tassi Spring during May 2004.

Taxon	Number
———	——
Insects	
Order Trichoptera	
Helicopsyche borealis	1
Order Ephemeroptera	
Tricorythodes sp.	1
Order Coleoptera	
Heterelmis, larva	1
Heterelmis, adult	1
Microcylloepus, larva	8
Microcylloepus, adult	3
Order Odonata	
Argia pulla	4
Order Hemiptera	
Ambrysus sp.	1
Order Heteroptera	
Nerthra sp.	1
Order Megaloptera	
Corydalus sp.	1
Order Diptera	
Polypedilum-flavum	2
Rheotanytarsus	9
Tanytarsus	4
Limonia sp.	1
Gastropods	
Pyrgulopsis bacchus	269
Physa/Physella sp.	1
Mites	
Oribatei sp. (genus key unavailable in English)	1
Fish	
Speckled dace (*Rhinichthys osculus*)	
Amphibians	
Red-spotted toad (*Bufo punctatus*)	
Southwestern toad (*Bufo microscaphus*)	

Springsnail Distribution, Abundance, and Habitat Preference

Pyrgulopsis bacchus occurred only in upper Tassi Spring where it inhabited approximately 65 m of the spring brook (see Figure 2). Its occupied habitat covered approximately 70 m^2, and its habitat extended from the spring source to approximately 10 m down the cascade before the spring brook entered the wash (see Figure 1, No. 5 and Table 1). *Physa* sp. was the only other mollusk observed in the system. It occupied only habitats in the wash and it was never syntopic with *P. bacchus*.

Mean springsnail density in the occupied portion of the spring brook was 29.0 individuals/25 cm^2 (range 0 – 106). Greatest density occurred in reaches between and 10 m and 40 m from the source (or upstream from the cascade) (see Figure 2). Qualitative surveys indicated that springsnails were scarce in portions of the area where dense arrow weed shaded the spring brook (e.g., in reaches between 20 m and 30 m from the source). Quantitative density estimates in this reach were minimal because arrow weed density was very high and it limited access to the spring brook.

Springsnails preferred deeper habitats (Figure 3) with slow current (Figure 4). Habitats less than 4 cm deep and swifter than 30 cm/sec were avoided. It preferred shaded habitats where sunlight penetrated onto the spring brook (e.g., sparsely shaded by willow trees or sparse arrow weed) and avoided areas that were densely shaded by grass and exposed to bright sunlight (Figure 5). It also preferred moderate water temperatures and avoided both cold and warm conditions (Figure 6). It occupied all of the substrate types occurring in upper Tassi Spring and showed no preference for any particular type (Figure 7). It preferred habitats with watercress and roots, avoided *Potomogeton* sp., and neither preferred or avoided green algae (Figure 8).

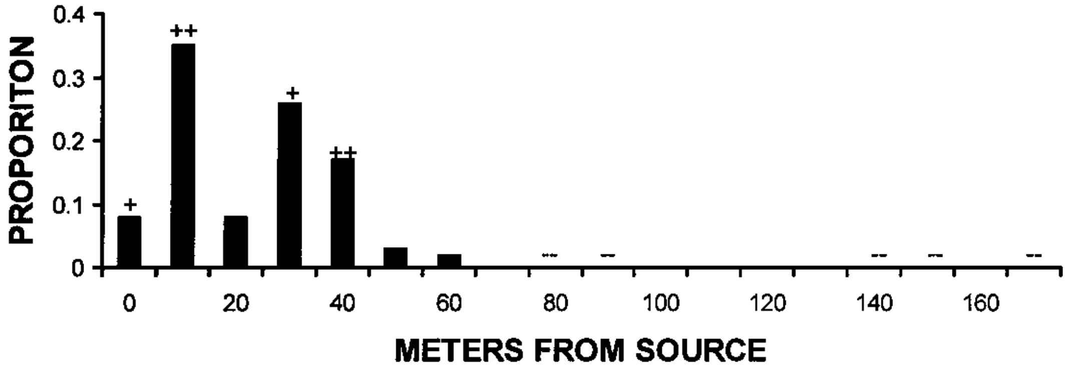

Figure 2. The distribution of *P. bacchus* along the upper Tassi Spring spring brook shown as the proportion of the total population estimate (N = 1647). Preference for each resource category is identified as '+' or moderate (values between 0.25 and 0.5), or '++' strong (values > 0.5), or '-' moderately avoided (values < -0.5) or '--' strongly avoided (values between -0.25 and -0.5). Absence of these identifiers indicates resources for which there was no preference or avoidance.

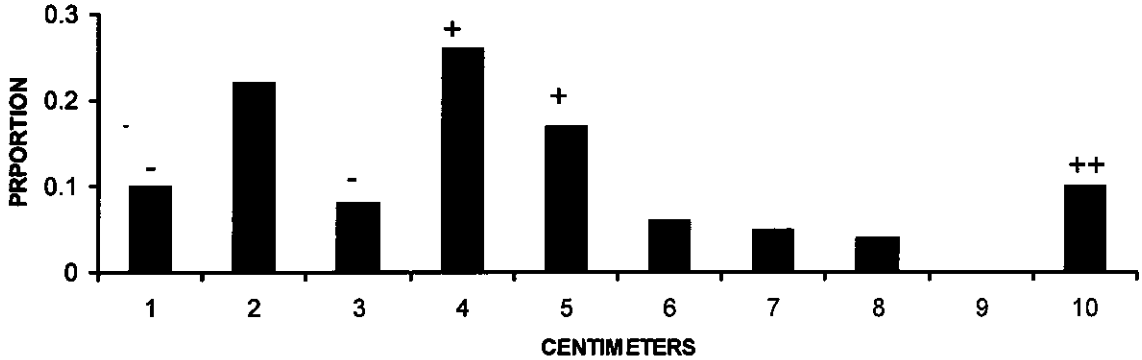

Figure 3. Water depth of habitat occupied by *P. bacchus* in upper Tassi Spring during May 2004. Identification of preference and avoidance of resource categories as described in Fig. 2.

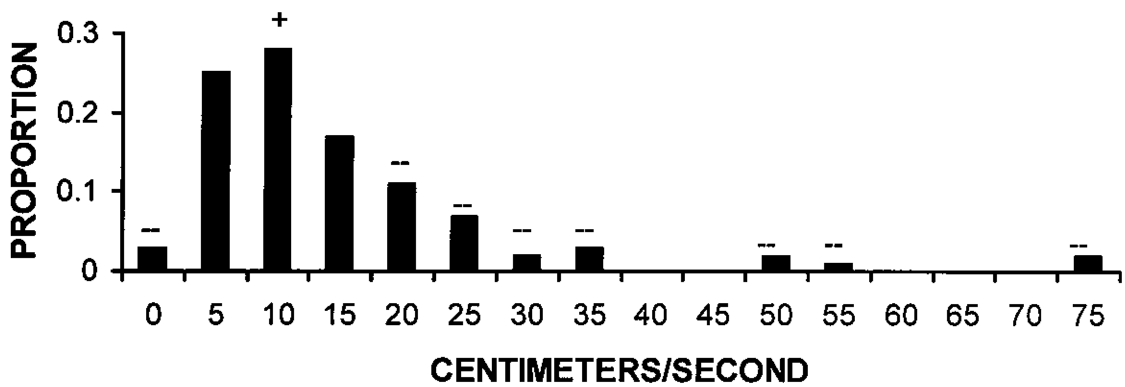

Figure 4. Mean water column velocities occupied, preferred and avoided by *P. bacchus* in upper Tassi Spring during May 2004. Identification of preference and avoidance of resource categories as described in Fig. 2.

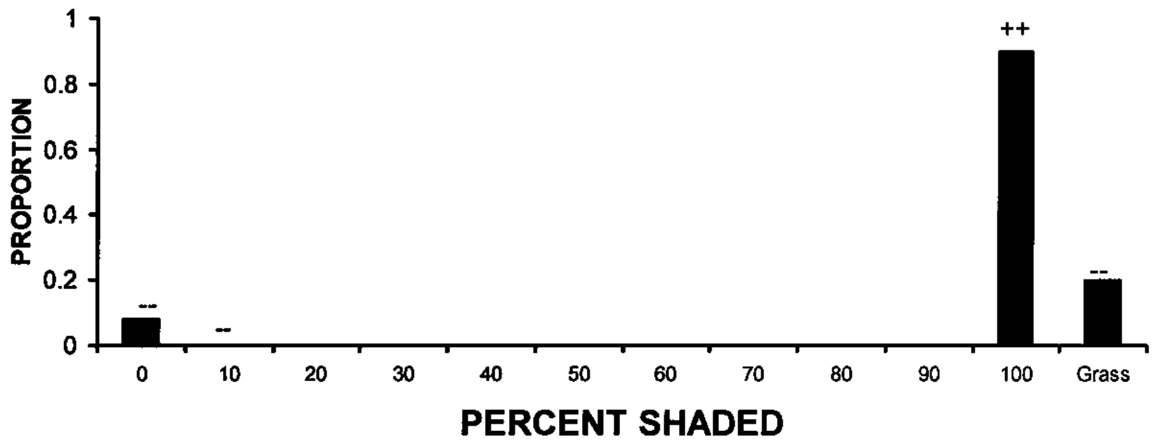

Figure 5. Percent shading of habitats occupied by *P. bacchus* in upper Tassi Spring during May 2004. Identification of preference and avoidance of resource categories as described in Fig. 2.

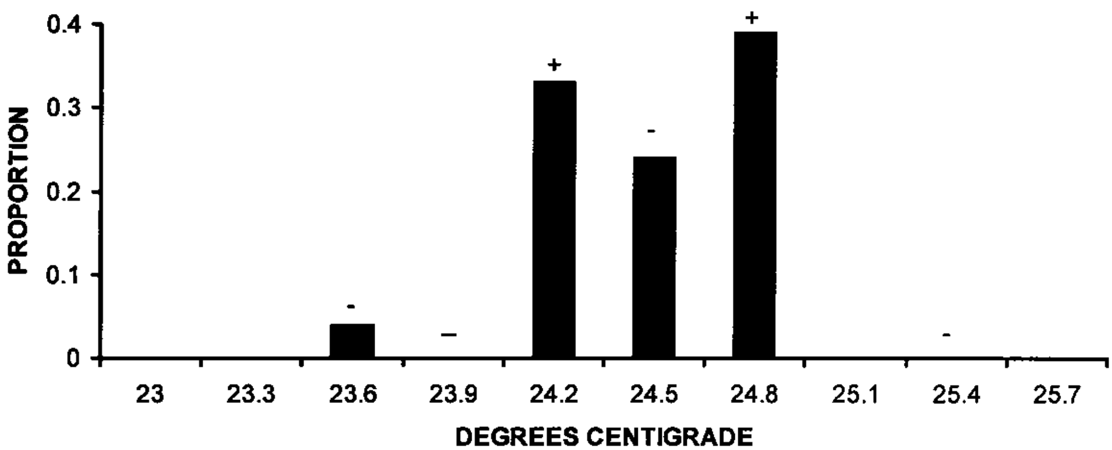

Figure 6. Water temperatures occupied, preferred and avoided by *P. bacchus* at Tassi Springs during May 2004. Identification of preference and avoidance of resource categories as described in Fig. 2.

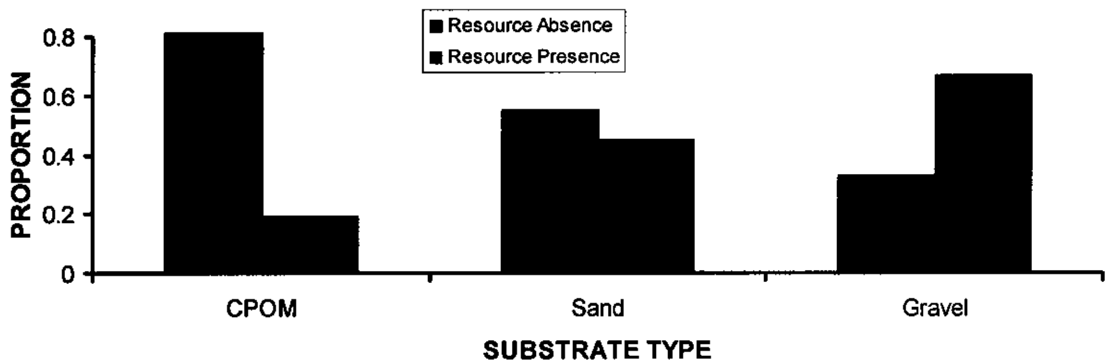

Figure 7. Proportion of *P. bacchus* occurring where CPOM, Sand, and Gravel occur and do not occur at Tassi Springs during May 2004. *P. bacchus* did not exhibit preference for any paraticular type of substrate.

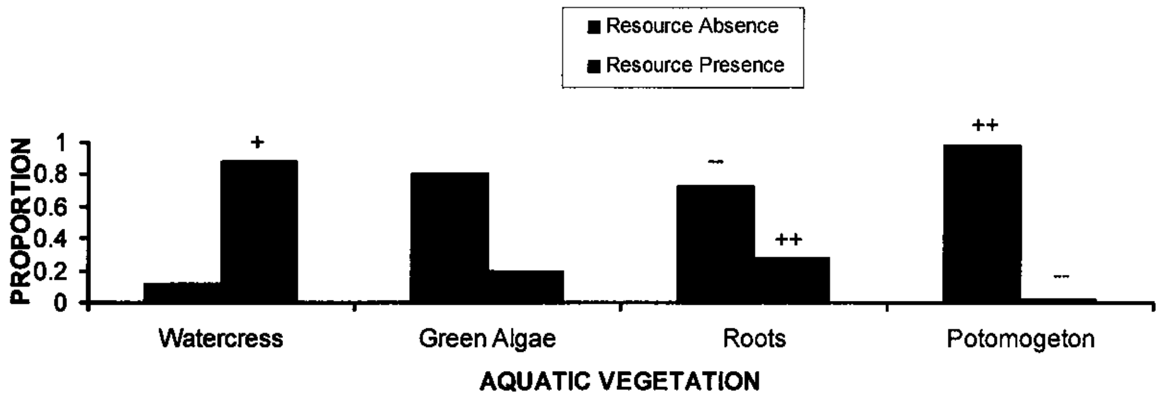

Figure 8. Proportion of *P. bacchus* occurring with submerged aquatic vegetation at Tassi Springs during May 2004. Identification of preference and avoidance of resource categories as described in Fig. 2.

DISCUSSION

Springs are small-scale aquatic systems that occur where ground water reaches the surface (Meinzer 1923). They range widely in size and morphology. Some dry each year, some dry only during extended droughts, while some persist for millennia. Thousands are scattered over all climates, elevations, and geological settings in the western U.S., where they are frequently the most common source of water. They support diverse aquatic communities and their riparian zones are habitat for numerous terrestrial species. Myers and Resh (1999) believed that these aquatic communities contribute substantially to the biodiversity of semi-arid landscapes. Springs also support riparian systems, which Thomas et al. (1979) estimated to be habitat for approximately 80 percent of all Great Basin terrestrial species. Saab et al. (1995) estimated that between 2/3 and 3/4 of non-game bird species in arid lands are associated with riparian habitats during breeding season. Springs in the western U.S. are also important for many crenobiontic invertebrates and vertebrates with limited distribution (e.g., Hubbs and Miller 1948, Hershler 1998, Baldinger et al. 2000, Shepard 1992, Schmude 1999, Wiggins and Erman 1987).

Springs support distinctive aquatic systems, and many studies have shown that they are influenced by geology, climate, and ground water hydrology (e.g., Freeze and Cherry 1979, McCabe 1998). Each spring is a unique assemblage of interacting biotic and abiotic factors, where water chemistry, temperature, and spring morphology most commonly influence aquatic biota (Resh 1983, Cushing 1996), and soil moisture, chemistry, and soil structure and particle size most strongly influence riparian systems (Knopf et al. 1988, Castelli et al. 2000). Springs are also distinctive because their chemistry, temperature, and flow regime are comparatively stable and minimally influenced by drought and runoff attributed to snow melt or rain (Mattson et al. 1995). Environmental variation in springs is lowest near the source and greatest downstream (McCabe 1998), which is contrary to lotic systems where environmental variation is greatest near the source and lowest downstream (e.g., Hynes 1970, Vannote et al. 1980). Aquatic communities in springs include many species that also occupy lotic and lentic systems, but they may also be inhabited by crenobiontics that are often weakly vagile and limited in their distribution (Taylor 1985, Hershler 1998, Schmude 1999). Their energy pathways also are distinct because organic material is usually limited near spring sources (except in some karst systems), but it rapidly increases downstream, where energy pathways are similar to low order streams (Thorup 1974). This is contrary to lotic systems, where organic material is not limited near the stream sources (Vannote et al. 1980). The diversity of aquatic species is usually lowest near spring sources, and increases downstream because of changes in thermal regime, suspended solids, water chemistry, and nutrient availability (Minckley 1963, Ward and Dufford 1979, Meffe and Marsh 1983). As with lotic systems, faunal diversity in springs may also be attributed to other factors such as physical habitat characteristics and microhabitat diversity (Williams 1991, Ferrington 1998). Although much is known about springs, few studies have examined spring-fed riparian systems and most ecological studies have examined springs that are minimally affected by disturbance.

Humans have used arid land wetlands since the early Holocene (e.g., Janetski and Madsen 1990). Intermittent settlement near wetlands and daily activities of hunting and gathering naturally tended to focus upon the biotic resources concentrated at these habitats. For a short period, before the region was settled by European immigrants, a few tribes also diverted water to irrigate land and increase production of commonly eaten native plants. The Fremont Tribe diverted from large rivers near the Wasatch Range (Madsen 1989) and Owens River Basin Paiutes diverted streams from the east flank of the Sierra Nevada (Steward 1933). In the Great Basin, wetlands also produced food such as Owens pupfish (*Cyprinodon radiosus*), Tahoe sucker (*Catostomus tahoensis*), cui-ui (*Chasmistes cujus*), Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), tui chub (*Gila bicolor*) and brine flies (*Ephydra hians*) that were eaten by Paiutes along the eastern Sierra Nevada (Steward 1933, Knack and Stewart 1984). Excavation of Fremont Tribe sites found that their diet included Utah sucker (*Catostomus ardens*), Utah chub (*Gila atraria*), and cutthroat trout (*Oncorhynchus clarki utah*) (Janetski 1990). Greenspan (1990) also documented fish remains from archaeological sites in the Great Basin of Oregon and Drews (1990) reviewed the dietary role of shellfish.

While Native Americans used and altered arid land wetlands, the major anthropogenically-induced changes to aquatic biogeography accompanied habitat alterations and non-native taxa introductions during the past 120 years. Since the beginning of the twentieth century, most rivers in the region have been dammed and diverted for irrigation, flood control, or power generation (La Rivers 1962, Sigler and Sigler 1987, Hendrickson and Minckley1984). Many springs and streams have been modified by livestock grazing, diversion, and ground water use (see Miller 1961, Dudley and Larson 1976, Fleischner 1994). Native aquatic biota have also been impacted by non-native fish species that were introduced for sport, pest management (Sigler and Sigler 1987), and unspecified recreational or commercial interests (Moyle 1984). Fisheries management agencies have also enhanced non-native sport fish populations by poisoning hundreds of miles of streams, causing the consequent reduction or elimination of native fish populations and macroinvertebrates (Moyle et al. 1986, Rinne and Turner 1991, Andersen and Deacon 1996). These habitat modifications and introductions have caused many populations to decline and driven some species to extinction (Minckley and Deacon 1968, Williams et al. 1985, Miller et al. 1989, Minckley and Douglas 1991).

The paucity of water in the desert has focused human use on springs in areas with sparse streams, rivers, and lakes. As a consequence, most springs have been modified by diversion, livestock trampling, and introduction of non-native species (Hendrickson and Minckley 1984, Sada and Vinyard 2002). Complete loss of aquatic communities occurs when springs are dried by diversion or excessive ground water pumping (Minckley and Deacon 1968, Brune 1975, Williams et al. 1985, Johnson and Hubbs 1989) or degraded by other cultural activities (Miller et al. 1989, Sada and Vinyard 2002).

Tassi Springs support comparatively diverse riparian and aquatic systems that change along the spring brook continuum from source to terminus. Similar to other arid land springs in the western U.S., upper reaches of spring brook are occupied by crenobiontic species that require stable environments and are relatively intolerant of disturbance or harsh conditions. Lower reaches (in the wash) are occupied by more tolerant species that persist through stressful environmental change (e.g., flooding, high water temperatures, etc.). The spring province is occupied by a diverse assemblage of aquatic vertebrates and invertebrates, including several crenobiontic species. The extensive riparian habitat that is supported by these springs and the occurrence of rare species all indicate that Tassi Springs support high quality and geologically persistent terrestrial and aquatic systems.

The size and persistence of Tassi Springs are reasons why this site is also historically important. Historical cultural activities such as diversion, dredging, spring box construction, ditching, and piping have altered its springs in a manner that is similar to human impacts observed at other springs over the past 120 years. The absence of information that describes pre-settlement condition of the springs prevents a quantitative assessment of physical and biological changes that have been caused by historical disturbance. As a consequence, it is not possible to determine the affect of these disturbances on spring ecology, but changes similar to these have resulted in loss of populations of rare species and extinction (see Miller 1961, Brune 1975, Shepard 1993, Hershler 1998, Sada and Vinyard 2002). Although none of the Tassi springs remain in natural condition, the upper and middle springs are in relatively good condition. They have not been recently disturbed, which has allowed them to 'naturalized' into relatively stable environments that are probably similar to historic conditions. Additionally, an extensive riparian forest has been maintained in the wash because discharge from the largest springs continues to flow. At this time, only the middle spring bears little resemblance to a natural spring because all of its water is piped to a trough.

These circumstances suggest that existing management of Tassi Springs is probably maintaining the most important biological values of the site. Altering management in ways that may decrease spring discharge, modify spring brooks, introduce non-native species, and or degrade water quality would decrease its environmental quality and possibly result in irrevocable biotic changes.

MANAGEMENT RECOMMENDATIONS

- Maintain upper Tassi Spring in existing condition. Although this spring is altered from its historic condition, attempts to redirect it toward its historic spring brook would constitute a disturbance that may extirpate rare species.
- Maintain existing conditions in existing spring brooks to maximize the diversity of aquatic and riparian habitats on the ranch.

- Initiate minor restoration of the upper spring brook by decreasing arrow weed density. Arrow weed is rapidly established in riparian systems following vegetation removal, and its density frequently prohibits establishment of other riparian species. Over several years, selectively remove arrow weed from the riparian system to allow additional sunlight to reach the spring brook and establish later seral stages of vegetation in the riparian community (e.g., willow dominated).
- Conduct taxonomic surveys to identify additional crenobiontic species.
- Install gauge(s) to monitor discharge and quantify temporal change in discharge rates.

SPRINGSNAIL MONITORING PROGRAMS

Resource monitoring programs should be designed to assess relationships between humans and the physical and biological components of environment that they affect. These relationships can then be placed in context of ecosystem health to determine how uses affect habitats and the abundance and distribution of plants and animals. They should be tailored to assess circumstances at particular locations and to assist with designing future management strategies (Hunsaker and Carpenter 1990). Monitoring programs vary in the amount of information that they accumulate, and they may range from cursory, ocular inventories to highly quantitative collection of data over long time periods.

Work with other springsnail species shows that seasonal variations in their abundance may exceed one order of magnitude (e.g., Sada 2000), and that each species occupies a specific microhabitat. Two basic monitoring programs are outlined below to assess the status of *P. bacchus* at Tassi Springs and identify changes in the spring environment. The first program is qualitative and focuses on springsnails and it can be conducted with very little training and specific equipment. the second program is highly quantitative and involves the intensive collection of ecological information. The second program is designed to assess spatial and temporal variation in springsnail abundance and the aquatic envirionment. Basic springsnail survey and preservation methods are also described in Appendix B.

It is the responsibility of surveyors to acquire any permits required to handle springsnails and to secure permission for access across non-federal lands. State wildlife agencies and National Park Service require a Scientific Collection Permit to collect, study, or display mollusks. Several people can usually collect under a single permit, but a permit usually includes the names and birth dates of each sub-permittee. Collection permits are granted by submitting a proposal that describes reasons for collecting, methods of take and handling, disposition of specimens, and the completion of an annual report.

Monitoring Method I is a qualitative assessment of *P. bacchus* status and distribution at Tassi Springs. Required equipment for this work includes a 6″ diameter kitchen sieve (mesh size ~ 1 mm), a 6″ plastic tray, field notebook, and digital camera. This assessment should be made annually, preferably during the spring (which was the season when surveys were conducted for this report), so that future levels of abundance and distribution can be compared to observations during May 2004. The survey should require no longer than one day to complete, and it should include the following activities:

1) Sample Tassi Springs using methods that discourage the accidental introduction of non-native animals. Follow protocols that are described in Appendix C
2) Establish at least 6 photo points showing the spring source and various portions of spring brook occupied by *P. bacchus*. Record the date photos were taken, GPS coordinates for each photo point, and if photo was taken looking upstream or downstream.
3) Conduct a general springsnail distribution survey using the sieve to sample the aquatic environment. Sample at least 10 points (that include ~ 0.10 m^2 of habitat) along the spring brook continuum from the source to the downstream extent of springsnails. At each point, gently work the sieve over the substrate and through vegetation for approximately 10 seconds to dislodge springsnails and cause them to fall into the sieve. Empty the contents into the plastic container that contains approximately 1 cm of spring water. Record only springsnail presence or absence, and do not sample intensively, this type of sampling will occur under item 3. Return the sample to the

spring brook. Continue sampling down the spring brook until springsnails are no longer found. Record GPS coordinates of the downstream extent of springsnails. See Appendix A for schematic drawings of a typical *Pyrgulopsis* shell.

4) Determine the springsnail abundance every 2.5 m downstream from the spring source by working the sieve over the substrate and through vegetation in a manner similar to Item 2 above. At each location, work the sieve for approximately 20 seconds and empty its contents into the plastic tray that contains approximately one cm of spring water. Count the number of springsnails in each sample, record this number, and the distance of the sample from the spring source. Return the sample to the spring brook. Do not let samples dry and cause springsnail mortality. This sampling provides an estimate of the number of individuals captured per unit of time through the occupied habitat. Record the downstream limit of springsnails and the last sample collected.
5) Identify any non-native aquatic species observed in the spring brook. Non-native species that are most likely to occur are mosquito fish, crayfish, red-rimmed thiara (a gastropod), and bullfrogs. These and other non-native species that are most likely to occur in the springs are shown in Appendix A.
6) Every 5 years, approximately 50 springsnails should be collected, relaxed, preserved (see Appendix B), and sent to specialist to verify their identity.
7) Collect a grab sample of the macroinvertebrate community and have it examined by a qualified taxonomist to determine if small, non-native macroinvertebrate species have been added to the community, or if the community is changing over time. Preserve this sample in 90 percent ethyl alcohol and label (see Appendix B for labeling instructions).

Monitoring Method II is a highly quantitative program to assess spatial and temporal variation in the spring environment and springsnail abundance. These surveys should be conducted if changes in the spring environment are anticipated (e.g., decreased discharge attributed to diversions, ground water pumping, or other habitat alterations) or if qualitative surveys indicate that springsnail abundance and distribution are decreasing. Sample methods and variables that should be measured or estimated during theses surveys are described in the report of *P. bacchus* surveys that were conducted during May 2004. These surveys should be conducted twice a year for a minimum of three years, and during May (for comparison with 2004 surveys) and during the winter (late December or early January) when springsnail abundance is lowest.

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Appendix A – Identification of Important Aquatic Animals

A Field Guide to Important Taxonomic Groups of Macroinvertebrates in Tassi Springs.

Periodic surveys of aquatic life at Tassi Springs should include observations that document the presence of several important native and non-native animals. These observations provide general information to assess the biological integrity of these springs by noting the disappearance of native species and/or the presence of introduced, non-native species that may compete or prey upon native species. Establishment of non-native species may cause declines in the status of native species. This field guide illustrates the general body shape of important native species that are known from Tassi Springs, and several non-native species that do not occur at Tassi Springs but they are known from other springs in the region (Table 1, Appendix A). These non-native species are most likely to be introduced into Tassi Springs.

Table 1-Appendix A. Important native species in Tassi Springs, and non-native species that are most likely to be introduced by people (no non-native species are currently known to occupy Tassi Springs). * = native species.

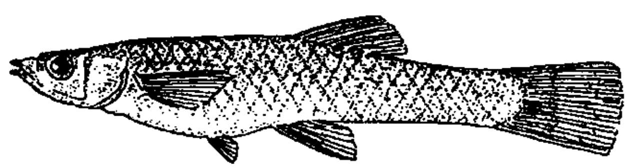
Springsnails (*Pyrgulopsis bacchus*)*
Riffle beetles (*Microcylloepus* sp.)*
Ceeping water bugs (*Ambrysus* sp.)*
Speckled dace (*Rhinichthys osculus*)*
Red-spotted toad (*Bufo punctatus*)*
Southwestern toad (*Bufo microscaphus*)*
Red-rimmed thiara (*Melanoides tuberculata*)
New Zealand mud snail (*Potamopygrus antipodarum*)
Goldfish (*Carassius auratus*)
Mosquitofish (*Gambusia affinis*)
Bullfrog (*Rana catesbeiana*)
Crayfish

Fish

Record 'unknown fish' if the species cannot be identified. Mosquito fish and goldfish are the most common non-native fish in springs, and they are comparatively easy to identify. Most people are familiar with goldfish from aquaria, but the mosquito fish is less well known. Goldfish and mosquito fish are not native to Tassi Springs. Speckled dace are native to Tassi Springs.

Mosquito fish (*Gambusia affinis*)

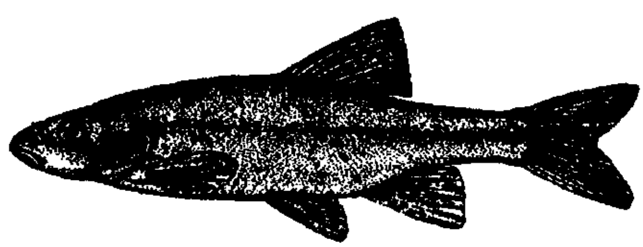
Mosquito fish are native to the southeastern U.S. and they have been introduced throughout the world as a biological control agent for mosquitoes. They are hardy, highly predatory, and feed on macroinvertebrates as well as fish eggs and larvae. While they occur throughout the water column, they are usually near the water surface. They are small (< 3 cm), gray, and easily identified because of their flattened head.



The Mosquito fish body form

Speckled dace (*Rhinichthys osculus*)

The speckled dace occupies a wide variety of spring, stream, river, and lake habitats and it is the most widely distributed native fish in western North America. A number of subspecies have been described throughout its range. It is colored tawny brown to green and reaches a maximum length of 8 cm. It is a native species in Tassi Springs.



Speckled dace body form

Amphibians

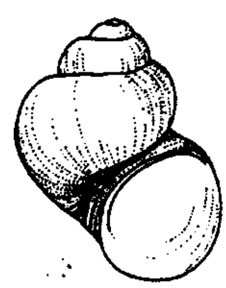
Two species of amphibians (red-spotted toads and southwestern toads) are known from Tassi Springs. They may be difficult to identify without training, and adults are typically seen only during nocturnal sampling. Tadpoles are typically more commonly seen, but it is also very difficult to identify amphibians by examining tadpoles. If tadpoles are seen, record 'unknown tadpoles during the surveys, and if adults are seen record 'unknown adult amphibian'. Bullfrogs have been recorded at Tassi Springs in the past, but they were not at there during the May 2003 surveys. No amphibians are illustrated in this field guide.

Mollusks

Currently the only two mollusks in Tassi Springs are springsnails and a wide-ranging species in the genus *Physa*. There are currently no non-native mollusks in Tassi Springs, but there are two species in the western U.S. that are most likely to be introduced. The red-rimmed thiara occurs in many springs throughout the southwestern U.S. and distribution of New Zealand mud snail is rapidly expanding. Only springsnails and the two most-likely to be introduced non-native species are shown.

Pyrgulopsis bacchus

Pyrgulopsis bacchus is small (2mm – 3.5mm shell height), darkly colored, and common in upper Tassi Springs.



General body form of *Pyrgulopsis bacchus*

Red-rimmed thiara (*Melanoides tuberculata*)

This mollusk was introduced into North America by the aquarium trade, and it has become widespread throughout the western U.S. It is native to Asia, parthenogenic (reproduces asexually), and it can survive long periods out of water. It can be easily transplanted, it is tolerant of harsh conditions, and it prefers warm water. It prefers fine substrates and slow water. It is easy to identify because its shape and color are distinctive and very different from all other mollusks in the western U.S. It is long (up to 2.5 cm) and conical, with body whirls terminating at a sharp point. It shell is slightly sculptured and it is coloration is an attractive and distinct modeled, reticulated mixture of tan and brown. Since these mollusks are easily transplanted, care should be taken to completely clean and inspect field gear to insure they are not carried and introduced into other springs.

The generalized body form of the red-rimmed thiara (measurement line = 1mm)

New Zealand mud snail (*Potamopygrus antipodarum*)

This mollusk was recently introduced into North America, probably as a passenger associated with sports fishing activities. It is parthenogenic and able to survive many days outside of the water. Therefore, it can be easily transplanted by swimmers, fisherman, or related activities.

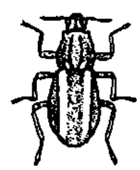
Photograph courtesy of http://www.esg.montana.edu/aim/mollusca/nzms/id2a.html.

Aquatic Insects

The benthic macroinvertebrate community in Tassi Springs includes at least 14 species of aquatic insects. Two of these are known to be crenobiontic, and they are illustrated below.

Riffle beetles (*Microcylloepus* sp., Family Elmidae)

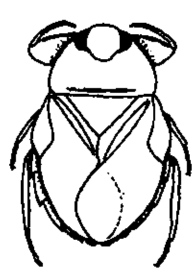
These beetles are flightless and unable to disperse away from Tassi Springs. They are small (< 3 mm long) and difficult to examine without magnification. They are easy to see in samples, however. These beetles are black or dark brown with long, spindly legs. They move slowly by crawling and they have weak swimming ability.



The generalized body form of a riffle beetle

Creeping water bugs (*Ambrysus* sp., Family Naucoridae)

Several genera of true bugs occur in springs throughout the western United States. The most common are *Ambrysus, Belostomatidae*, and *Pelocoris*. Each of these genera has crenobiontic species, but only a species of *Ambrysus* is known from Tassi Spring. These predators are approximately 5 mm to 10 mm long.

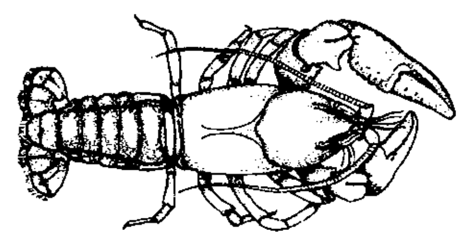


The generalized body form of a creeping water bug

Crustaceans

Crayfish

Crayfish are not native to Tassi Springs, but they have been introduced into many springs throughout the southwest. Since people enjoy eating crayfish and can live out of water for extended periods, they have been transplanted into many springs. The likelihood that they will be introduced into Tassi Springs is high.



Schematic drawing of a Crayfish

Appendix B. Springsnail Collection and Preservation Methods

Surveying and collecting springsnails is challenging because it requires specific techniques that relax, and preserve animals for identification and archival. These techniques must be followed to identify most species of springsnails; identification of many other gastropod groups is possible by examining shell morphology, and these techniques are unnecessary. If springsnails cannot be properly relaxed and preserved for some reason (e.g., lack of equipment), specimens may be either dried or preserved in 75% ethyl alcohol and sent to either Dr. Robert Hershler at the Smithsonian Institution or Dr. Donald Sada at the Desert Research Institute. This provides a record of springsnails at a spring, and they may be properly collected during a later visit. Mollusk collection and preservation methods are described below.

Equipment

Equipment that is necessary for mollusk collection includes:

- 1-quart glass Mason jars with clean lids. Clean, without residue from soap or other chemicals. Each jar can be used for only one collection, so, carry a number of jars if several collections are to be made in a single day.
- Specimen labels prepared from write-in-the-rain paper. Label should record, at least, date, location information (State, County, spring or site name, Township, Range, Quarter Section), field note number, and collector.
- $\leq$ 1 mm mesh kitchen sieve (finer mesh may be necessary to collect some hydrobiid species). The size of a sieve is relatively unimportant, but the most convenient sizes range from 2 inches to 6 inches in diameter. They can be purchased at most grocery stores.
- Fine-tipped forceps.
- "Tupperware" container for sandwiches (measuring approx. 6 in.X 6 in.X 1 in.). Clean, without soap or chemical residue.
- Ice chest and ice if living specimens are to collected for identification or species confirmation.
- Reagent grade menthol crystals.
- 4% Formalin.
- 75 % ethyl alcohol
- Screw-top glass vials (approximately 10 mm wide X 30 mm tall).

Surveyor Skills

Personnel doing these surveys must be trained to recognize springsnails (See Appendix A for guidance). Training that is more thorough than what is described in Appendix A should be provided for individuals that are not familiar with molluscs. Each surveyor should have sufficient knowledge and experience to demonstrate skills in executing these survey methods and in finding and recognizing springsnails. They should be trained for these abilities by person(s) knowledgeable of mollusk taxonomy and survey methods.

Training objectives are to: (1) recognize adults of the most common springsnail genera in the area to be surveyed; and (2) accurately implement survey and collection protocols. Training should include one day in a classroom for instruction to recognize the most common mollusk taxonomic groups, and to learn survey and handling and preserving methods. This should be followed by a day of field training in survey methods and identifying mollusks.

Collection and Preservation

Aquatic mollusks occupy a wide diversity of habitats in a spring, and their abundance varies widely. They may be very abundant or very scarce. Widespread, common species may occur in any type of habitat in the spring (e.g., gravel, aquatic vegetation, backwaters, riffles, etc.). Springsnails are typically most abundant near spring sources and they decrease downstream habitats. Consequently, springsnail surveys should focus on habitats near spring sources.

Springsnails are most easily collected by roiling substrates and vegetation to dislodge macroinvertebrates and capture them as they drift downstream into the kitchen sieve. Minimize the amount of debris, substrate, etc. that is collected. Empty the sieve into a Tupperware container and inspect for mollusks. When springsnails are common, a collection can be made in several minutes. When they are scarce, it will take much longer (maybe up to 1 hr.). An adequate collection includes approximately 100 individuals because species can be determined only by examining the male genitalia of sexually mature individuals (hence not every animal can be used for identification) and all specimens will not be adequately relaxed (which is necessary for identification). If larger mollusks are present in the sample, they should be removed and relaxed separately.

Following collection, all other macroinvertebrates should be removed and returned to the spring, and the sample must be kept cool and springsnails alive. This is best accomplished by keeping the collection in a Mason jar that is filled (to within 1 in. of the top) with high quality water from the spring inhabited by the collected animals. Do not use water from any other source!!! Place the collection in a cooler that with sufficient ice to keep the sample cool until the end of the field day. Menthol crystals should also be kept in the cooler because menthol has a very low boiling point and must be kept cold for crystals to remain intact.

At the end of the day, prepare material for relaxation and preservation. Do not keep samples in the cooler for more than 12 hrs. Samples must first be thoroughly cleaned to remove extraneous matter such as other macroinvertebrates (e.g., aquatic insects, amphipods, flatworms, oligochaete worms, etc.) and organic material. This is necessary because springsnails must be relaxed in a clean environment that is unaffected by other macroinvertebrates (that may die or remain active and disturb springsnails during relaxation) or decaying organic material (which will decrease dissolved oxygen concentrations and water quality). Any movement or irritation caused by these other animals will also prevent effective relaxation of snail specimens.

After cleaning, decant water from the Mason jar until approximately 1 inch of clear, well-oxygenated water remains in the bottom. Attempt not to evenly distribute springsnails across the bottom of the jar. Place the jar in a dark area where it will not be disturbed and where the temperature for the next 12-14 hours will range between 19°C - 22 °C. Next, add menthyl. Pulverize enough crystals (usually in the palm of your hand) to thoroughly cover the surface of water that lies over the relaxing snails. Let the samples sit over night, and after 12 – 14 hours gently pour off the water/crystals. The collection is now 'relaxed'. Fix (preserve) the collection by very gently (do not agitate snails!!!) adding 4% formalin (this step is necessary because many species will contract back into their shells if they are placed directly into alcohol.) In 1-2 days, replace the formalin with 75% ethyl alcohol. For long-term preservation, the specimens should be placed in 70% ethyl alcohol- 15% glycerin- 15% water, and buffered to pH 7. Many different relaxants and preservatives may be appropriate in various circumstances. Pennak (1989) and Araujo et al. (1995) provide a general discussion of mollusk preservation.

Sieved, cleaned and concentrated samples should be placed in glass vials that contains a label showing where, when, and who made the collection. Field labels made using pencil are satisfactory, but an indelible ink is recommended for long-term storage of preserved specimens. If the ink leaches when the label is immersed in alcohol, this may be prevented by dipping the label in dilute acetic acid (vinegar). Labels printed by computers or photocopied are suitable and stable in alcohol years or longer.

Samples and field data sheets should be sent to Dr. R. Hershler (Smithsonian Institution) or Dr. D. Sada (Desert Research Institute, Reno, NV) for identification, verification, and archiving. All spring information should be collected in a format that is consistent with the Springs Database and submitted to D. Sada for inclusion in this Database.

Appendix C. Preventing Inter-Wetland Translocation of Foreign Material into Springs

Each isolated, arid land wetland is occupied by a distinctive aquatic community. While inter-wetland translocation of vertebrates and invertebrates may occur via natural factors, such as transport via waterfowl and mammals, it is also caused by human activities such as recreational bathing, wildlife management, release of aquarium life, and scientific investigation. When caused by humans, translocation frequently results in establishment of non-native species, which has typically been detrimental to native fauna and ecosystem health. Preventing translocation of vertebrates is relatively easy because it requires a conscious effort to provide suitable habitat during transport (ergo sufficient amounts of water to permit respiration and prevent over heating). Translocation of macroinvertebrates and disease may occur more readily because many forms are able to live outside of water for extended periods of time.

This is a Standard Operation Procedure that describes mechanical and chemical methods to prevent accidental translocations that may occur during spring surveys. These methods must be employed upon completion of surveys at each isolated site and before additional surveys are conducted. During these surveys, there are two types of isolated sites: 1) individual, isolated springs, and 2) spring provinces where there is either continuous or periodic (e.g., seasonal) connectivity between springs that naturally permits inter-spring movement of life. These methods must be used following the survey of each of these wetland types, therefore, between surveys of isolated springs or, if springs are connected, before springs that are outside of the immediate province are surveyed.

Equipment

- 10 % Clorox solution
- Contained within a leak-proof, plastic bottle (approximately 250 ml that can be carried to remote sites, or 1 L that can be carried in a vehicle).
- Toothbrush
- Scrub brush
- Size is relatively unimportant, but the bristles should be stiff and durable. A small brush (e.g., 2 cm X 7 cm) may be carried to remote sites, and larger brushes may be used when there is vehicle support.

Methods

Every precaution should be taken to avoid wadding and getting shoes wet, which should be easily accomplished because most arid land springs are small and extensive biological sampling is not an element of the protocol. When wading is necessary, rubber boots must be worn (either hip boots or 'irrigator boots'). Upon completing the survey, they should be rinsed in water from the surveyed spring to remove mud, vegetation, and all other material. Dry the boots, then wash boots in the Clorox solution, and dry again before entering another spring. Precautions should also be taken when shoes are kept dry and wading does not occur. This can be accomplished by using a small scrub brush to buff the soles and sides of shoes and remove all material that may have been gathered from the spring.

Equipment used to collect biological samples is the most likely translocation vector. After completing surveys at each isolated site, all equipment must be: 1) vigorously shaken to remove as much material as possible, 2) treated with Clorox by either dipping into a container and/or using a toothbrush scrub surfaces and clean crevices where macroinvertebrates may be hidden, and 3) dried in the sun before initiating subsequent spring surveys.