FINAL REPORT

Project: The Blue Point Pyrgulopsis: Population Density, Distribution, and Sensitivity to Exotic Fishes

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SUMMARY

- This research project focused on an aquatic springsnail of conservation concern, *Pyruglopsis coloradensis*, and a co-occurring springsnail, *Tryonia infernalis*. Both species are endemic to Blue Point Spring in Lake Mead National Recreation Area.
- The objectives were to assess seasonal distribution, relative abundance, and habitat associations of these springsnails over one year.
- Three sampling strategies were used to determine distributions and relative abundances: kicknet sampling of natural substrate, artificial substrate samplers (small ceramic tiles), and vegetation samplers.
- Morphology was used to identify springsnails to species on tile and vegetation samples; a comparative assessment using mitochondrial sequence data was conducted to ensure accuracy.
- To better understand the findings from sampling data, habitat variables were measured and experiments conducted to assess: colonization rates on tile samplers, speed of springsnail movement, fish predation, and effects of fish exclusion.
- A total of 976 samples were processed across sampling approaches (kicknet, tile, and vegetation) from which 98792 springsnails were isolated.
- Both species reached high densities in the source pool (first 10 meters of the spring), with seasonal high densities based on tile samples ranging from 10044–78844/m^2 for *P. coloradensis* and 6044–26311/m^2 for *T. infernalis.*
- Seasonal high abundances were in December for *P. coloradensis* and October for *T. infernalis*, with low points in March and May, respectively.
- Both species were not common below the source pool, with very low numbers in tile and kicknet samples.
- Exotic fish were present below the source pool, and convict cichlids appear to be a major factor limiting springsnail abundances immediately below the source pool.
- Maintaining integrity of the gauging station weir as a fish barrier to the source pool appears to be paramount to maintaining abundances of *P. coloradensis* and *T. infernalis* in the presence of exotic fish at Blue Point Spring.

BACKGROUND

Blue Point Spring is a unique groundwater-dependent aquatic and riparian environment within the arid landscape of the eastern Mojave Desert. The spring is located on National Park Service (NPS) lands in southern Nevada within Lake Mead National Recreation Area (Figure 1). The spring emerges from carbonate bedrock at a fairly high discharge rate, around 935L/min, with a source temperature of 30 °C year-round (Bailard and Moret 2017, Pohlmann et al. 1998). The resulting stream flows for several kilometers towards Lake Mead, although only intermittently on the surface (see Bradford et al. 2004). The spring has been a component of the landscape for a considerable period of time, with early spring deposits dated to middle to lower Pleistocene (≤ 2.6 million years; see Hershler et al. 2015), plenty of time for evolutionary processes such as speciation to occur. The unique nature of Blue Point Spring is exemplified by the diversity of rare aquatic and amphibious organisms that occupy the site (Hershler et al. 2015). The research we describe herein was focused on one such endemic species – the aquatic springsnail, *Pyrgulopsis coloradensis* (commonly referred to as the Blue Point Pyrg; Hershler 1998). This species was of particular conservation concern because while reported to be relatively abundant in recent years, the population appears to have been limited at times in the past and has even gone undetected during searches (USFWS 2017, Sada 2017).

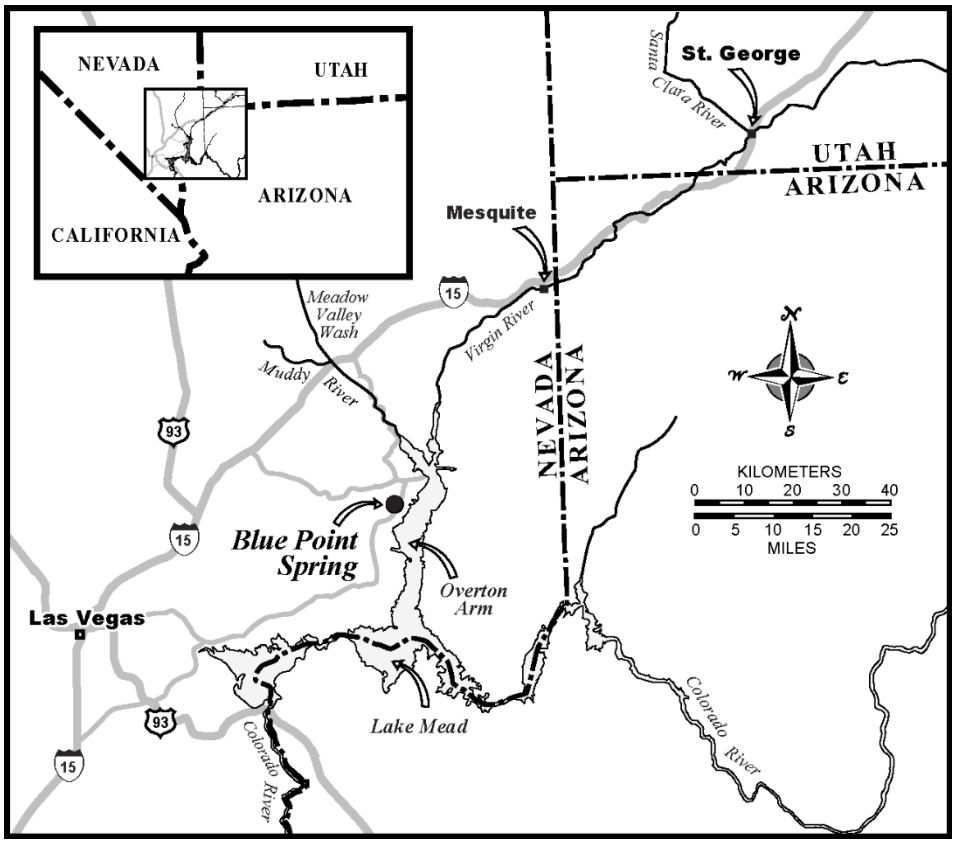

Figure 1. General location of Blue Point Spring.

There are two other species of springsnails endemic to Blue Point Spring: *Tryonia infernalis* and an unnamed *Assiminea* species that appears closely related to *Assiminea infima* (Hershler et al. 2015). Springsnails in the genera *Pyrgulopsis* and *Tryonia* are aquatic and their populations are often concentrated near springheads (Brown et al. 2008, Hershler 1998). We suspected that the distribution of *T. infernalis* would overlap considerably with that of *P. coloradensis*, and we included *T. infernalis* in our assessments (see below). *Assiminea*, however, are amphibious organism and typically occur in emergent vegetation along the banks of streams (Hershler et al. 2015). Because of this habitat difference, we did not include the *Assiminea* sp. as a focal species for assessment, although it was occasionally found in our samples.

Generalities about the natural histories of springsnails in the genera *Pyrgulopsis* and *Tryonia* are often assumed, but research into the ecology of specific species (autecology) yields considerable idiosyncrasy. This does not seem particularly surprising given that *Pyrgulopsis* and *Tryonia* have diversified extensively across western North America; *Pyrgulopsis* in particular is the largest genus of springsnails, consisting of 140 identified species (Hershler et al. 2016). Springsnails are generally quite small, and at Blue Point Spring, *P. coloradensis* adults only average around 1.31 mm in shell height (Hershler 1998) and large adult *T. infernalis* range up to 2.8 mm in height (Hershler et al. 2015). Most *Pyrgulopsis* and *Tryonia* are assumed to be annual species (Hershler 1994), with continuous recruitment (reproduction) in warm water springs (Brown et al. 2008).

Pyrgulopsis and *Tryonia* species are generally assumed to be grazers of periphyton (e.g., Mladenka and Minshall 2001), and despite their small size may play an important role in community-level interactions (Brown et al. 2008). This would seem particularly the case for species and populations that reach high densities on the order of 10,000 per square meter (e.g., Mladenka and Minshall 2001) or more; for example, *P. bernardina* has been estimated at up to 374,000 individuals per square meter (Malcom et al. 2005). The restriction of many *Pyrgulopsis* and *Tryonia* species to areas near springheads has been assumed to result from narrow physiological tolerances (Hershler 1998, Brown et al. 2008), with spring sources providing uniform flows, thermal stability, and low oxygen environments (Brown et al. 2008). Other factors that appear to influence these springsnails (reviewed in Brown et al. 2008) that may be important at Blue Point Spring include substrate type and shading.

Blue Point Spring contains a number of non-native aquatic species that may pose a threat to springsnails, either through predation or competition. The spring was used in the late 1950s to raise tropical aquarium fishes (Deacon et al. 1964), and exotic fish populations exist in the system, including convict cichlids (*Amatitlania nigrofasciata*), short finned mollies (genus *Poecilia*), and mosquitofish (*Gambusia affinis*). There are statements in the literature that the convict cichlids and mosquitofish consume *Pyrgulopsis* and *Tryonia* species (e.g., Brown et al. 2008; Sada 2017). Contemporary observations of colored aquarium sand and occasionally other tropical fish species indicate fish dumping by visitors is a reoccurring event (JRJ observations).

Blue Point Spring has also been invaded by an exotic aquatic snail, the red-rimmed melania (*Melanoides tuberculate*; Hershler et al. 2015). This invasive snail can reach high abundance and there is concern about potential negative impacts on native springsnails (Rader et al. 2003).

Objectives. – Effective conservation and management of species often requires species-specific knowledge, particularly of natural history, and the requirement for such information has been argued for springsnails specifically (Brown et al. 2008, Hershler et al. 2014). Without an understanding of species biology, generalizations based on similar species often become the bases of management actions. This is a reasonable approach, but in most cases probably should only be a temporary measure prior to acquiring information on the species of concern. The study described herein was conducted in response to resource management concerns at Lake Mead National Recreation Area regarding a lack of information on *P. coloradensis*, relevant for its management and the management of the broader aquatic system at Blue Point Spring. Our objectives were to better understand the distribution, seasonal relative abundance, and habitat associations of this springsnail, along with the co-occurring species *T. infernalis*.

General Approaches. – We used three sampling strategies to determine distributions and relative abundances for these species: kicknet sampling of natural substrate, artificial substrate samplers (e.g. small ceramic tiles), and vegetation samplers. We had observed *P. coloradensis* utilizing vegetation as a substrate, and therefore created novel vegetation samplers to assess distribution and abundance of springsnails on vegetation, relative to our other sampling approaches. We used a morphological protocol to identify springsnails species for data collected from the artificial substrate and vegetation samplers, and assured the efficacy of the morphological protocol through a comparative assessment using mitochondrial sequence data. To better understand the finding from our sampling, we also conducted experiments to assess colonization rates by *P. coloradensis* and T. *infernalis* of our artificial substrate samplers, as well an observational assessment of the speed of springsnail movement. We investigated the impact of exotic fishes on springsnail abundance by conducting a fish exclusion experiment within the spring at the scale of our artificial substrate samplers. We also conducted an observational study of fish predation on springsnails in the system.

METHODS

Study area. – We focused research efforts on the upper 20 linear meters of the spring where the majority of springsnails, particularly *P. coloradensis*, have been previously documented to occur (Bailard and Moret 2017). We conducted sampling starting at meter 0.5 below the uppermost springhead and continued downstream at 1-meter intervals to meter 19.5 (each of these meters are referred to hereafter as sampling locations; Figure 2). An important feature across this stretch of stream was a weir constructed as a gauging station at meter 10 (Figure 3). The metal and

cement weir backs up the flow over the springhead area and produces a water drop (~ 0.5 m) that prevents upward movement of fish. At present fish do not occur in the upper 10 m of the spring, although fish were documented there for a period in 2007.

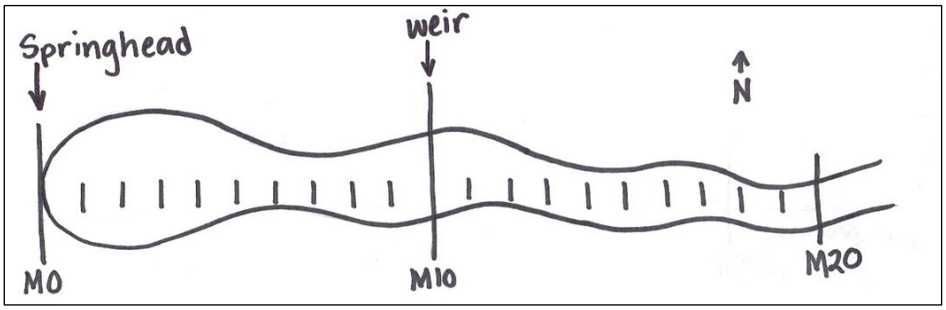

Figure 2. Schematic of study area. Dash lines indicate sampling locations at meter intervals.

Direct substrate samples. – We collected kicknet samples at the center of the stream at each sampling location. These substrate samples allowed direct assessment of the relative abundances of springsnails on the streambed. We used a modified kicknet approach similar to that described by Bailard and Moret (2017). Specifically, we agitated a 10 × 10 cm area of substrate for 30 seconds using a 10 cm wide paintbrush, and collected the displaced substrate in a mesh net (10 × 7 cm, 250 micrometer mesh). Kicknet samples were collected over 4 time points coinciding with summer, fall, winter, and spring seasons (Table 1). We limited this sample approach to 4 events to minimize disturbance and springsnail take (see Martinez and Sorensen 2007 for concerns with such sampling).

Table 1. Timeline of field sampling for springsnails by approach.

Date	Tiles	Vegetation	Kicknet	Fish Exclusions
Aug 14–16, 2018	Yes	Yes	Yes	-
Oct 19–20, 2018	Yes	Yes	Yes	-
Dec 14–15, 2018	Yes	Yes	Yes	-
Feb 01–02, 2019	Yes	Yes	-	Yes
Mar 29–30, 2019	Yes	Yes	Yes	Yes
May 24–25, 2019	Yes	Yes	-	Yes
July 19–20, 2019	Yes	Yes	-	Yes
Sept 13–14, 2019	Yes	Yes	-	Yes

Artificial substrate samples. – We constructed tile samplers from unsealed Saltillo clay tiles, cut to 7.5 × 7.5 cm. To hold tiles in place within the stream, we drilled a hole diagonally through one corner of each tile into which we inserted a zip tie to allow anchorage to a plastic stake inserted in the streambed. We placed tiles in pairs (Figure 4), with replicate pairs at each meter on the north and south sides of the stream. The stream tends to be narrow (~ 0.35 – 3 m wide) resulting

in more shading on the south portions of the streambed than on the north. We intended this sampler placement to allow any potential variance in shading to be incorporated into an overall assessment of relative springsnail abundances. Tile samples consisted of the material we scraped from the upper surface of each tile with a putty knife every 8-weeks. We then replaced each tile on the streambed until the next sampling period.

Figure 3. Weir located at meter 10, but extending across meter 9 where only kicknet samples were collected. Exotic fish occupied the spring below the weir, but had not been observed above the weir for more than a decade.

Vegetation samples. – We developed a novel approach to assessing springsnail abundance on native vegetation without directly harvesting vegetation in the study area, as vegetation was not always present or abundant at each sampling location. We constructed vegetation samplers out of an emergent native sedge (*Scirpus americanus*) collected from above the waterline and downstream of the study area. Each sampler consisted of 7 stems each ~ 0.5 cm in width and cut to 20 cm in length. We tied the stems to a bamboo stake and then submerged the sampler into the steam as much as possible given stream depth (Figure 4). Vegetation samplers were installed

near the tile samplers, but with only one sampler on the north and south sides of the stream. We collected the sedge stems from samplers every 8 weeks at the same time as the tiles.

Figure 4. Images showing a set of paired tile samplers (left) and a vegetation sampler prior to installation (right). Springsnails (small black objects) can be seen on the tiles.

Sample processing. – Laboratory assessment of springsnail samples has been recommended by numerous studies to increase accuracy of counts, which can be very difficult under field conditions due to the small size of springsnails and frequent presence of debris and other material in samples (Bailard and Moret 2017, Lysne et al. 2007, Mladenka and Minshall 2001). We stored all samples in 80% ethanol prior to laboratory analysis. We isolated and identified springsnails under 10–80x magnification using a dissecting microscope (Zeiss SteREO Discovery V8). Sample analysis was multi-staged. We first isolated springsnails from other material. We then counted springsnails, counting only those snails that we determined to be alive at the time of collection, as determined by a clearly visible visceral mass inside the shells. Shells from dead snails were also very brittle and often broken. We conducted each processing step twice for quality assurance.

Species identification. – We identified springsnails to species using characteristics of shell morphology for the tile and vegetation samples; we did not identify species in the kicknet

samples because the process was time consuming and the kicknet sample sizes were excessively large. Previous studies had indicated the feasibility of using shell morphology to differentiate between genera of springsnails (Bichain et al. 2007). We used three main characteristics: shell aperture, shell whorl, and suture between shell whorls. Our criteria were based on previously published scanning electron micrographs and descriptions of *P. coloradensis*, *T. infernalis*, and *A. infima* (Hershler 1998, Hershler et al. 1987, Hershler et al. 2015). We easily differentiated the *Assiminea* species from the other two species based on its broadly conical shell, markedly less convex whorls, and more obtuse suture contour between teleoconch whorls. We discerned *P. coloradensis* based on its ovate-conic shell, highly convex whorls, and more circular aperture, whereas the shell of *T. infernalis* was fairly narrow and turriform, with slightly more convex whorls and an aperture noticeably greater in height than width. At very small sizes the characteristics became increasingly ambiguous and we were unable to resolve individual identifications.

We conducted an assessment of our accuracy in identifying adult *P. coloradensis* and *T. infernalis* by comparing results from morphological assessments to mitochondrial DNA sequence data. We first identified 30 representative adult springsnails from the study area to species using our morphological approach. This assessment was conducted independently by two researchers without discrepancy. We then isolated genomic DNA from the samples by first mechanically crushing the shells and then using a QIAGEN DNeasy® Blood & Tissue Kit following the spin-column protocol for animal tissues. We used primers COIL1492 and COIH2390 from Liu et al. (2001) for PCR and sequencing. We accomplished amplification in 35 cycles using a QIAGEN® Multiplex PCR Kit at a 62 °C annealing temperature. We purified PCR fragments using either an Omega Bio-tek E.Z.N.A.® Cycle Pure Kit or Exo-SAP-IT™ from Applied Biosystems. We then sequenced these individuals for a 658 bp fragment of the cytochrome c oxidase subunit I gene (COI; Liu et al. 2001), using an ABI 3130 genetic analyzer and ABI BigDye Terminator v3.1 sequencing chemistry. For genetic identification, we used reference sequences for *P. coloradensis* (KP899919) and *T. infernalis* (KP899916–KP899918) available in GenBank from Hershler et al. (2015).

Fish exclusions and observation. – To assess the impact of excluding exotic fish at a small spatial scale on springsnail abundance, we constructed fish exclusion cages at the scale of the tile samplers. These were constructed from plastic mesh with 2 mm openings, small enough to exclude the fish that were present but large enough to allow springsnails to easily move through (Figure 5). Cages were installed on half of each tile pair starting at the end of sampling in December 2018 (Table 1). We extended the caging along the upper 10 m of stream, even though fish were not present, in order to assess potential impact that the cages themselves may have on springsnail use of tiles.

Figure 5. Plastic cage with tile inside. Several mosquitofish (*Gambusia affinis*) can be seen swimming around the cage.

We also conducted an observational study of fish predation on springsnails. We placed tiles colonized by springsnails (predominantly, *P. coloradensis* and *T. infernalis*) into areas of the spring where fish were prevalent (below the weir) and used an underwater video camera (AKASO Brave 4) to film fish interaction with springsnails on the tiles. We conducted timed trials of variable lengths of 5, 15 and 30 minute durations. We then assessed the videos to identify the fish species present, record the number of springsnails consumed by each fish species, as well as determine the movements of springsnails off of the tiles. We conducted our observations over two dates to take advantage of times when cichlid fish were naturally absent in the immediate area of our feeding trials; the cichlids exhibited guarding behavior when present, which appeared to reduce opportunities for mollies and mosquitofish to interact with the tiles.

Springsnail movement. – We evaluated the movements of *P. coloradensis* and *T. infernalis* using an observational approach. Early in the study, we had observed fast recolonization of scraped tiles by springsnails, largely in under 24 hours. We assessed recolonization rates more directly during October 2019 by placing 2 seasoned tiles into each of two areas of the spring where springsnails were likely abundant (at meters 2 and 5, between sampling locations). We then

counted the number of individual snails seen on each tile after 2 hours. At the end, we used our morphological protocol to identify the species of a semi-random subsample of 20 or 25 springsnails collected from across each tile.

To gain an idea of the speed of springsnail travel, we followed active individuals on a tile placed in the spring using time-lapse photography. We then collected each springsnail to identity the species in the laboratory using our morphological protocol. We used the program ImageJ to track movement of each springsnail over 35 seconds (a starting image and one image every 5 seconds for a total of 8 images) and measure the distance traveled as a polyline. This time was appropriated for movements on our 7.5 x 7.5 cm tiles.

Habitat associations. – During each sampling event, we collected variables for water chemistry and stream morphology. We used an YSI (ProPlus Polarographic DO/pH/Conductivity Quatro) to measure water temperature, pH, percent dissolved oxygen, and conductivity (µS/cm) at each sampling location. We directly measured abiotic variables of stream morphology including: width, water depth at each sample, and deepest point across the stream width at each sampling meter. We estimated stream flow using a categorical index (pool, glide, riffle, or rapid), and substrate type using a modified Udden-Wentworth grain-size scale (boulder > 256 mm, cobble 256–32 mm, pebble 32–2 mm, sand 2.0–0.625 mm, silt 0.625–0.0039, clay < 0.0039). We estimated vegetation percent cover above and below the waterline at each sampling location within a 25 × 25 cm quadrat, utilizing calibration squares.

Except for stream width and deepest point, the other variables were measured at sampling points within the sampling location, thus generally 3 data points were taken at each meter on the north, south, and center of the stream. In some cases, where the stream was too narrow, the vegetation sampling quadrats overlapped and at those points only one or two estimates were made. For this report, many of the variables were averaged, either as the average of the points within a sampling location (meter) or across sampling periods (see Results).

Statistical approaches. – Our sampling designed for tile and vegetation samples was focused on paired samples, with repeated measures from the sampling time points. For the preliminary analyses presented in this report, we used a series of Paired Sample *t* Tests. In several cases, we conducted preliminary assessments to determine if the replicate samples could be combined for subsequent assessments. In a few cases, samples were lost or damaged between time periods, and in those cases the associated samples were discarded from analyses. Kicknet sampling was invasive, so we did not replicate samples within the sampling locations, but samples were repeated during seasonal time points. In this report, we describe and qualitatively assess the abiotic variables and vegetation cover data collected during sampling to determine those likely to impact springsnail distributional abundance at Blue Point Spring, prior to planned multivariate

analyses. For some habitat variables that appeared to be potentially important factors for springsnail abundance, we generated preliminary information using Pearson's correlations.

RESULTS

Across the year of sampling and sampling types (kicknet, tiles, vegetation), we processed a total of 976 samples from which we isolated 98792 springsnails. Our morphological assessment of species identity was 97% accurate when compared to sequence data. In that comparison, only 1 of 30 springsnails examined was misidentified by morphology, and the researchers had indicated uncertainty regarding that particular snail. From the sequence data, 22 of the 30 springsnails were *P. coloradensis*, including 3 novel haplotypes, and 8 were *T. infernalis*, including one novel haplotype (novel compared to the data available from Genbank). Using morphology to identify springsnails from our tile and vegetation samples, we identified combined totals of 41474 *P. coloradensis*, 18395 *T. infernalis*, 9 *Assiminea* sp., 2214 very small unidentified juveniles, and 137 adults that were too damaged to identify. The samples of *Assiminea*, unidentified juveniles, and unidentified adults represented a small percentage (0.036) of springsnails from the tile and vegetation samples, and we excluded these data from assessments.

Direct substrate samples. – In general, our kicknet samples of substrate contained relatively very high numbers of springsnails (Figure 6). The total number of springsnails collected from our kicknet samples was highest in August with 8863 springsnails, and lowest in March with 2531 springsnails. Abundances were highest in the source pool above the weir (meters 0.5–9.5) and declined sharply below the weir; this distributional pattern was repeated across our tile and vegetation samples (see below). Unlike the tile and vegetation samplers, we were able to acquire kicknet samples along the cement apron of the weir at meter 9.5.

Artificial substrate samples. – We used half our replicate tiles (half of each tile pair) for the fish exclusion experiment (see below) starting after our sampling in December 2018 (Table 1), but before doing so we assessed whether there was any difference between the replicates. When we compared the replicate tiles across all sampling locations for the months of August, October, and December, there was no significant difference in the sum of abundances of all springsnails between the replicates ($df = 113$, $t = -0.256$, $p = 0.779$).

We assessed potential differences in abundances on the north and south sides of the spring (excluding tiles used for the cage experiment) by species across all sampling time points. When assessed across the entire study area (meters 0.5–19.5), we found no significant difference for *P. coloradensis* ($df = 148$, $t = 0.424$, $p = 0.672$), but a significant difference for *T. infernalis* ($df = 148$, $t = -2.226$, $p = 0.028$). To further explore the difference in *T. infernalis*, we partitioned the data for this species above and below the weir and re-ran the analyses separately for each area. The abundances of *T. infernalis* on the north versus south sides of the spring above the weir were

significantly different ($df = 71$, $t = -2.334$, $p = 0.022$). In the source pool, *T. infernalis* averaged 26.29 (± 32.802 SD) individuals on north tiles and 38.47 (± 46.483 SD) on the south tiles, ~ 1.46X higher on south tiles. Below the weir, we found no significant difference ($df = 76$, $t = 1.631$, $p = 0.107$). Based on these results, data from north and south tiles below the weir were combined for each species in the subsequent analyses below.

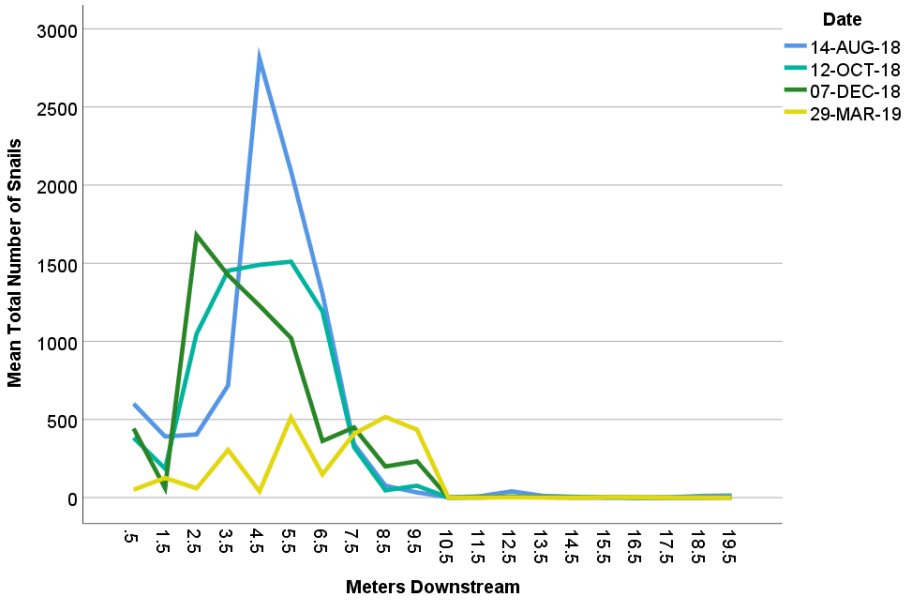

Figure 6. Total number of springsnails in kicknet samples at Blue Point Spring, Nevada.

When we compared abundances of the two springsnail species above the weir, there was significantly more *P. coloradensis* than *T. infernalis* on both north and south tiles (north $df = 71$, $t = 5.430$, $p < 0.001$; south $df = 70$, $t = 7.227$, $p < 0.001$). The north tiles contained an average of 116.18 (± 145.391 SD) *P. coloradensis* versus 26.30 (± 32.802 SD) *T. infernalis*, ~ 4.42X more *P. coloradensis* on north tiles. The south tiles averaged 109.80 (± 91.975 SD) *P. coloradensis* versus 38.47 (± 46.483 SD) *T. infernalis*, a difference of ~ 2.85X more *P. coloradensis* on south tiles. Below the weir, the difference was reversed and there were significantly more *T. infernalis* than *P. coloradensis* ($df = 76$, $t = -3.147$, $p = 0.002$), although both species were scarce across meters 10.5–19.5, with tiles averaging less than a single snail of either species across seasonal samples.

The distribution of *P. coloradensis* on tiles tended to peak around meters 2.5 to 3.5, with highest abundances in October and December (Figure 7). A tile sampler in December at meter 2.5 contained an average of 443.5 individuals. The highest numbers of *P. coloradensis* on tile samplers occurred in December, when across the source pool (meters 0.5–8.5), a tile contained an average of 229.39 (± 194.600 SD) of these springsnails. Over the seasonal samples, *P. coloradensis* was relatively abundant in the source pool with shifting peak areas. In the months

of March and May, when abundance on tiles was lowest, peak numbers occurred closer to meters 6.5 to 7.5 (Figure 7). Within the source pool in March, the average number of this springsnail on tiles was only 28.61 (± 24.162 SD). Below the weir, *P. coloradensis* occurred infrequently and in very low numbers on tiles across seasons (Figure 7).

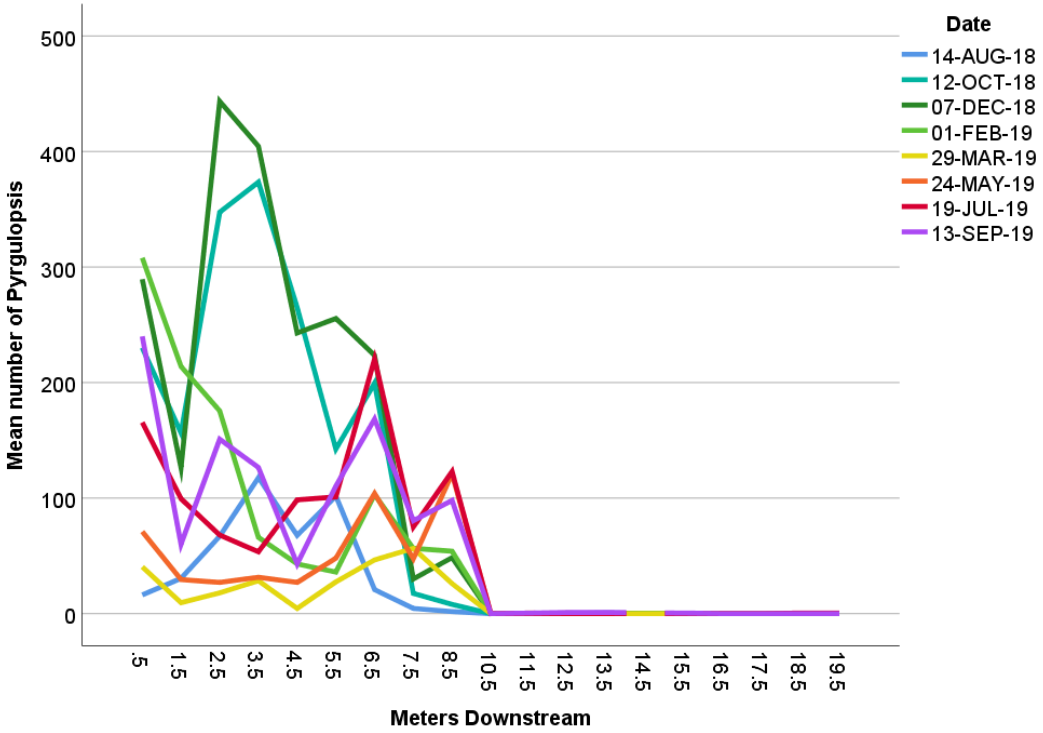

Figure 7. Average numbers of *Pyrgulopsis coloradensis* in tile samples at Blue Point Spring, Nevada.

Tryonia infernalis distribution on tiles in the source pool above the weir (meters 0.5–8.5) tended to peak at meter 5.5, and this pattern was fairly consistent between July and December, excluding our first sample collection in August 2018 (Figure 8). A tile sampler at meter 5.5 in October contained an average of 148 individuals. Across the source pool, the highest numbers of *T. infernalis* on tiles were in October, with an average of 57.80 (± 56.858 SD) per tile. The lowest numbers were in March and May, with an average of only 10.56 (± 13.764 SD) individuals per tile in March. The numbers of *T. infernalis* on tiles below the weir were relatively low (Figure 8).

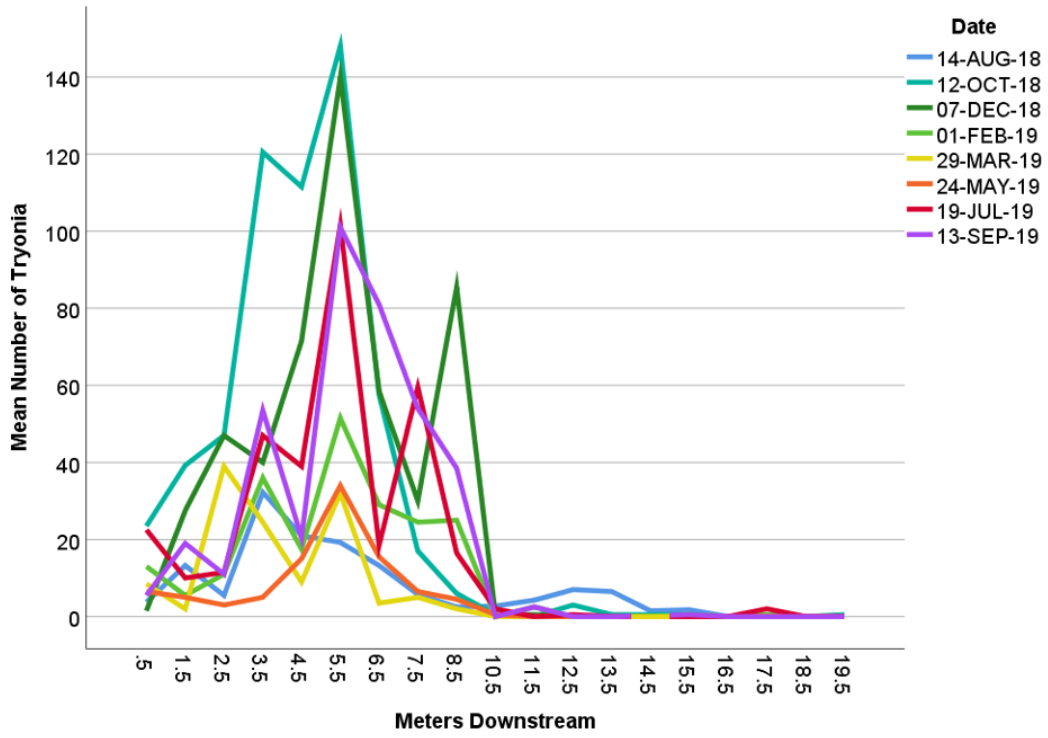

Figure 8. Average numbers of *Tryonia infernalis* in tile samples at Blue Point Spring, Nevada.

Vegetation samples. – There was no significant difference between the numbers of *P. coloradensis* or *T. infernalis* in vegetation samples from the north and south edges of each sampling location in the source pool (meters 0.5–8.5; *Pyrgulopsis df* = 65, t = -0.875, p = 0.385; *Tryonia df* = 65, t = 0.089, p = 0.929). Subsequently, we combined these data per meter by species for the ensuing analyses (below). As observed in the data from tiles, there was significantly more *P. coloradensis* than *T. infernalis* in vegetation samples (*df* = 70, t = 8.113, p < 0.001). In general, vegetation samples in the source pool contained an average of 130.39 (± 86.08 SD) *P. coloradensis* versus 48.94 (± 42.32 SD) *T. infernalis*, a difference of ~ 2.66X more *P. coloradensis* in samples.

On the vegetation samplers, *P. coloradensis* abundance tended to peak at meter 2.5 fairly consistently over the sampling periods (Figure 9), with the highest numbers of individuals from July through October. A vegetation sampler in August at meter 2.5 contained an average of 394 *P. coloradensis*. Across the source pool, the highest numbers of *P. coloradensis* in vegetation samples were in July, with an average of 171.67 (± 114.888 SD) individuals per sample. Numbers of individuals in vegetation samples were much lower in February through May, with the lowest average of 75.94 (± 52.417 SD) individuals per sample in March.

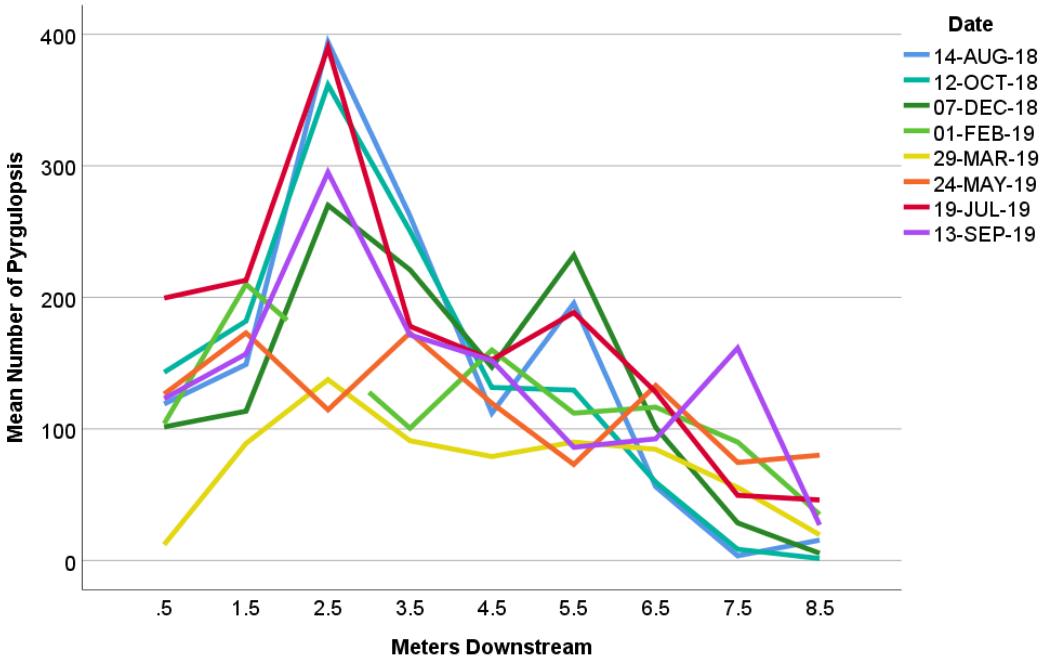

Figure 9. Average numbers of *Pyrgulopsis coloradensis* in vegetation samples at Blue Point Spring, Nevada.

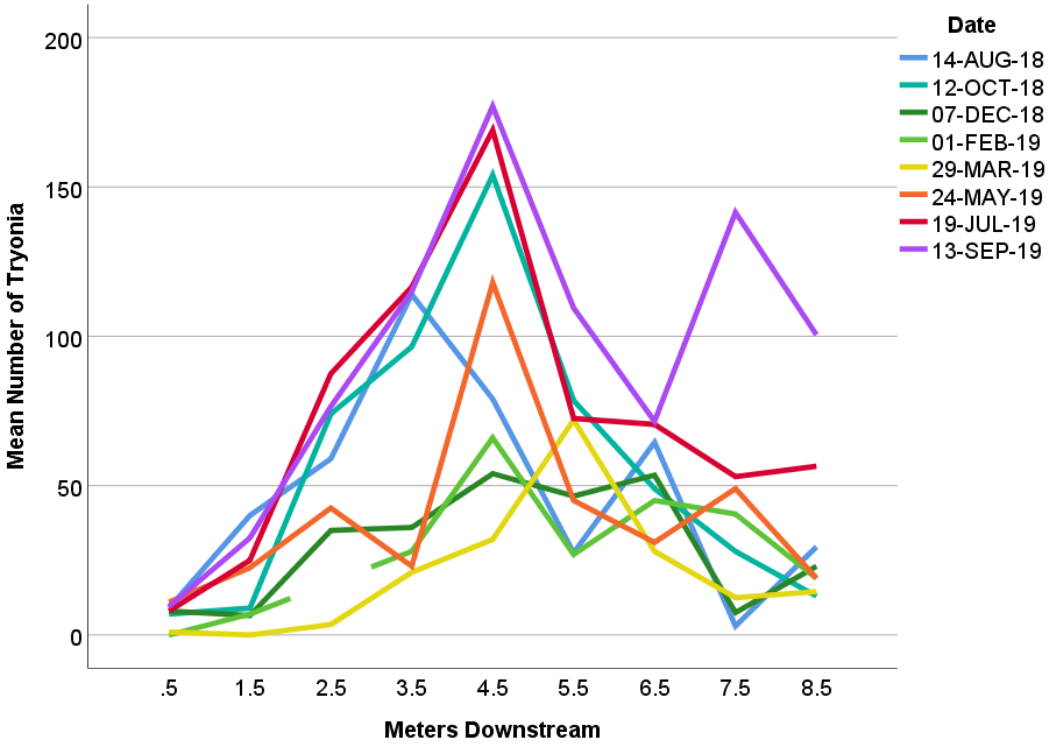

Figure 10. Average numbers of *Tryonia infernalis* in vegetation samples at Blue Point Spring, Nevada.

The distribution of *T. infernalis* in vegetation samples from the source pool, tended to peak around meter 4.5, with the highest numbers of individuals observed in July, September and October, although the samples from August 2018 showed somewhat lower numbers (Figure 10). A vegetation sampler at meter 4.5 in September contained an average of 177 *Tryonia*. Across the source pool, the highest numbers of *T. infernalis* in vegetation samples were in September, with an average of 92.61 (± 87.818 SD). *Tryonia infernalis* was at its lowest abundances in December through March, averaging only 23 (± 28.696 SD) individuals per sample in March.

Fish exclusions. – Above the weir (meters 0.5-8.5) where there were no fish present, we observed a significant difference in abundances of *P. coloradensis* on caged and uncaged tiles ($df = 89$, $t = -2.497$, $p = 0.014$). On average there were 86.72 (± 75.98 SD) *P. coloradensis* on exposed tiles versus 68.00 (± 63.64 SD) on caged tiles, a difference of ~ 1.2X more individuals on exposed tiles. We also observed a significant difference for *T. infernalis* ($df = 89$, $t = 7.883$, $p < 0.001$), with an average of 25.41 (± 30.64 SD) of this springsnail on exposed tiles versus 63.44 (± 49.24 SD) on the caged tiles, ~ 2.5X more individuals on caged tiles.

We also observed a significant difference in abundances between caged and uncaged tiles below the weir for both *P. coloradensis* ($df = 93$, $t = 3.965$, $p < 0.001$) and *T. infernalis* ($df = 93$, $t = 2.539$, $p = 0.013$). On average, *T. infernalis* continued to occur in lower numbers on exposed tiles (0.24 ± 0.888 SD) versus caged tiles (7.67 ± 28.621 SD), but while the numbers were relatively small, the caged tiles had ~ 32X more of this springsnail than uncaged tiles in the lower segment of the spring. *Pyrgulopsis coloradensis* also had higher numbers on caged versus uncaged tiles below the weir – a telling reversal of the pattern above the weir. The average numbers of this springsnail on tiles were small, with only 0.11 (± 0.343 SD) individuals on exposed tiles versus 1.06 (± 2.413 SD) on the caged tiles, but this represented ~ 9.6X more *P. coloradensis* on caged tiles.

In the source pool, we observed no significant difference in the numbers of *P. coloradensis* or *T. infernalis* in caged samples from the north and south edges of the spring (*P. coloradensis,* $df = 44$, $t = 2.009$, $p = 0.051$; *T. infernalis,* $df = 44$, $t = 0.147$, $p = 0.884$), indicating that numbers of springsnails could be averaged across meter by species for subsequent analysis. When we compared the number of *P. coloradensis* to *T. infernalis* on cages in the source pool, there were no significant differences ($df = 44$, $t = 0.412$, $p = 0.682$).

Below the weir there were significant differences for both species when comparing cages on the north to south banks (*P. coloradensis*, $df = 46$, $t = 2.097$, $p = 0.042$; *T. infernalis,* $df = 46$, $t = 2.199$, $p = 0.033$). We found an average of 1.45 (± 3.084 SD) *P. coloradensis* in the northern caged samples versus 0.68 (± 1.400 SD) in the southern caged samples, ~ 2.1X more individuals on the north side. For *Tryonia*, caged samples averaged 13.13 (± 5.757 SD) on the north side versus 2.21 (± 6.057 SD) on the south, ~ 5.9X more individuals on the southern caged tiles.

When we compared the numbers of each species on either side of the spring below the weir, there was significantly fewer *P. coloradensis* than *T. infernalis* on both northern caged tiles and southern caged tiles (north $df = 46$, $t = -2.166$, $p = 0.036$; south $df = 46$, $t = -2.038$, $p = 0.047$). In general, the north caged tiles contained an average of 1.45 (± 3.084 SD) *P. coloradensis* versus 13.13 (± 39.466 SD) *T. infernalis*, ~ 9.1X more *T. infernalis*. The south caged samples averaged 0.68 (± 1.400 SD) *P. coloradensis* versus 2.21 (± 6.057 SD) *T. infernalis*, ~ 3.25X more individuals.

Fish predation. – We collected a total of 40 minutes of observational data on mosquitofish when cichlids and mollies were not present in the immediate area where we conducted the feeding trials. At those times, mosquitofish interacted with the tiles we placed into the spring, but no springsnails were consumed by these fish (Table 2). We collected 85 minutes of observations when all three fish species were present, and over that time cichlids consumed 537 springsnails from the tiles. There were few mollies and cichlids observed during feeding trials. Although the cichlids exhibited guarding behavior over tiles when present, we did observe one springsnail consumed by a molly and another consumed by a mosquitofish while cichlids were in the vicinity (Table 2).

Table 2. Observational assessment of fish predation on springsnails (predominantly, *Pyrgulopsis coloradensis* and *Tryonia infernalis*) under natural conditions. Convict cichlids and mollies were not present (NP) in the immediate area where the feeding trials were being conducted on October 5, 2019. All three fish species were present on October 13, 2019.

Date	Trial Duration	No. of Springsnails	Consumed by			Snails Moved Off or Unaccounted
			Mosquitofish	Mollies	Cichlids	
10/05/2019	10 min	46	0	NP	NP	2
	10 min	48	0	NP	NP	3
	5 min	43	0	NP	NP	1
	5 min	40	0	NP	NP	3
	5 min	25	0	NP	NP	0
	5 min	16	0	NP	NP	1
10/13/2019	30 min	106	1	1	89	12
	30 min	104	0	0	87	17
	15 min	350	0	0	238	0
	5 min	169	0	0	81	27
	5 min	49	0	0	42	7

Springsnail movements. – We examined springsnail movement from two perspectives: rate at which tiles were colonized and measurement of species-specific speeds of travel. Our assessment of colonization of seasoned tiles showed that after 2 hours at meter 2 an average of 192 springsnails colonized the tile, and at meter 5 an average of 39.5 springsnails colonized the tile. At these rates, during the month of October it would take between 2.12 and 5.02 hours for a tile at meter 2 to be fully recolonized after scraping (based on average abundances on monitoring tiles at meters 1.5 and 2.5 during October), and between 17.46 and 27.06 hours for recolonization at meter 5 (based on average abundances on tiles at meters 4.5 and 5.5 during October). Our morphological assessment of a subset of the springsnails on these tiles showed that *Pyrgulopsis* and *Tryonia* had colonized in roughly equivalent numbers.

From our use of time-lapse photography, we determined the travel speed of 28 active individual springsnails, 19 *Pyrgulopsis* and 9 *Tryonia*. *Pyrgulopsis* individuals averaged an active speed of 0.709 centimeters per minute on clay tile, with the fastest observed *Pyrgulopsis* moving at a speed of 1.222 cm/min. *Tryonia* individuals averaged a speed of 0.932 cm/min on clay tile, with the fastest individual moving at 1.982 cm/min.

Habitat data. – Our measure of spring width reflected the wider nature of the source pool above the weir (meters 0.5–8.5) and the narrower stream below the weir (meters 10.5–19.5). All the widest points were in the source pool above the weir (Figure 11). Similarly, water depth at each sampling location and the deepest point per meter were predominately associated with the source pool, except at meter 10.5 directly below the plunging water from the weir (Figure 12). Water flow was classified as a glide in the source pool and either a riffle or a rapid below the weir, depending on turbulence (Table 3). Dominant substrate type reflects the nature of the system above and below the weir (Table 3). The substrate above the weir was dominated by silt, with some cobble and vegetation. The substrate below the weir generally consisted of larger particles (sand/pebbles) and cobble.

Preliminary correlations were run to compare the average number of *P. coloradensis* and the average number of *T. infernalis* at each meter across seasons, to measurements of spring width and deepest point. Neither spring width nor depth were significantly correlated with abundance of either springsnail species in the source pool. When the entire study area was assessed, both spring width and spring depth were modestly correlated with *P. coloradensis* (spring width, $r = 0.695$, $p = 0.001$; spring depth, $r = 0.635$, $p = 0.004$) and *T. infernalis* (spring width, $r = 0.701$, $p = 0.001$; spring depth, $r = 0.534$, $p = 0.019$). These patterns probably simply reflect higher abundances of these springsnails in the wider and deeper source pool, and lower abundances in the stream below.

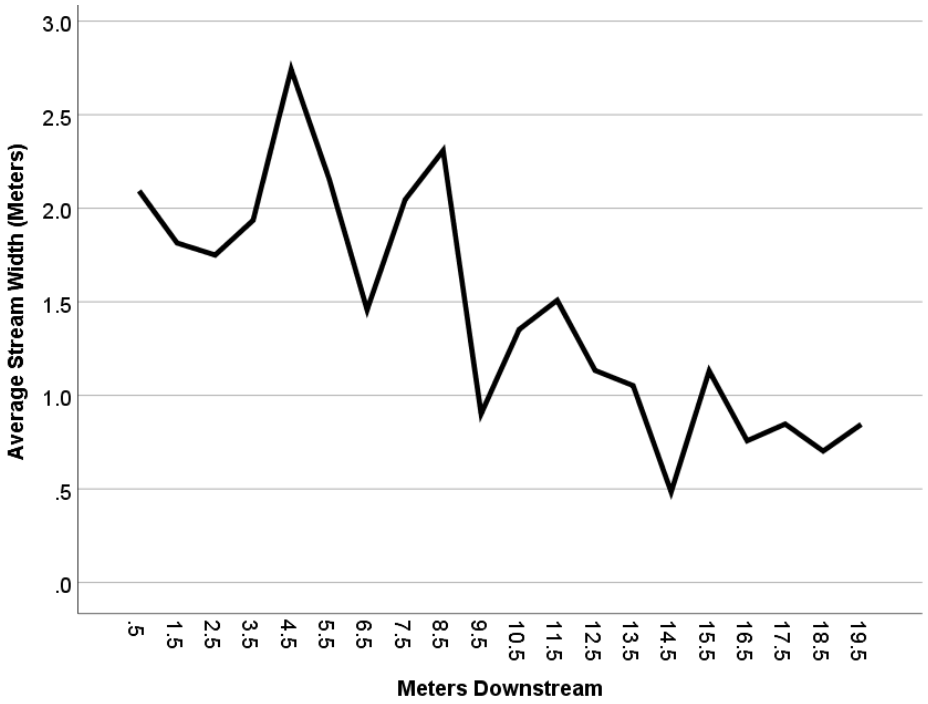

Figure 11. Spring width at water line along the upper 20 m of Blue Point Spring, Nevada. Values are the average of measurements taken across 8 sampling periods over a year.

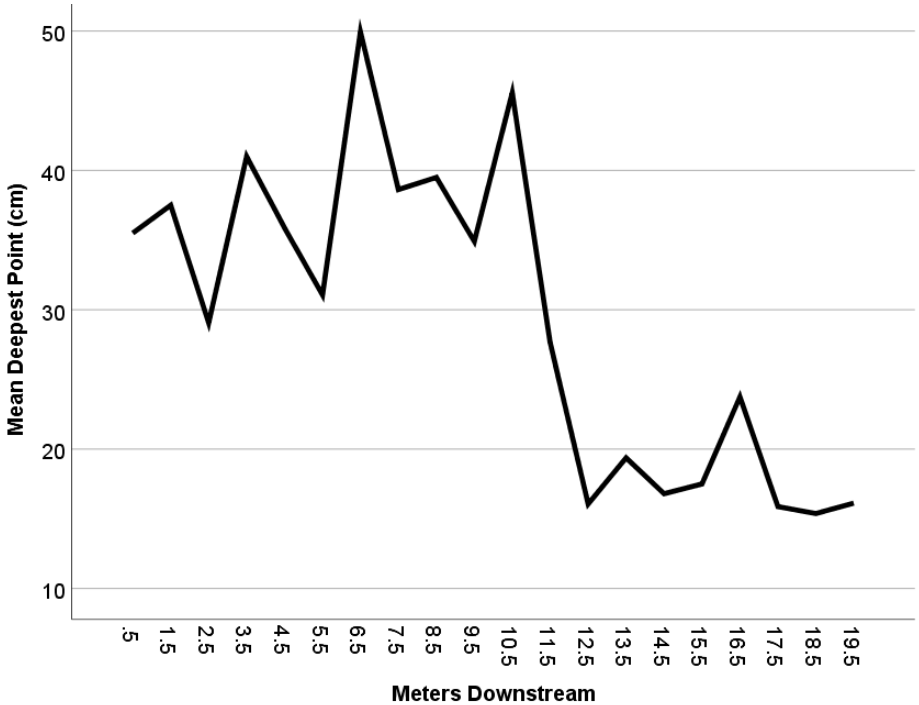

Figure 12. Spring depth at deepest point per meter along the upper 20 m of Blue Point Spring, Nevada. Values are the average of measurements taken across 8 sampling periods over a year.

Table 3. Description of water flow index category and substrate type along the upper 20 m of Blue Point Spring, Nevada. Substrate reflects the dominate type at each meter.

Meter	Water Flow	Substrate Type
0.5	glide	silt
1.5	glide	silt
2.5	glide	silt/cobble
3.5	glide	silt/cobble
4.5	glide	silt/root mat
5.5	glide	silt/root mat
6.5	glide	silt/cobble
7.5	glide	silt
8.5	glide	silt
9.5	glide	concrete
10.5	rapid	cobble
11.5	rapid	pebble/cobble
12.5	riffle	sand/pebble
13.5	riffle	sand
14.5	riffle	silt
15.5	riffle	sand/pebble
16.5	riffle	silt
17.5	rapid	cobble
18.5	riffle	sand/pebble
19.5	riffle	sand/pebble

Vegetation cover above the water varied across the study area, with very high cover (~ 80%) at meter 0.5 at the top of the source pool, but generally lower vegetation cover across the rest of the source pool. Below the weir vegetation cover was generally high (Figure 13). Vegetation cover over tile samplers on the north and south sides of the stream appears fairly similar along most meters except meters 5.5 to 8.5, where percent cover is greater on the south side of the spring (Figure 14).

Preliminary correlations were run to compare the average numbers of *P. coloradensis* and average numbers of *T. infernalis* at each meter to measurements of average percent vegetation cover above the water line. Regardless of whether assessments were parsed by source pool or the entire study area, *P. coloradensis* show no significant correlation to vegetation cover, and *T. infernalis* was only weakly correlated with vegetation cover over the entire study area (r = -0.175, p = 0.033).

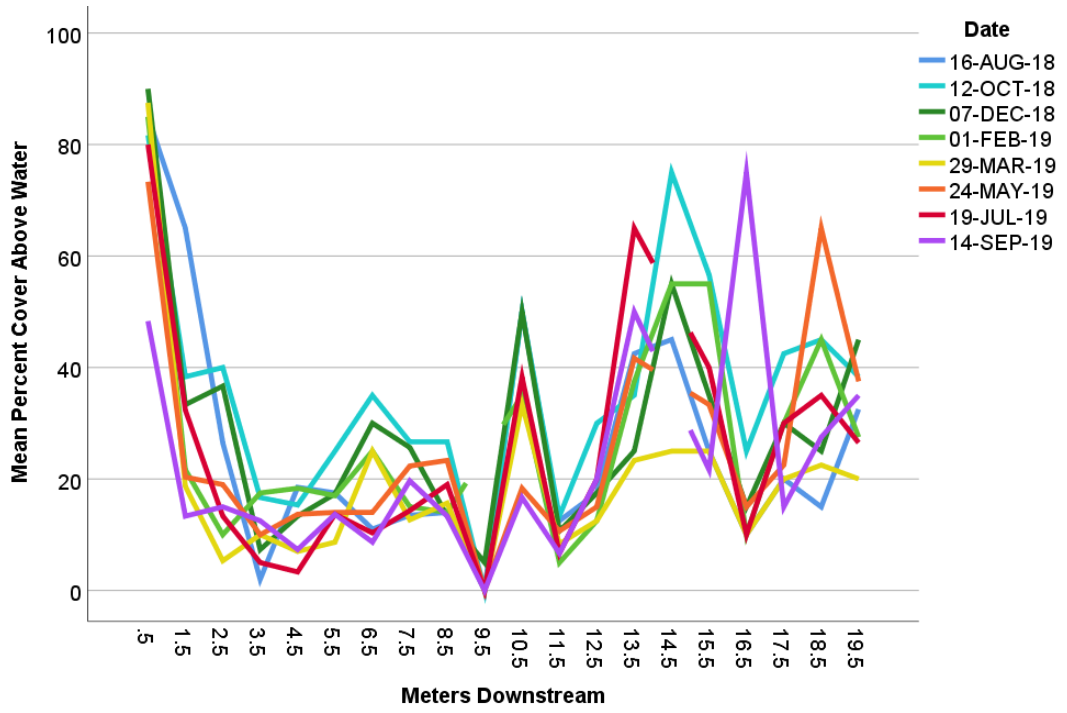

Figure 13. Vegetation cover above the water line along the upper 20 m of Blue Point Spring, Nevada. Values are the average of measurements taken across the spring.

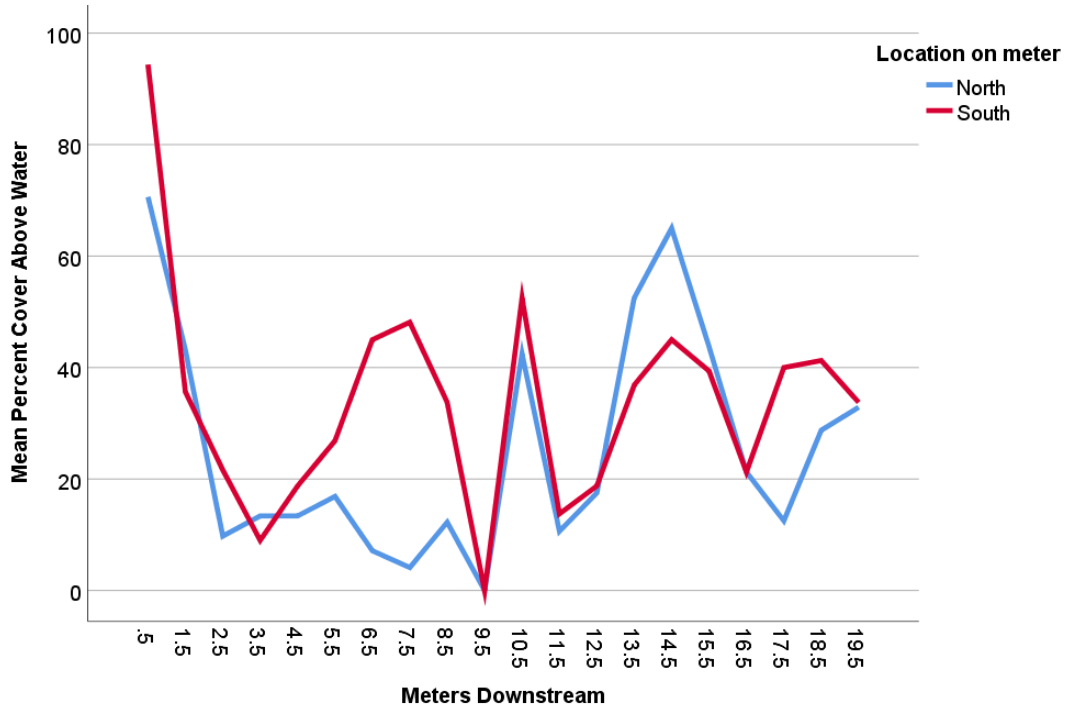

Figure 14. Vegetation cover above the water line along the upper 20 m of Blue Point Spring, Nevada. Values are the average of measurements taken across 8 sampling periods over a year.

Across the study area, pH varied seasonally as well as across meters. In the months of March–early September, pH was ~ 0.1 higher than in late September–February (Figure 15). These periods roughly reflect warmer and cooler seasons. Across seasons, pH was ~ 0.1 lower in the source pool than in the stream below the weir.

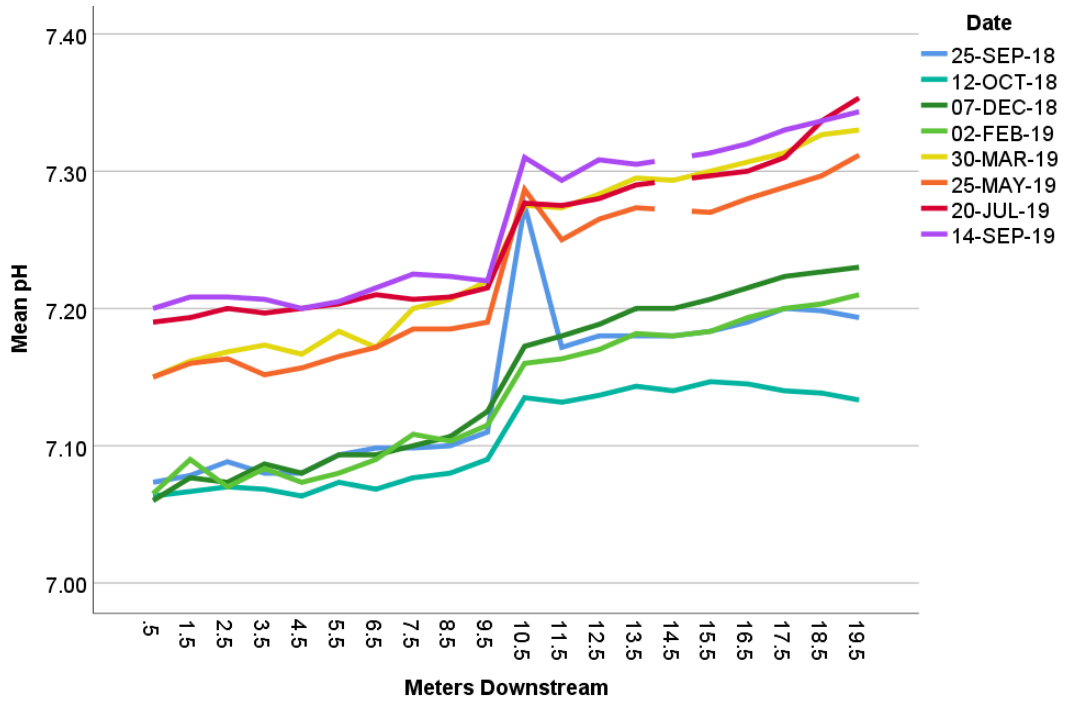

Figure 15. Measures of pH along the upper 20 meters of Blue Point Spring, Nevada. Values are the average of 3 measurements taken across the spring (north, south, and center).

Water temperature had a relatively consistent profile of variation across the study area, with low temperatures generally occurring at the upper end of the source pool at meters ≤ 2.5 and peak temperatures around meters 4.5–7.5, likely reflecting large thermal vents at those locations (Figure 16). Seasonal variation in temperature was seen more in the stream below the weir, with December through March having overall cooler water temperatures, but the overall differences in temperature across the study area and seasons were < 0.5 °C.

We did not measure any clear pattern of seasonal variation in dissolved oxygen data, but there was a large effect across the lower meters from the water plunging over the weir. Percent dissolved oxygen averaged 24.71% in the source pool, and jumped to an average of 50.21% in the stream below the weir (Figure 17). Our measure of conductivity were relatively high which was not unexpected. Over the study area the values were generally flat, but they varied inconstantly across sampling periods (range of averaged values: 4498–5071 µS/cm).

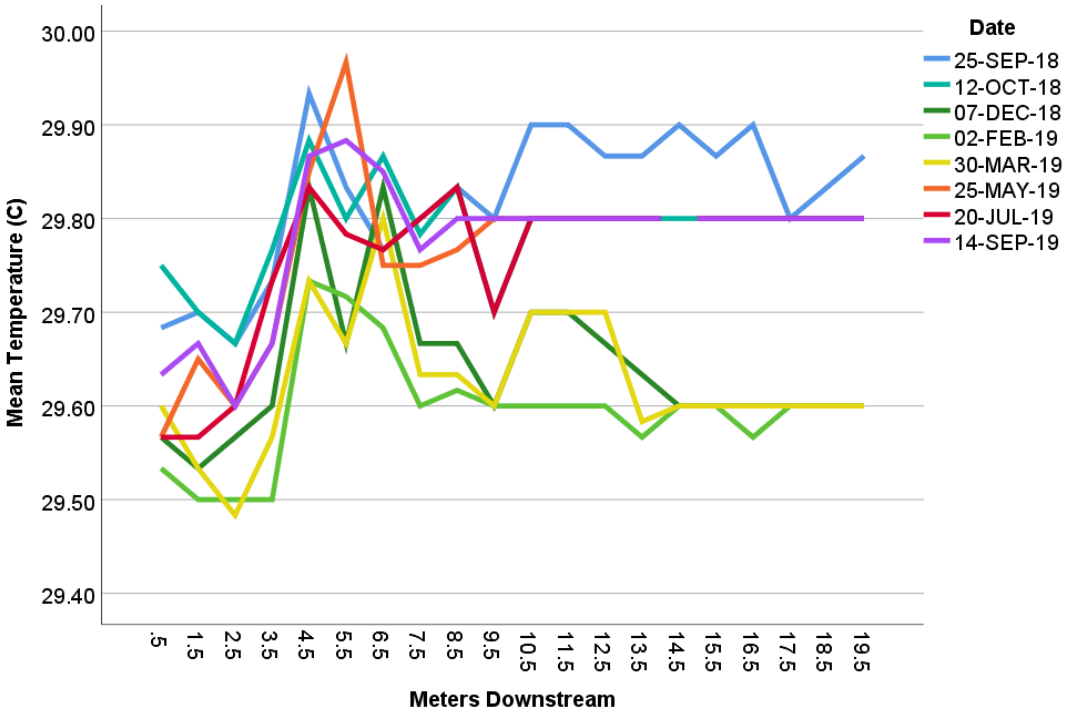

Figure 16. Temperature along the upper 20 meters of Blue Point Spring, Nevada. Values are the average of 3 measurements taken across the spring (north, south, and center).

Preliminary correlations were run to compare the average number of *P. coloradensis* and the average number of *T. infernalis* at each meter to measurements of pH, temperature, and percent dissolved oxygen. *P. coloradensis* was weakly correlated with pH in the source pool (r = -0.323, p = 0.006) and modestly across the entire study area (r = -0.522, $p < 0.001$). *T. infernalis* was only correlated with pH across the entire study area (r = -0.420, $p < 0.001$). Abundance of *P. coloradensis* was not significantly correlated with temperature, but *T. infernalis* showed a weak correlation across the source pool (r = 0.306, p = 0.009). Percent dissolved oxygen was weakly correlated with *P. coloradensis* in the source pool (r = -0.306, p = 0.009) and modestly across the entire study area (r = -0.641, $p < 0.001$). Dissolved oxygen was modestly correlated with *T. infernalis* across the entire study area (correlation coefficient = -0.542, $p < 0.001$).

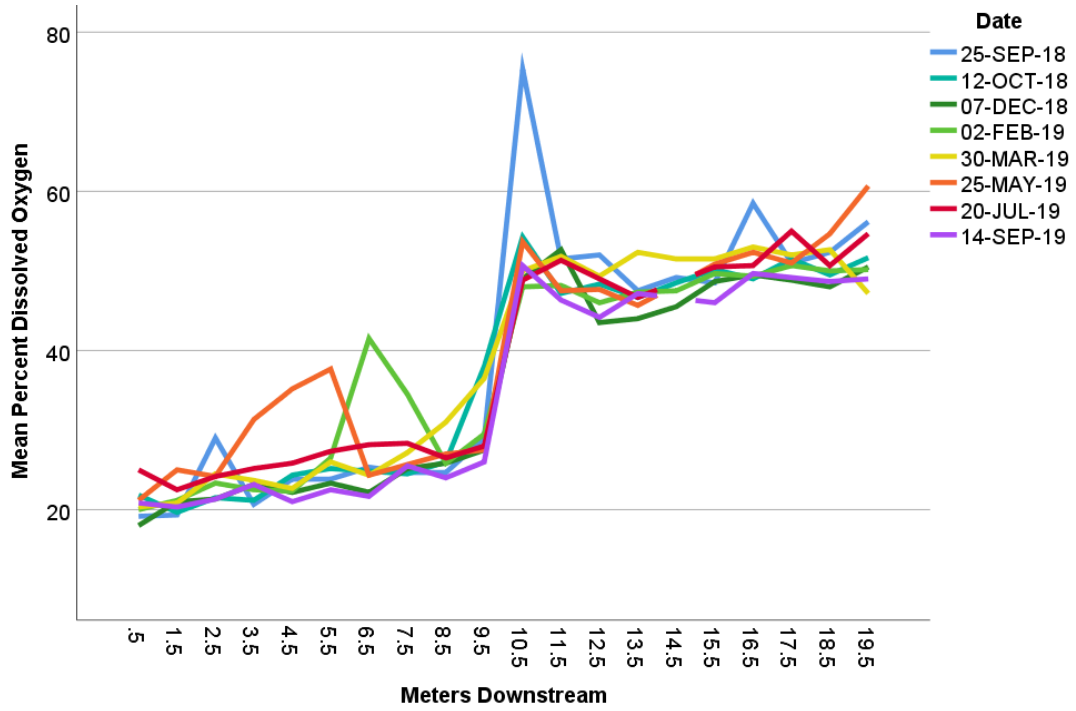

Figure 17. Dissolved oxygen along the upper 20 meters of Blue Point Spring, Nevada. Values are the average of 3 measurements taken across the spring (north, south, and center).

DISCUSSION

Springsnail distributions and abundances. – The NPS Mojave Desert Network Inventory and Monitoring Program has conducted benthic macroinvertebrate sampling at Blue Point Spring (Bailard and Moret 2017). Their single time point sampling is intended to be conducted on intervals over years. That monitoring has provided valuable data for this study, particularly showing the concentration of aquatic springsnails in the main source pool. Our study focused on characterizing the seasonal distributions and relative abundances of *P. coloradensis* and *T. infernalis* over the course of an annual cycle. We also found that the distributional abundances of both *P. coloradensis* and *T. infernalis* at Blue Point Spring were primarily constrained to the source pool, consisting of the first 10 m above the weir. Very few individuals of either species were found below the weir. This pattern was evident in both the kicknet and tile samples which extended across the entire upper 20 meters of the spring.

Both species could reach high densities within areas of the source pool, particularly around meters 2.5–3.5 for *P. coloradensis* and meter 5.5 for *T. infernalis.* Within the source pool (meters 0.5 –8.5), we observed seasonal high densities on our tile samples ranging from 10044–78844/m^2 for *P. coloradensis* and 6044–26311/m^2 for *T. infernalis.* To provide a rough estimate of the total numbers of these springsnails in the source pool, we extrapolated from the seasonal numbers of

these springsnails (averaged over tiles by meter) across rough measurements of the source pool derived from our measurements of spring width. From this exercise, we estimated the population of *P. coloradensis* across meters 0.5–8.5 to contain ~ 89551 individuals at a low point in March, and ~ 730881 at a high point in December. For *T. infernalis* we estimated the population at ~ 35264 at a low point in May, and as many as ~ 212161 in October. These estimates are likely conservative (low). Springsnails of this type can move within porous areas of the substrate (Brown et al. 2008), and our estimates were based on solid tile surfaces. Other three-dimensional factors of environment that were not accounted for and would likely inflate the estimates included: the banks of the spring pool, rocks, logs, and vegetation; our vegetation samples indicated that these springsnails utilize the underwater surfaces of emergent vegetation in numbers approaching those on tiles. Nevertheless, the estimates clearly indicate that even at low points, the populations of these two species were quite high over the sampling period.

Although both species of springsnail are most abundant in the source pool, the distributions of *P. coloradensis* and *T. infernalis* showed some differences. Across the tile and vegetation samples, the two species tended to have relatively consistent peaks of abundance at different meters within the source pool. While these species clearly overlap in space, there appears to be some partitioning by species, probably reflecting habitat preferences. In the source pool, *T. infernalis* shows higher abundance on exposed tiles on the south bank, higher abundance on caged tiles, and peaks of abundances that generally correspond with higher vegetation cover on the south bank of the spring. Collectively, these observations point to a potential positive influence of shading on *T. infernalis* abundance. *Pyrgulopsis coloradensis* did not show differences in abundances on north versus south tiles, but showed overall lower abundance on caged tiles than on exposed tiles, further evidence of potential differences in site selection.

Impact of exotic fishes. – The presence of fish has been suggested as a substantial threat to springsnails at Blue Point Spring. Our observational study of fish predation provided insights on the negative impact of exotic fish on *P. coloradensis* and *T. infernalis*. During feeding trials, we observed one mosquitofish consume one springsnail, but the rest of the mosquitofish observed showed little interest in springsnails. We had few observations of mollies during the feeding trials, but only a single springsnail was consumed by these fish. Our observations do not exonerate mosquitofish, nor potentially mollies, from direct impacts on springsnail populations, most importantly because we did not address the potential consumption of springsnail eggs or very small juveniles by these fish. Nevertheless, the likely impact of mosquitofish, and possibly mollies, seems limited. Our observations on convict cichlids, however, demonstrated a high likelihood for a large negative impacts of these fish on springsnail numbers. During feeding trials, individual convict cichlids hovered around the feeding tile consuming large numbers of springsnails.

Our finding from the experiment with fish cages on tile samplers were also consistent with the expectation that the cages would provide some level of protection from fish predation. The cages increase abundances of both springsnail species on the tile samplers below the weir where fish were present. This was particularly true for *T. infernalis*, where we observed a large increase (~32X) in springsnail abundances on caged tiles compared to exposed tiles. The impact was also evidenced by *P. coloradensis* which demonstrated lower abundance on caged tiles compared to exposed tiles in the source pool, but the opposite pattern below the weir. We speculate that the cages increased shading which resulted in the decreased abundance of *P. coloradensis* on caged tiles where fish were absent, but where fish were present, the cages provided protection from fish predation. The level of protection, however, was small because of the small cage size, which was on the scale of an individual tile. Our observations of movements by *P. coloradensis* and *T. infernalis* show that that these springsnails can move at rates > 1 cm/minute. Springsnails probably moved in and out of the cages, resulting in refuges from predation that were too small to greatly impact relative abundances.

Other habitat variables. – Several of the habitat variables measured showed significant but low to modest correlations with the abundance of one or both springsnail species. Most of the variables, however, showed substantial changes across the study area associated with the weir. The source pool was generally wider, deeper, and the majority of the substrate was siltier than the stream below the weir. The pool also had areas of higher temperature, but the differences in temperature (< 0.5 °C) across the study area was minimal. Dissolved oxygen and pH were also both lower in the source pool than downstream, but the change in pH (~ 0.1 pH) does not appear to be biologically relevant to these springsnails. *Pyrgulopsis* species have been shown to be associated with lower dissolved oxygen levels, and the difference in dissolved oxygen caused by the turbulent flow of water over the weir was substantial. Another complex variable of some importance, as discussed above, appeared to be shading which was represented by sample locations on the north (less shaded) and south (more shaded) sides of the spring, as well as vegetation cover above the waterline. The complex nature of habitat variables requires a multivariate approach to modeling which has not yet been conducted, but the association of most of the variables with the weir and the presence of fish will likely limit the usefulness of such an exercise. Based on the feeding trails and fish exclosure experiment, the presence of fish, particularly convict cichlids, seems to be a major limiting factor for both *P. coloradensis* and *T. infernalis* in the system.

Management implications. – Integrity of the gauging station weir as a fish barrier appears to be paramount to maintaining the abundances of *P. coloradensis* and *T. infernalis* populations in the presence of exotic fish at Blue Point Spring. Fish were able to get over or bypass the weir in 2007 when they were observed in the source pool (JRJ observations). Attempts were made at that time to trap the fish out of the pool, but after several month, the abundance of fish in the source pool did not appear to decline and the effort was abandoned because fish were thought to be able

to somehow get past the weir. At that time, the weir showed some bypass in flow and was later repaired (we have not been able to confirm the date of repair). Fish in the source pool eventually disappeared, although we are not clear as to when, but fish have not been present above the weir for many years. Maintenance of the weir should be a management priority.

Eradication of fish would also likely have a positive effect on *P. coloradensis* and *T. infernalis* distributions below the weir. There have been discussions amongst resource managers and one of the authors (JRJ) about creating a second fish barrier and potentially eradicating fish over a segment of the stream. There is an elevational change in the stream ~120 meters downstream from the springhead that has promise as a site for construction of a barrier (at an old earthen dam; see aerial image in Bailard and Moret 2017); the level of effort required for such construction at that location has not been determined, but may be substantial. Fish eradication also may be successful if the effort only removes cichlid fish from the stream, a potentially easier endeavor than removing all fish. Convict cichlids appeared at Blue Point Spring sometime between 1963 (Deacon et al. 1964) and 1980 (Courtenay and Deacon 1983).

Another action that could potentially improve conditions for springsnails below the weir would be to modify the weir in a way that reduces the turbulence of water flowing over the structure to reduce the large increase in dissolved oxygen in the steam directly below the weir. As noted above, the weir is associated with several changes in the spring environment, and the jump in dissolved oxygen is substantial. Many *Pyrgulopsis* and *Tryonia* species are associated with low oxygen levels, and decreasing oxygen levels in the stream directly below the weir could potentially expand the numbers of *P. coloradensis* and *T. infernalis* in that area.

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LITERATURE CITED

Bailard, J.L., and G.J.M. Moret. 2017. Mojave Desert Network selected large springs: Blue Point Spring benthic macroinvertebrate and springsnail data 2012 to 2015. Natural Resource Data Series NPS/MOJN/NRDS–2017/1124. National Park Service, Fort Collins, Colorado.

Bichain, J.-M., P. Gaubert, S. Samadi, and M.-C. Boisselier-Dubayle. 2007. A gleam in the dark: phylogenetic species delimitation in the confusing spring-snail genus *Bythinella* Moquin-Tandon, 1856 (Gastropoda: Rissooidea: Amnicolidae). Molecular Phylogenetics and Evolution 45:927–941.

Bradford, D.F., J.R. Jaeger, and R.D. Jennings. 2004. Population status and distribution of a decimated amphibian, the relict leopard frog (*Rana onca*). The Southwestern Naturalist 49:218–228.

Brown, K.M., B. Lang, and K.E. Perez. 2008. The conservation ecology of North American pleurocerid and hydrobiid gastropods. Journal of the North American Benthological Society 27:484–495.

Courtenay, W.R.jr., and J.E. Deacon. 1983. Fish introductions in the American Southwest: a case history of Rogers Spring, Nevada. Southwest Naturalist 28:221–224.

Deacon, J.E., C. Hubbs, and B.J. Zahuranec. 1964. Some effects of introduced fishes on the native fish fauna of Southern Nevada. Coepia 1964:384–388.

Hershler, R. and D. W. Sada. 1987. Springsnails (Gastropoda: Hydrobiidae) of Ash Meadows, Amargosa Basin, California-Nevada. Proceedings of the Biological Society of Washington 100:776–843.

Hershler, R. 1994. A review of the North American freshwater snail genus *Pyrgulopsis* (Hydrobiidae). Smithsonian Contributions to Zoology 544.

Hershler, R. 1998. A systematic review of the hydrobiid snails (Gastropoda: Rissooidea) of the Great Basin, western United States. Part I. Genus *Pyrgulopsis*. The Veliger 41:1–132.

Hershler, R., H.-P. Liu, and J. Howard. 2014. Springsnails: a new conservation focus in Western North America. BioScience 64:693–700.

Hershler, R., H.-P. Liu, and J.S. Simpson. 2015. Assembly of a micro-hotspot of caenogastropod endemism in the southern Nevada desert, with a description of a new species of *Tryonia* (Truncatelloidea, Cochliopidae). ZooKeys 492:107–122.

Hershler, R., H.-P. Liu, and L.E. Stevens. 2016. A new springsnail (Hydrobiidae: *Pyrgulopsis*) from the Lower Colorado River basin, northwestern Arizona. Western North American Naturalist 76:72–81.

Liu, H.P., R. Hershler, and F.G. Thompson. 2001. Phylogenetic relationships of the Cochliopinae (Rissooidea Hydrobiidae): an enigmatic group of aquatic gastropods. Molecular Phylogenetics and Evolution 21:17–25.

Lysne, S.J., L.A. Riley, and W.H. Clark. 2007. The life history, ecology, and distribution of the Jackson Lake springsnail (*Pyrgulopsis robusta* Walker 1908). Journal of Freshwater Ecology 22:647–653.

Malcom, J., W.R. Radke, and B.K. Lang. 2005. Habitat Associations of the San Bernardino Springsnail, *Pyrgulopsis bernardina* (Hydrobiidae), Journal of Freshwater Ecology 20:71–77.

Martinez, M.A. and J.A. Sorensen. 2007. Effect of sampling without replacement on isolate populations of endemic aquatic invertebrates in central Arizona. Journal for the Arizona-Nevada Academy of Science 39:28–32.

Mladenka, G.C. and G.W. Minshall. 2001. Variation in the life history and abundance of three populations of Bruneau hot springsnails (*Pyrgulopsis bruneauensis*). Western North American Naturalist 61:204–212.

Pohlmann, K.R., D.J. Campagna, J.B. Chapman, and S. Earman. 1998. Investigation of the origin of springs in the Lake Mead National Recreation Area. Publ. No. 41161, Desert Research Institute, Las Vegas, Nevada.

Rader, R.B., M.C. Belk, and M.J. Keleher. 2003. The introduction of an invasive snail (*Melanoides tuberculata*) to spring ecosystems of the Bonneville Basin, Utah. Journal of Freshwater Ecology, 18:4, 647-657.

Sada, D. 2017. Environmental and biological factors influencing Great Basin and surrounding areas springsnail (Gastropoda: Rissooidea) abundance and distribution. Desert Research Institute Division of Hydrologic Sciences, report prepared for U.S. Fish and Wildlife Service, Reno Fish and Wildlife Office, and Southern Nevada Fish and Wildlife Office, Reno and Las Vegas, NV.

USFWS - U.S. Fish and Wildlife Service. 2017. Endangered and Threatened Wildlife and Plants; 12-Month Findings on Petitions to List 25 Species as Endangered or Threatened Species. Federal Register (October 5, 2017) 82, No. 192:46618–46645.