White Sands Pupfish Conservation Plan

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U.S. Army White Sands Missile Range
Environmental Stewardship Branch
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and

Holloman Air Force Base
49th Civil Engineer Squadron
Environmental Flight

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Author's Biography

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John S. Pittenger studied biology, chemistry, and aquatic ecology at Northern Illinois University, where he received a B.S. in Biological Sciences in 1982, and fishery science at New Mexico State University, where he received an M.S. in 1986. He has worked in the Southwest since 1988 and in New Mexico since 1989. His work experience has included positions with the U.S. Fish and Wildlife Service, the U.S. Forest Service, and the New Mexico Department of Game and Fish. He has worked as a private consultant since 1998. John has been involved with conservation of White Sands pupfish since 1994. His early work on the species included coordinating with White Sands Missile Range on protecting habitats of the pupfish from feral horse impacts, reestablishing the interagency conservation team and reviving the cooperative agreement for conservation of the species, developing and implementing the initial monitoring plan for the species, and conducting research on the pupfish and its habitats. Subsequent work on White Sands pupfish included revision of the monitoring plan and evaluation of potential natural refuge sites for the Malpais Spring population. He has also worked on other native fish species in New Mexico including Gila trout, Rio Grande chub, Rio Grande cutthroat trout, spikedace, loach minnow, headwater chub, and native fishes of the Canadian River drainage. John has also worked extensively in wetland habitats in New Mexico and Arizona, including monitoring wetland vegetation and wetland hydrology, developing and implementing wetland mitigation and restoration plans, assessing wetland hydrology and hydric soils, and conducting floral and faunal surveys of wetland habitats including surveys for federal listed species such as southwestern willow flycatcher, narrow headed gartersnake, northern Mexican gartersnake, and Chiricahua leopard frog.

Summary

Introduction White Sands pupfish (*Cyprinodon tularosa*) is endemic to the Tularosa basin in south-central New Mexico. The two native populations of the species, at Salt Creek and Malpais Spring, occur entirely within White Sands Missile Range (WSMR), and introduced populations are also found at Mound Spring on WSMR and at Lost River and Bradford Spring on adjacent Holloman Air Force Base (HAFB). The Lost River population may periodically expand onto adjacent White Sands National Monument during wet periods. White Sands pupfish is a Department of the Army At-Risk Species (critically imperiled category) and is listed by New Mexico as threatened. Also, the U.S. Fish and Wildlife Service determined in 2009 that federal listing of the species may be warranted, and a status review of the species is scheduled for completion in 2016. The purpose of this conservation plan is to identify actions that can be implemented on WSMR and HAFB to improve the security of the species.

Natural History White Sands pupfish is the only inland occurrence of the maritime clade of *Cyprinodon*. The ancestor of White Sands pupfish likely gained access to the Tularosa Basin from the Gulf of Mexico approximately 1.8 million years ago, when the ancestral Rio Grande flowed through Fillmore Pass into the Tularosa and Hueco basins and on to the Gulf of Mexico. Subsequently (on a geological time scale), the ancestral Rio Grande changed course to the west following land surface elevation at the Artillery Range fault. The Tularosa Basin then became a closed, interior drainage basin.

The two native populations of White Sands pupfish (Salt Creek and Malpais Spring) likely were isolated in the Holocene with development of a warmer and drier climate and concurrent contraction and isolation of aquatic habitats in the Tularosa Basin. It is estimated that the two native populations diverged 3,250 to 11,000 years ago. The two native populations are considered to be evolutionarily significant units, which are unique evolutionary lineages that should be preserved.

White Sands pupfish is typically abundant where it is found. The influence of habitat attributes such as wetland vegetation density, open-water area, water depth, variation in salinity or water temperature, and other factors on distribution and abundance of the species are not well known. White Sands pupfish appear to move and change behavior in response to changes in water temperature. For example, White Sands pupfish appear to move toward the headspring at Malpais Spring in the winter, and may form large schools at water temperatures ranging from 4^{o}C (39.2^{o}F) to 15^{o}C (59^{o}F).

White Sands pupfish is broadly omnivorous. Chironomid larvae and cyclopoid copepods are common food items of White Sands pupfish at Malpais Spring. At Salt Creek, emerging dipterans and ceratopogonid larvae are most common in the diet. Diatoms and detritus are also common in the diet of White Sands pupfish at both Malpais Spring and Salt Creek. Feeding activity likely occurs when water temperatures are between 4^{o}C and 40^{o}C (39.2^{o}F to 104^{o}F).

Like other *Cyprinodon* species, White Sands pupfish apparently has a resource-based polygonous breeding system. In such systems, males establish breeding territories on suitable substrates, gravid females visit territories and spawn with the resident male, and there may be some degree of indirect parental care by the resident male guarding the territory. However, territorial males and other pupfish invading the territory may cannibalize eggs. Virtually all spawning activity takes place on male territories. Microtopographic diversity in a spawning territory may confer protection of the demersal, cryptic eggs by allowing them to remain undetected by predators. Suitable breeding habitat may be limited even in large habitats. Reproductive behavior is initiated in late spring when water temperature approaches 11ºC (52ºF) to 15ºC (59ºF) and daylight periods begin to lengthen.

Average number of mature ova per female has been reported as 33.3 per female at Malpais Spring and 43.3 per female at Salt Creek. Mortality rates appear to be high in White Sands pupfish. Estimates of age-specific survivorship have been reported as 1.56 percent from young-of-year to Age I, 1.56 from Age I to Age II, and 8.76 percent from Age II to Age III. Causes of mortality include cannibalism, predation (*e.g.* wading birds and waterfowl, black-necked gartersnake), disease, and habitat dessication.

The two native populations occupy habitats that differ in water chemistry, hydrologic characteristics, and other features. Salt Creek is primarily a lotic system, but also has features of standing water habitats in large pools along the stream channel and playas at the mouth of the drainage. Malpais Spring has lotic features in spring run and marsh channels. Large areas of lentic habitat occur in the extensive marsh and the *laguna* at the south end of that site.

Habitats of White Sands pupfish in the northern Tularosa Basin are sustained by two hydrologic systems: 1) the surface water runoff system; and 2) the sulfate-laden groundwater system. The runoff system responds with a short time lag to precipitation events, whereas the groundwater system has a longer time-lag response to precipitation. Groundwater in the Tularosa Basin moves generally southward and toward the basin away from the mountain-front aquifer recharge zone. The mountain-front recharge zone is characterized by alluvial fans composed of coarse, permeable sediments. Sulfur isotope analysis indicates that the main sulfate sources for springs in the northern Tularosa Basin is likely dissolution of the San Andres and Artesia formations of the middle Permian strata in the surrounding mountains.

Perennial flow in Salt Creek is maintained by groundwater discharge from the alluvial aquifer. Groundwater input, in the form of springs and seeps, occurs throughout the reach of Salt Creek from the headwaters downstream to the vicinity of a head-cut waterfall above the Range Road 316 crossing. Stream flow in this reach, a distance of approximately 12.1 stream-km (7.5 stream-mi), roughly doubles from the headwaters to the waterfall. Groundwater input to the stream appears to cease near the Route 316 crossing, where the stream begins to lose surface flow. Salt Creek becomes an intermittent stream below this point downstream to where the valley opens up into broad alkali flats, a distance of approximately 17.5 stream-km (10.9 stream-mi). Salt Creek often dries completely in the lower end through the alkali flats to near the mouth of the stream in a playa above Big Salt Lake, a distance of approximately 8.5 stream-km (5.3 stream-mi). Rainfall-runoff events cause short-term increases in stream flow in Salt Creek, and provide the main source of surface water flow in lower Salt Creek.

Groundwater discharge at Malpais Spring is from a regional aquifer that consists of Quaternary and Tertiary bolson fill and stream channel sediments buried under the Carrizozo lava flow. Groundwater discharge from Malpais Spring maintains a large inundated marsh area that is occupied by White Sands pupfish. Marsh habitat at Malpais Spring fluctuates with variation in precipitation and ranges from about 65 ha (160 ac) to a maximum of about 363 ha (897 ac). The minimum habitat area is maintained by flow from the headspring and seeps, while maximum areal extent of wetland habitat at the site is a function of groundwater discharge combined with direct precipitation input to the wetland.

Salinity is markedly higher in Salt Creek than at Malpais Spring, although there is a general trend of increasing salinity with increasing distance from headwaters or headspring at both habitats. From 1996 to 2007, mean salinity was 28,492 mg/L in the middle reach of Salt Creek, while at the spring outflow at Malpais Spring it was 4,931 mg/L. Salinity has ranged from 12,700 to 38,100 mg/L in Salt Creek, with the lowest values recorded below the headcut waterfall and the highest values recorded from the lower reaches of Salt Creek. Salinity is relatively constant at the spring outflow at Malpais Spring, and ranged from 4,580 mg/L to 5,500 mg/L from 1911 to 2000 at the spring outflow. Salinity levels at the southern end of the wetland are generally double that of the spring outflow.

The contributions of major ions to total salinity also varies between the Salt Creek and Malpais Spring. Anions at Malpais Spring are dominated by sulfate and chloride with very little contributed by bicarbonate-carbonate. Cations at Malpais Spring are dominated by calcium, followed by sodium and then magnesium. In contrast, anions at Salt Creek are dominated by chloride followed by sulfate. Similar to Malpais Spring, bicarbonate-carbonate is insignificant. Cations at Salt Creek are dominated by sodium, with potassium being present only at very low levels. Sodium is followed by calcium and then by magnesium.

Conservation Analysis The goal of conservation of White Sands pupfish is to ensure the long-term viability of the two native populations (Salt Creek and Malpais Spring). The Malpais Spring and Salt Creek populations of White Sands pupfish may be quite large numerically, but they are geographically restricted. Viability of populations with these attributes is influenced primarily by environmental uncertainty and catastrophic factors. Consequently, viability of the Salt Creek and Malpais Spring populations depends primarily upon maintaining large populations that are spatially distributed throughout suitable habitats, and protecting against catastrophic events.

Environmental uncertainties that may influence the viability of White Sands pupfish populations include events such as changes in weather or climatic patterns, introduction of non-native competitors, predators, or diseases, and changes in habitat structure associated with vegetation dynamics. Catastrophic events may include prolonged and severe drought, extreme floods, toxic chemical spills, and hybridization (*e.g.* introduction of sheepshead minnow or non-native pupfish).

Three objectives were developed to address maintenance of large, spatially distributed populations at Salt Creek and Malpais Spring, and establishment and maintenance of natural refuge populations as a hedge against catastrophic events. These objectives are to: 1) maintain the spatial distribution of pupfish at Salt Creek and Malpais Spring; 2) maintain abundance within the natural range of variation; and 3) establish and maintain natural refuge populations. Potential stressors that may influence the status of White Sands pupfish include diminished discharge from springs and seeps, saltcedar persistence and growth, installation activities, density of marsh vegetation, loss of genetic integrity, and introduction of nonnative aquatic biota.

Conservation actions that have been implemented to date for the species have included signing of a cooperative agreement for conservation of White Sands pupfish and establishment of an interagency White

Sands pupfish conservation team (1995 and renewed in 2006), removal of artificial barriers to fish movement in Salt Creek at Range Roads 6 (in 1993) and 316 (in 2013), removal of feral horses from White Sands Missile Range (completed in 1999), annual monitoring (initiated in 1995), installation of stream discharge gages on Salt Creek (1995) and Malpais Spring outflow (2003), closure of roads in habitat of White Sands pupfish along Lost River on HAFB (1997), establishment of restricted-use essential habitat and limited use areas for pupfish on White Sands Missile Range and Holloman Air Force Base (1995), prohibition of transport of live nonnative aquatic organisms and their introduction into aquatic habitats on White Sands Missile Range and Holloman Air Force Base (1995). and evaluation of natural refuge sites for the Malpais Spring population (2010).

Conservation Actions Eight categories of conservation actions were developed to address factors potentially affecting the status of White Sands pupfish. These categories of conservation actions are:

1) establish Malpais Spring refuge populations;
2) improve population and habitat monitoring;
3) control saltcedar;
4) refine delineation of aquifer recharge zones;
5) develop an ecological restoration and management plan for Malpais Spring;
6) review installation activities to avoid or reduce potential impacts;
7) reduce potential for land-based chemical spills; and
8) conduct research in support of conservation.

Conceptual designs are provided for restoration of spring habitats to make them suitable as natural refuge sites for the Malpais Spring population. Restoration actions include geomorphic modifications, vegetation management, and removal of nonnative aquatic biota. Spring habitats identified as potential natural refuge sites for the Malpais Spring population include North Mound Spring, Mound Spring, South Mound Spring, and Barrel Spring. Recommendations are provided for improving population and habitat monitoring, which include increasing spatial coverage of sample sites and conducting monitoring on a biennial schedule with three sample surveys per site. Development of an ecological restoration and management plan for Malpais Spring is discussed in the context of the history of anthropogenic changes at the site, the probable natural geomorphic conditions at the site, and potential natural factors influencing wetland vegetation dynamics.

Appendices Two appendices are included in the Conservation Plan. Appendix A is an analysis of long-term trends in catch per unit effort and an assessment of the current monitoring program. Recommendations provided in Appendix A for improving the monitoring program included increasing the spatial distribution of sampling sites, increasing sampling occasions during each survey from one sample to three per site, and moving from an every-year sampling schedule to sampling sites every other year.

Appendix B is a cursory analysis of vegetation change at Malpais Spring and Salt Creek using aerial imagery from 1985 and 2012. The Malpais Spring analysis indicated that the area of open-water habitat at that site decreased approximately 53 percent since completion of feral horse removal in 1999. The Salt Creek analysis indicated that the spatial distribution and extent of saltcedar has changed little since 1985, which suggests that site-specific control activities may be effective.

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1.0 Introduction

White Sands pupfish (*Cyprinodon tularosa*) is endemic to the Tularosa basin in south-central New Mexico (Figure 1). The two native populations of the species occur entirely within White Sands Missile Range (WSMR), a military installation encompassing approximately 9,422 km^2 (3,638 mi^2) that is administered by the U.S. Department of the Army. Introduced populations are also found on WSMR as well as on adjacent Holloman Air Force Base (HAFB), which is administered by the U.S. Department of the Air Force. One introduced population found on HAFB may periodically expand onto adjacent White Sands National Monument during wet periods. White Sands National Monument is administered by the National Park Service.

White Sands pupfish is a Department of the Army At-Risk Species (critically imperiled category) and is listed by New Mexico as threatened. Also, the U.S. Fish and Wildlife Service determined in 2009 that federal listing of the species may be warranted, and a status review of the species is scheduled for completion in 2016.

A cooperative agreement for conservation of White Sands pupfish was executed in January 1995. Signatories to the agreement were the New Mexico Department of Game and Fish, the U.S. Fish and Wildlife Service, White Sands Missile Range, Holloman Air Force Base, and White Sands National Monument. The agreement included a conservation plan for the species that was revised in 1994 (Pittenger and Propst, 1994). The cooperative agreement was renewed in 2006.

The purpose of this conservation plan is to identify actions that can be implemented on WSMR and HAFB to improve the security of the species. The plan is organized into six chapters, including this introduction and a list of literature cited. The principal chapters of this plan are as follows.

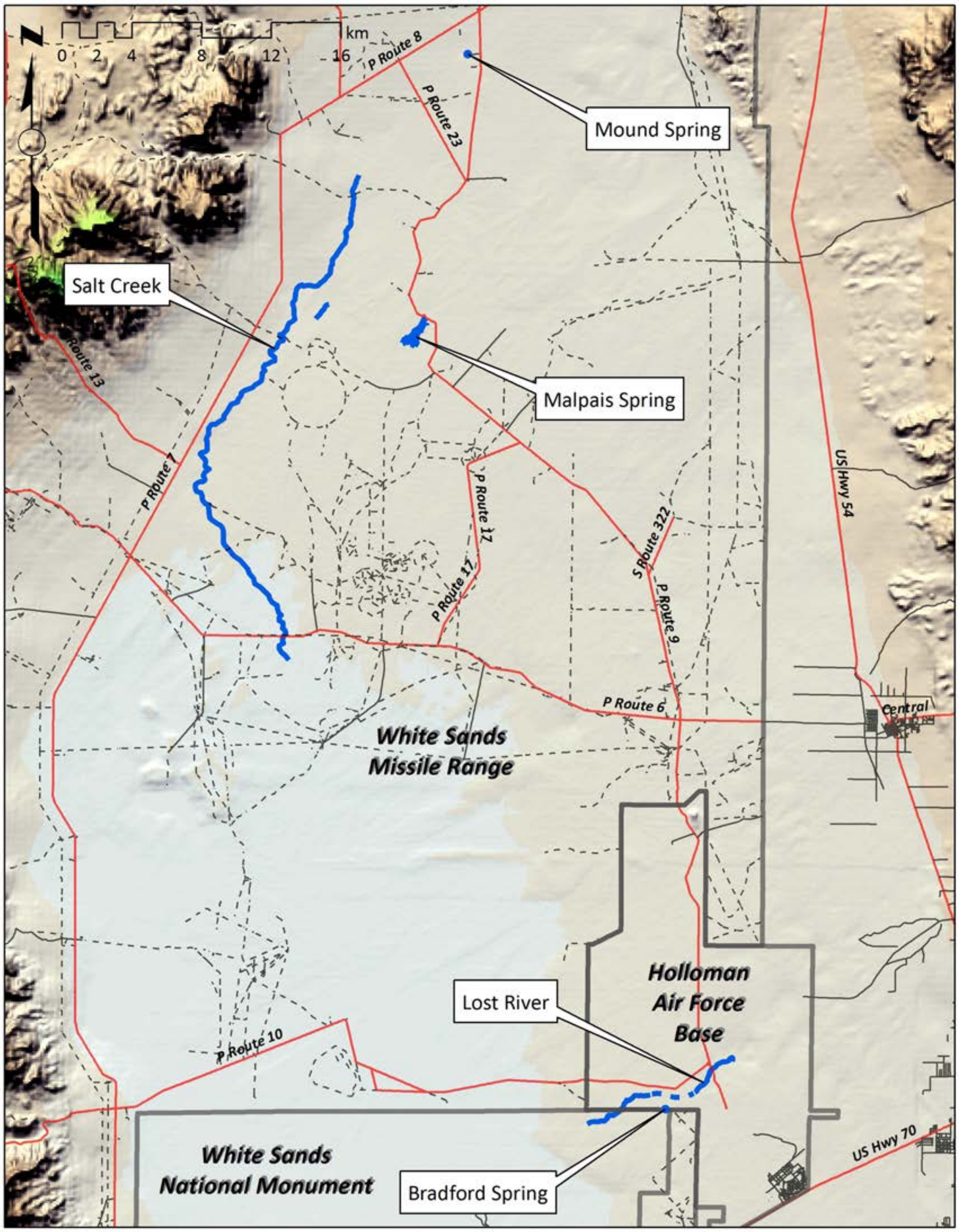
- Chapter 2 is a summary of the natural history of White Sands pupfish and provides the necessary background information for evaluating the current status of the species, identifying factors potentially affecting its persistence or security, and informing the development of conservation actions.

- Chapter 3 presents a conservation analysis of White Sands pupfish that includes a conservation goal, objectives, a conceptual ecological model for the species that describes vulnerabilities and stressors, a discussion of conservation efforts to date, and an assessment of the current status of the species.

- Chapter 4 follows with descriptions of conservation actions that target specific stressors or vulnerabilities.

- Chapter 5 includes a schedule for implementation of conservation actions.

Figure 1. Installation boundaries and extant populations of White Sands pupfish. The two native populations are in Salt Creek and at Malpais Spring. The Lost River, Bradford Spring, and Mound Spring populations are introduced and were established with pupfish from Salt Creek.



2.0 Natural History of White Sands Pupfish

2.1 Systematics, Biogeography, and Species Description

2.1.1 Systematics and Biogeography

The genus *Cyprinodon* (Order Cyprinodontiformes, Family Cyprinodontidae) includes approximately 50 species, of which about 30 occur in arid regions of southwestern North America (Miller, 1981; Minckley *et al*., 1991; Echelle *et al*., 2005; Martin and Wainwright, 2013; Froese and Pauly, 2014). Divergence among the five major clades of *Cyprinodon* likely began in the late Miocene to early Pliocene, about five to six million years ago (Echelle *et al*., 2005).

The maritime clade of pupfish (which includes *C. tularosa*, the only inland occurrence of this clade) and the southern Great Plains - northern Chihuahuan Desert clade (which includes *C. pecosensis*, *C. bovinus*, *C. rubrofluviatilis*, and *C. elegans*) likely diverged at the beginning of the Pliocene, about 5.2 million years ago (Echelle *et al*., 2005). Estimated time of vicariance for White Sands pupfish is approximately 1.8 million years ago (Figure 2; Echelle *et al*., 2005). The scenario for divergence of *C. tularosa* from *C. variegatus* involves upstream movement of the ancestral pupfish from the marine environment into the Tularosa basin from the Gulf of Mexico followed by geographic isolation of the basin. Hawley (1975: 146) first hypothesized a potential geologic history describing such a scenario, whereby the ancestral Rio Grande flowed through Fillmore Pass into the Tularosa and Hueco basins and on to the Gulf of Mexico.

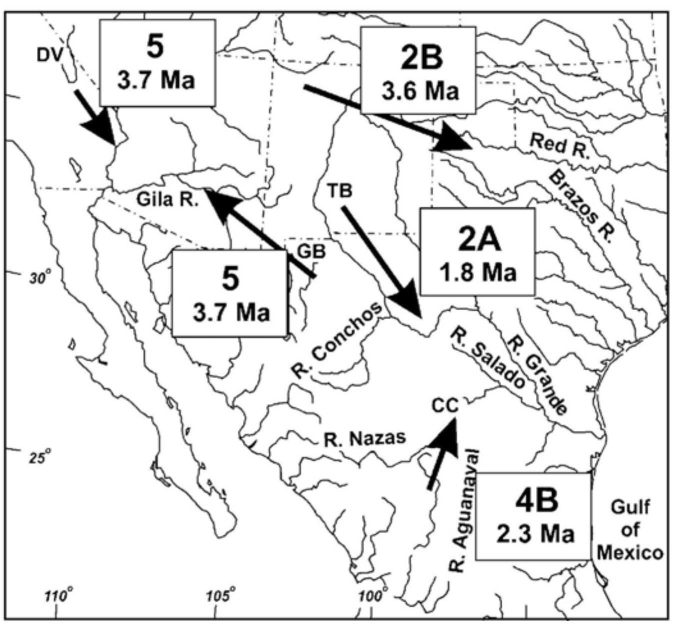


Figure 2. Hypothetical paleosystems and estimated times of vicariance for pupfish in each system. Arrows indicate paleosystems. The Tularosa basin is indicated by **TB**. Pupfish clades discussed in the text are: **2A** = maritime (which includes *C. tularosa*); **2B** = southern Great Plains-northern Chihuahuan Desert. Figure excerpted from Echelle and others (2005: Figure 4).

Other pupfish clades shown in the figure are: **4B** = old Río Nazas; **5** = western. Other basins shown in the figure are: **CC** = Cuatro Ciénegas; **GB** = Guzmán basin; **DV** = Death Valley system.

The ancestral upper Rio Grande had formed by late Pliocene time (*ca*. five million years ago; Mack *et al*., 2006) with development of an axial drainage connecting a chain of structural depressions that extended from southern Colorado to northern Mexico (Gile *et al*., 1981: 32). This ancestral river likely terminated in a series of sink areas near El Paso, collectively referred to as the Lake Cabeza de Vaca basin. Extensive fluvial deposits in south-central New Mexico indicate that the upper Rio Grande terminated in these southern sink areas from the late Pliocene to the middle Pleistocene (Gile *et al*., 1981: 32-34).

Continued deposition of fluvial sediments likely aggraded the river to the level of Fillmore Gap, leading to avulsions of the ancestral river and spillover into the Tularosa basin (Mack *et al*., 1997). Spillovers occurred periodically from 2.6 to 0.78 million years ago, and perhaps earlier (Mack *et al*., 2006). During these spillover periods, the river "swept northward into the southern Tularosa basin, before ultimately flowing southward into the Hueco basin" (Mack *et al*., 2006). This spillover drainage system may have continued southeast through the Presidio bolson and joined with the ancestral Rio Conchos to flow on to the Gulf of Mexico approximately 2.25 million years ago (Gile *et al*., 1981: 48; Gustavson, 1991).

Flow of the ancestral Rio Grande into the Tularosa basin was terminated by subsequent elevation of the Artillery Range fault (Mack *et al*., 2006) and uplift of the Organ and Franklin mountains (Mack *et al*., 1997), which directed flow back into the Mesilla basin. Integration of the ancestral upper Rio Grande with the lower river valley to form the present Rio Grande valley system occurred in early middle Pleistocene, approximately 0.78 million years ago (Hawley, 1975; Gile *et al*., 1981; Mack *et al*., 2006).

2.1.2 General Species Description

White Sands pupfish was described in 1975 from specimens collected between 1927 and 1950 at Malpais Spring and Salt Creek (Miller and Echelle, 1975). Similar to other pupfish, *C. tularosa* is a small-bodied, stocky fish with an upturned, protractile jaw. Typical maximum total length is about two inches (50 mm).

Male White Sands pupfish develop brilliant coloration during breeding (*i.e.* nuptial coloration). Nuptial male coloration is characterized by deep metallic or iridescent blue dorsolaterally, grayish blue laterally, light blue on throat and chin, and whitish to orange color on the abdomen (Figure 3). The distal half of the dorsal fin is bright yellow-orange to deep orange and the proximal portion is dusky. The anal fin is dusky proximally and orange distally, the pectoral and pelvic fins are yellowish to orange, and the caudal fin is light yellow with a black terminal band (Miller and Echelle, 1975; Sublette *et al*., 1990: 252; Propst, 1999: 69).

Females, juvenile males, and non-breeding males are olivaceous dorsally, whitish to grayish blue laterally, white ventrally, sides with dark vertical bars or blotches, a black ocellus near the posterior base of the dorsal fin, yellowish pectoral and pelvic fins, and whitish to colorless anal and caudal fins (Figure 3).

White Sands pupfish is distinguished from Pecos pupfish by having the breast and abdomen fully scaled or almost so (Miller and Echelle, 1975). Similar to *C. tularosa*, *C. variegatus* also has a fully scaled abdomen.

Figure 3. White Sands pupfish nuptial male. Photo courtesy of Doug Burkett, ECO Inc.

2.2 Conservation Genetics

2.2.1 Isolation of Native Populations

The two native populations of White Sands pupfish (Salt Creek and Malpais Spring) likely were isolated in the Holocene with development of a warmer and drier climate and concurrent contraction and isolation of aquatic habitats in the Tularosa basin. Pittenger and Springer (1999) speculated that the populations were isolated following dessication of Lake Otero and emplacement of the Carrizozo lava flow 5,900 to 4,500 years ago (Dunbar, 1999). Stockwell and others (2013) estimated that the two native populations diverged 3,250 to 11,000 years ago, using Approximate Bayesian Computation analysis.

Lake Otero, which comprised a shallow, saline lake or series of lakes and wetlands, occupied the floor of the Tularosa basin from *ca*. 45,000 to 28,000 years ago, as evidenced by a record of accumulating lacustrine sediments during that period (Lucas and Hawley, 2002; Allen *et al*.,2009). A period of erosion of lacustrine deposits occurred from *ca*. 28,000 to 25,000 years ago, followed by repeated episodes of increased precipitation and runoff from surrounding watersheds, enhanced fluvial activity, and freshening of the lake system from *ca*. 24,500 to at least 15,500 years ago (Allen *et al*., 2009). Maximum lake elevation occurred during this period, when surface water elevation reached *ca*. 3,960 ft (1,207 m) and the lake covered at least 287 mi^2 (745 km^2; Allen *et al*., 2009). During this high-stand period, the fluvial systems of the ancestral Carrizozo drainage and Three Rivers were integrated with the lake (Allen *et al*., 2009). Dense stands of emergent aquatic vegetation were present around the margins of the lake during this time.

With onset of severe drought from 15,000 to 14,000 years ago the lake shrank or desiccated completely, likely followed by lake expansion from 14,000 to 12,500 years ago. During this wet period there was an extensive fluvio-deltaic complex along the northern margins of Lake Otero with wetlands encompassing at least 19 mi^2 (50 km^2; Allen *et al*., 2005). Drying and deflation of the lake basin likely began again *ca*. 12,000 to 11,000 years ago, with a second generation of deflation beginning about 7,000 years ago which lowered ground water levels on the basin floor by *ca*. 10 m (Allen *et al*., 2009). The ancestral Carrizozo drainage was filled by the Carrizozo lava flow (Weir, 1965: 26) *ca*. 5,900 to 4,500 years ago (Dunbar, 1999; Love *et al*., 2012). Wetlands stalled the progress of the Carrizozo lava flow at the present-day location of Malpais Spring (Love *et al*., 2012).

Deflation of the desiccated lake basin *ca*. 12,000 to 11,000 years ago and again *ca.* 7,000 years ago likely resulted in channel incision in contributing drainage channels. This channel incision, coupled with filling and capping of the ancestral Carrizozo drainage by the lava flow 5,900 to 4,500 years ago may have resulted in hydrologic disconnection of the Salt Creek and Malpais Spring aquatic habitats.

2.2.2 Genetic Characteristics

The first genetic analysis of White Sands pupfish was conducted by Echelle and others in 1987 using allozyme electrophoresis. They examined 28 gene loci and found only two that were polymorphic: phosphogluconate dehydrogenase (Pgdh-A) and creatine kinase (Ck-C; Echelle *et al*., 1987). The Pgdh-A locus was highly variable among the four populations examined (Salt Creek, Malpais Spring, Mound Spring, and Lost River). The Salt Creek and Lost River populations were found to be most similar, while the Mound Spring population was the most divergent of the four populations (Echelle *et al*., 1987).

It was speculated that the spatial heterogeneity observed in White Sands pupfish could be the result of geographic isolation of the populations, but it was noted that a similar degree of heterogeneity was observed in *C. elegans* in a recently interconnected habitat (Echelle *et al*., 1987).

The findings of Echelle and others (1987) were subsequently reaffirmed (Stockwell and Mulvey, 1998; Stockwell *et al*., 1998), and two additional polymorphic loci were found: hexokinase (Hk-A) and xanthine dehydrogenase (Xdh-A). There were fixed differences in allele frequencies at allozyme locus Hk-A and two DNA microsatellite loci, WSP-02 and WSP-11 (Stockwell *et al*., 1998). Malpais Spring was the most divergent population and the Salt Creek, Mound Spring, and Lost River populations were most similar (Table 1).

The genetic analyses supported the interpretation of historical data that the Lost River and Mound Spring populations were introduced (Pittenger and Springer, 1999), and were established with fish from Salt Creek. Subsequent analyses found no signs of genetic divergence between the Mound Spring and Salt Creek populations (Heilveil and Stockwell, 2007; Collyer *et al*., 2011).

Based on their analysis, Stockwell and others (1998) concluded that loss of either the Salt Creek or Malpais Spring genotypes would result in a corresponding loss of 32 to 36 percent of the allelic diversity found in White Sands pupfish. Stockwell and others (1998) designated the Malpais Spring and Salt Creek populations as evolutionarily significant units (ESU) and this distinction was reaffirmed in subsequent genetic analyses (Heilveil and Stockwell, 2007). The ESU designation was made under the assumption that the two populations have been isolated since the end of the Pleistocene and thus constitute unique evolutionary lineages that should be preserved (Stockwell *et al*., 2013).

Table 1. Genetic distance among four populations of White Sands pupfish, excerpted from Stockwell and others (1998). The results above the diagonal are arc distance (D_{arc}) for allozyme data. The results below the diagonal are the pairwise distance metric (R_{ST}) values for microsatellite data. The values in bold indicate the pairwise comparisons of Malpais Spring with the other three populations.

	Malpais Spring	Salt Creek	Mound Spring	Lost River
Malpais Spring	---	**0.530**	**0.530**	**0.564**
Salt Creek	**0.652**	---	0.145	0.122
Mound Spring	**0.795**	-0.014	---	0.217
Lost River	**0.605**	-0.016	-0.017	---

2.3 Distribution and Abundance

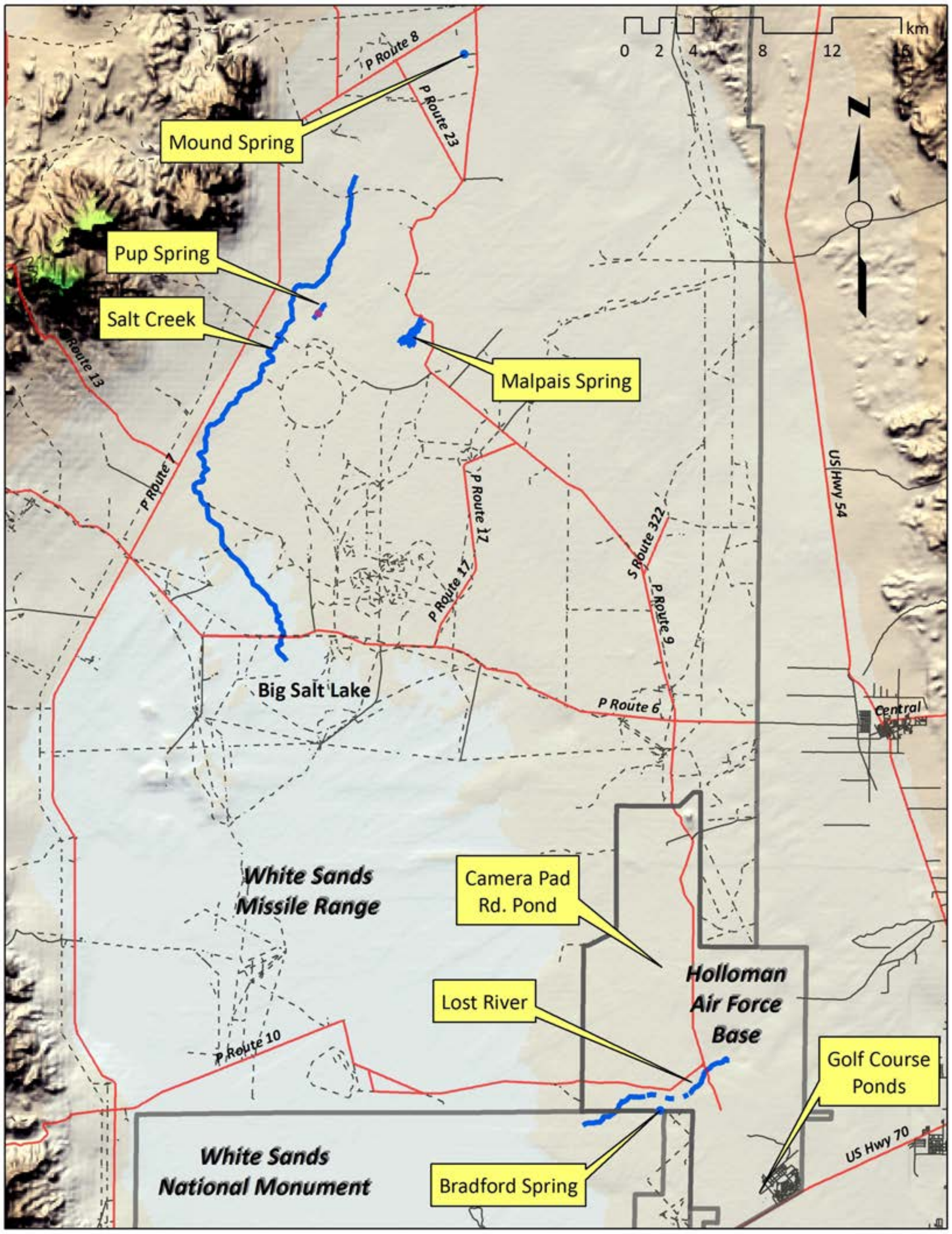
2.3.1 Historic and Current Distribution

The current distribution of White Sands pupfish includes the two native populations (Salt Creek and Malpais Spring) and introduced populations at Mound Spring, Lost River, Bradford Spring, and possibly the golf course ponds at Holloman Air Force Base (Figure 4).

White Sands pupfish is the only fish species native to the Tularosa basin, and the current distribution of the species is confined to the basin (Sublette *et al*., 1990: 254; Propst, 1999: 69). When the species was described in 1975, it was known to occur at Malpais Spring, "ponds" located approximately 2.6 km (1.6 mi) south of the spring, and at Salt Creek. However, Miller and Echelle (1975) noted that other populations could occur in aquatic habitats that had not been sampled up to that time.

Turner (1987) reported that *C. tularosa* occurred in Malpais Spring, Salt Creek and Mound Spring and that a presumably introduced population occupied a short (*ca*. less than 100 m [330 ft]) section of Malone Draw on Holloman Air Force Base (Figure 4). Sublette and others (1990: 253) described the distribution of *C. tularosa* as including "Malpais Spring and its outflow and Lost River (including Malone Draw) in Otero County; Salt Creek in Sierra County; and Mound Springs in Lincoln County." They presumed the population at Lost River to be introduced based on genetic analysis by Echelle and others (1987), and indicated that the Mound Spring population was the most genetically dissimilar (again, based on Echelle *et al*., 1987).

Figure 4. Current distribution of White Sands pupfish. The two native populations are in Salt Creek and at Malpais Spring. The Lost River, Bradford Spring, and Mound Spring populations are introduced and were established with pupfish from Salt Creek. The introduced population at Camera Pad Road Pond is extirpated and the status of pupfish introduced to the HAFB golf course ponds is unknown. See text for discussion.



Pittenger and Springer (1999) assessed all known aquatic habitats, both artificial and natural, in the upper Tularosa basin and found the distribution of *C. tularosa* to include Salt Creek from below Salt Springs downstream to Big Salt Lake, Malpais Spring from the headspring downgradient to a terminal laguna, both ponds at Mound Spring, and Lost River from the confluence of Ritas and Malone draws downstream to the terminus of the drainage in the gypsum dunes of White Sands National Monument (Figure 4). Analysis of historical accounts, interviews, and collection records, and field notes indicated that only the Malpais Spring and Salt Creek populations were native. Documentation was found in William J. Koster's field notes of stocking of approximately 30 *C. tularosa* into Lost River on 29 September 1970 (Pittenger and Springer, 1999). Historical evidence indicated that fish were absent from Mound Spring prior to 1967, when the spring was excavated by dragline, and that fish were stocked into the resulting pond sometime between 1967 and 1973. Subsequent genetic analysis supported this conclusion (Stockwell *et al*., 1998). White Sands pupfish have recently been documented from Pup Spring in Upper Basin Draw, which is a tributary to Salt Creek (Figure 4).

Populations of *C. tularosa* at the golf course ponds, Camera Pad Road Pond, and Bradford Spring, all located on Holloman Air Force Base (Figure 4), were established by unregulated translocations on 18-21 December 2006 by Dr. Craig Stockwell, North Dakota State University (NDSU), using pupfish from a terminated common-garden experiment. Founding population sizes ranged from 500 to 6,774. The translocated fish were all of Salt Creek lineage (C. Stockwell, NDSU, pers. comm., 6 April 2010). Camera Pad Road pond was found to be dry on 28 May 2014 and the population there is extirpated. White Sands pupfish persist at Bradford Spring and were observed there on 28 May 2014, but the status of the translocated population at the golf course ponds is unknown (J. S. Pittenger, *in litt*.). Populations of White Sands pupfish introduced to a pond south of Alamogordo in 1973, using fish from Salt Creek, and to another pond at Valley of Fires State Park in 1977, using fish from Malpais Spring, are extirpated (Jester and Suminski, 1982; J. S. Pittenger, *in litt.*).

2.3.2 Abundance

Turner (1987) reported densities of White Sands pupfish ranging from 10.08 to 30.01 pupfish/m^2 in the spring outflow at Malpais Spring and 1.18 to 7.40 pupfish/m^2 in Salt Creek downstream from the Range Road 316 crossing at an old road crossing. Lowest densities were observed in early summer and peak densities occurred in early fall. Biomass estimates ranged from 7.7 to 19.2 g/m^2 at Malpais Spring and 1.0 to 4.6 g/m^2 at Salt Creek (Turner, 1987). Jester and Suminski (1982) reported a density of 12.5 pupfish/m^2 from a now-extirpated population at a temporary pond near Alamogordo.

Relative abundance of White Sands pupfish at two monitoring sites on Salt Creek was correlated with stream flow indices (Pittenger *et al.*, 2015). Relative abundance at the Salt Springs site, located in the headwaters of Salt Creek, was highly positively correlated with annual growing degree days (Pittenger, *in litt.*) and previous winter flow conditions. Hydrology of the Salt Springs site is controlled by spring inputs and, to a lesser extent, storm water runoff. In contrast, relative abundance at the Range Road 316 site was strongly correlated (negatively) with the number of zero-flow days and (positively) with mean monthly flow. Hydrology at the Range Road 316 site is influenced by upstream spring inputs and storm-water runoff.

Abundance of White Sands pupfish at Malpais Spring appears to be influenced primarily by wetland vegetation dynamics prior to and following removal of feral horses (Pittenger, *in litt*.). However, the observed variation in relative abundance from 1995 through 2014 may also be the result of changes in distribution of pupfish or capture probability that are associated with wetland vegetation dynamics at the site.

Dense wetland vegetation has been identified as unsuitable habitat for pupfish (Kennedy, 1977; Itzkowitz, 2010), although Kodric-Brown (1978) did report establishment of pupfish breeding territories on subsurface mats formed by rooted aquatic vegetation. Turner (1987: 87-88) noted that White Sands pupfish was rare in habitat with dense emergent wetland vegetation at Malpais Spring. However, this assertion was based on visual observation and Turner (1987: 87) did note that visual detection of pupfish in such habitats was "difficult to impossible."

An inverse relationship between wetland vegetation density and abundance of desert fishes has been inferred in some studies (*e.g.* Winemiller and Anderson, 1997; Kodric-Brown and Brown, 2007). In a spring-fed marsh situation similar in nature to Malpais Spring, Johnson and others (2013) found that Amargosa pupfish (*C. nevadensis*) was significantly more abundant in marsh habitats with lower emergent vegetation cover and more open-water area compared to sites with high plant cover and little or no open-water habitat. Similarly, pupfish abundance in the outflow of Jackrabbit Spring (Ash Meadows National Wildlife Refuge) declined concurrent with an increase in the density of common reed and bulrush in the spring outflow channel, and increased stream shading by saltcedar (Scoppettone *et al*., 2013).

Itzkowitz (2010) reported marked declines in Leon Springs pupfish (*Cyprinodon bovinus*) at Diamond Y Spring concurrent with increases in bulrush (*Schoenoplectus* sp.). It was suggested that population decline was due to loss of suitable spawning habitat because male pupfish establish breeding territories at unvegetated sites. Addition of cement substrates increased breeding sites and an increase in abundance was subsequently noted (Itzkowitz, 2010). Kennedy (1977) stated that distribution of *C. bovinus* at Diamond Y Spring was restricted by dense and extensive growths of bulrush.

2.4 Population Trend

Annual monitoring of population trend conducted since 1995 has not shown any persistent pattern of decline or increase of White Sands pupfish at monitoring sites at Malpais Spring and Salt Creek. As discussed above in section 2.3.2, annual variation in relative abundance is correlated with environmental factors including flow conditions and temperature at Salt Creek and wetland vegetation dynamics at Malpais Spring. Population-wide trend assessment is hindered by the limited spatial coverage of monitoring sites (see Appendix A for a review and assessment of the monitoring program).

Annual monitoring data from 2008 to 2013 showed no trend at either of the two sample sites located on Salt Creek (Appendix A, section A.2.1). However, the 2008-2013 data set indicated a significant increase in pupfish catch per unit effort at the Upper Marsh site at Malpais Spring, but no trend at the Middle Marsh site at Malpais Spring (Appendix A, section A.2.1). These results disagree with the findings presented in recent annual monitoring reports (Wick and Caldwell, 2012; Caldwell, 2014) because the analysis in Appendix A is based on total catch per unit effort, while the monitoring reports examined mean catch per unit effort. As discussed in Appendix A, regression analysis using mean catch per unit effort (*i.e.* grouped data) may result in biased regression coefficients and loss of information (Freund, 1971; Appendix).

The introduced population at Mound Spring has experienced abrupt declines followed by gradual recovery. Population declines were associated with periodic fish kills caused by overabundance of a digenetic trematode (Pittenger and Springer, 1996:5).

2.5 Activity Patterns and Movements

Seasonal changes in abundance were observed at Malpais Spring that indicated potential movement of fish toward the more thermally stable headspring area in the winter and out into the wetland and terminal laguna in the spring (Pittenger and Springer, 1996: 12). Concentration of pupfish in pool habitats in the winter was also observed in Salt Creek (J. S. Pittenger, *in litt*.). Dispersal of predominately smaller fish was observed in Salt Creek in the summer concurrent with expansion of available habitat from surface runoff (Pittenger and Springer, 1996: 16). The dispersal was from core population areas to more marginal habitats.

Pupfish are known to move in response to changes in water temperature. For example, Barlow (1958) found desert pupfish (*C. macularius*) became inactive or avoided habitats with water temperatures warmer than 36ºC. Dissolved oxygen concentration and salinity did not appear to influence movement (Barlow, 1958). Young pupfish typically move to habitats with higher water temperatures compared to adult pupfish (*e.g.* Lowe and Heath, 1969). Baugh and Deacon (1983) reported movement of pupfish (*C. diabolis*) to and from a shallow shelf at Devil's Hole in response to changes in solar insolation, which influences water temperature (Hausner *et al*., 2013). Pupfish moved off of the shallow shelf during periods of high light intensity on both diel and seasonal time frames. In contrast, however, movements by Quitobaquito pupfish (*C. macularius eremus*) from a thermally stable spring habitat to an adjacent pond were virtually nonexistent (Douglas *et al*., 2001).

2.6 Habitat

The two native populations of White Sands pupfish occupy habitats that differ in water chemistry, hydrologic characteristics, and other features. Salt Creek is primarily a lotic system, but also has features of standing water habitats in large pools along the stream channel and playas at the mouth of the drainage. Malpais Spring has lotic features in spring run and marsh channels. Large areas of lentic habitat occur in the extensive marsh and the *laguna* at the south end of the site.

2.6.1 Hydrology

Habitats of White Sands pupfish in the northern Tularosa Basin are sustained by two hydrologic systems (Allen *et al*., 2005): 1) the surface water runoff system; and 2) the sulfate-laden groundwater system. The runoff system responds with a short time lag to precipitation events, whereas the groundwater system has a longer time-lag response to precipitation.

<u>2.6.1.1 Aquifer Characteristics</u> Weir (1965: 9-11) estimated that approximately 554,850,000 m^3 (450,000 acre-feet) of precipitation falls annually in the Tularosa basin, but due to the high evaporation rate only about five percent of the precipitation that falls throughout the basin reaches the basin-fill aquifer (Weir, 1965: 29; Huff, 2005). Contemporary annual recharge is very small compared to the existing volume of water stored in the basin-fill and bedrock aquifers (Weir, 1965: 30).

Groundwater in the Tularosa basin moves generally southward and toward the basin away from the mountain-front aquifer recharge zone (Figure 5; Huff, 2005). The mountain-front recharge zone is characterized by alluvial fans composed of coarse, permeable sediments (Weir, 1965: 26-30; Orr and Myers, 1986). The basin floor area likely contributes very little to recharge of the basin-fill aquifer due to the relative impermeability of thick strata consisting of clays and other fine-grained sediments (Weir, 1965: 30; Orr and Myers, 1986), low precipitation, and the high rate of evaporation rate on the basin floor (Huff, 2005).

The basin-fill aquifer is recharged by infiltration of intermittent surface-water flows into coarse sediments near the proximal end of alluvial fans and also subsurface flow along stream channels with larger catchments (Huff, 2005: 4). Huff (2005) identified catchment basins in the mountains and hills surrounding the basin that potentially contribute to the basin-fill aquifer based on the work of Waltemeyer (2001; Figure 5). However, Waltemeyer (2001) did not evaluate numerous catchments in the San Andres Mountains that drain to Salt Creek (*e.g*. the area between catchments 43 and 44 in Figure 5). Furthermore, no basin-fill aquifer recharge catchments were identified in the northern third of the basin (Figure 5; Huff, 2005: 6).

Fryberger (2001) noted that the Carrizozo lava flow "blocks runoff from the west, which ponds at the edge of the flow, then percolates downward," suggesting that principal recharge of the Malpais alluvial aquifer is from the catchments west of the lava flow (Figure 5). The effect, if any, of the expanse of watershed in the northern third of the basin on recharge of the Malpais alluvial aquifer is unknown.

There are several large drainages, including mountain-front systems at the north end of the Sacramento Mountains, that empty into basin-fill on the east and northwest sides of the Carrizozo lava flow (Figure 6). Relatively large catchments including Harkey Draw, Nogal Arroyo, White Oaks Draw, Ancho Gulch, Largo Canyon, and Harvey Draw drain from basin highlands to the upper half of the Carrizozo lava flow in the northern part of the Tularosa basin (Figure 6).

Huff (2005) estimated recharge of the basin-fill aquifer to be approximately 143,000 m^3/day (42,320 acre-feet/yr). Roughly 88 percent of the total recharge leaves the basin-fill aquifer as evapotranspiration (Huff, 2005). Estimated maximum evapotranspiration in the basin is 0.0033 meters/day (4 ft/yr) and maximum depth at which evapotranspiration occurs is 4.5 m (15 ft; Huff, 2005: 4).

Huff (2002) reported apparent age of groundwater in the southeastern area of the basin ranging from 1,000 to 10,000 years old, with older water associated with deeper and thus longer flow paths. Apparent age in the uppermost 100 m (328 ft) of the basin-fill aquifer ranged from 1,534 to 8,019 years old, while the apparent age of groundwater from 200 to 300 m (656 to 984 ft) depth was 9,188 years old (Huff, 2002).

Subsurface movement of groundwater from bedrock aquifers, including the sedimentary San Andres and Artesia formations, to the basin-fill alluvial aquifer is indicated by the slope of the water table (Weir, 1965: 27). Sulfur isotope analysis indicates that the main sulfate sources for springs in the northern Tularosa basin is likely dissolution of the San Andres and Artesia formations of the middle Permian strata (Szynkiewicz *et al*., 2009).

Figure 5. Basin-fill aquifer recharge catchments delineated in the northern Tularosa basin (from Huff, 2005: 6). Catchments are (counterclockwise from north): **46** (Taylor Canyon); **45** (Red Canyon); **44** (Oscura Mountains backslope); **43** (Thurgood Canyon); **42** (Good Fortune and Dry canyons); **12** (Fresnal and La Luz canyons); **11** (Nogal and Tularosa canyons); **10** (Rinconada Canyon); **9** (Three Rivers Canyon); **1-8** (White Mountain Wilderness west-side drainages). Not all catchments are characterized (see Waltemeyer, 2001).

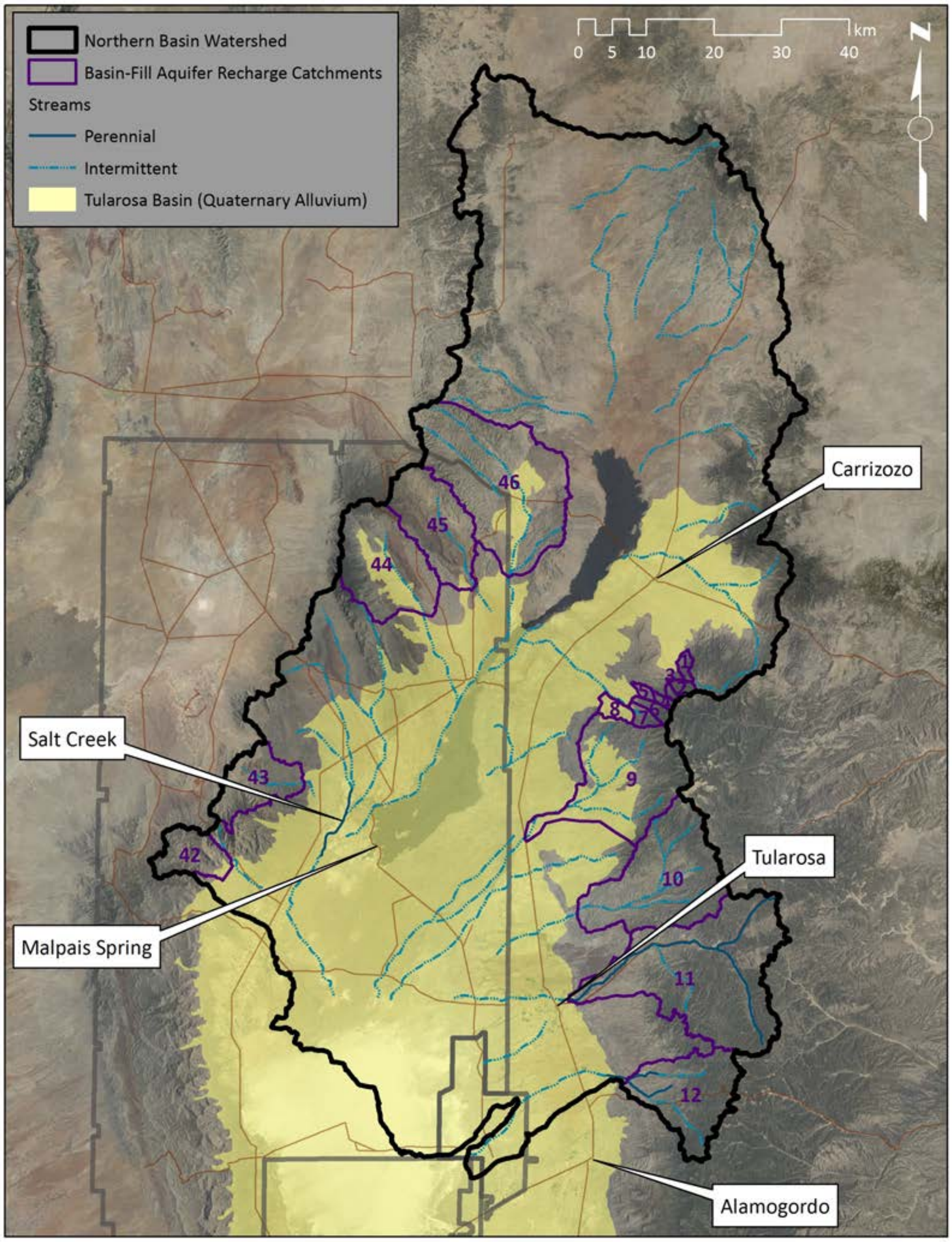
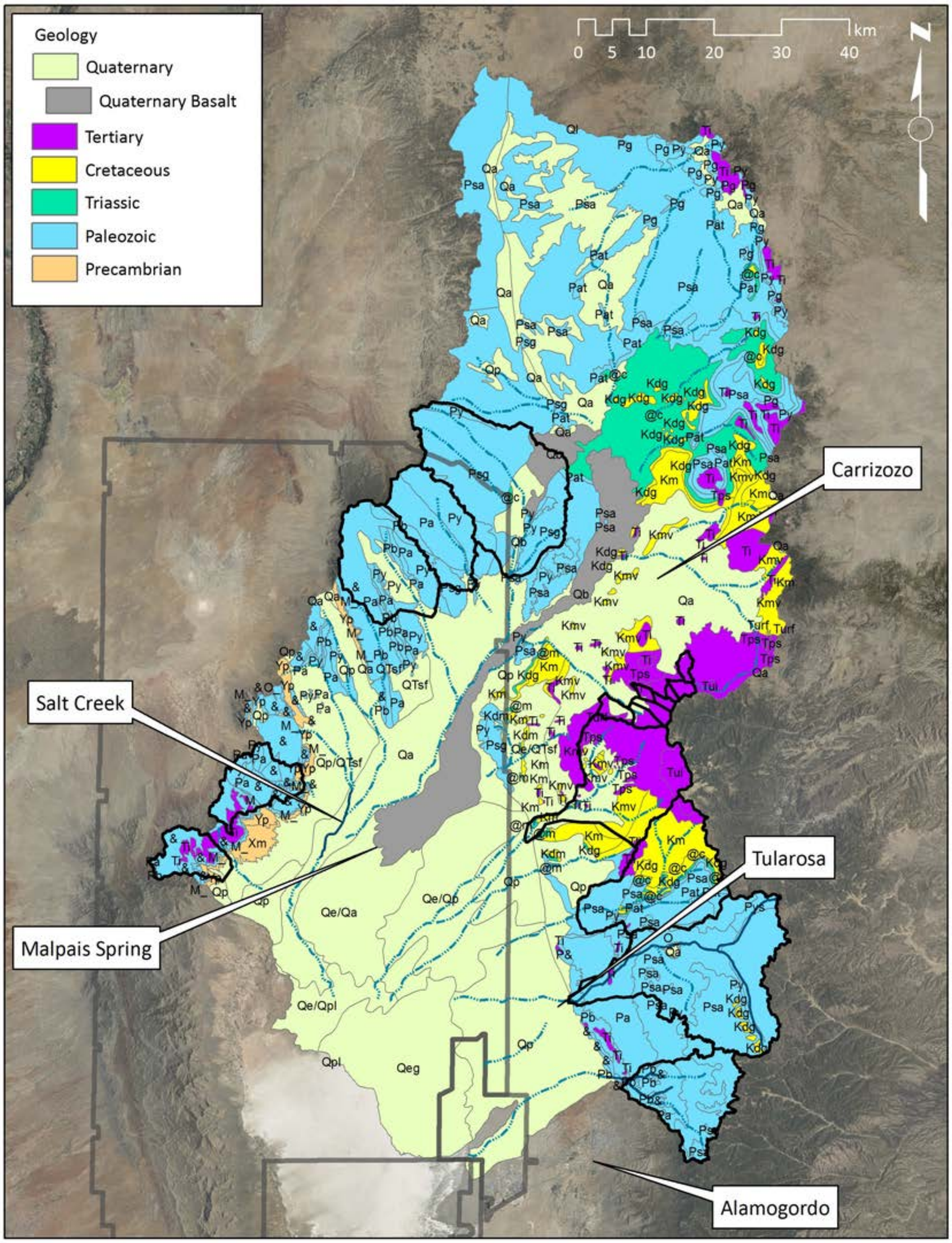


Figure 6. Geology of the northern Tularosa basin. The mountain-front recharge catchments delineated by Waltemeyer (2001) and used by Huff (2005) in modeling groundwater recharge and flow are outlined in black.



Groundwater from the alluvial aquifer is discharged into Salt Creek, at Malpais Spring, and at the Mound Springs. The Mound Springs apparently are the result of decreased permeability along a fault zone (Weir, 1965: 26), which forces water to the surface. Groundwater gradients in the basin range from approximately 18.9 to 37.9 m/km (100 to 200 ft/mi) on the margin of the basin to approximately 6.1 m/km (20 ft/mi) in the center of the basin (Weir, 1965: 26-27).

2.6.1.2 Salt Creek Perennial flow in Salt Creek is maintained by groundwater discharge from the alluvial aquifer (Weir, 1965: 37). Groundwater input, in the form of springs and seeps, occurs throughout the reach of Salt Creek from the headwaters downstream to the vicinity of a head-cut waterfall (Figure 7). Stream flow in this reach, a distance of approximately 12.1 stream-km (7.5 stream-mi), roughly doubles from the headwaters to the waterfall (Myers and Naus, 2004)

Groundwater input to the stream appears to cease near the Route 316 crossing (Figure 7), where the stream begins to lose surface flow. Salt Creek becomes an intermittent stream below this point downstream to where the valley opens up into broad alkali flats, a distance of approximately 17.5 stream-km (10.9 stream-mi; Figure 7). Salt Creek often dries completely in the lower end through the alkali flat to near the mouth of the stream in a playa above Big Salt Lake (Figure 7), a distance of approximately 8.5 stream-km (5.3 stream-mi).

In the upper section of Salt Creek, groundwater input maintains a stable aquatic habitat with substantial volume (Figure 8). Hourly monitoring of water level in representative pool habitat in the headwaters of Salt Creek showed that pool depth did not drop below 71 cm (28 in; Figure 9). In comparison, representative pool habitat in the lower section of Salt Creek (Figure 10) exhibited marked fluctuations in depth, with a several-day period of no standing water in the late summer (Figure 9).

Rainfall-runoff events cause short-term increases in stream flow in Salt Creek. Maximum recorded peak flow at the stream gage near Range Road 316, which began operation on 31 August 1995, was 10.65 m^3/s (376 cubic feet per second [cfs]) on 7 May 2007 (U.S. Geological Survey, 2014*a*). In contrast, the peak flow events in 1997 ranged from a high of 0.76 m^3/s (27 cfs) to 0.03 m^3/s (1.1 cfs; Figure 9). Rainfall-runoff events provide the main source of surface water flow in lower Salt Creek in the summer months (Figure 8). In the absence of rainfall-runoff events, the lowermost reaches of Salt Creek cease to flow and often dry in the summer. In contrast, groundwater inputs in the upper section of Salt Creek serve to maintain aquatic habitat during hot, dry periods.

Figure 7. Hydrologic features of Salt Creek.

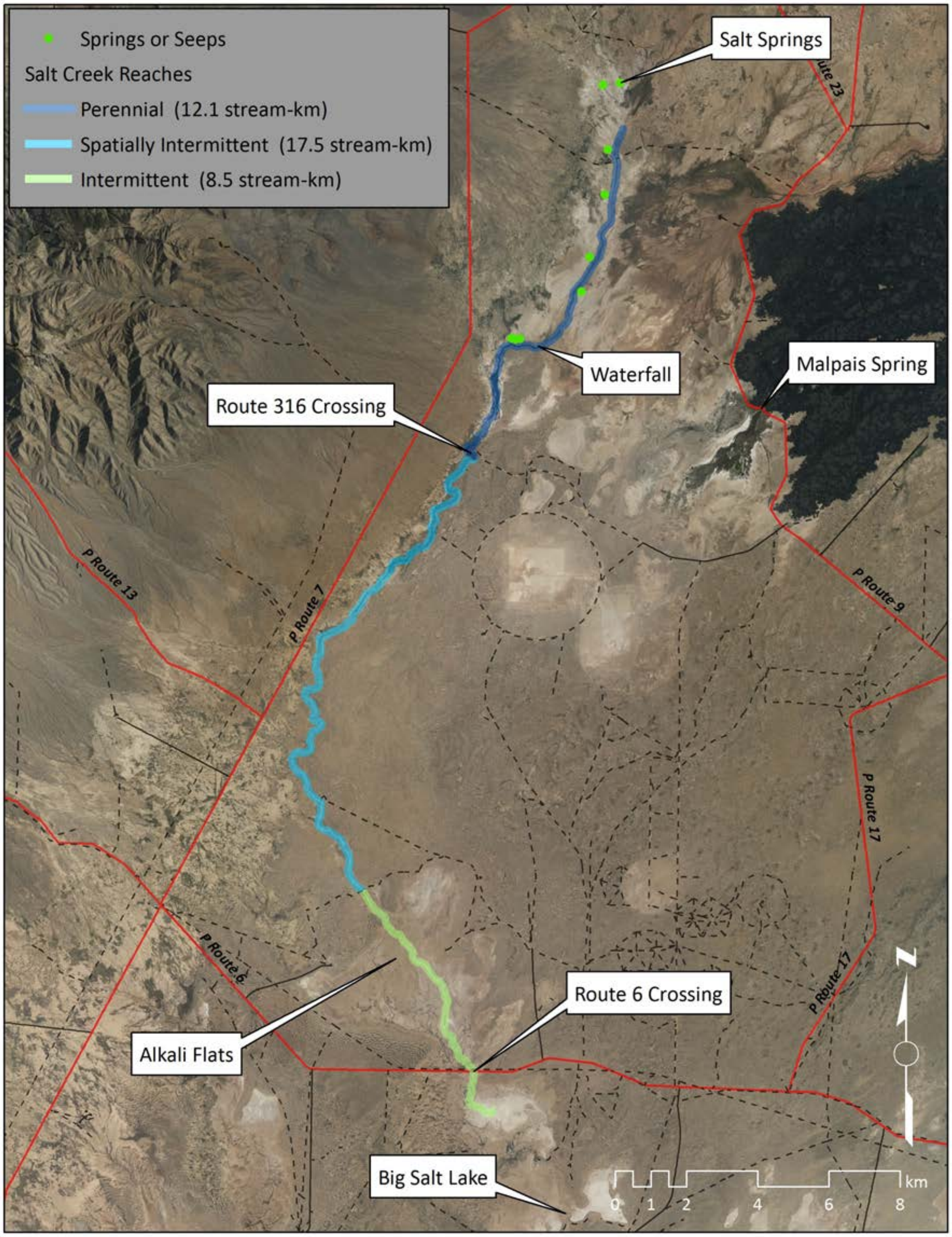


Figure 8. Pool habitat in the headwaters of Salt Creek. Photo by J. S. Pittenger, 23 April 2014.

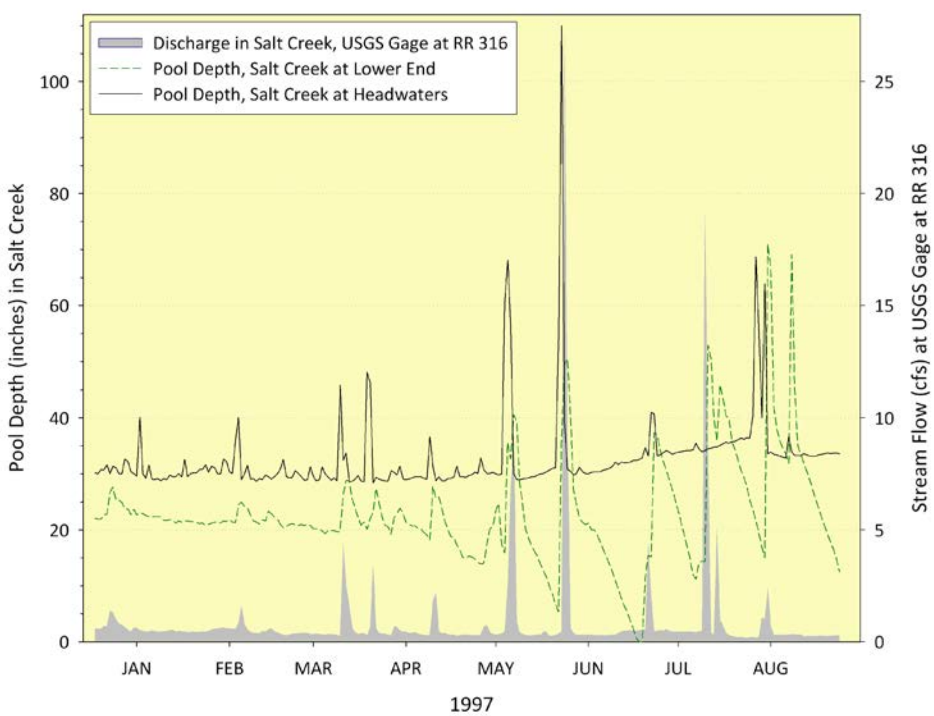


Figure 9. Water level in pool habitat in upper and lower sections of Salt Creek (J. S. Pittenger, *in litt.*). Discharge is shown as the gray-shaded area (U.S. Geological Survey, 20014*a*).

Figure 10. Habitat at the lower end of Salt Creek about the Route 6 crossing. Photo by J. S. Pittenger, 23 April 2014.

2.6.1.3 Malpais Spring Groundwater discharge at Malpais Spring is from a regional aquifer that consists of Quaternary and Tertiary bolson fill and stream channel sediments buried under the Carrizozo lava flow (Myers *et al*., 2008). Early reports estimated discharge from Malpais Spring to be 0.09 m^3/s (1,500 gal/min; Weir , 1965: 50) to 0.13 m^3/s (2,000 gal/min; Meinzer and Hare, 1915: 300). A continuous-flow stream gage was installed by the U.S. Geological Survey in July 2003 in the spring outflow channel and was operated until 4 April 2012. Mean daily discharge at the stream gage varied from 0.02 to 0.14 m^3/s (0.64 to 4.9 cfs; Figure 11; U.S. Geological Survey, 2014*b*).

However, total flow from the large seep at the head spring area cannot be measured because it is diffuse and widespread. Therefore, the gage data only represent part of the actual flow to the wetland ®. Myers, WSMR, pers. comm., 3 May 2010). Annual maximum mean daily flows measured at the Malpais Spring gage from 2004 through 2011 ranged from 0.08 to 0.14 m^3/s (2.7 to 4.9 cfs; U.S. Geological Survey, 2014*b*). Variation in discharge from the spring appears to be influenced primarily by precipitation and resulting recharge of the buried stream-channel alluvial aquifer (Myers and Naus, 2004; Myers *et al*., 2008).

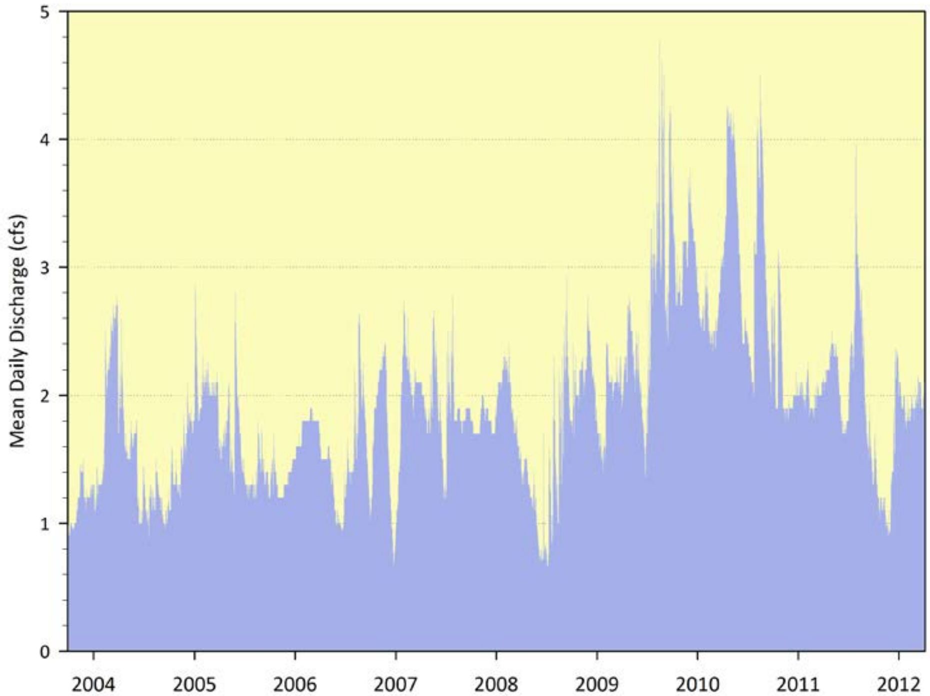
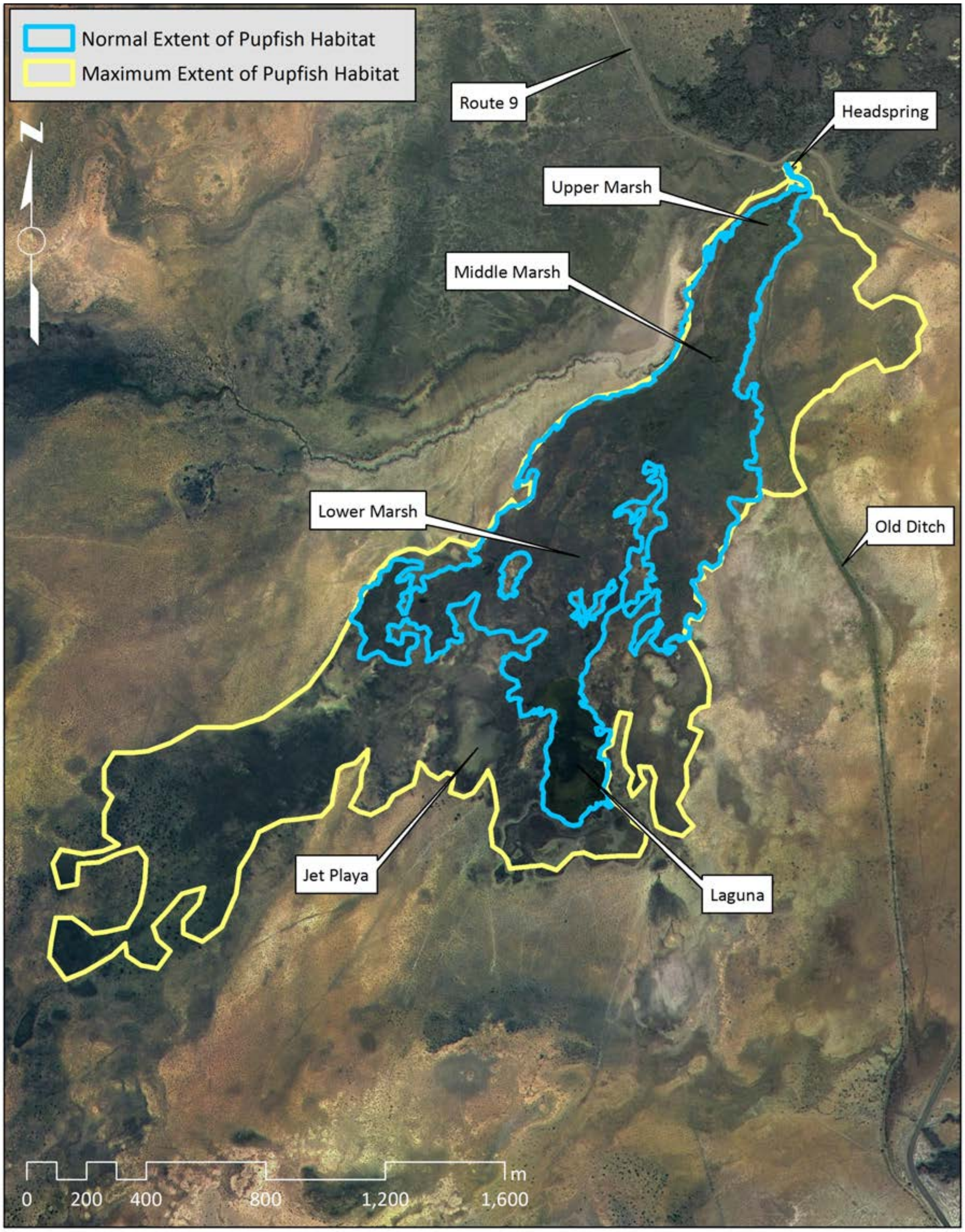


Figure 11. Mean daily discharge at Malpais Spring. Period of record is 1 October 2003 through 4 April 2012 (U.S. Geological Survey, 2014*b*).

Groundwater discharge from Malpais Spring maintains a large inundated marsh area that is occupied by White Sands pupfish. Habitat for White Sands pupfish at Malpais Spring ranges from about 65 ha (160 ac) to a putative maximum of about 363 ha (897 ac; Figure 12; J. S. Pittenger, *in litt.*).

The minimum habitat area is maintained by flow from the headspring and seeps, while maximum areal extent of wetland habitat at the site is a function of groundwater discharge combined with direct precipitation input to the wetland. As discussed above, precipitation may also have a rapid-recharge effect on the buried alluvial aquifer. This may result in increased discharge from the headspring associated with precipitation events.

Figure 12. Extent of pupfish habitat at Malpais Spring.



The headspring, outflow, and upper marsh habitats at Malpais Spring (Figure 13) are relatively stable in terms of areal extent and water depth, due to the controlling influence of discharge from the spring. Water-level fluctuations become more pronounced with distance from the headspring. The maximum observed water-level fluctuation in the middle marsh area in 1995 was 6.1 cm (0.20 ft; Figure 14). At the southern end of the Malpais Spring habitat, maximum observed water-level fluctuation at the laguna (Figure 15) was 24.4 cm (0.8 ft; Figure 14). In 1996, water-level monitoring showed a similar trend at the laguna. Here there was a maximum water-level decline of 0.67 feet from late winter to early summer (Figure 16). Onset of summer thunderstorms in late July brought on an increase in water level at the laguna (Figure 16).

Depth and current velocity measurements were made at selected sampling locations at Malpais Spring in March 1995 (J. S. Pittenger, *in litt.*). Mean depth in the spring outflow-upper marsh was 23.8 cm (N = 14, s.d. = 11.5). Mean current velocity the outflow-upper marsh area was 1.6 cm/s (N = 14, s.d. = 1.6). In the middle marsh (Figure 17), mean depth was 29.1 cm (N = 12, s.d. = 13.2) and mean current velocity was 1.1 cm/s (N = 12, s.d. = 1.1). In the lower marsh, mean depth was 17.3 cm (N = 15, s.d. = 4.0) and mean current velocity was 1.2 cm/s (N = 15, s.d. = 1.7). Mean depth in the laguna was 36.0 cm (N = 5, s.d. = 3.4). The laguna apparently was present at least as long ago as 1947, as indicated by Koster's collection of pupfish from pond habitat located "about 2.6 km south of Malpais Spring" (Miller and Echelle, 1975).

Figure 13. Open-water habitat in the upper marsh at Malpais Spring. View is looking west. Photo by J. S. Pittenger, 24 April 2014.

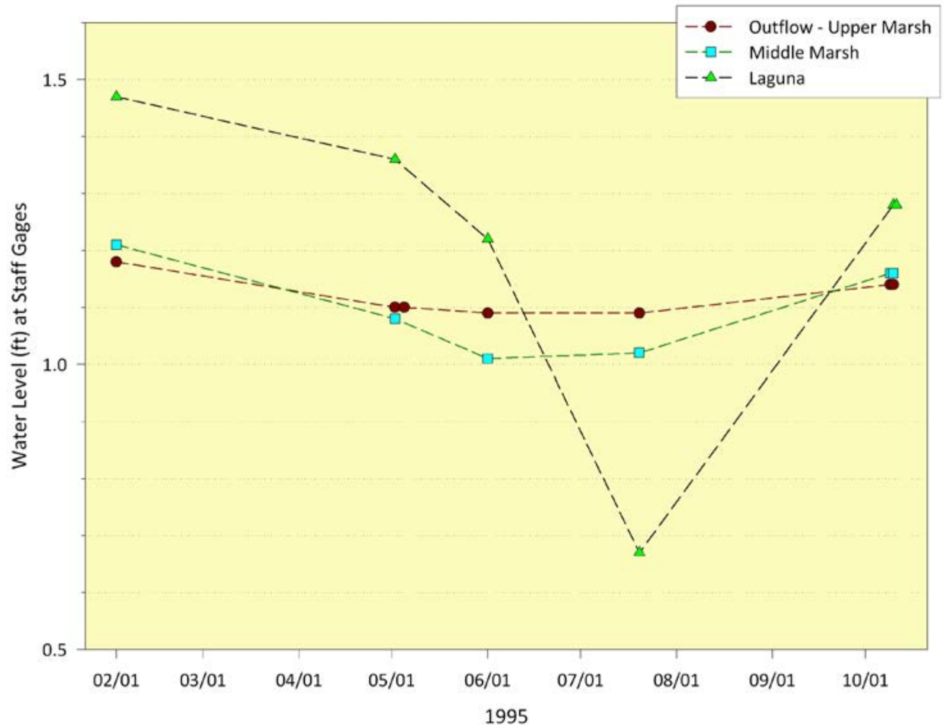


Figure 14. Seasonal water-level variation at three locations at Malpais Spring. Data are from periodic staff-gage readings made in 1995 (J. S. Pittenger, *in litt.*). Locations are shown in Figure 13.

Figure 15. The laguna at the south end of the Malpais Spring wetland. View is looking south from the northeast edge of the laguna. Photo by J. S. Pittenger, 25 April 2014.

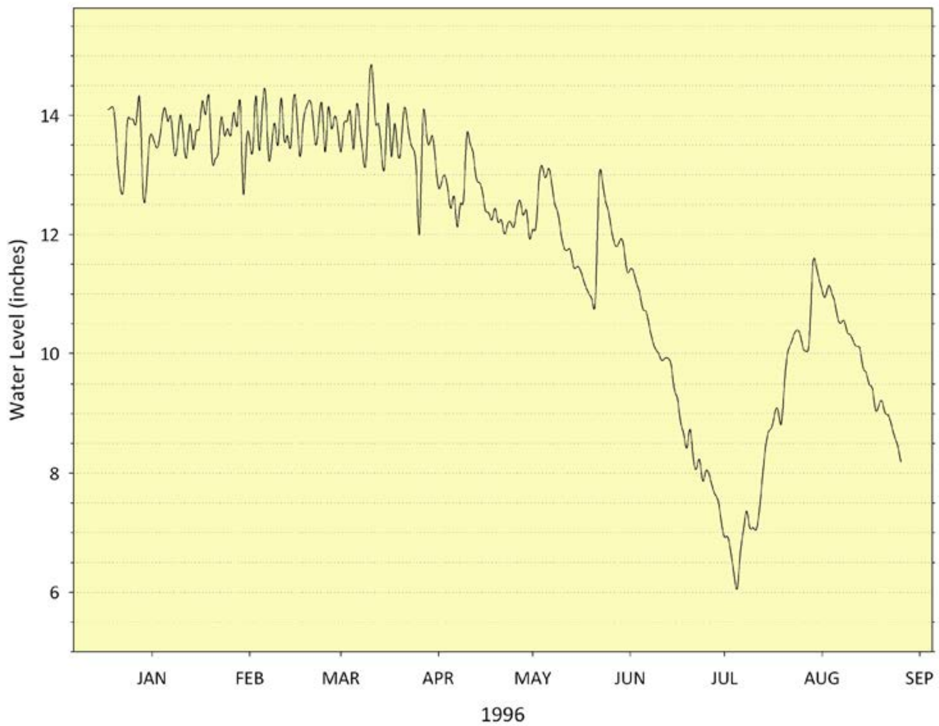


Figure 16. Seasonal water-level variation at the Malpais Spring laguna. Data are from hourly water-level monitoring with a datalogging, submersible pressure transducer in 1996 (J. S. Pittenger, *in litt.*).

Figure 17. Habitat in the middle marsh at Malpais Spring. View is looking south. Photo by J. S. Pittenger, 24 April 2014.

2.6.2 Water Quality

In general, water temperature characteristics are similar throughout the Malpais Spring and Salt Creek habitats (Figure 18). However, the concentrations of ions (Figure 18) and ionic composition of waters at the two habitats differ markedly.

2.6.2.1 Water Temperature Water temperature is relatively stable in the headwaters of Salt Creek and, more so, in the spring outflow at Malpais Spring (Figure 18). In 1995, the average diel water temperature fluctuation in the headwaters of Salt Creek was 9.4ºC (16.9ºF) and the maximum diel water temperature fluctuation was 18.4ºC (33.1ºF). In contrast, water temperature fluctuation in the lower reach was much greater, consistent with the increased variability associated precipitation-driven hydrology of the reach (see section 2.6.1.2).

While average diel water-temperature fluctuation in the lower reach of Salt Creek in 1995 was the same as the headwaters 9.4ºC (16.9ºF), maximum diel water-temperature fluctuation was considerably higher 32.3ºC (58.14ºF; Figure 19). Maximum hourly water temperature recorded at the headwaters in 1995 was 34ºC (93.2ºF), while the maximum recorded in the lower reach was 37.5ºC (99.6ºF; J. S. Pittenger, *in litt.*). Minimum hourly water temperature recorded in the headwaters in 1995 was -1.9ºC (28.5ºF) and minimum temperature recorded in the lower reach in 1995 was 0ºC (32ºF; Figure 19).

The spring outflow at Malpais Spring maintains relatively constant water temperature, as expected in a groundwater discharge-dominated habitat (Figure 19). Average diel water-temperature fluctuation at the upstream end of the spring outflow channel, just below the headspring pool, was only 1.0ºC (1.8ºF) in 1995. The maximum diel water-temperature fluctuation in the outflow channel in 1995 was 1.6ºC (2.9ºF; Figure 19). Maximum hourly water temperature in the spring outflow in 1995 was 18ºC (64.4ºF) and minimum temperature was 16.9ºC (62.4ºF).

The downstream end of the Malpais Spring marsh is marked by a *ca*. 15-acre ponded area referred to as the laguna (see figures 12 and 15). Water temperature variation is much higher at this location than at the spring outflow, with variability similar to the lower reach of Salt Creek (Figure 19). Average diel water-temperature variation at the laguna in 1995 was 7.6ºC (13.6ºF) and the maximum recorded diel fluctuation was 20.9ºC (37.6ºF; Figure 19). In 1995, the maximum hourly water temperature recorded at the laguna was 38ºC (100.4ºF) and the minimum hourly temperature was 1.7ºC (35.1ºF; Figure 19).

2.6.2.2 Salinity of Surface Water Wetzel (1983: 179) stated that, with respect to limnology and conditions for aquatic life, “salinity is the correct term for the ionic composition” of aquatic habitats and is best expressed as “the sum of the ionic composition of the eight major cations and anions in mass or milliequivalents per liter.”

Salinity is markedly higher in Salt Creek than at Malpais Spring, although there is a general trend of increasing salinity with increasing distance from headwaters or headspring at both habitats (Myers and Naus, 2004; Myers *et al*., 2008). From 1996 to 2007, mean salinity was 28,492 mg/L in the middle reach of Salt Creek (n = 12, s.d. = 13,543), while at the spring outflow at Malpais Spring it was 4,931 mg/L (n = 11, s.d. = 124; U.S. Geological Survey, 2009*a* and 2009*b*).

Figure 18. Specific conductance and water temperature in native habitats of White Sands pupfish. Both parameters were measured contemporaneously during each sample. Box plots show the median value as the horizontal line in the middle of each box. The upper and lower borders of the box denote the 75th and 25th percentile, respectively. The upper and lower whiskers represent the 90th and 10th percentiles, respectively. Outliers are shown as circles. Sample sizes for each site are indicated on the plots. Measurements were made in the field during all four seasons from 1993 to 1996 using a Yellow Springs Instruments® meter calibrated to local elevation.

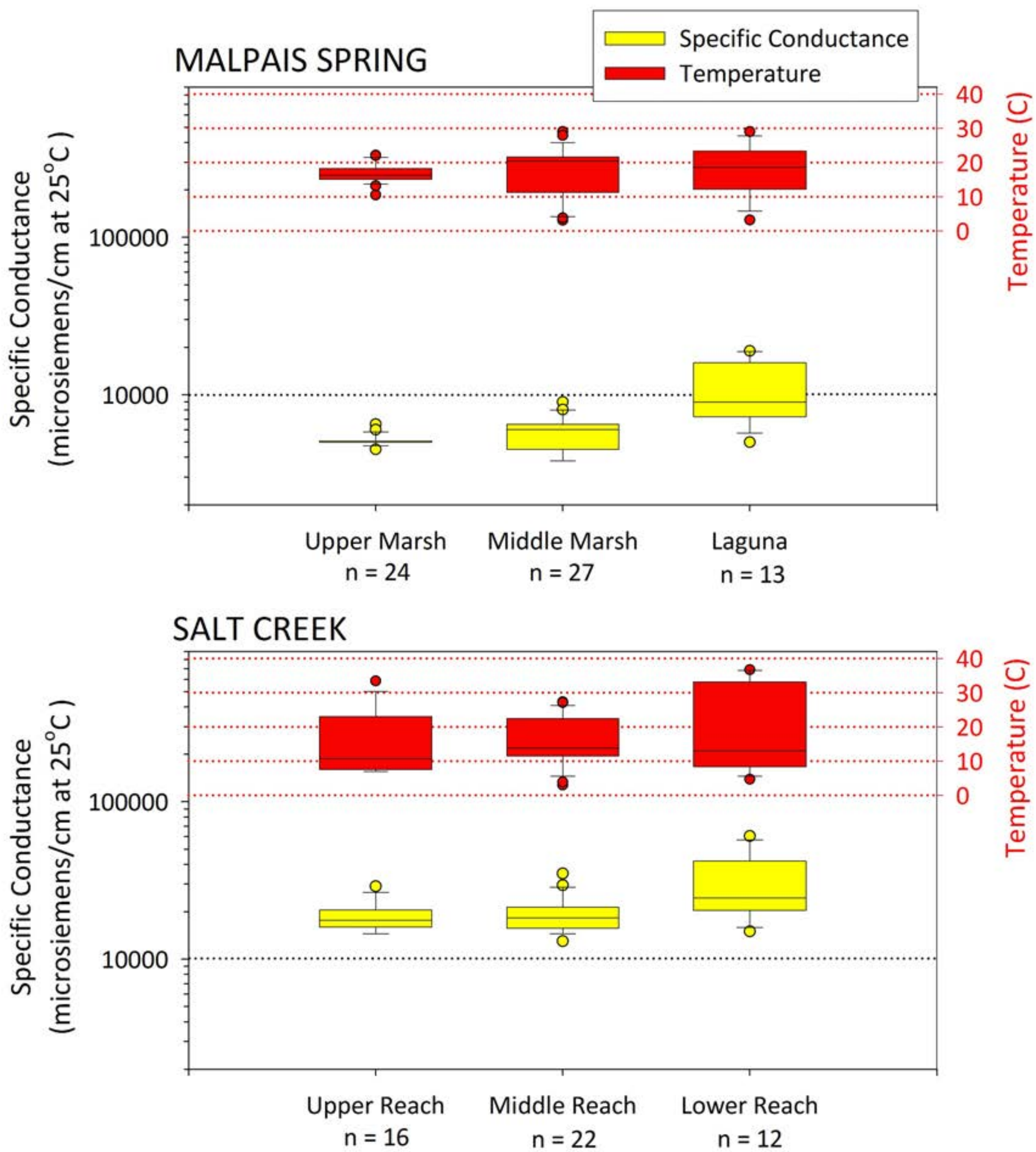
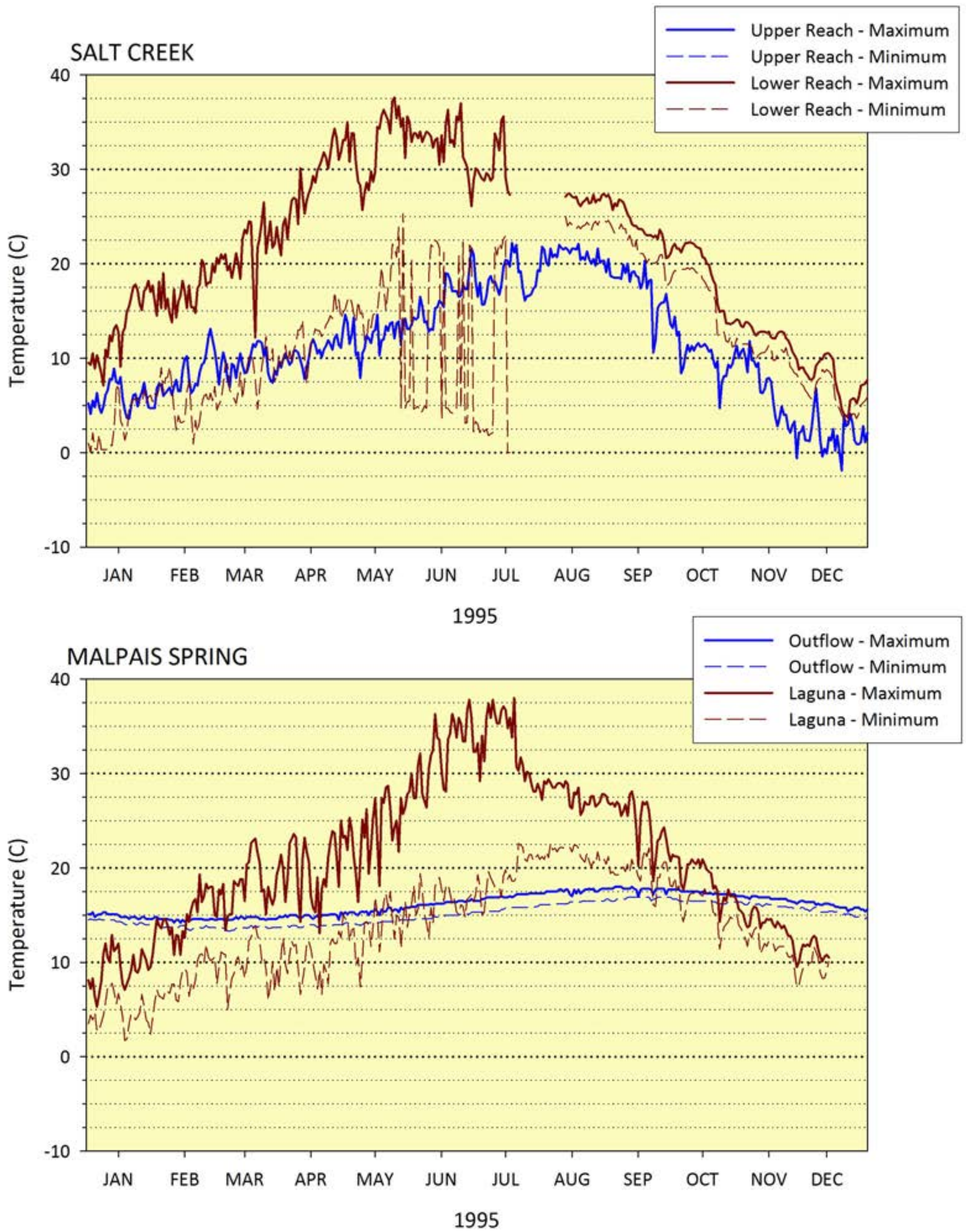


Figure 19. Seasonal water temperature variation at Salt Creek and Malpais Spring. Data are from hourly datalogging thermograph readings in 1995 (J. S. Pittenger, *in litt.*). The aberrant minimum temperatures in the lower reach of Salt Creek from May through July were the result of stream drying and resulting instrument malfunction.



Not only are mean salinity values different, but the fluctuations in salinity also appear to be substantially greater at Salt Creek compared to Malpais Spring. Myers and Naus (2004) reported a salinity range of 12,700 to 38,100 mg/L in Salt Creek, with the lowest values recorded in the middle reach, below the headcut waterfall, and the lower reach of Salt Creek having the highest salinity. Field measurements of specific conductance corroborate this trend (Figure 18).

Salinity is relatively constant at the spring outflow at Malpais Spring. Myers and Naus (2004) reported salinity ranging from 4,580 mg/L to 5,500 mg/L from 1911 to 2000 at the spring outflow. However, salinity levels at the southern end of the wetland are generally double that of the spring outflow (Myers *et al*., 2008). Again, field measurements of specific conductance further testify to this trend (Figure 18).

The contributions of major ions to total salinity also varies between the two habitats (Figure 20). Anions at Malpais Spring are dominated by sulfate (50 to 60 percent) and chloride (40 to 50 percent) with very little contributed by bicarbonate-carbonate (Figure 20). Cations at Malpais Spring are dominated by calcium (40 to 60 percent), followed by sodium (35 to 55 percent), and then magnesium (10 to 20 percent; Figure 20). Thus, ion dominance at Malpais Spring can be expressed as follows.

Anions: $SO_4 \succ Cl \gg CO_3\text{-}HCO_3$
Cations: $Ca \geq Na \succ Mg$

In contrast, anions at Salt Creek are dominated by chloride (65 to 80 percent), followed by sulfate (20 to 35 percent; Figure 20). Similar to Malpais Spring, bicarbonate-carbonate is insignificant. Cations are dominated by sodium (70 to 85 percent), potassium being present only at very low levels. Sodium is followed by calcium (15 to 30 percent) and then by magnesium (five to 15 percent; Figure 20). Ion dominance at Salt Creek can be expressed as follows.

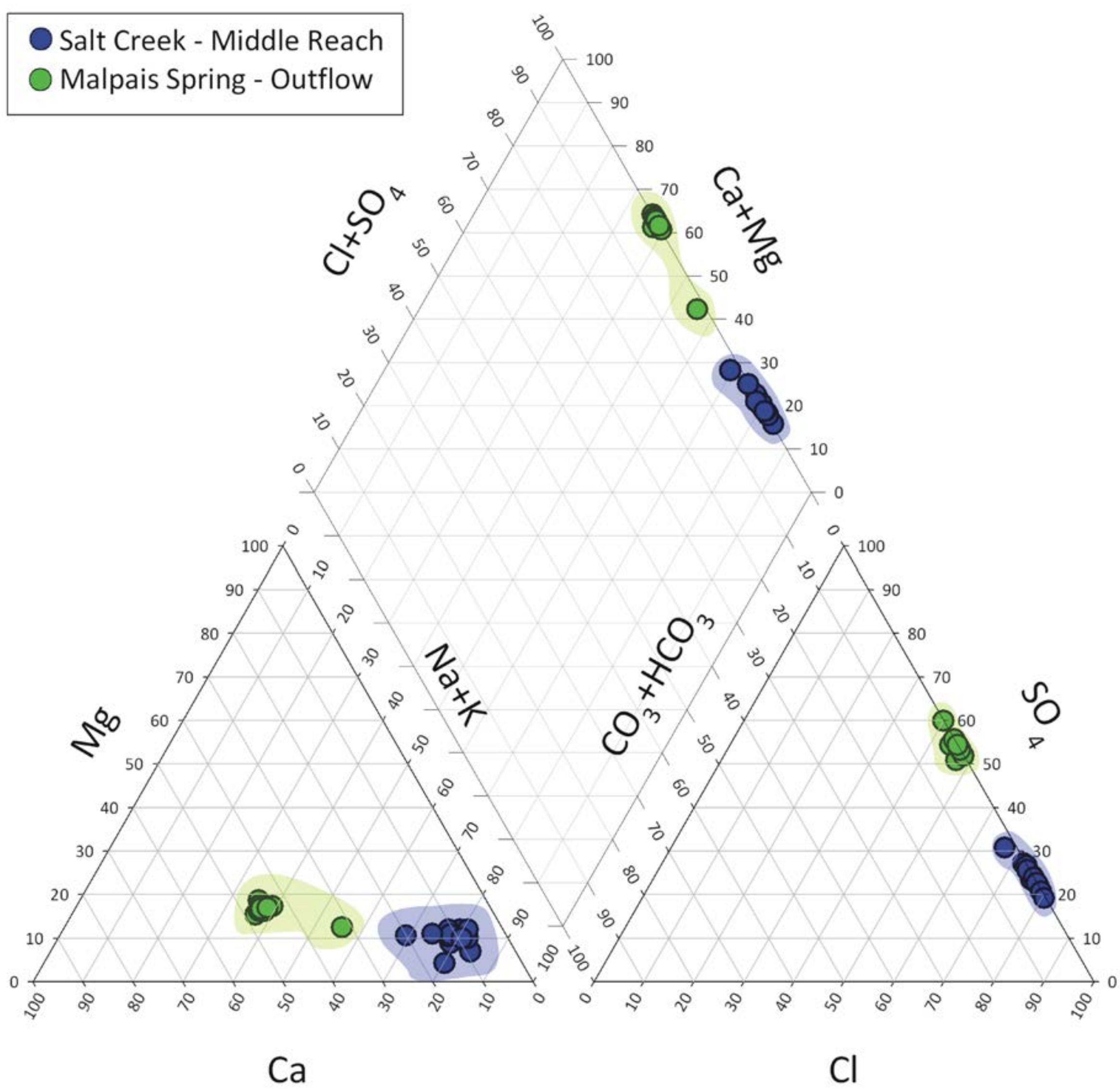
Anions: $Cl \succ SO_4 \gg CO_3\text{-}HCO_3$
Cations: $Na \succ Ca \succ Mg$

In summary, calcium, the dominant cation at Malpais Spring, is replaced by sodium at Salt Creek. Sulfate, the dominant anion at Malpais Spring, is replaced by chloride at Salt Creek (Figure 20).

<u>2.6.2.3 Field Measurements of pH and Dissolved Oxygen</u> The pH of surface water in Salt Creek is alkaline. Average pH at a site in the middle reach of Salt Creek from 1995 through 2007 was 9.0, with a maximum of 9.8 and a minimum of 8.0 (n = 12; U.S. Geological Survey, 2009*a*). In contrast, the pH of water in the outflow at Malpais Spring is near neutral. Field measurements of pH made from 1982 to 2007 (n= 12) averaged 7.5, with a maximum of 8.0 and a minimum of 7.3 (U.S. Geological Survey, 2009*b*). Dissolved oxygen concentrations in both upper Salt Creek and Malpais Spring are typically well above 80 percent saturation (U.S. Geological Survey, 2009*a* and 2009*b*; J. S. Pittenger, *in litt.*).

<u>2.6.2.4 Trace Elements</u> Boron and strontium are present in surface waters of Malpais Spring and Salt Creek at relatively high concentrations. At Malpais Spring, mean concentration of boron in 12 unfiltered samples was 218.75 ì g/L and 239.67 ì g/L in six unfiltered samples (U.S. Geological Survey, 2009*b*). Boron concentrations were markedly higher in Salt Creek, where the average of 12 filtered samples was 1,263 ì g/L and 1,170 ì g/L in six unfiltered samples (U.S. Geological Survey, 2009*a*).

Figure 20. Piper diagram of major ion concentrations at Salt Creek and Malpais Spring. Data are from Turner (1987), n = 2, and from the U.S. Geological Survey (2009*a* and 2009*b*), n = 12. Data were converted from mg/L to milliequivalents/L following Hem (1985). The triangle plot in the lower right shows the relative contribution, as percent of total, of three major anion groups: chloride (Cl^-), sulfate ($SO_4^=$), and bicarbonate-carbonate ($CO_3^=$-HCO_3^-). The triangle plot in the lower left shows the relative contribution, as percent of total, of three major cation groups: calcium (Ca^{++}), magnesium (Mg^{++}), and sodium-potassium (Na^+-K^+). The upper, central, diamond-shaped plot combines the data of the two lower plots by extending the points in the lower triangles to the point of intersection in the center field.



Mean strontium concentration was 11,458.53 ì g/L in 12 filtered samples from Malpais Spring and 11,816.67 ì g/L in six unfiltered samples (U.S. Geological Survey, 2009*d*). As with boron, concentrations of strontium were higher in Salt Creek. The mean strontium concentration in 12 filtered samples from Salt Creek was 21,789.17 ì g/L and 20,766.67 in nine unfiltered samples (U.S. Geological Survey, 2009*a*).

Lithium was present at relatively high concentrations in Salt Creek, with a mean concentration in 12 unfiltered samples of 2,047.5 ì g/L and 1,792 ì g/L in 10 unfiltered samples (U.S. Geological Survey, 2009*a*). In contrast, lithium concentrations were quite low in Malpais Spring (*i.e.,* less than 60 ì g/L; U.S. Geological Survey, 2009*b*).

<u>2.6.2.5 Nitrogen and Phosphorus</u> Combined nitrogen compound concentrations are slightly different at Malpais Spring and Salt Creek. Mean ammonia (as NH_4) concentrations are similar at both habitats ($\bar{x}$ = 0.13 mg/L at Malpais Spring and $\bar{x}$ = 0.16 mg/L at Salt Creek). However, nitrate (NO_3) is higher at Malpais Spring ($\bar{x}$ = 11 mg/L) than at Salt Creek ($\bar{x}$ = 1.47 mg/L). Conversely, nitrite (NO_2) is lower at Malpais Spring ($\bar{x}$ = 0.01 mg/L) compared to Salt Creel ($\bar{x}$ = 0.11 mg/L; U.S. Geological Survey, 2009*a* and 2009*b*).

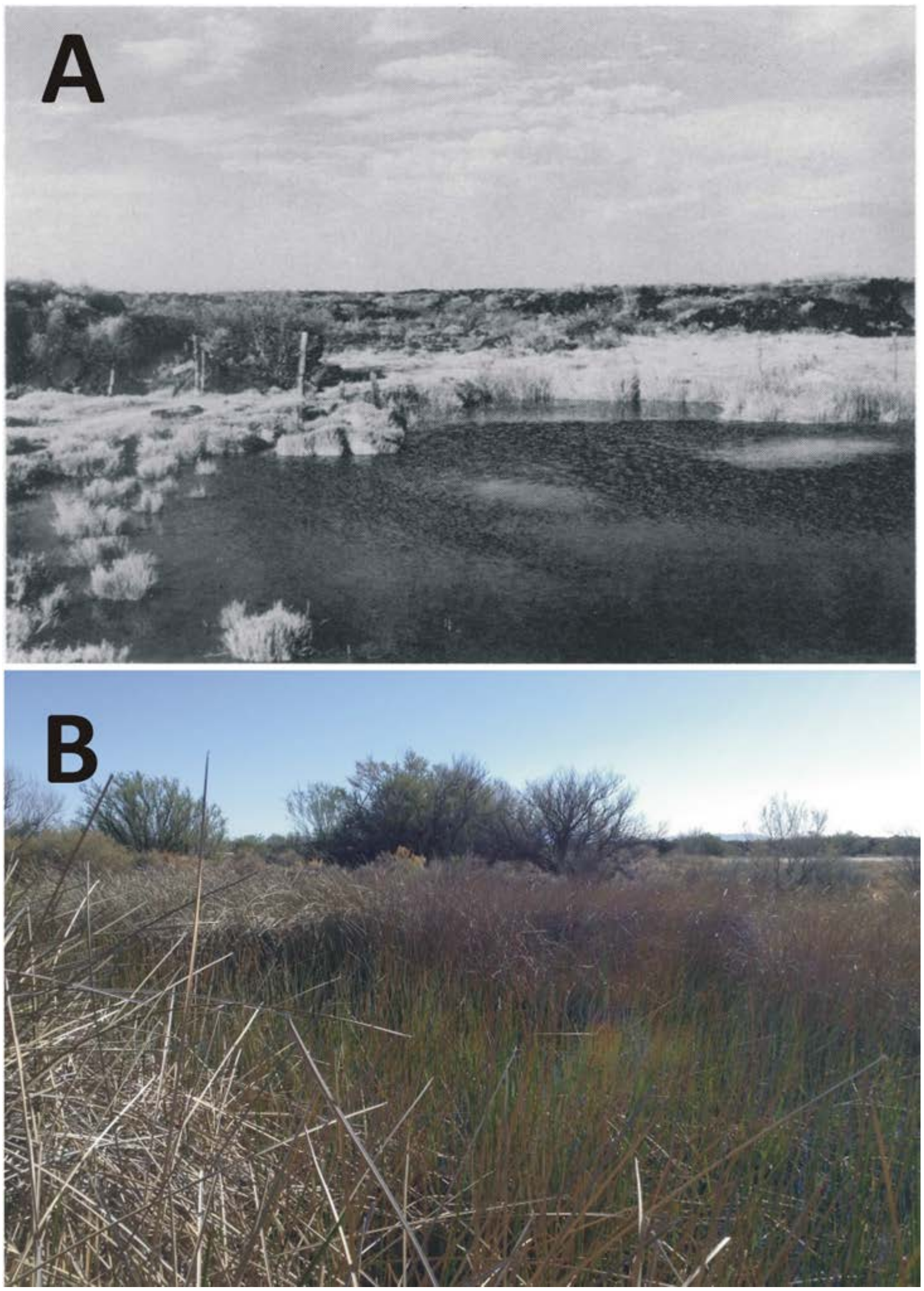
Phosphorus typically is the most important nutrient limiting productivity in freshwater ecosystems (Wetzel, 1983: 255). Mean orthophosphate concentration in four samples taken from Malpais Spring was 0.07 mg/L (U.S. Geological Survey, 2009*b*). Another five samples had less than 0.01 mg/L and another two had less than 0.02 mg/L. At Salt Creek, average orthophosphate concentration from nine samples was 0.01 mg/L (U.S. Geological Survey, 2009*a*). These data are consistent with qualitative observations that the Malpais Spring habitat is more productive than Salt Creek.

2.6.3 Riparian and Wetland Vegetation

<u>2.6.3.1 Malpais Spring</u> The headspring pool at Malpais Spring is surrounded by a dense growth of saltcedar (*Tamarix* sp.) and the spring pool margins are vegetated with dense growths of chairmaker's bulrush (Sivinski and Tonne, 2011: 81). Saltcedar is also found along the spring outlet channel and around the margins of the wetland, particularly in the southeast lobe of the wetland, east of the laguna and along the old ditch that runs south from the headspring (see Figure 12). As recently as 1950, saltcedar was absent from Malpais Spring including the headspring pool, which was fenced to exclude livestock, and the spring outflow (Figure 21; Miller and Echelle, 1975). Photos of the headspring and outflow in Turner (1987) show very little saltcedar growth in the area. Consequently, it appears that much of the increase in density and spatial extent of saltcedar at Malpais Spring has occurred over the last 30 years.

The extensive inundated wetland habitat at Malpais Spring is dominated by chairmaker's bulrush (*Schoenoplectus americanus*), beaked spikerush (*Eleocharis rostellata*), and saltgrass (*Distichlis stricta*; Sivinski and Tonne, 2011: 76-81). Stands of common reed (*Phragmites australis*) occur primarily in platform marshes northwest and southwest of the Malpais Spring wetland (Allen *et al*., 2005; Sivinski and Tonne, 2011: 81). A small stand of common reed is found in the upper marsh. Allen and others (2005) referred to the existing wetland as "artificial," implying that the relict west-flowing gypsum distributary stream was the original outlet of Malpais Spring.

Figure 21. Salt cedar invasion at Malpais Spring, 1950 to 2014. The 1950 photo (A) was taken on 5 March 1950 and is excerpted from Miller and Echelle (1975: Figure 3). The 2014 photo (B) is approximately the same view as the 1950 photo and was taken by J. S. Pittenger on 27 March 2015. The view in both photos is looking east from the west side of the headspring.



Turner (1987) also reported sago pondweed (*Stuckenia pectinata*) and the alga muskgrass (*Chara* sp.) from the headspring and outflow channel at Malpais Spring.

The density of emergent wetland vegetation has steadily and markedly increased at Malpais Spring following completion of removal of feral horses from WSMR in 1999. The areal extent of open-water habitat in the wetland has concurrently decreased substantially, particularly in shallow-water areas around the margin of the wetland where horse trails were extensive prior to removal of feral horses (see Appendix B).

<u>2.6.3.2 Salt Creek</u> The reach of Salt Creek from the headwaters downstream to the waterfall is characterized by a relatively narrow, incised channel. Thick cover of saltgrass in saturated soils or shallow inundation grows on either side of the channel with patchy, small stands of saltcedar. Extensive algal mats are common in this reach. Saltcedar forms a dense and continuous thicket along the stream channel downstream from the waterfall to the confluence of Malpais Draw, where saltcedar stands thin out. Dense saltcedar along both banks continues downstream from the confluence, beyond the Route 316 crossing, to approximately 0.5 stream-km below the cable-crossing site. From this point downstream to the mouth of Salt Creek at the playa near Big Salt Lake, saltcedar occurs as patchy small stands and isolated plants. With the exception of saltgrass growing in shallow areas along the edge of the stream, emergent wetland vegetation is absent from Salt Creek.

Miller and Echelle (1975) noted that in 1950 there was no saltcedar along Salt Creek in the vicinity of the present-day Route 316 crossing. Habitat along the stream at this location was described as a stream with "slight to no current, flows through an alkaline channel flanked by 1 to 1.6 m mud and grass banks ... varied from 4 to 12 m wide ... about 30 m below our station the creek enters a small canyon with rather high banks; this section is deeper with mesquite overhanging the banks."

Turner (1987: 15) did not report any aquatic plants as occurring in Salt Creek. Saltgrass is common along the banks of Salt Creek and typically occurs as an emergent plant in shallow water along the margins of the stream, particularly upstream from the Route 316 crossing (see Figure 8).

2.6.4 Aquatic Invertebrates

Turner (1987: 105) reported nine aquatic invertebrate taxa from Malpais Spring and six from Salt Creek. The aquatic amphipod *Gammarus lacustris* was the most abundant taxa at Malpais Spring, followed by tubificid worms, planaria, physid snails, and chironomid larvae (Turner, 1987: 107). The most abundant aquatic invertebrate taxa at Salt Creek were midge larvae (Chironomidae), damselfly larvae (*Enallagma*), tubificid worms, and water boatmen (*Trichocorixia*).

Hoover and others (1999) reported 16 taxa from Salt Creek including a physid snail, the cladoceran *Oxyurella*, an amphipod (*Gammarus fasciatus*), water boatmen (*Trichocoria verticalis* and other species), six beetle taxa, ceratopogonid larvae, midge larvae (Chironomidae), mosquito larvae (Culicidae), and another unidentified dipteran larvae.

Twenty-five taxa were reported from Malpais Spring by Hoover and others (1999). These taxa included Turbellaria, physid snail, six cyclopoid crustaceans, the amphipod *Gammarus fasciatus*, five odonates, five hemipterans, three beetle taxa (Coleoptera), and four Diptera taxa.

Kritsky and Stockwell (2005) described a new species of monogenean ectoparasite (*Gyrodactylus tularosae*) from the skin of White Sands pupfish from Salt Creek, and Hershler and others (2002) described a new species of aquatic snail (*Juturnia tularosa*) from Salt Creek.

2.7 Food Habits

Similar to Pecos pupfish (Davis, 1981), White Sands pupfish is broadly omnivorous (Suminski, 1977; Hoover *et al*., 1999). Terry (1971) found algae, diatoms, and detritus to be the most common items in stomachs of White Sands pupfish collected from Salt Creek. These food items were found in all of 50 pupfish examined. Invertebrates were found in 20 percent of the pupfish examined.

Hoover and others (1999) found chironomid larvae (29 percent) and cyclopoid copepods (21 percent) to be the most common food items of White Sands pupfish at Malpais Spring. At Salt Creek, emerging dipterans (55 percent) and ceratopogonid larvae (28 percent) were most common in the diet. Diatoms and detritus were also common in the diet of White Sands pupfish at both Malpais Spring and Salt Creek (Hoover *et al*., 1999).

Hoover and others (1999: 29) found White Sands pupfish at Malpais Spring to feed primarily on chironomid larvae (29 percent of all food items found in stomach content samples), followed by cyclopoid copepods (21 percent), diatoms (10 percent), detritus (eight percent), and ostracods (seven percent; Table 2). At Salt Creek, principal food items were emerging dipterans (55 percent) and ceratopogonid larvae (28 percent; Table 2).

Diet varied at Malpais Spring with distance from the headspring (Hoover *et al*., 1999: 30). Diatoms and detritus were important near the headspring (49 percent of all food items found in stomach content samples), but these items decreased to 26 percent in the upper marsh and 12 percent in the middle marsh areas. Benthic macroinvertebrates increased in importance in the diet with distance from the headspring. At Salt Creek, emerging Diptera were very important in the diet in the upper reach (89 percent of all food items found in stomach content samples), but were only a minor component in the lower reach of the stream (nine percent). Diatoms replaced emerging dipterans as the principal food item of White Sands pupfish in lower Salt Creek.

Table 2. Diet of White Sands pupfish at Malpais Spring and Salt Creek. Excerpted from Hoover and others (1999: 30). Values are percent of total number of food items in samples.

Food Item	Malpais Spring	Salt Creek
Diatoms	10.4	6.5
Detritus	8.2	7.1
Cladocera	1.9	0.3
Copepoda	21.1	0.3
Ostracoda	7.1	---
Amphipoda	1.3	0.1
Ephemeroptera	0.4	---
Odonata	0.2	---
Hemiptera	---	0.1
Chironomidae (larvae)	28.7	1.7
Ceratopogonidae (larvae)	0.1	28.1
Diptera (pupae and adults)	2.4	55.0
Sand	3.4	0.3

Omnivory of White Sands pupfish is consistent with studies of food habitat of other pupfish species. For example, Kennedy (1977) reported that diatoms, algae, amphipods, gastropods, ostracods, beetles, and seeds were the principal food items in gut of *Cyprinodon bovinus* (Leon Springs pupfish), and that the species commonly engaged in pit-digging and plowing behavior apparently to locate buried food items in soft substrates. Echelle (1973) reported similar feeding behavior in Red River pupfish. Naiman (1975) found blue-green algae and detritus to be the most common food items consumed by Amargosa pupfish (*C. nevadensis*) in a thermal stream where aquatic invertebrates were uncommon.

Although the feeding periodicity of White Sands pupfish has not been investigated (Hoover *et al*., 1999) it is likely similar to other pupfish species. Amargosa pupfish actively foraged throughout the day (Naiman, 1975). Feeding acts by Red River pupfish (*C. rubrofluviatilis*) in saline stream habitat consisted mostly of bottom-oriented acts including "Nipping" and "Digging" movements as well as substrate "Plowing" (Echelle, 1973).

Feeding by Red River pupfish occurred at water temperatures ranging from 4ºC and 40ºC (39.2ºF to 104ºF; Echelle, 1973). On warm days feeding increased from dawn to midday and remained high until nightfall. Little to no feeding occurred at night. At temperatures ranging from 4ºC (39.2ºF) to 15ºC (59ºF) pupfish formed large schools. Schooling diminished at higher temperatures but persisted to some degree among females and nonbreeding males. Schooling pupfish stopped to feed and would defend small feeding territories, circular in shape and slightly larger in diameter than the length of the fish.

Feeding persisted at single sites for 15 to 30 minutes or longer. At temperatures near 30ºC (86ºF) as much as 50 percent or more of the population was defending feeding territories (Echelle, 1973).

2.8 Breeding Biology

Like other *Cyprinodon* species (Barlow, 1961; Echelle, 1973; Kodric-Brown, 1977 and 1978), White Sands pupfish likely has a resource-based polygonous breeding system. In such systems, males establish breeding territories on suitable substrates, gravid females visit territories and spawn with the resident male, and there may be some degree of indirect parental care by the resident male guarding the territory. However, territorial males and other pupfish invading the territory may cannibalize eggs. Similarities among species of *Cyprinodon* indicate that behavior, with the exception of courtship, is conservative in the genus, from an evolutionary perspective (Liu, 1969).

Virtually all spawning activity takes place on male territories (Kodric-Brown, 1983). Microtopographic diversity in a spawning territory may confer protection of the demersal, cryptic eggs by allowing them to remain undetected by predators (Kodric-Brown, 1983). Suitable breeding habitat may be limited even in large habitats. For example, Kodric-Brown (1978, 1983) found that only about 10 percent of suitable habitat for Pecos pupfish (*C. pecosensis*) at a limnocrene pond was high-quality breeding habitat, and that there was intense competition for suitable breeding sites.

Reproduction of *C. rubrofluviatilis* may occur throughout the year but is concentrated in the period from March to July, with greatest reproduction occurring in the spring (Echelle, 1973; Lee *et al*., 2014). Males established territories on the bottom of the stream in water up to 50 cm (19.7 in) deep, over sandy shoals and in small coves with little to no current. Territories were also established in deeper water (1 m [3.3

ft]) over large boulders and heaps of woody debris. Pecos pupfish were found to breed in water depths ranging from 2 cm (0.8 in) to no more than 2 m (6.5 ft; Kodric-Brown, 1978).

Male Red River pupfish consistently began defending territories when water temperature during daylight hours approached 11ºC (51.8ºF) to 15ºC (59ºF; Echelle, 1973). Males moved into deeper water at night in the beginning of the breeding season and then would return to shallow water territories when water temperature began to reach 8ºC (46.4ºF) to 10ºC (50ºF) in the morning. It appeared that males returned to the same breeding territories day after day. Later in the breeding season males stayed on territories during all hours of the day and night, but when water temperatures neared 33ºC (91.4ºF) to 35ºC (95ºC), pupfish defended only the centers of territories or left and went to deeper water. Males rested on the bottom in their territories at night. Similarly, Kennedy (1977) found two diurnal peaks (mid-morning and late afternoon) in spawning activity by Leon Springs pupfish, apparently related to water temperature.

Male pupfish reproductive success is related to the type of breeding substrate (Barlow, 1961;Echelle, 1973; Kodric-Brown, 1977; Leiser and Itzkowitz, 2003). Breeding territories are typically established on sites with unvegetated substrate that has some microtopographic heterogeneity (*e.g.* Kodric-Brown, 1977 and 1978; Leiser and Itzkowitz, 2003). Strong fidelity is exhibited by males to established breeding territories throughout the breeding season (Echelle, 1973; Kodric-Brown, 1977 and 1978). Non-territorial, smaller males may engage in satellite positioning and sneak spawning to attempt to accrue some reproductive opportunities in the presence of larger, territorial males (Leiser and Itzkowitz, 2002 and 2003).

2.9 Demography

Jester and Suminski (1982) reported age-specific fecundity of White Sands pupfish from an introduced (now extinct) population in a pond (Table 3). Fecundity increased with age class, with a grand mean of 1,140 ova per female (Table 3). Young-of-year females developed ova in August and September but did not spawn until late the following spring.

Table 3. Age-specific fecundity of female White Sands pupfish. Excerpted from Jester and Suminski (1982).

Age Group	Mean Ova Count (Range)
I	810 (729 to 3,319)
II	1,134 (237 to 6,609)
III	1,693 (414 to 3,301)
Grand Mean	1,140

Jester and Suminki (1982) reported counts of all ova prior to and through the spawning season, and found that the number of ova became smaller as the season progressed. In contrast to the total fecundity data reported by Jester and Suminski (1982), Hoover and others (1999: 26-27) reported mature ova counts, or clutch size, averaging 33.3 per female at Malpais Spring and 43.3 per female at Salt Creek.

Age-specific survivorship estimated by Jester and Suminski (1982) from the introduced population is shown in Table 4. Survivorship from young-of-year to Age II was estimated to be 0.02 percent Jester and Suminski, 1982: 51; 468 Age II/1,920,056 YOY = 0.0002).

In Devil's Hole, a small, closed system, mortality of *C. diabolis* was related to water level, which in turn influenced primary productivity and oxygen concentration (Chernoff, 1985). Hoover and others (1999) speculated that food resources in the closed Mound Spring habitat may limit abundance of White Sands pupfish. In open systems, emigration may be an important mechanism in regulating pupfish populations.

Table 4. Age-specific survivorship of White Sands pupfish. From Jester and Suminski (1982).

Age Group	Percent Surviving to Next Age Group
YOY to I	1.56%
I to II	1.56%
II to III	8.76%
III to IV	19.51%

McMahon and Tash (1988) reported density-dependent emigration in an experimental system using *C. macularius*. Emigration may be an important regulatory mechanism in both the Malpais Spring and Salt Creek populations. Downstream dispersal or movement of primarily small White Sands pupfish has been observed in Salt Creek (J. S. Pittenger, *in litt*.). Pupfish that move or are flushed into the downstream intermittent reach of Salt Creek may experience high or complete mortality as habitat contracts or dessicates entirely during dry periods. Similarly, primarily small pupfish are often observed in the shallow margins of the Malpais Spring wetland (Figure 22). However, this may not be a result of population regulation via behavioral spacing (McMahon and Tash, 1988), but rather the result of small fish seeking warmer water temperatures and food resources (Hoover *et al*., 1999: 24).

Figure 22. School of small pupfish in shallow water at Malpais Spring. Water depth was *ca*. 6 cm (2.4 in) at the location, which was on the periphery of standing water in the marsh. Photo by J. S. Pittenger, 24 April 2014.

2.10 Community Ecology

2.10.1 Predation

White Sands pupfish may be preyed upon by wading birds and waterfowl such as snowy egret (*Egretta thula*), white-faced ibis (*Plegadis chihi*), great blue heron (*Ardea herodias*), mallard (*Anas platyrhynchos*), American coot (*Fulica americana*), black-necked stilt (*Himantopus mexicanus*), American avocet (*Recurvirostra americana*), and others. Tiger salamander (*Ambystoma tigrinum*) was observed in the lower pond at Mound Spring in the past (J. S. Pittenger, *in litt.*), and would likely prey on pupfish. Additionally, black-necked gartersnakes (*Thamnophis cyrtopsis*) have been collected in minnow traps in the Malpais Spring wetland (J. Pittenger, pers. obs.). Black-neck gartersnake would likely prey on White Sands pupfish (see Burkett, 2008: 53; Degenhardt *et al.*, 1996: 313).

Mosquitofish (*Gambusia affinis*) had a significant negative effect on population size and biomass of White Sands pupfish in an experimental situation (Rogowski and Stockwell, 2006). Predation by mosquitofish on eggs and juvenile pupfish was suspected as the causative mechanism. While mosquitofish does not occur in native habitats of White Sands pupfish, populations of the species are established in the Tularosa basin, such as the HAFB wastewater effluent wetlands and golf course ponds, and at Barrel Spring on WSMR.

Crayfish may also have a significant negative effect on White Sands pupfish through predation, particularly at higher densities (Rogowski and Stockwell, 2006). While crayfish are not currently found in native habitats of White Sands pupfish, populations of introduced crayfish are known from the basin (*e.g.* Barrel Spring on WSMR).

Other nonnative aquatic organisms that occur in the basin and that, if introduced into suitable pupfish habitats, may prey on White Sands pupfish include green sunfish (*Lepomis cyanellus*) and largemouth bass (*Micropterus salmoides*). Both of these species have been found in aquatic habitats in the basin in the past.

2.10.2 Competition

No other fish species are present in native habitats of White Sands pupfish. Kilburn (2012) reported significant overlap in food consumed by introduced crayfish (*Procambarus clarkii*) and two species of pupfish at Ash Meadows National Wildlife Refuge in Nevada. Also, crayfish depressed aquatic invertebrate species richness (Kilburn, 2012). Martin and Saiki (2005) found desert pupfish were most abundant in habitats where nonnative fish were absent or present in very low numbers, suggesting that competition with or predation by nonnative fish was an important influence on pupfish.

2.10.3 Disease

Abrupt decline of the introduced population at Mound Spring was observed in 1995 and was attributed to a fish kill caused by an overabundance of a digenetic trematode (Pittenger and Springer, 1996:5). No such incidents have been documented in the two native populations. The monogenean ectoparasite *Gyrodactylus tularosa*, which is found at both Salt Creek and Malpais Spring, did not appear to affect survival, growth, or fat content of White Sands pupfish (Vinje, 2007).

3.0 Conservation Analysis

3.1 Conservation Goal

The goal of conservation of White Sands pupfish is to ensure the long-term viability of the two native populations (Salt Creek and Malpais Spring).

Population viability is influenced by four categories of factors (Shaffer, 1981):

1. Demographic uncertainty;
2. Environmental uncertainty;
3. Natural catastrophes; and
4. Genetic uncertainty.

The Malpais Spring and Salt Creek populations of White Sands pupfish are numerically quite large (see section 2.3.2), but they are geographically restricted. Viability of populations with these attributes is influenced primarily by environmental uncertainty and catastrophic factors (*e.g.* Murphy *et al*., 1990). Consequently, viability of the Salt Creek and Malpais Spring populations depends primarily upon maintaining large populations that are spatially distributed throughout suitable habitats, and protecting against catastrophic events.

Environmental uncertainties that may influence the viability of White Sands pupfish populations include events such as changes in weather or climatic patterns, introduction of non-native competitors, predators, or diseases, and changes in habitat structure associated with vegetation dynamics. Catastrophic events may include prolonged and severe drought, extreme floods, toxic chemical spills, and hybridization (*e.g.* introduction of sheepshead minnow or non-native pupfish).

3.2 Conservation Objectives

The following objectives address maintenance of large, spatially distributed populations at Salt Creek and Malpais Spring, and establishment and maintenance of natural refuge populations as a hedge against catastrophic events.

3.2.1 Maintain the Spatial Distribution of Pupfish at Salt Creek and Malpais Spring

Maintenance of large, persistent populations of White Sands pupfish at Salt Creek and Malpais Spring requires adequate spatial distribution of pupfish throughout the extent of suitable habitat at both sites.

Fluctuations in the areal extent of aquatic habitat occupied by White Sands pupfish at Salt Creek and Malpais Spring occur in response to climatic variation (see sections 2.6.1.2 and 2.6.1.3). Long-term monitoring of spatial distribution of White Sands pupfish has not been conducted, so there is a lack of baseline data to allow for assessment of distribution changes.

This objective requires preventing habitat loss due to human actions, and ecological restoration of habitat that has been degraded. Additionally, ecological management measures that would make habitats more resilient to the effects of climate change would help to maintain adequate spatial distribution of pupfish.

3.2.2 Maintain Abundance within the Natural Range of Variation

This objective requires maintenance of pupfish abundance within the natural range of variation. Specifically, this objective is directed at the ability to detect sustained population declines, identify the causes of decline, and then intervene with corrective actions to address causative factors.

Relative abundance of White Sands pupfish (*i.e.* catch per unit effort) is monitored annually as an index of absolute abundance (see Appendix A). The current monitoring program has several shortcomings that limit the ability to assess population trend. However, recommendations are provided in Appendix A to address these shortcomings and improve the ability of the monitoring program to detect changes in relative abundance with specified power and significance level criteria.

3.2.3 Establish and Maintain Natural Refuge Populations

Natural refuge populations are defined here as natural habitats that can support adequate numbers of pupfish to allow for repopulation of native habitats in the event of catastrophic loss or decline. These refuge populations would not constitute repatriation of the species to sites within its historic range where it has been eliminated, because there is no evidence that White Sands pupfish has suffered range contractions or local extirpations. The refuge populations are intended to be large and self-sustaining, and serve as genetic replicates to preserve genetic diversity. Refuge sites would be regularly replenished with fish from the parent population to ensure that adequate numbers, genetic integrity, and genetic diversity are maintained. Ideally, refuge sites for a native population should support a collective population size of at least 1,000 fish. Currently, the only refuge population is Lost River, which is occupied by pupfish translocated from Salt Creek. The genetic integrity of this refuge population is maintained by regular infusions of fish from the parent population at Salt Creek (*e.g.* Caldwell, 2014: 3).

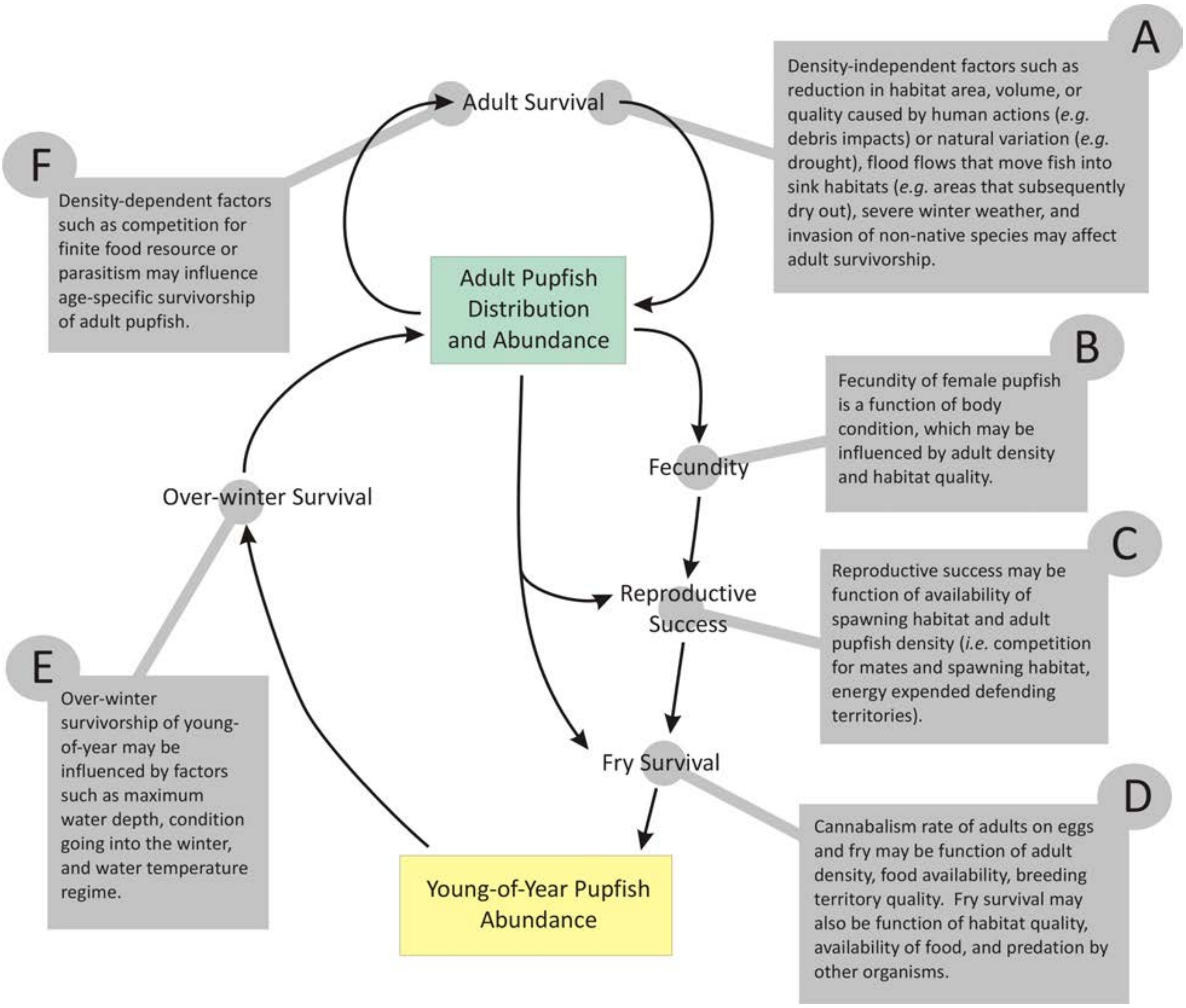
3.3 Identification of Potential Stressors

3.3.1 Conceptual Model of Factors Affecting White Sands Pupfish

A conceptual model of factors affecting the abundance and distribution of White Sands pupfish in a particular habitat is shown in Figure 23. The factors are arranged in groups labeled A through G that influence particular aspects of the life history of White Sands pupfish. Pupfish abundance and distribution is linked to habitat size and available resources (A in Figure 23) as indicated by large fluctuations in population size in highly variable habitats (McMahon and Tash, 1988).

Factors that influence fecundity, reproductive success, and egg and fry mortality have a major influence on abundance of White Sands pupfish (B, C and D in Figure 23). A primary factor affecting egg production by female White Sands pupfish is fish size (Jester and Suminski, 1982; Hoover *et al*., 1999). Consequently, habitats that support pupfish with high condition factor would have high fecundity (Hoover *et al*., 1999). Condition factor may also be influenced by population density (McMahon and Tash, 1988). Additionally, factors including water temperature (Shrode and Gerking, 1977) and salinity (Gerking and Lee, 1980) may influence oogenesis.

Figure 23. Conceptual model of factors affecting White Sands pupfish distribution and abundance. See text for discussion of each factor.



The most vulnerable part of the *Cyprinodon* life cycle is the egg stage, during which mortality is high (Kodric-Brown, 1983). In *C. tularosa* the mortality rate from egg to age 1 may reach 98 percent (Jester and Suminski, 1982). Consequently, habitats that have optimal temperature and salinity conditions for oogenesis and that promote high condition factor would be expected to foster good reproductive performance compared to habitats with suboptimal conditions.

Reproductive success (C in Figure 23) is influenced by the availability of suitable habitat for establishment of male breeding territories (Kodric-Brown, 1977 and 1978), quality of the breeding habitat (Kodric-Brown, 1983; Leiser and Itzkowitz, 2003), and competition for breeding habitat (Leiser and Itzkowitz, 2003). Breeding substrate is a critical resource (Kodric-Brown, 1978), and higher reproductive success is associated with breeding territories established on sites with high microtopographic complexity (Kodric-Brown, 1977 and 1983).

Although pupfish may inhabit a seemingly expansive habitat, suitable sites for establishment of breeding territories may be quite limited. For example, Kodric-Brown (1978) found that breeding territories were established in only about 10 percent of habitat occupied by the species at Mirror Lake in Bottomless Lakes State Park. Itzkowitz (2010) reported that an increase in bulrush (*Schoenoplectus*) density reduced spawning areas for Leon Springs pupfish (*Cyprinodon bovinus*), which resulted in population decline.

Survival of larval pupfish (D in Figure 23) may be influenced by predation (*e.g.* Gido *et al*., 1999), availability of escape cover (Deacon *et al*., 1995), food availability (Mapula, 2011), dissolved oxygen concentration, water temperature, and salinity. Deacon and others (1995) reported that production of larval Devils Hole pupfish was highest in habitats with shallow water and abundant algae, which produced substantial diel variation in dissolved oxygen concentration.

Over-winter mortality of pupfish (E in Figure 23) may be high, particularly among fry (McMahon and Tash, 1988). This was observed during winter monitoring of White Sands pupfish when dead fish, primarily small individuals, were frequently observed on the bottom of pools in Salt Creek (J. Pittenger, *in litt*.). McMahon and Tash (1988) reported that mortality was greatest after rapid temperature declines of greater than 5^{o}C in 48 hrs. Mortality increased with decreasing condition factor, which in turn was associated with high population density (McMahon and Tash, 1988).

Adult survival may be influenced by population density (F in Figure 23), particularly in habitats that are closed to emigration. McMahon and Tash (1988) found that closed experimental habitats experienced high population density followed by declines in reproduction, increased mortality, and starvation. The Salt Creek population is open to regulation of population density by emigration, particularly to marginal, sink habitats in the downstream reaches. Similarly, habitat at Malpais Spring expands with precipitation input, which creates ephemeral playa habitats on the margins of the wetland. Similar to Salt Creek, population density at Malpais Spring may be regulated by emigration of pupfish to those ephemeral, sink habitats.

3.3.2 Potential Stressors

3.3.2.1 Diminished Discharge from Springs

Native populations of White Sands pupfish, and natural refuge populations, may be affected by reduction in overall habitat size. As described in section 2.6.1, core habitats in Salt Creek and at Malpais Spring are maintained by spring and seep discharge from the basin-fill aquifer. Discharge from springs that maintain core habitats may be affected by reduced recharge of the basin-fill aquifer or increased groundwater pumping.

Recharge of the basin-fill aquifer is a function of precipitation, subsequent runoff and subsurface flow in catchment basins, and infiltration at mountain-front recharge zones (see Figure 5). However, as discussed in section 2.6.1.1, the role of several rather large catchments in the northern part of the Tularosa basin on recharge of the Malpais Spring alluvial aquifer is not known.

Climate projections indicate that winter temperatures may increase 0.5°C to 1.5°C in the next 20 years, while summer temperatures may increase 0.5°C to 2°C over the same period (Figure 24; Intergovernmental Panel on Climate Change, 2013). Temperature change projected for 2046-2100 during winter months is an additional 1°C to 3°C, and for summer months the change is projected to be another 1°C to 4°C (Figure 24). Projections of precipitation changes show no change from 1986-2005 conditions except for a 10- to 20-percent reduction for April-September 2081-2100 in the 25th percentile model run (Figure 25; Intergovernmental Panel on Climate Change, 2013).

The climate change projections suggest that although precipitation patterns may not change substantively compared to 1986-2005 conditions (Figure 25), air temperature is likely to increase, both in summer and winter months (Figure 24). Consequently, evaporation rates in the Tularosa basin would be expected to increase. This would likely reduce the amount of water available for recharge as well as increase water loss. The groundwater flow model developed by Huff (2005) used a maximum evapotranspiration rate of 0.33 cm 90.13 in) per day and a maximum depth from which evapotranspiration would occur of 4.5 m (14.8 ft). Huff (2008) estimated that 88 percent of the total recharge to the basin-fill aquifer was lost to evapotranspiration. This volume would be expected to increase with higher air temperatures, resulting in accelerated water loss from aquatic habitats and, potentially, diminished spring flow.

Huff (2005) simulated groundwater flows into and out of the basin-fill aquifer under various return-flow scenarios with projected groundwater withdrawals in the basin in the vicinities of Tularosa, Alamogordo, and Holloman Air Force Base. The model simulations did not show any changes in the basin-fill aquifer water-level contours in Salt Creek, Malpais Spring, Lost River, or Mound Springs areas through 2040. However, the model did not appear to address the northern part of the basin (*i.e.* from Oscura northward).

3.3.2.2 Saltcedar Persistence and Growth As discussed in section 2.6.3, saltcedar has invaded wetland and riparian habitats of White Sands pupfish relatively recently. Saltcedar may impact pupfish habitat by water loss through evapotranspiration, physical changes to habitat structure, and productivity.

Kennedy and others (2005) found that the density of native pupfish increased following removal of saltcedar along a desert spring brook. This positive effect was caused by a reduction of stream shading, which resulted in increased algal production. Development of dense stands of saltcedar in pupfish habitats may reduce aquatic habitat through increased use of water by the plants (*e.g.* Sala *et al.*, 1996; Devitt *et al*., 1997). Saltcedar often replaces native woody riparian species through competitive exclusion (Busch and Smith, 1995).

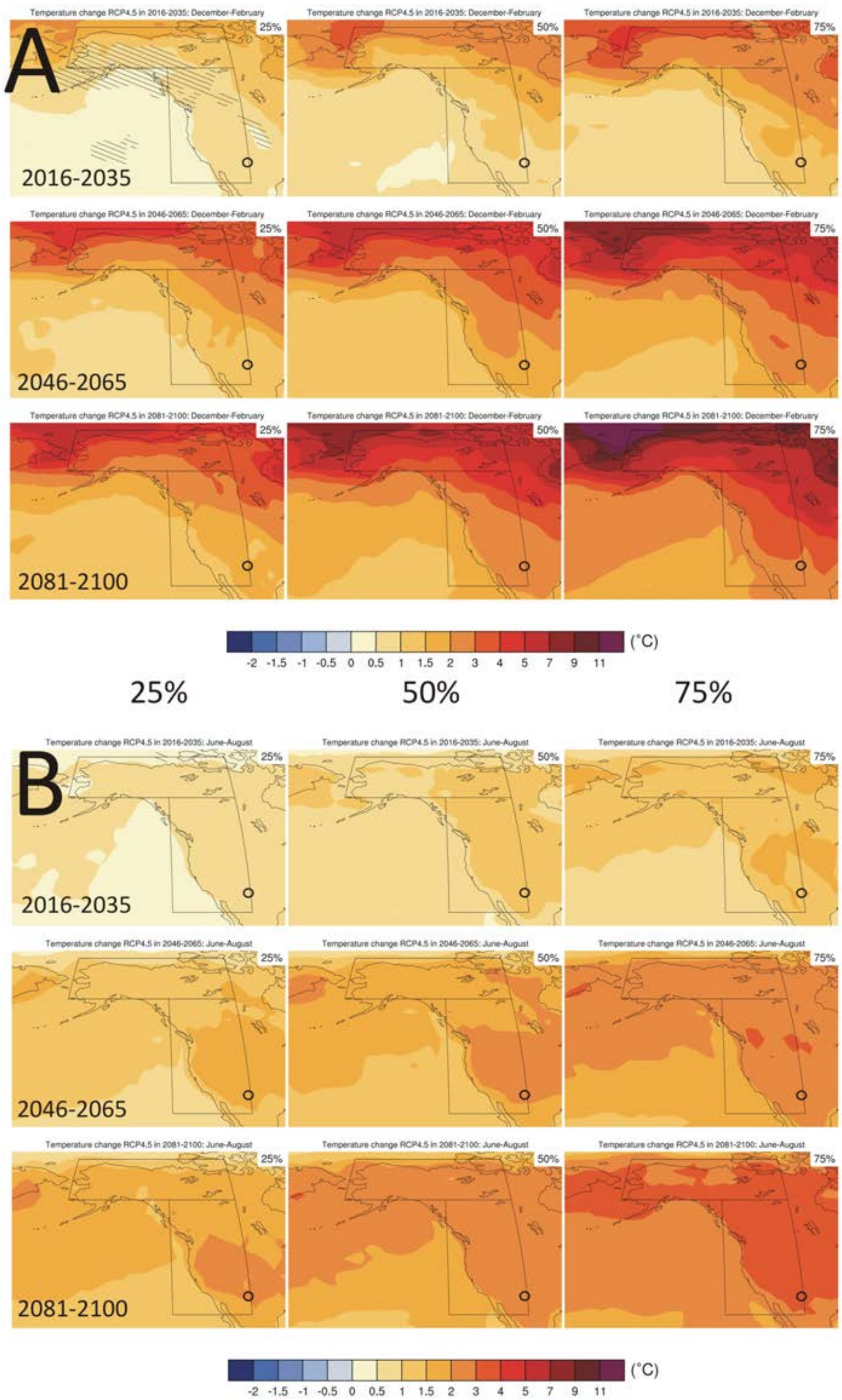


Figure 24. Projected temperature changes during winter (A) and summer (B) months in 2015-2035, 2046-2065, and 2081-2100 (rows) relative to 1986-2005. The 25th, 50th, and 75th percentiles of the distribution of model runs (columns). Hatching denotes no change from 1986-2005. Excerpted from Intergovernmental Panel on Climate Change (2013: figures AI.16 and AI.17). The small circle in each map shows the approximate location of the Tularosa basin.

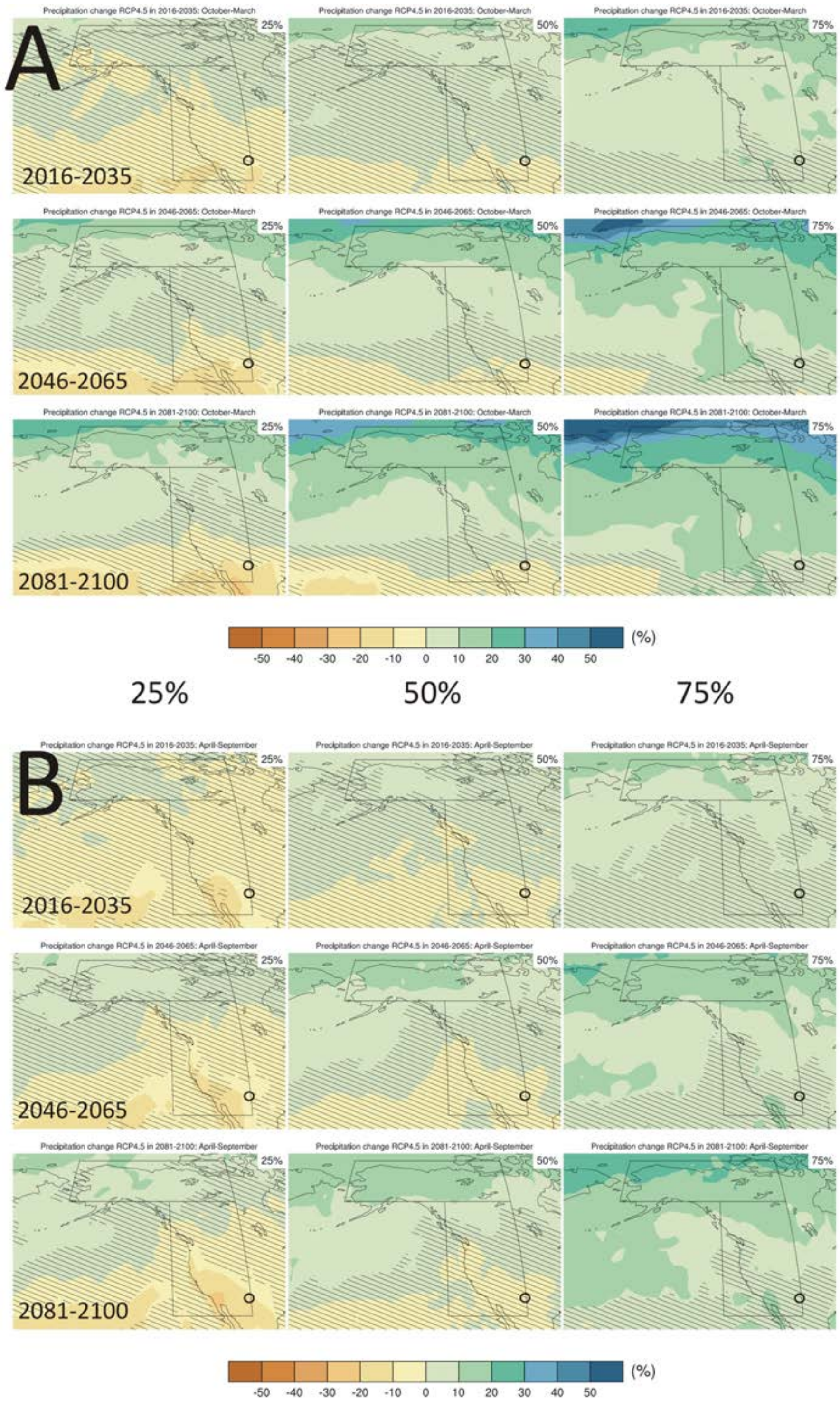


Figure 25. Projected precipitation changes during winter (A) and summer (B) months in 2015-2035, 2046-2065, and 2081-2100 (rows) relative to 1986-2005. The 25th, 50th, and 75th percentiles of the distribution of model runs (columns). Hatching denotes no change from 1986-2005. Excerpted from Intergovernmental Panel on Climate Change (2013: figures AI.18 and AI.19). The small circle in each map shows the approximate location of the Tularosa basin.

Water use by individual saltcedar plants is similar to other phreatophytes (Nagler *et al*., 2003). However, the scale of water use at the stand level is determined by total leaf area (Anderson, 1982), so very dense stands of saltcedar have higher water use than native species such as mesquite, which does not grow in stands as dense as saltcedar and which has lower leaf area index (Sala *et al*., 1996).

Dahm and others (2002), using an eddy covariance approach, found saltcedar stands on Rio Grande floodplain had ET rates ranging from 1110 to 1220 mm/yr (43.7 to 48.0 in/yr). Similarly, Devitt and others (1998), using micrometeorological methods, reported ET of 1500 mm/yr (59.0 in/yr) during a wet year and 750 mm/yr (29.5 in/yr) during a dry year. Water use by saltcedar varies with depth to water table (Horton *et al*., 2001). Saltcedar growing in close proximity to streams or in areas with shallow water tables transpire more water than plants growing farther from the stream or where the water table is deeper (Devitt *et al*., 1997).

Saltcedar is common along the banks of Salt Creek, where it forms dense monoculture stands, particularly from the waterfall about the Route 316 crossing downstream to the vicinity of the cable-crossing site (section 2.6.3.2). Development of dense saltcedar stands likely altered channel morphology of Salt Creek, primarily by narrowing the stream channel (Hereford, 1984). For example, Miller and Echelle (1975) noted that the channel of Salt Creek at the Route 316 crossing was “4 to 12 m wide.” Channel width is narrower now, ranging from about 1.5 to 2.5 m (5 to 8 ft). The narrower channel is more likely to concentrate the force of flood flows, which may result in increased displacement of pupfish downstream.

Saltcedar also creates more canopy cover and stream shading than native vegetation along Salt Creek, which may reduce water temperature compared to sites without saltcedar canopy shading. In addition to potential effects on water temperature, canopy shading by saltcedar may depress primary productivity and shift dominant organic matter input to strongly pulsed allochthonous input associated with seasonal leaf fall (Kennedy and Hobbe, 2004, Kennedy *et al*., 2005). Kennedy and others (2005) found that pupfish relied heavily on algae-derived carbon and not saltcedar-derived carbon.

Saltcedar leaf litter apparently has similar nutritive value to native deciduous riparian plants, but the saltcedar litter degrades more rapidly and leaf packs are not persistent (Going and Dudley, 2008; Moline and Poff, 2008). Bailey and others (2001) reported lower species richness and decreased abundance of aquatic macro-invertebrates on leaf packs of saltcedar compared to leaf packs of native deciduous riparian species.

Saltcedar may increase soil salinity on floodplains (Merritt and Shafroth, 2013; Ladenburger *et al*., 2006; but see Bagstad *et al*., 2006 and Imada *et al*., 2013). However, other factors such as naturally saline parent material and the frequency and extent of floodplain inundation by high flows also influence soil salinity on floodplain sites (Glenn *et al*., 2012; Merritt and Shafroth, 2013).

<u>3.3.2.3 Installation Activities</u> Installation activities have the potential to impact White Sands pupfish through physical impacts to pupfish habitat, alteration of hydrology, or degradation of water quality. For example, Turner (1987) evaluated the potential effect of herbicide treatments within weapon impact targets near pupfish habitat. The practice of using herbicides to remove vegetation from weapon impact targets has since been abandoned in favor of physical removal (*i.e.* surface grading or scalping) in specific areas.

Debris impacts or introduction of contaminants from debris into waters occupied by pupfish is another potential impact. However, field work conducted throughout pupfish occupied habitat since 1994 has not identified any substantive debris impacts in pupfish habitats, or any water quality impacts associated with debris.

White Sands Missile Range has delineated White Sands pupfish essential habitat, where activities are prohibited unless specifically authorized after review, and limited use areas that buffer these essential habitats. Proposed installation activities are reviewed by the Environmental Stewardship Branch to ensure that pupfish habitats are protected. White Sands Missile Range consults and coordinates with the New Mexico Department of Game and Fish and the U.S. Fish and Wildlife Service on activities that may potentially affect White Sands pupfish and its habitat to ensure protection of the species. However, there is still the potential for unintentional impacts to pupfish habitat from installation activities.

3.3.2.4 Density of Marsh Vegetation As discussed in section 2.3.2, aquatic habitats with dense emergent wetland vegetation do not appear to be used to a great extent by pupfish. Marsh vegetation at Malpais Spring has increased markedly since removal of feral horses was completed in 1998 (see section 2.6.3.1 and Appendix B). Similar responses of marsh vegetation to removal of grazing have been reported in other arid land aquatic habitats, along with negative consequences for native aquatic biota inhabiting those systems (*e.g.* Kodric-Brown and Brown, 2007; Stacey *et al*., 2011: 30). Reduced primary production due to shading (Scoppettone *et al*., 2013; Johnson *et al*., 2013) and reduction in suitable spawning habitat (Itzkowitz, 2010) have been forwarded as the causes of pupfish decline following increased marsh vegetation density.

3.3.2.5 Loss of Genetic Integrity The Salt Creek and Malpais Spring populations have been determined to be evolutionarily significant units (Stockwell *et al*., 1998). Consequently, maintaining the genetic integrity of these two populations is important. Due to the proximity of the two populations and the history of unregulated translocations (see section 2.3.1), there is the potential for mixing of the two ESUs in one or both of the native habitats.

Introduction of nonnative sheepshead minnow (*C. variegatus*) has resulted in introgression of populations of Pecos pupfish (Childs *et al*., 1996) and Leon Springs pupfish (Echelle and Echelle, 1997). Sheepshead minnow is not known to occur in the Tularosa basin, but it has advanced upstream into the Pecos River in New Mexico (Echelle *et al*., 1997).

Barriers to fish movement may prevent gene flow in a population, resulting in adverse genetic effects (Martin, 2010). For example, Stockwell and Mulvey (1996) found that White Sands pupfish upstream from the Route 316 crossing in Salt Creek had lower allelic diversity than fish downstream from the crossing. At the time of that study, the Range Road 316 crossing was a perched culvert that prevented upstream movement of pupfish.

3.3.2.6 Introduction of Nonnative Aquatic Biota As discussed in section 2.10, White Sands pupfish populations would likely be detrimentally affected by introduction and establishment of nonnative species such as western mosquitofish and crayfish (Rogowski and Stockwell, 2006). Mosquitofish are abundant in the HAFB wastewater effluent wetlands and golf course ponds. However, these habitats are geographically removed, by a considerable distance, from the native populations. The Lost River population, located on HAFB, is much closer to the effluent wetlands and the golf course ponds.

Mosquitofish can tolerate high levels of salinity (39 to 58.8 parts per thousand; Chervinski, 1983), similar to high salinities recorded in Lost River and Salt Creek. Kendall and Schwartz (1964) found that the 33 ppt salinity caused rapid mortality of the crayfishes *Orconectes virilis* and *Cambarus bartonii*. Both species appeared to be able to persist in up to 6ppt salinity.

Toxins produced by golden alga *Prymnesium parvum* have caused large-scale fish kills in the Pecos River in New Mexico and Texas since the1980s (Rhodes and Hubbs, 1992). Dispersal of the alga may occur via waterfowl movements or wind currents. Pupfish habitats in the Tularosa basin have similar water chemistry to the Pecos River, and it is conceivable that the alga could enter the basin (Carman, 2010).

3.4 Conservation Efforts to Date

Following is a chronological account of the major actions implemented for conservation of White Sands pupfish.

- 1990 The initial conservation plan for the species was developed.
- 1993 The Route 6 crossing over Salt Creek was reconstructed, allowing for fish movement through the crossing.
- 1994 The conservation plan was revised.
- 1994 WSMR completed an environmental assessment for reduction of the feral horse population.
- 1995 A cooperative agreement was executed with the New Mexico Department of Game and Fish, U.S. Fish and Wildlife Service, White Sands Missile Range, Holloman Air Force Base, and White Sands National Monument as signatories. The agreement defined essential and limited use habitat areas with restrictions on activities (*e.g.* off-road vehicle use) that may occur in those areas for protection of pupfish habitat. The agreement also established the interagency White Sands Pupfish Conservation Team and agency responsibilities, which included annual monitoring by the New Mexico Department of Game and Fish.
- 1995 The feral horse population on WSMR was reduced by over 80 percent, springs were fenced to exclude horses, which were causing adverse impacts to pupfish habitat at Malpais Spring and Mound Springs as well as fishless springs.
- 1995 Annual monitoring of White Sands pupfish populations was initiated.
- 1995 Essential habitat and limited use areas were established on White Sands Missile Range, which delineated areas where installation activities are prohibited or restricted unless specifically authorized. Essential habitat on White Sands Missile Range includes Salt Creek, the Mound Spring complex, and Malpais Spring and the associated wetland. Essential habitat includes these areas and a 100 m buffer on each side of the center line of stream channels or around the perimeter of spring pools and wetlands. All non-emergency activities are prohibited in essential habitat. Limited use areas on White Sands Missile Range consist of lands adjacent to essential habitat. Limited use areas are managed to ensure that degradation of essential habitat does not occur.

- 1995 White Sands pupfish essential habitat and limited use areas were established on Holloman Air Force Base. Essential habitat on Holloman Air Force Base includes the channel of Malone Draw and Lost River and a corridor along the channel extending 100 m from either side of the center of the stream channel. All non-emergency activities are prohibited in essential habitat, with the exception of use of existing unimproved and improved roads. Limited use areas on Holloman Air Force Base include Ritas, Pruess, and Carter draws and their tributaries. These areas are managed to prevent degradation of essential habitat.

- 1995 The transport of live nonnative aquatic organisms and their introduction into aquatic habitats on White Sands Missile Range and Holloman Air Force Base was prohibited.

- 1995 A stream discharge gage was installed on Salt Creek just upstream from the Route 316 crossing on 30 August, in cooperation with the U.S. Geological Survey.

- 1995 Collection and analysis of water quality samples from Salt Creek was initiated by WSMR.

- 1996 The feral horse herd on WSMR was reduced to approximately 500 horses.

- 1996 Signs were installed at pupfish habitats on WSMR with information on regulations governing collection of the species and protection of its habitat.

- 1996 Research on White Sands pupfish was initiated at experimental ponds set up on HAFB

- 1997 Selected unimproved roads in essential habitat and limited use areas were closed and signing was installed at HAFB to protect habitat of White Sands pupfish in the Lost River drainage.

- 1997 WSMR published a supplement to the 1995 environmental assessment, proposing to remove all feral horses.

- 1998 All feral horses were removed from WSMR.

- 1998 Barrel Spring was treated with rotenone in an attempt to remove nonnative fish. A single application was made that resulted in eradication of green sunfish and largemouth bass, but mosquitofish and nonnative crayfish persisted at the site.

- 1998 Research on genetic characteristics of White Sands pupfish populations was published, which recognized two evolutionarily significant units for the species (Salt Creek and Malpais Spring).

- 1998-1999 The status of extant populations was investigated and the native status of Salt Creek and Malpais Spring was confirmed, as was the introduced status of Mound Spring and Lost River populations.

- 2001 A bibliography of geology and hydrology references, which included 590 citations of papers pertinent to habitats of White Sands pupfish, was prepared by WSMR.

- 2003 A stream discharge gage was installed at the main spring outflow channel at Malpais Spring, in cooperation with the U.S. Geological Survey.

- 2005 Research on the morphological divergence of native and recently established populations of White Sands pupfish was published.

- 2006 The interagency cooperative agreement was renewed, which including continuation of essential habitat and limited use area designations and prohibition of transport or introduction of live nonnative aquatic organisms on White Sands Missile Range and Holloman Air Force Base.

- 2006 Research on potential impacts of exotic species on populations of White Sands pupfish was published.

- 2009 The monitoring plan for White Sands pupfish was revised.

- 2010 WSMR completed an evaluation of potential natural refuge sites for the Malpais Spring population.

- 2013 Research on the divergence time of the Salt Creek and Malpais Spring populations was published.

- 2013 The perched culvert at the Route 316 crossing of Salt Creek was replaced with a bridge that allowed for movement of pupfish through the crossing.

- 2014 A compilation of hydrologic data from 1911 through 2008 for aquatic habitats of the Tularosa basin, including habitats of White Sands pupfish, was prepared by the U.S. Geological Survey in cooperation with WSMR.

- 2015 WSMR funded the U.S. Geological Survey to replace and reactivate the stream discharge gages on Salt Creek and at Malpais Spring; the work will be completed in the summer of 2015.

3.5 Population Status

The most recent status report for White Sands pupfish (Caldwell, 2014) indicated that both the Malpais Spring and Salt Creek populations met conservation objectives and were therefore considered secure. However, continued low catch rates at the middle marsh site at Malpais Spring were noted and investigation of cause (*i.e.* artifact of reduced sampling efficiency or true change in abundance) was recommended. Pupfish were observed throughout the three reaches of Lost River, which met conservation objectives for that population.

The issue of how to manage the Mound Spring population, which was established with fish from Salt Creek, has not been resolved. The genetic management plan for replicate populations recommended removal of the existing population at Mound Spring and establishment of a refuge population for Malpais Spring at the site (New Mexico Department of Game and Fish, 2006). The proximity of a Salt Creek-derived pupfish population to Malpais Spring raises concerns about potential unregulated translocations between the sites, which could conceivably result in introgression of the Malpais Spring population.

4.0 Conservation Actions

Eight categories of conservation actions were developed to address the factors identified in Chapter 3 that potentially affect the conservation status of White Sands pupfish.

These action categories, and the factors they address, are summarized below in Table 5. A discussion of each action category follows in sections 4.1 through 4.8.

Table 5. Conservation actions and stressors that they address.

Conservation Action	Potential Stressor					
	Diminished spring discharge	Salt cedar persistence and growth	Installation activities	Marsh vegetation dynamics	Loss of genetic integrity	Introduction of nonnative aquatic biota
Establish Malpais Spring refuge populations	X	X	X	X	X	X
Improve population and habitat monitoring	X	X	X	X	X	X
Develop ecological restoration plan for Malpais Spring				X		
Control saltcedar	X	X				
Refine delineation of aquifer recharge zones	X					
Review installation activities to avoid or reduce impacts			X			
Reduce potential for land-based chemical spills					X	
Conduct research in support of conservation	X	X	X	X	X	X

4.1 Establish Malpais Spring Refuge Populations

An evaluation of potential natural refuge sites for the Malpais Spring population was completed in 2010 (Pittenger, 2010). The evaluation concluded with three top-ranked sites: North Mound Spring, Mound Spring, and Barrel Spring. All three sites are well-matched with Malpais Spring in terms of water chemistry, which is an important consideration for establishing refuge populations (Karam *et al*., 2012). The latter two sites would require removal of existing fish populations, while North Mound Spring is fishless. Consequently, North Mound Spring was recommended as the best choice for immediate use as a natural refuge for the Malpais Spring population.

Pupfish use of spring pool habitat is restricted to relatively shallow water margins. For example, Itzkowitz (2010: 11) reported that Leon Springs pupfish were found only from 0-50 cm (19.7 in) 0-depth in the deep Lower Monsanto pool and Diamond Y Spring, and most were in the upper 15 cm (5.9 in) of water. Consequently, the relatively small pool at North Mound Spring may not support a very large population. Therefore, other spring sites including Mound Spring, South Mound Spring, and Barrel Spring should be considered for restoration and eventual use as additional natural refuge sites for the Malpais Spring population.

4.1.1 North Mound Spring

North Mound Spring is a fishless limnocrene located at the north end of the chain of mound springs (Meinzer and Hare, 1915: 52-53; Figure 26). The spring pool was enlarged and deepened by excavation at least once in the past. The current surface area of the spring pool is approximately 205 m^2 (2,206 ft^2), and the pool has a maximum depth of 244 cm (8.0 ft) and a mean depth of 91 cm (3.0 ft; Pittenger, 2010).

Because the spring pool is fishless, the site would not require any substantial restoration work prior to translocation of pupfish. Some vegetation management is recommended, however, prior to translocation. This includes removal of a small number of saltcedar plants (see section 4.3 below) from the spring mound and mechanical removal of emergent wetland vegetation on the shallow shelf at the north edge of the pond (Figure 27). Emergent wetland vegetation has colonized the shelf over the last five years (Figure 27).

Emergent wetland vegetation at North Mound Spring consists mainly of saltmarsh bulrush (*Bolboschoenuus maritimus*). The vegetated area encompasses approximately 10 m^2 (108 ft^2). Saltmarsh bulrush may be controlled by removing entire plants including (and especially) rhizomes and corms (*e.g.* Albert, 2005), from which the plants may re-sprout vigorously (Kantrud, 1996). The majority of root material is typically within 20 cm (8 in) of the surface, but there may be root material as deep as 60 cm (24 in; Kantrud, 1996). Consequently, shallow (not to exceed 60 cm [24 in]), scraping excavation of the vegetated portion of the inundated shelf area using a mini excavator with a wide bucket should be conducted to remove bulrush. Testing with a shovel to determine the required depth of excavation to remove bulrush should be conducted first to avoid over-excavation of the clay shelf, and to minimize the area disturbed. After vegetation is removed, rocks may be placed at various depths on the shelf to enhance spawning habitat (Watters *et al*., 2003).

Figure 26. Location of potential natural refuge sites for the Malpais Spring population.

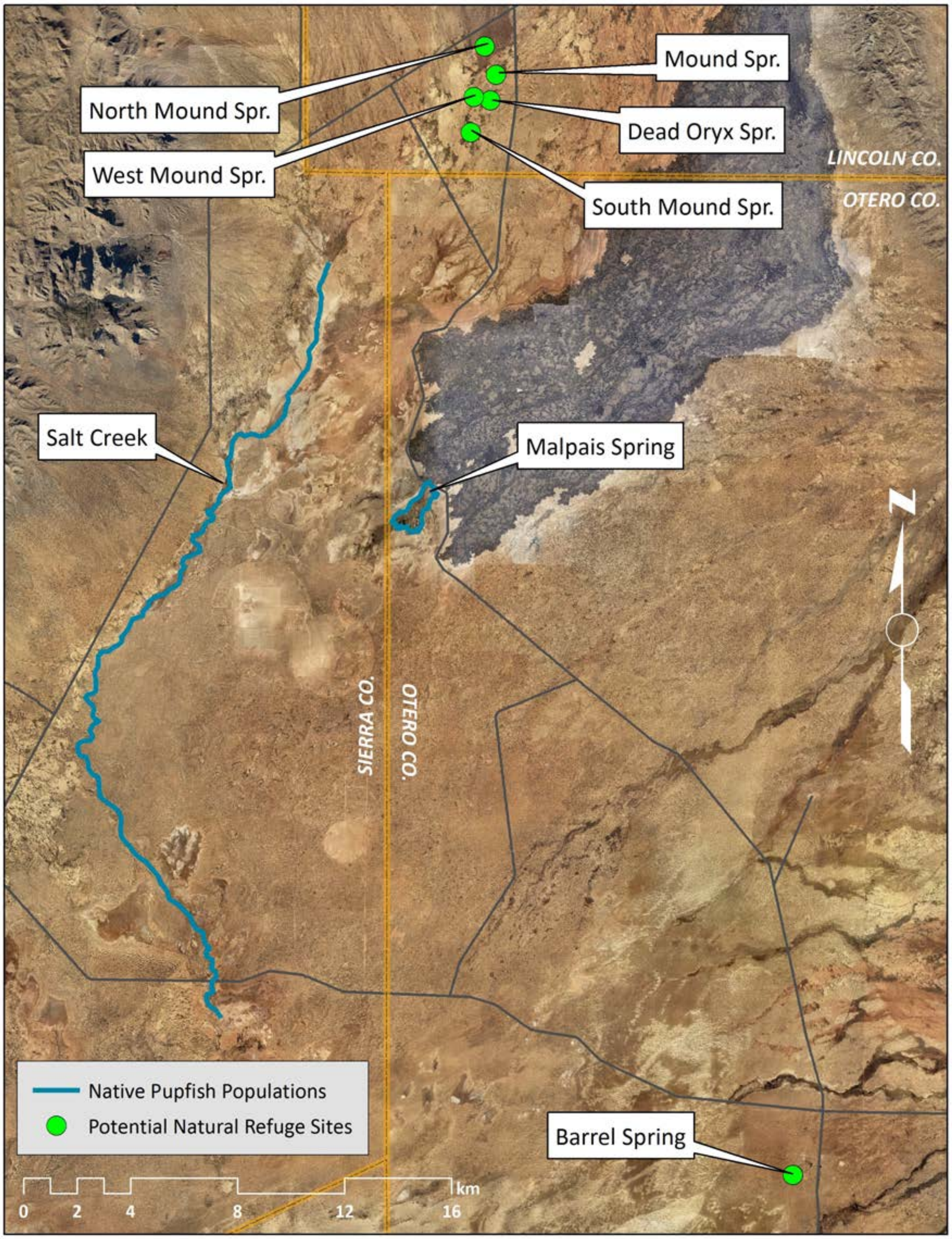
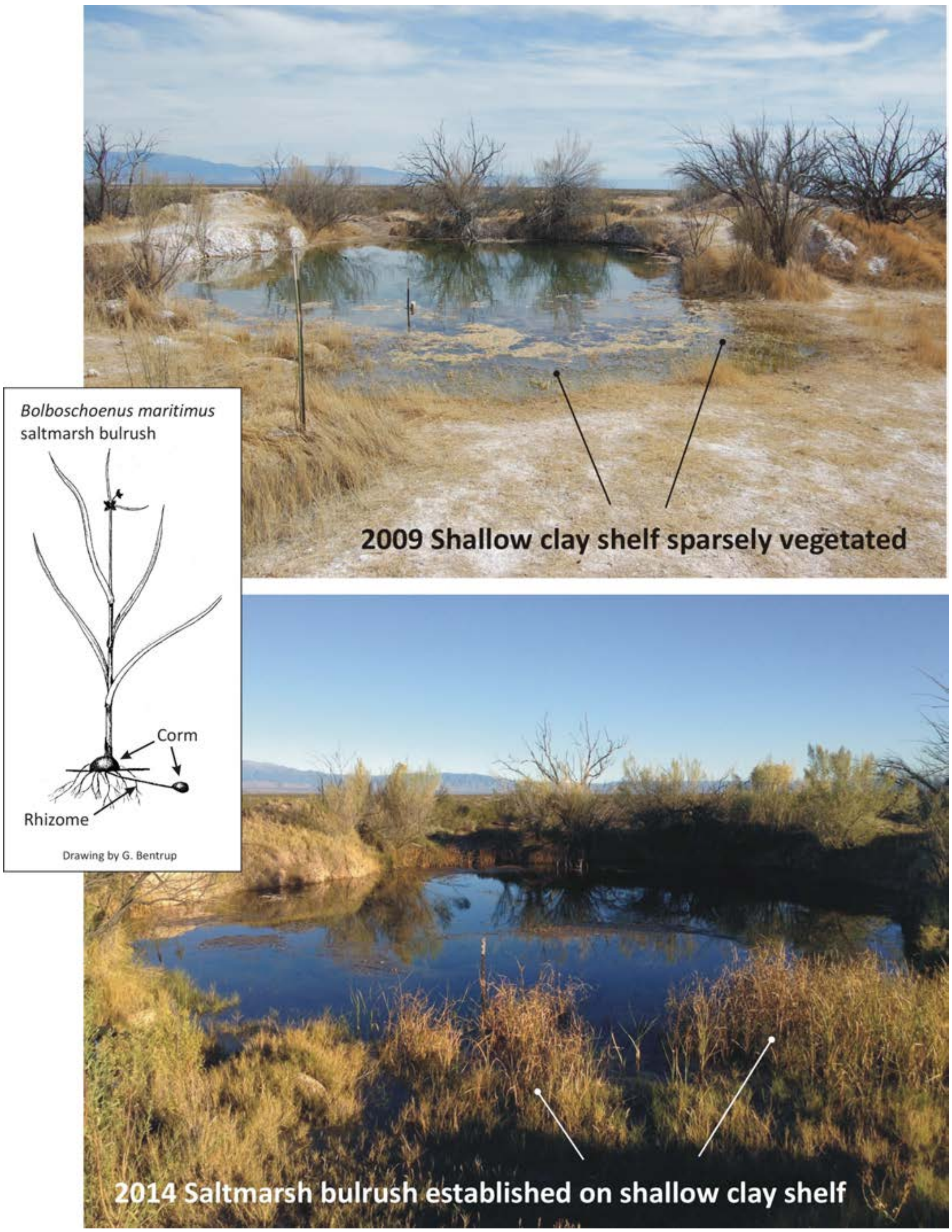


Figure 27. View south across North Mound Spring pool showing establishment of saltmarsh bulrush (*Bolboschoenus maritimus*) on shallow clay shelf on north edge of pool (photos by J. S. Pittenger, upper photo taken on 3 March 2009 and lower photo taken on 23 April 2014). The inset shows botanical features of saltmarsh bulrush, with rooting structures (corms and rhizome) noted.



Following restoration work, pupfish should be translocated to North Mound Spring. Finger and others (2013) recommended founding new refuge populations with 30 to 50 fish and translocating up to 10 migrants per generation (year) among stable populations, and maximizing habitat area and quality. The *Genetic Management Plan for Replicate Populations of White Sands Pupfish* (New Mexico Department of Game and Fish, 2006) specifies that at least 200 pupfish should be collected from Malpais Spring and placed in North Mound Spring. Pupfish for translocation to North Mound Spring should be collected from the south wetlands and *laguna* at Malpais Spring to capture the range in allelic diversity in the population (Stockwell and Mulvey, 1998; New Mexico Department of Game and Fish, 2006).

Maintenance and monitoring of North Mound Spring following restoration and translocation of pupfish should be conducted. Maintenance should consist of periodically raking the shallow shelf area on the north side of the pond to remove emergent wetland vegetation and prevent re-establishment of dense stands of saltmarsh bulrush or other wetland plants. Maintenance should also include removal of saltcedar seedlings or sprouts through hand-pulling or other treatment (see section 4.3 below). Genetic maintenance of the North Mound Spring refuge population should consist of moving 25 fish annually from Malpais Spring to the site (New Mexico Department of Game and Fish, 2006).

Monitoring should include installation of a datalogging thermograph (*e.g.* Onset HOBO Water Temperature Pro® v2) on the clay shelf and a water-level datalogger (*e.g.* Solinst Model 3001LTC Levelogger® Junior, which measures water level, temperature, and conductivity at user-specified time intervals) in the deep portion of the pool (*e.g.* on the south edge of the pond). The water-level datalogger should be surveyed to establish elevation relative to a site benchmark. Hourly datalogging intervals would be sufficient for both water level and water temperature. Dataloggers should be downloaded regularly (*e.g.* on a quarterly basis) and data should be analyzed and presented in annual monitoring reports. Monitoring of the translocated population should also be conducted, with monitoring methods (*i.e.* sampling techniques, frequency of sampling) determined by the Conservation Team.

4.1.2 Mound Spring

Mound Spring (Figure 26) contains a population of White Sands pupfish founded with fish from Salt Creek. However, Mound Spring is a poor ecological replicate for Salt Creek and is of little use as a natural refuge for that stream-dwelling population (Collyer *et al*., 2007 and 2011). The spring pond and overflow pond at Mound Spring were excavated in 1967, and pupfish were stocked into the ponds sometime between 1967 and 1973 (Pittenger and Springer, 1999). Genetic analysis has confirmed that the pupfish stocked into Mound Spring were from Salt Creek (Stockwell *et al*., 1998).

The spring pool, or upper pond, at Mound Spring (Figure 28) has a surface area of approximately 965 m^2 (0.24 ac), maximum depth of approximately 4.8 m (15.8 ft), and mean depth of approximately 1.7 m (5.6 ft). The approximate volume of the spring pool is 1,640 m^3 (1.33 acre-feet). The lower pond (Figure 28), which is fed by seepage and overflow from the spring pool, has a surface area of approximately 1,217 m^2 (0.30 ac), maximum depth of approximately 4.4 m (14.4 ft), and mean depth of approximately 2.8 m (9.2 ft). The approximate volume of the lower pond is 3,408 m^3 (2.76 acre-feet).

Figure 28. Aerial view of Mound Spring, 2012.

If genetic testing indicates that the Mound Spring population does not contain unique alleles, the site should be renovated to remove the existing population prior to being stocked with pupfish from Malpais Spring (New Mexico Department of Game and Fish, 2006).

Preparatory habitat restoration work should be conducted before beginning renovation treatments. Habitat restoration at Mound Spring should include removal of saltcedar from the site (see section 4.3 below), removal of emergent wetland vegetation from the perimeter of the spring pool, and dredging of the lower pond.

Emergent wetland vegetation in the spring pool consists of southern cattail (*Typha domingensis*) and beaked spikerush (*Eleocharis rostellata*) that form a band up to 3.0 m (10 ft) wide along the approximately 125 m (410 ft) perimeter of the pool, constituting a total area of approximately 375 m^2 (0.1 ac) . These emergent wetland plants can be removed using the same technique described above in section 4.1.1.1 for removing saltmarsh bulrush from North Mound Spring. A tracked mini excavator fitted with a wide bucket should be used to scrape and remove the top 30 to 45 cm (12 to 18 in) of sediment along with the vegetation, thereby removing rhizomes from which the plants may re-sprout (Beule, 1979).

The lower pond should be dredged with a large excavator or similar piece of equipment to reduce the dense growth of stonewort (*Chara vulgaris*), accumulated organic sediments, and cattails around the pond, which are concentrated along the southeast edge of the pond. Assuming an average depth of excavation of 0.6 m (2 ft), approximately 730 m^3 (955 yd^3) of sediment and associated plant material would be removed from the lower pond. After vegetation removal and dredging is completed, rocks may scattered over the substrate in shallow water of both ponds to enhance spawning habitat.

Removal of the existing population would be conducted through piscicide treatments using rotenone, following the label requirements and EPA-approved standard operating procedures (Finlayson *et al*., 2010). Rotenone application would not be conducted until the preparatory habitat restoration work described above is completed, and suspended sediment has settled in both ponds.

Rotenone would be applied by backpack sprayer in liquid form (1 part CFT Legumine to 10 parts water) and in powder-gelatin-sand mixture form, both of which would be used to achieve a concentration not to exceed 0.2 parts per million active rotenone, pursuant to label specifications. Based on the pond volumes described above, 1.67 L (0.44 gal) of CFT Legumine (5% active ingredient) would be required for a single treatment of the spring pool and 3.44 L (0.91 gal) would be required for a single treatment of the lower pond with resulting concentrations of 0.1 ppm rotenone (active ingredient).

Rotenone should be allowed to detoxify naturally, which would take no longer than one month. Efficacy of treatments should be assessed by sampling and/or snorkel survey. If fish are still present after the first rotenone treatment, subsequent treatments should be made until both ponds are determined to be fishless. It is expected that no more than three treatments would be required to remove all fish. Treatments should be conducted during the period from the beginning of April to the end of September.

Following confirmation that fish have been eradicated and the aquatic invertebrate community has recovered (no sooner than the next spring following completion of renovation treatments), at least 200 pupfish from Malpais Spring should be translocated to the spring pool and another 200 to the lower pond at Mound Spring (New Mexico Department of Game and Fish, 2006).

Maintenance and monitoring of Mound Spring following restoration and translocation of pupfish should be conducted. Maintenance should consist of periodically raking shallow pond margins to remove emergent wetland vegetation and prevent re-establishment of dense stands of cattail or other wetland plants. Maintenance should also include removal of saltcedar seedlings or sprouts through hand-pulling or other treatment (see section 4.3 below). Genetic maintenance of the Mound Spring refuge population should consist of moving 25 fish annually from Malpais Spring to the site (New Mexico Department of Game and Fish, 2006).

Monitoring should include installation of a datalogging thermographs (*e.g.* Onset HOBO Water Temperature Pro® v2) at approximately 30 cm (12 in) depth in both ponds. Water-level dataloggers (*e.g.* Solinst Model 3001LTC

Levelogger® Junior) should be installed in each pond in deep-water areas (*e.g.* on the northeast side of the spring pool and east side of the lower pond). The water-level dataloggers should be surveyed to establish elevation relative to a site benchmark. Hourly datalogging intervals would be sufficient for both water level and water temperature. Dataloggers should be downloaded regularly (*e.g.* on a quarterly basis) and data should be analyzed and presented in annual monitoring reports. Monitoring of the translocated population should also be conducted, with monitoring methods (*i.e.* sampling techniques, frequency of sampling) determined by the Conservation Team.

4.1.3 South Mound Spring

South Mound Spring is a fishless limnocrene located at the south end of the chain of mound springs (Meinzer and Hare, 1915: 52-53; Figure 26). Excavation and modification of the spring pool has occurred in the past. Prior to completion of feral horse removal in 1999, the spring pool banks were breached by horse trampling and the spring pool was drained. Continued horse use of the site changed the spring pool to a muddy wallow. Following removal of feral horses, the spring pool was partially restored.

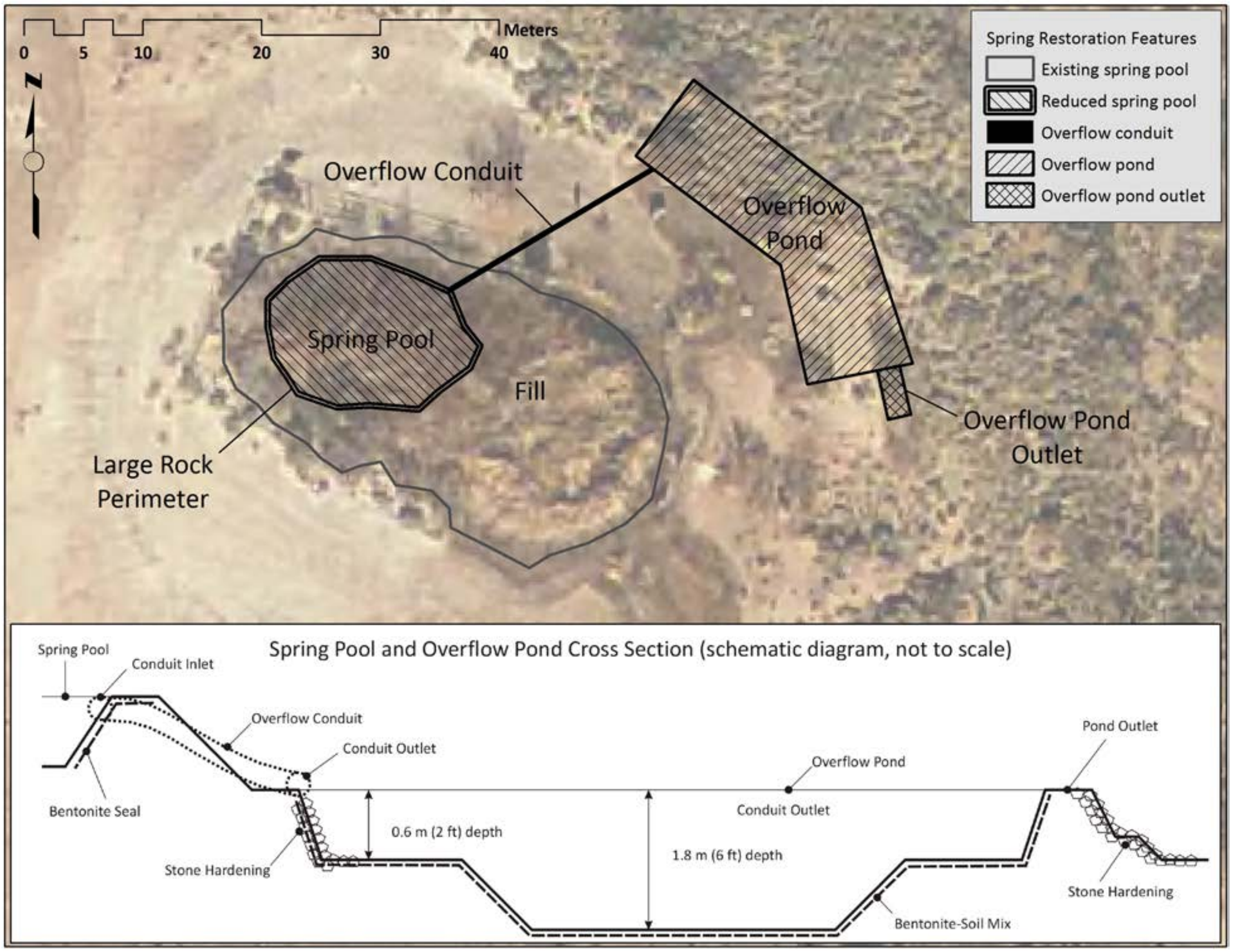
Presently, the surface area of the spring pool is approximately 681.2 m^2 (0.17 ac), and the pool has a maximum depth of 56 cm (22 in) and a mean depth of 20 cm (8 in; Figure 29). The shallow spring pool is completely filled in with a very dense stand of broadleaf cattail (*Typha latifolia*; Sivinski and Tonne, 2011: 87). The spring overflows the rim of the pool on the east side. The overflow is not confined to a channel and spreads out as it flows down the slope of the spring mound to seep into the desert floor at the base of the mound.

Aquatic habitat at South Mound Spring currently would not support a population of White Sands pupfish because of the shallow water depth and extremely dense stand of broadleaf cattail that occupies the entire spring pool. Consequently, aquatic habitat should be restored to make the site suitable for pupfish (Watters *et al.*, 2003; Abele, 2011: 25; Stacey *et al*., 2011: 30; Nevada Springs Restoration Workshop Committee, 2012). Restoration should consist of saltcedar removal and control (see section 4.3 below), removal of vegetation and organic sediment from the spring pool, reducing the surface area of the spring pool and raising the pool banks, installation of an overflow conduit, and construction of an overflow pond fed by the conduit.

Emergent wetland vegetation and accumulated litter and organic debris should be removed from the spring pool with careful, shallow, scraping excavation, as described above for North Mound and Mound springs. A mini excavator with a wide bucket should be used to pull sediment and vegetation back from the center of the pool to the edge. Maximum depth of excavation should be no more than 0.61 m (2 ft), and particular care should be taken around the spring vent.

The spring pool surface area should be reduced (*e.g.* Settevendemio, 2014) by placing large rock around the smaller spring pool perimeter, then placing fill (Figure 29). The rim of the spring pool should be raised approximately 30 to 60 cm (1 to 2 ft), using soil from the overflow pond excavation area (Figure 29). The purpose of reducing the surface area of the spring pool is to increase depth to reduce the potential for emergent wetland vegetation establishment and reduce evaporation. The spring pool surface area should be reduced to approximately 160 to 175 m^2 (0.04 ac).

Figure 29. Conceptual aquatic habitat restoration design for South Mound Spring.



The spring vent area, which may be indicated by a zone of roiling sand or deep, flocculant sediment, should be marked with flagging prior to excavation. Use of a suction dredge in the spring vent area may be warranted during removal of vegetation and organic sediments, particularly if there is no clear zone of roiling sand indicating the precise location of the vent. Although many of the mound springs have been excavated in the past, there is the potential to adversely affect the spring by blocking the spring orifice or throat (Queensland Wetlands Programme, 2005; Unmack and Minckley, 2008; Haynes, 2008).

An overflow pond with a minimum surface area of 200 m^2 (0.05 ac) should be constructed immediately east of the spring pool. Recommended pond dimensions are a rectangle measuring at least 20 m (66 ft) by 10 m (33 ft). The pond should be constructed with relatively steep banks and a shallow-water shelf around the perimeter with water depths ranging from 20-60 cm (8-24 in) and a maximum depth of approximately 1.6 m (6 ft; Figure 29). A conduit (*e.g.* 10-cm [4-in] diameter Corex® solid drain pipe) should be installed to convey overflow from the spring pool to the overflow pond. The spring pool rim should be level to ensure that all overflow is conveyed through the conduit to the overflow pond. The area around the conduit inlet should be sealed with bentonite clay to prevent piping and leaking around the inlet. The conduit outlet area in the overflow pond should be hardened with small stone (*e.g.* D_{50} = 5 cm [2 in]) to prevent erosion (Figure 29). Also, a hardened outlet should be constructed on the pond bank opposite the overflow conduit to establish maximum surface water elevation in the overflow pond (Figure 29). Transplanting of saltgrass rhizomes may be conducted to establish vegetation on the overflow pond banks and the overflow pond outlet area.

Before water is let into the overflow pond, sodium bentonite should be applied to the pond basin to ensure that the basin holds water. Soils at and around South Mound Spring are mapped as Mimbres-Chutum-Ybar complex, 0 to 5 percent slopes (Natural Resources Conservation Service, 2015). This soil unit has an average clay content of 27.3 percent and saturated hydraulic conductivity of 3.54 ì m/sec (Natural Resources Conservation Service, 2015). Based on these data, approximately 1 kg (2 lbs) of sodium bentonite should be applied per 0.1 m^2 (1 ft^2). If the overflow pond is constructed with a surface area of 200 m^2 (2,153 ft^2), approximately 2,000 kg (4,409 lbs) of sodium bentonite would be required. Soil testing should be conducted to determine actual clay content of the soil, which may reduce the amount of sodium bentonite required to seal the overflow pond basin. Sodium bentonite should be applied with a fertilizer spreader or similar device to ensure even distribution, raked into the soil, moistened, and compacted before water is let into the overflow pond basin. Resulting pond volume, assuming a surface area of 200 m^2 (0.05 ac) and a mean depth of 1.2 m (4 ft) would be approximately 240 m^3 (0.2 ac-ft or 65,170 gal).

The overflow pond should be allowed to stabilize and develop for at least one year following completion of restoration before pupfish are translocated. Following stabilization of hydrology and development of an adequate food base, at least 200 pupfish from Malpais Spring should be translocated to the overflow pond (New Mexico Department of Game and Fish, 2006).

Maintenance and monitoring of South Mound Spring following restoration and translocation of pupfish should be conducted. Maintenance should consist of periodically raking the spring pool to remove emergent wetland vegetation and prevent re-establishment of dense stands of cattail or other wetland plants. The overflow pond should also be kept free of dense emergent plant growth by hand removal and raking. Maintenance should also include removal of saltcedar seedlings or sprouts through hand-pulling or other treatment (see section 4.3 below). Genetic maintenance of the South Mound Spring refuge population should consist of moving 25 fish annually from Malpais Spring to the site (New Mexico Department of Game and Fish, 2006).

Monitoring should include installation of a datalogging thermograph (*e.g.* Onset HOBO Water Temperature Pro® v2) at approximately 30 cm (12 in) depth in the overflow pond. A water-level datalogger (*e.g.* Solinst Model 3001LTC Levelogger® Junior) should be installed in the overflow pond. The water-level datalogger should be surveyed to establish elevation relative to a site benchmark. Hourly datalogging intervals would be sufficient for both water level and water temperature. Dataloggers should be downloaded regularly (*e.g.* on a quarterly basis) and data should be analyzed and presented in annual monitoring reports. Monitoring of the translocated population should also be conducted, with monitoring methods (*i.e.* sampling techniques, frequency of sampling) determined by the Conservation Team.

4.1.4 Barrel Spring

Barrel Spring (Figure 26) consists of an excavated spring pool and a man-made, U-shaped, overflow pond immediately north of the spring pool. The north pond, which is fed by the spring pool through drain tiles, is shallow and choked with southern cattail (Sivinski and Tonne, 2011: 89). The site contains mosquitofish (*Gambusia affinis*) and red swamp crayfish (*Procambarus clarkii*), both of which are nonnative species.

Aquatic habitat restoration would be required before Barrel Spring is suitable as a natural refuge site for the Malpais Spring population. Restoration would consist of saltcedar removal and control (see section 4.3 below), filling of the overflow pond, removal of emergent aquatic vegetation from the spring pool, and renovation of the spring pool to remove mosquitofish and crayfish.

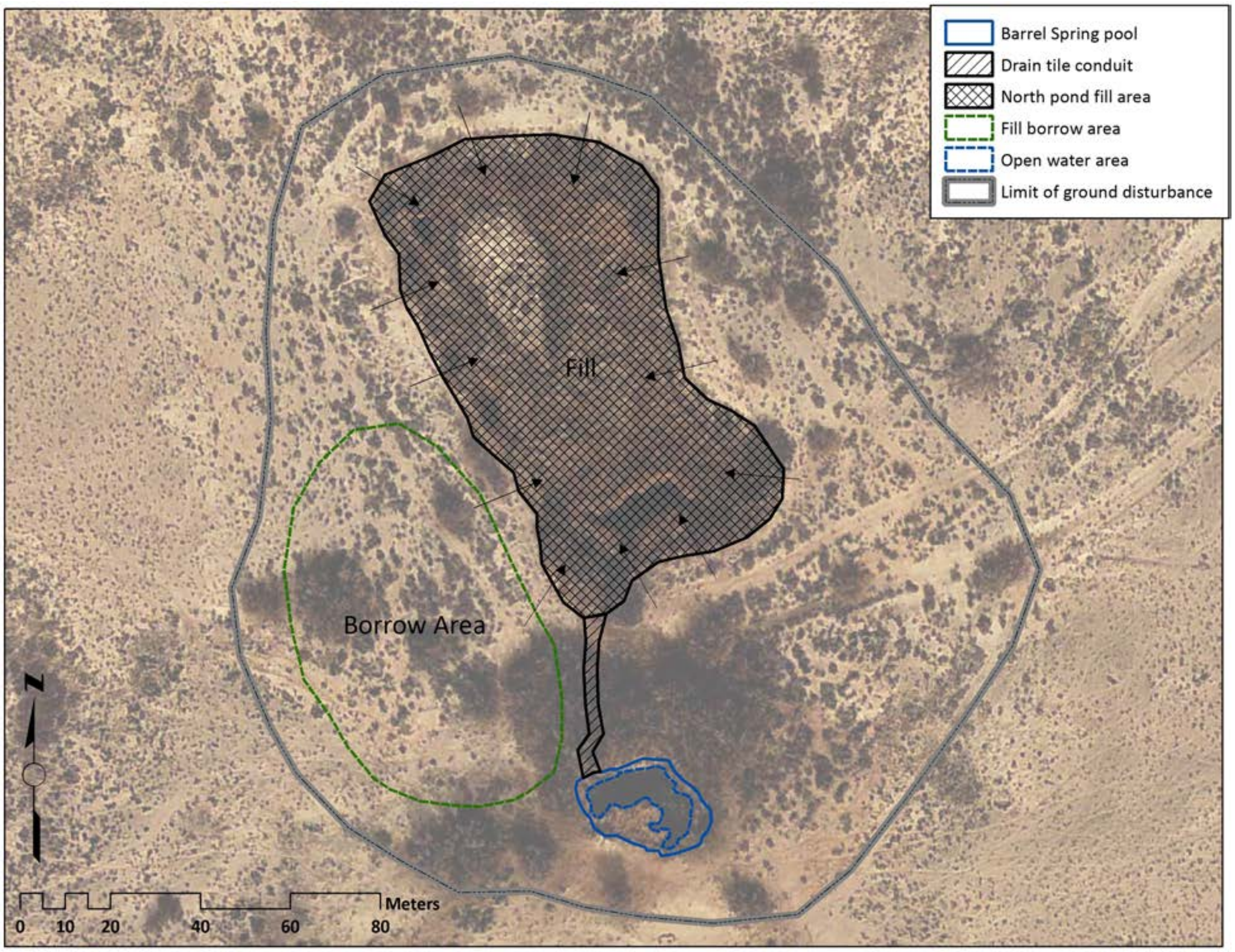
Saltcedar removal should be conducted first to remove the on-site seed source, given that restoration would involve substantial ground disturbance. Saltcedar should be removed from within the ground-disturbance limits by extracting whole plants with a tracked excavator or similar piece of equipment, or by other methods, as described below in section 4.3.

Following removal of saltcedar, the conduit from the spring pool to the overflow pond should be collapsed and compacted, and the overflow pond should be filled with soil. Borrow material should be obtained from the high ground surrounding the pond and an area immediately southwest of the overflow pond (Figure 30). The resulting depression created by excavation of borrow material may then be used as a basin to accept water during pumping of the spring pool for renovation to remove mosquitofish and crayfish, if pumping is conducted to lower water levels during mosquitofish and crayfish control efforts (see below). Soil fill should be compacted in lifts to eliminate subsurface voids. Assuming a surface area of 5,460 m^2 (1.35 ac) and an average fill depth of 1.8 m (6 ft), a total of 9,828 m^3 (12,874 yd^3) would be required to fill the overflow pond area. The filled area and borrow sites should be revegetated with native species.

After the overflow pond is filled with earth, emergent wetland vegetation around the perimeter of the spring pool should be removed by shallow, scraping excavation to a maximum depth of 0.6 m (2 ft). The emergent wetland vegetation removal area is approximately 253.5 m^2 (0.06 ac), which would result in removal of approximately 152.1 m^3 (200 yd^3) of organic sediment and associated plant material. The pool banks should be compacted with the backside of the bucket of a backhoe or small tracked excavator to collapse, as much as possible, crayfish burrows (Hyatt, 2004: 32).

The spring pool should be encircled with a continuous perimeter fence constructing with 61 cm (24 in) high aluminum roll flashing to contain crayfish following completion of saltcedar removal, filling and compaction of the overflow pond, emergent wetland vegetation removal from the spring pool, and compaction of the spring pool banks. The bottom of the perimeter fence should be buried at least 15 cm (6 in) into the ground.

Figure 30. Conceptual diagram of aquatic habitat restoration at Barrel Spring.



Following installation of perimeter fencing and settling of suspended sediments in the spring pool, efforts to eradicate mosquitofish and, hopefully, red swamp crayfish, should commence. If practicable, the spring pool volume should be diminished as much as possible by pumping water out of the spring pool and into the borrow area depression created during backfilling of the overflow pond. Rotenone should then be applied to the spring pool, following the label requirements and EPA-approved standard operating procedures (Finlayson *et al*., 2010), at the maximum allowable concentration of 0.2 ppm active ingredient (*i.e.* 4 ppm of 5-percent active ingredient product, such as CFT Legumine).

The first application of rotenone should be conducted using powder-gelatin-sand mixture, followed by surface application using backpack sprayer with rotenone in liquid form (1 part CFT

Legumine to 10 parts water), both of which should be used to achieve a concentration of 0.2 parts per million active rotenone, which is the maximum allowable concentration pursuant to label specifications. Assuming a spring-pool volume of 407 m^3 (0.33 ac-ft; surface area = 407 m^2 [0.1 ac], mean depth = 1 m [3.3 ft]), 1.67 L (0.44 gal) of 5-percent rotenone liquid or 1.61 kg (3.54 lbs) of 5-percent rotenone powder would be required for a single treatment of the spring pool with a resulting concentration of 0.2 ppm rotenone.

Crayfish likely will attempt to escape the water during rotenone treatments (Hyatt, 2004). Therefore, crayfish should be collected during rotenone treatments using dip nets, being careful not to disturb the substrate of the spring pool, and by hand from the banks. All crayfish collected should then be destroyed. Trapping of crayfish using baited minnow traps (3.17-mm [1/8-in] mesh) should also be conducted between rotenone treatments to reduce the population as much as possible.

Rotenone should be allowed to detoxify naturally, which would take no longer than one month. Efficacy of treatments should be assessed by sampling and/or snorkel survey. If fish and/or crayfish are still present after the first rotenone treatment, subsequent treatments should be made until the spring pool is determined to be fishless and devoid of crayfish. It is expected that numerous treatments would be required, and elimination of crayfish may not be possible. Treatments may be conducted throughout the year.

Following confirmation that nonnative fish and crayfish have been eradicated and the spring pool aquatic invertebrate community has recovered (no sooner than the next spring following completion of renovation treatment), at least 45 pupfish from Malpais Spring would be translocated to Barrel Spring. If crayfish have not been eradicated, the Conservation Team would make a determination on whether or not to stock pupfish into Barrel Spring. In the event that crayfish persist at the site, it may provide an opportunity to experimentally assess the effect of red swamp crayfish on White Sands pupfish.

Maintenance and monitoring of Barrel Spring following successful restoration and translocation of pupfish should be conducted. Maintenance should consist of periodically raking the spring pool to remove emergent wetland vegetation and prevent re-establishment of dense stands of cattail or other wetland plants. Maintenance should also include removal of saltcedar seedlings or sprouts through hand-pulling or other treatment (see section 4.3 below). Genetic maintenance of the Barrel Spring refuge population should consist of moving 25 fish annually from Malpais Spring to the site (New Mexico Department of Game and Fish, 2006).

Monitoring should include installation of a datalogging thermograph (*e.g.* Onset HOBO Water Temperature Pro® v2) at approximately 30 cm (12 in) depth. A water-level datalogger (*e.g.* Solinst Model 3001LTC Levelogger® Junior) should be installed in the spring pool. The water-level datalogger should be surveyed to establish elevation relative to a site benchmark. Hourly datalogging intervals would be sufficient for both water level and water temperature. Dataloggers should be downloaded regularly (*e.g.* on a quarterly basis) and data should be analyzed and presented in annual monitoring reports. Monitoring of the translocated population should also be conducted, with monitoring methods (*i.e.* sampling techniques, frequency of sampling) determined by the Conservation Team.

4.2 Improve Population and Habitat Monitoring

Population monitoring (including a genetics component) should continue, with consideration of possible improvements or adjustments to the monitoring protocol that would improve the spatial distribution of monitoring sites and ability to detect changes in the status of the native populations (see Appendix A). Adjustments to the current sampling design may include increasing spatial coverage of sampling sites, increasing the number of sampling occasions from one to three per survey, and sampling each site every other year instead of every year (see analysis and recommendations in Appendix A).

The Conservation Team should review all available data and deliberate on potential adjustments to come to agreement on what changes should be made and when they should be made. Monitoring of habitat attributes should be enhanced, and operation of stream gages at Salt Creek and Malpais Spring should be continued. Additional monitoring instruments, such as water-level and conductivity dataloggers should be installed at various locations to enable detection of habitat changes and correlation with climatic variables.

4.3 Control Saltcedar

Although the concept of increased stream flow with removal of saltcedar is equivocal, Wilcox and others (2006) indicated that potential for increased water yield is highest in shrubland situations where groundwater is close to the surface. This is the case throughout habitats of White Sands pupfish. Consequently, removal of saltcedar may have beneficial effects such as expanding the length of perennial stream habitat in Salt Creek downstream from Route 316.

4.3.1 Spring Habitats

Saltcedar infestations at spring habitats are characteristically small areas with dense stands (*e.g.* the headspring at Malpais Spring). Saltcedar at spring sites would best be controlled using individual plant cut-stump and foliar treatments (U.S. Forest Service, 2012: 5-6). The optimum time for application of either treatment is during the spring (March through May) using triclopyr (*e.g.* Garlon 3A) and in the fall (late August through October) using imazapyr (*e.g.* Arsenal, Polaris, Habitat; C. Rodden, WSMR, pers. comm., 8 May 2015). Spring habitats where saltcedar control should be implemented include North Mound Spring, Mound Spring, West Mound Spring, Dead Oryx Spring, South Mound Spring, Malpais Spring, Barrel and Guilez springs, and Bradford Spring (Table 6).

Table 6. Saltcedar control areas at spring sites.

Spring Site	Treatment Area Size	
	Hectares	**Acres**
North Mound Spring	0.13	0.32
Mound Spring	1.46	3.61
West Mound Spring	0.03	0.07
Dead Oryx Spring	0.03	0.07
South Mound Spring	1.94	4.79
Malpais Spring - Headspring	0.63	1.56
Malpais Spring - Old Outflow	5.79	14.31
Malpais Spring - Old Ditch	5.83	14.41
Malpais Spring - Marsh	2.25	5.56
Barrel and Guilez Springs	32.29	79.79
Bradford Spring	0.93	2.30
Total	**51.31**	**126.79**

The cut-stump method involves cutting saltcedar as close to the ground and as horizontal to the ground as possible, brushing off residual sawdust, then applying herbicide with a paintbrush, hand-held sprayer, or backpack sprayer. Herbicide should be applied immediately following cutting (*i.e.* within 15 minutes). Recommended herbicide is a solution of imazapyr (*e.g.* Arsenal) mixed with bark or crop oil at a ratio of 33:67 herbicide:oil when sprayers are used and a ratio of 50:50 when a paintbrush is used. A blue indicator dye is recommended to mark treated stumps. Follow-up treatments using foliar application of imazapyr with a backpack sprayer are typically necessary to achieve complete control. Cut-stump treatment may also be the best method of control in the upper reaches of Salt Creek, where saltcedar is patchy, and along the upper and middle reaches of Lost River.

Foliar spray treatments may also be used, particularly for small plants (*i.e.* less than 1.5 m [5 ft] tall) and relatively small areas (U.S. Forest Service, 2012: 6). The foliar spray method involves spraying individual plants with a 1 percent imazapyr solution with a nonionic surfactant (0.25 percent by volume) and a blue indicator spray dye. Spraying is conducted to achieve complete coverage of the foliage, particularly the terminal ends of all branches, to the dripping point. The interior of the plant is also thoroughly sprayed. Spraying should be conducted when wind speed is low, relative humidity is high, and air temperature is low.

4.3.2 Salt Creek and Lost River

Control of the extensive, dense stands of saltcedar along Salt Creek and Lost River would be best achieved by helicopter application of imazapyr (U.S. Forest Service, 2012: 6-7). Treatments should be conducted from late August through October (C. Rodden, WSMR, pers. comm., 8 May 2015), ideally when temperatures are moderate 15.5ºC to 25.7ºC (60ºF to 80ºF), relative humidity is 65 to 90 percent, and wind speeds are 4.8 to 11.3 km/hr (3 to 7 mph; U. S. Forest Service, 2012: 7). Cut-stump treatment, as described for spring sites, may be the best method of control in the upper reaches of Salt Creek, where saltcedar is patchy, and along the upper and middle reaches of Lost River.

Saltcedar control treatments along Salt Creek should be conducted on an experimental and incremental basis. Treatments should begin in the headwaters and then proceed downstream. Monitoring of water quality and the response of White Sands pupfish and Tularosa springsnail should be conducted to ensure that there are no adverse effects of the treatments (see section 4.3.3 below).

Saltcedar control areas along Salt Creek and Lost River were calculated based on a 100-m (328-ft) buffer on each side of the stream channel, except for the Salt Creek headwaters area (Table 7). A large polygon was delineated around the Salt Creek headwaters area where saltcedar is widely distributed, apparently due to past land disturbance. Consequently, the Salt Creek headwaters is the largest saltcedar control area. Salt Creek proper was divided into three sections for the purpose of delineating saltcedar control areas. The upper section of Salt Creek is defined as the reach from Salt Springs downstream to Range Road 316, the middle section is defined as the reach from Range Road 316 downstream to the alkali flat above Range Road 6, and the lower section is defined as the reach from the alkali flat downstream to Big Salt Lake (Table 7).

Lost River was divided into three sections for the purpose of delineating saltcedar control areas (Table 8). The upper section Lost River is divided into an upper section includes Ritas and Malone draws from the HAFB boundary downstream to Range Road 9, the middle section extends from Range Road 9 downstream to the playa causeway, and the lower section extends from the causeway downstream to the HAFB boundary in the gypsum dunes (Table 8).

Table 7. Saltcedar control areas along Salt Creek.

Stream Site	Treatment Area Size	
	Hectares	Acres
Salt Creek - Headwaters	1,274.5	3,149.4
Salt Creek - Upper Section	242.1	598.3
Salt Creek - Middle Section	350.5	866.2
Salt Creek - Lower Section	171.8	424.4
Total	**2,309.0**	**5,038.3**

Table 8. Saltcedar control areas along Lost River.

Stream Site	Treatment Area Size	
	Hectares	Acres
Lost River - Upper Section	281.3	695.0
Lost River - Middle Section	26.3	65.0
Lost River - Lower Section	57.6	142.4
Total	**365.2**	**902.4**

4.3.3 Monitor Saltcedar Control Treatments

Monitoring of the effects of saltcedar control treatments on aquatic habitat and biota should be conducted. Monitoring measures may include water quality, water level or discharge, sensitive taxa (*e.g.* White Sands pupfish, Tularosa springsnail), channel geomorphology, and other parameters that the Conservation Team considers to be important. Baseline conditions should be measured prior to implementation of control treatments.

4.4 Refine Delineation of Aquifer Recharge Zones

Current knowledge indicates habitats are supplied by the basin-fill aquifer, which is recharged primarily, if not wholly, by infiltration at mountain-front alluvial fans. Waltemeyer (2001) did not include several large catchments in the San Andres Mountains that potentially recharge the basin-fill aquifer in the Salt Creek drainage or in catchments north of Oscura that drain the west side of the Sacramento Mountains. These latter catchments drain into the Carrizozo lava flow and may be important with respect to the buried alluvial Malpais Spring aquifer. Investigations should be conducted to refine the delineation and characteristics of recharge zones for springs discharging from the basin-fill aquifer that sustain habitats of White Sands pupfish.

Salt Creek may be more sensitive than Malpais Spring to changing climate and possible reduction of mountain-front recharge because the perennial reach of the stream has a smaller mountain catchment area, and only about one percent of total annual precipitation is estimated to recharge the basin-fill aquifer from these catchments (Huff, 2005: 23).

4.5 Develop an Ecological Restoration and Management Plan for Malpais Spring

As with most desert spring systems in the American Southwest (Minckley *et al.*, 1991;

Unmack and Minckley, 2008), Malpais Spring has been extensively modified by humans. The natural west-flowing spring outflow was altered and flow was directed into ditches to provide water and irrigate forage for livestock. Natural disturbance regimes and vegetation were altered by introduction of saltcedar and grazing by cattle and feral horses. The continued increase in density of emergent wetland vegetation at the site, and ensuing potential negative impacts on White Sands pupfish, indicate that restoration action is warranted (*e.g.* Scoppettone *et al*., 2005; U.S. Fish and Wildlife Service, 2009: F-5 through F-14; Settevendemio, 2014).

An ecological restoration plan is needed for Malpais Spring to clearly identify restoration goals, reference conditions, restoration concepts, and restoration measures to achieve the goals. The restoration plan should be developed using established guidelines for restoration projects (SERI Science and Policy Working Group, 2004; Clewell *et al*., 2005).

The goal of ecological restoration at Malpais Spring should be to restore aquatic habitat for White Sands pupfish to natural structure and function, to the maximum extent possible. In particular, goals of restoration at Malpais Spring should include:

- an aquatic ecosystem with sufficient resiliency to endure normal periodic stress events (such as drought); and
- an aquatic ecosystem that is self-sustaining in that it has the potential to persist indefinitely under existing environmental conditions and evolve in response to changing environmental conditions

The focus of restoration should be on spring geomorphology and wetland vegetation dynamics, as discussed below.

4.5.1 Spring Geomorphology

The natural outflow of Malpais Spring apparently was west-southwest from the headspring (Figure 31). A remnant, sinuous channel is visible on the ground and on aerial imagery leading from the headspring in a southerly direction toward what is now a large playa, then continuing on westward (Figure 31).

Since prehistoric times, Malpais Spring and its associated wetland have been subject to considerable anthropogenic alteration. Human occupation of the shores of the Salina de San Andres (Wessel, 2010) suggest that discharge from Malpais Spring flowed almost due west. By the mid 1820s, this westerly flow was fixed by excavation of a small ditch to convey spring flow to the *salina* for the purpose of salt collection (Wessel, 2010) and a wetland existed west of the spring (Pittenger and Springer, 1999).

By 1946, discharge from Malpais Spring was re-routed via a ditch southward to a livestock range camp. Following establishment of White Sands Missile Range, a new ditch was constructed that conveyed all of the spring discharge due south to a pond called Denver Tank, where water was pumped out for construction activities. This ditch channeled almost all of the spring flow to the tank until 1984, when a plug was placed in the ditch and all spring discharge was directed south into the current wetland area (Turner, 1987). The existing, extensive wetland south of the spring was considered “artificial” by Allen and others (2005), reflecting the human modification of spring discharge patterns.

Figure 31. Malpais Spring, showing the presumed natural, remnant outflow channel. The aerial photo inset shows the headspring area and the man-made ditches that altered the natural spring outflow, and the ground-level inset photo shows the remnant channel. Imagery is from 2012.

Increasing vegetation density and associated accumulation of plant litter and organic sediments has apparently reduced surface-water slope along the existing, man-made flow path. This is evidenced by increased depth of the pool at the end of the man-made channel (Figure 32). The area of shallow inundation north of the pool is increasing, indicating that flow may be shifting north and west. A noticeable reduction in flow velocity in the man-made outflow channel has occurred as a result of the reduced water-surface slope, which has led to an increase in emergent wetland vegetation in the outflow channel (see discussion below of "lodging" in section 4.5.2 Wetland Vegetation Dynamics). Consequently, there appears to be a positive feedback loop leading to increasing emergent wetland vegetation density and concurrent loss of open-water habitat.

Restoration of spring geomorphology should evaluate returning spring outflow to the historic channel (such as has been done at springs on Ash Meadows National Wildlife Refuge), as well as options for effectively maintaining flow and aquatic habitat with existing spring outflow geomorphology. Some of the baseline data required for this evaluation include:

- comprehensive review and synthesis of historic information on spring geomorphology;
- detailed topographic mapping;
- soil mapping and analysis;
- mapping of existing vegetation; and
- assessment of pupfish habitat use.

Reference conditions for restoration of spring geomorphology at Malpais Spring shoud be based on pupfish habitat use and probable natural conditions.

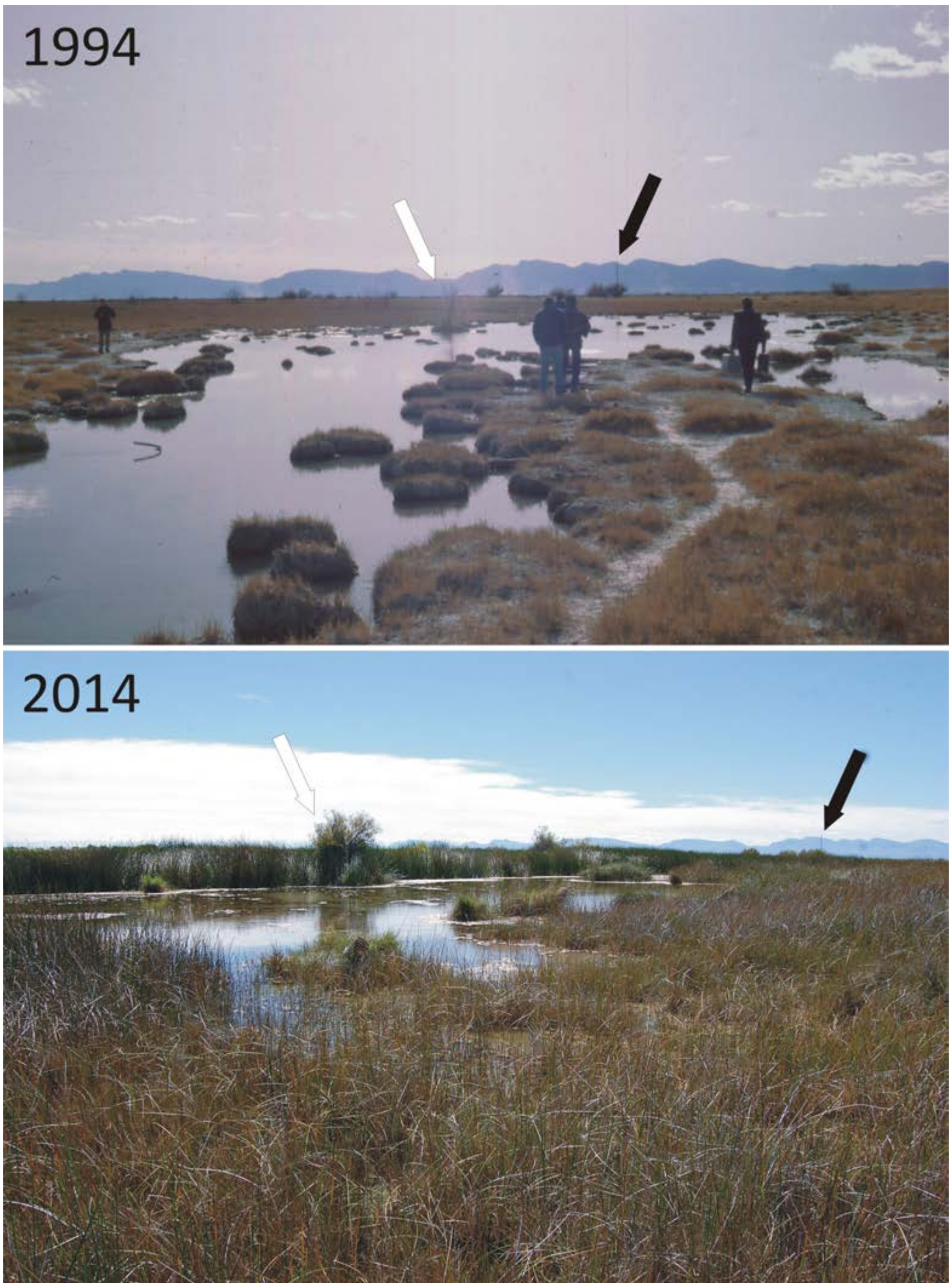
4.5.2 Wetland Vegetation Dynamics

The second major component of ecological restoration at Malpais Spring should address natural processes influencing wetland vegetation dynamics. Emergent wetland vegetation density has increased steadily since removal of feral horses from WSMR, which was completed by 1999, with concomitant loss of open water habitat (see Appendix B). Processes that historically may have influenced wetland vegetation at Malpais Spring include grazing and trailing by large herbivores, fire, and water flow patterns.

The present-day Chihuahuan desert scrub and desert grassland vegetation in the Tularosa basin developed approximately 18,000 years before present (Dick-Peddie, 1993: 16). However, subsequent climatic fluctuations would have influenced the character and extent of aquatic and wetland habitats in the basin. Approximately 12,000 years before present (ybp), the climate became warmer and drier. There was another marked reduction in winter precipitation approximately 8,000 ybp, and another xeric shift in climate approximately 5,000 to 4,000 ybp. The climate then cooled up to approximately 2,500 ybp before temperatures increased up to approximately 800 ybp. Climate conditions have been relatively stable for roughly the last 600 years (Dick-Peddie, 1993: 17). Consequently, *ca.* 1300 to present is a plausible reference-condition time frame for evaluation of natural processes influencing wetland vegetation dynamics.

4.5.2.1 Grazing Kodric-Brown and Brown (2007) documented rapid increases in wetland vegetation following removal of domestic livestock grazing, with ensuing adverse effects of aquatic habitat and native fishes at arid land spring sites.

Figure 32. Pool at the end of the Malpais Spring outflow channel in 1994 and 2014. The black arrow points to the same telephone pole in both photos, and the white arrow points to the same saltcedar in both photos. Views are looking southwest, and direction of flow is from the lower left toward the center in each photo. Photos by J.S. Pittenger.



They contended that domestic livestock grazing essentially simulated natural disturbance regimes associated with native, now extinct, large herbivores. Large herbivores such as mammoth and bison, became extinct *ca.* 13,000 to 12,000 ybp concurrent with the onset of a warmer and drier climate. Evidence of large herbivores, such as bison, in the Tularosa basin in recent time (*i.e.* the last 1,000 years) is lacking in the archaeological record (Gibbs, 2003). Even if they occurred in the Tularosa basin in recent times, there does not appear to be compelling evidence that bison would have influenced wetland vegetation to the same degree as domestic livestock (*e.g.* Kohl *et al*., 2013). Consequently, a strong case for herbivory as a major natural factor in wetland vegetation dynamics under the current climatic regime is difficult to support. Intensive livestock grazing occurred throughout desert grasslands in southern New Mexico beginning *ca*. 150 years ago (Dick-Peddie, 1993: 18).

Livestock ranching commenced in the Tularosa basin in the 1880s (Gibbs, 2003: 3-14), and was discontinued with establishment of White Sands Missile Range. Following abandonment of ranching, a feral horse population burgeoned (Figure 33). This chronology strongly suggests that cattle and feral horse grazing is not simulative of a natural disturbance regime influencing wetland vegetation dynamics at Malpais Spring under current climatic conditions.

4.5.2.2 Fire As with herbivory, there does not appear to be compelling evidence for fire as a major factor influencing emergent wetland vegetation dynamics at Malpais Spring. Prior to widespread degradation of desert grassland vegetation by livestock grazing, fire may have been a relatively common occurrence.

Figure 33. Feral horses at the Malpais Spring marsh in 1995. Photo by J. S. Pittenger.

Fire could have spread into the emergent wetland vegetation of Malpais Spring during dry times. However, fire is unlikely to have created or maintained open-water areas in the Malpais Spring marsh because the likely response of rhizomatous wetland vegetation (such as bulrush) to burning is an increase in density (*e.g.* Young, 1987; Thullen *et al*., 2002; Austin *et al*., 2007; Flores *et al*., 2011). For example, a history of regular winter burning of a wetland along the Little Colorado River resulted in formation of dense, monotypic stands of *Schoenoplectus americanus* that persist at the site years after the cessation of burning (J. Pittenger, *in litt*.).

4.5.2.3 Hydrology Current velocity, wave action, and water depth have major influences on wetland vegetation dynamics. Emergent wetland plant species such as bulrushes are excluded from habitat where critical depth-velocity thresholds are exceeded, through bending and rupturing of the plant stem - a condition referred to as "lodging" (*e.g.* Duan *et al*., 2007). Groeneveld and French (2007) reported a depth-velocity envelope that resulted in exclusion of *Scirpus acutus*, with a critical threshold of **uD/d** = 12.8 where **u** = average velocity acting on a plant stem (m/sec), **D** = local depth of flow (m), and **d** = plant stem diameter at point of attachment (m). Exceeding this threshold prevented encroachment of *S. acutus* in an active channel.

Johnson and others (2013: 33) described a situation of shifting flow patterns in marsh habitat on relatively flat terrain at Ash Meadows National Wildlife Refuge. Emergent wetland vegetation growth and litter accumulation caused changes in patterns of spring flow. A similar situation likely is occurring at the artificial outflow channel of Malpais Spring, where *Schoenoplectus americanus* has steadily increased in density over the last 20 years (Figure 34). It seems likely that increased bulrush density downstream and concurrent litter accumulation is creating a backwater effect, flattening the water slope from the spring to the backwater pool, resulting in lower flow velocity. The lower flow velocity may be the key factor in the increase in bulrush in the outflow channel. Minckley and others (2013) reported recovery of narrow, constrained channels in a *cienega* system following cessation of livestock grazing, suggesting that open-water channel habitat does not require herbivore pressure for its maintenance.

Wave action and deep, soft sediments may also prevent the encroachment of emergent wetland vegetation through lodging (Schutten and Davy, 2000). The persistent open-water habitat at the south end of the Malpais Spring marsh (Laguna Cachorrito; Figure 35), as well as small, persistent open-water pools in the wetland, may be the result of such a mechanism.

Restoration of factors influencing emergent wetland vegetation dynamics should include:

- development of appropriate reference conditions;
- thorough analysis of the factors mentioned above (as well as other factors that may be identified), and the interaction among factors (*e.g.* precipitation, spring discharge, hydrodynamics); and
- development and evaluation of restoration options.

Returning flow to the historic, natural channel may provide hydrologic conditions that could control the distribution of emergent wetland vegetation, both in the channel and in relatively large, lacustrine habitats that are now ephemeral.

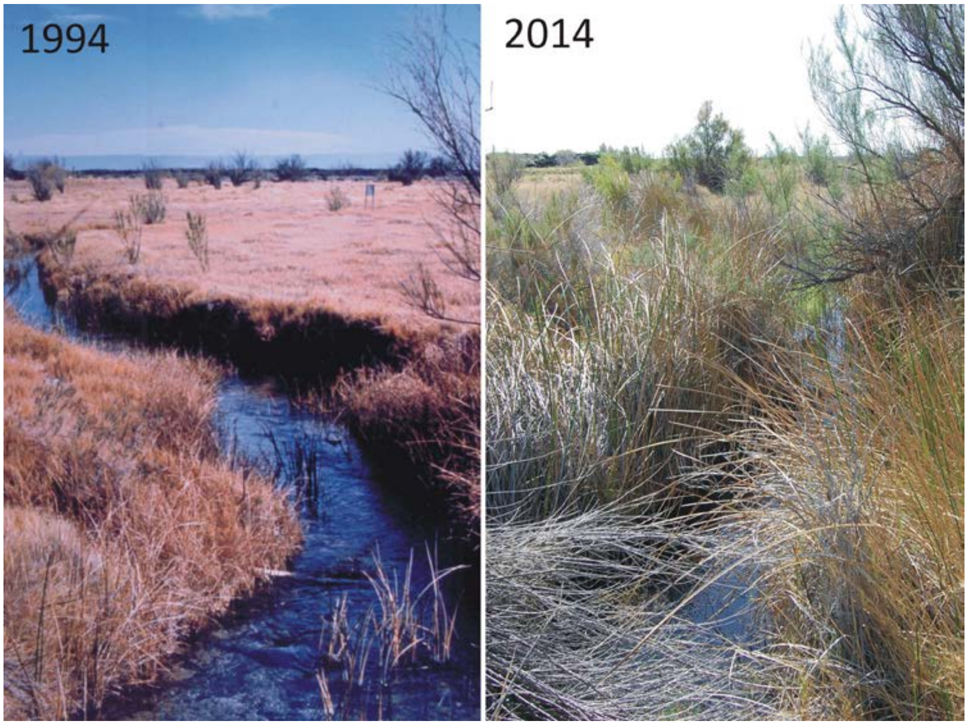


Figure 34. The Malpais Spring outflow channel in 1994 and 2014. Photos by J. S. Pittenger.

Figure 35. Absence of emergent wetland vegetation in Laguna Cachorrito at the south end of the Malpais Spring marsh, April 2014. Photo by J. S. Pittenger.

4.6 Review Installation Activities to Avoid or Reduce Potential Impacts

The Environmental Stewardship Branch at White Sands Missile Range and the Environmental Flight at Holloman Air Force Base review proposed activities for potential to impact natural and cultural resources, including potential effects on White Sands pupfish. Installation activities are carefully regulated in zones designated as essential habitat and limited use areas.

There have not be any substantive impacts to habitat of White Sands pupfish from installation activities since the initial execution of the *Cooperative Agreement for Protection and Maintenance of White Sands Pupfish* in 1995. Activities that may potentially impact pupfish essential habitat or limited use areas include installation and repair of utility lines, management of impact areas and debris fields, recovery efforts, road and infrastructure construction and maintenance, training activities, and management of storm-water runoff.

4.7 Reduce Potential for Land-Based Chemical Spills

The potential for vehicle accidents on Route 9 at the Malpais Spring headspring has been recognized for some time. Additional road-side signs warning vehicle operators of the sharp curve at the headspring were installed in 2014 along the approaches to the spring.

An accident could result in introduction of toxic substances to the spring and cause a fish kill. The potential for accidents may be reduced by milling rumble strips in the road way on either side of the sharp turn at the headspring and by installing signs with flashing lights on either side of the headspring curve.

A program to inform and educate Missile Range users should be formulated and implemented to increase awareness of the need to slow down before entering the sharp turn at the headspring. This program should include transport of military targets back and forth from Oscura Bombing Range and Red Rio by Holloman Air Force Base. Military training activities should be directed to use other routes or security personnel should be posted to keep traffic slow and controlled.

4.8 Conduct Research in Support of Conservation

Relatively little research has been conducted on the basic ecology and life history of White Sands pupfish (see Chapter 2). Research on the following topics would provide useful information for improving understanding of White Sands pupfish and what is needed to conserve the species:

- relationship between catch per unit effort (*C/f*) and absolute abundance (in order for *C/f* to be an accurate index of absolute abundance, there must be a linear relationship between the two variables);
- activity patterns and movements (*e.g.* do pupfish exhibit significant seasonal movements at Malpais Spring);
- habitat use (*e.g.* what habitat features are most selected by pupfish at Malpais Spring and Salt Creek);
- food habits (*e.g.* how does diet vary with habitat conditions at Salt Creek and Malpais Spring);

- breeding biology (*e.g.* annual ovarian cycle and reproductive traits in Malpais Spring and Salt Creek populations);

- demography (*e.g.* factors influencing age-specific survival at Salt Creek and Malpais Spring) ; and

- community ecology (*e.g.* do giant water bugs [Belostomatidae] exert significant predation pressure on White Sands pupfish at Malpais Spring, and does this vary with habitat conditions such as wetland vegetation density).

There are certainly many other research topics that would provide information useful to conservation of White Sands pupfish, in addition to the general topics listed above. The Conservation Team should identify priority research topics and discuss funding sources and research partners.

A useful framework for focusing and prioritizing research could be developed using a Bayesian belief network approach (*e.g.* Ellison, 2004; Marcot *et al*., 2006; Peterson *et al*., 2008; Wade, 2000). This approach could be used to further identify and refine key factors affecting White Sands pupfish (refer back to Figure 23), and thereby guide prioritization of research.

5.0 Implementation Schedule

The table below describes a general schedule for implementation of the conservation actions described in Chapter 4. The first column lists the conservation actions. The second column identified the entities responsible for implementation (WSMR = White Sands Missile Range, HAFB = Holloman Air Force Base, NMGF = New Mexico Department of Game and Fish, USFWS = U.S. Fish and Wildlife Service). The third column shows the priority or importance of the conservation action (*i.e.* 1 indicating the highest priority) and the anticipated year of implementation. Priority is indicated to allow for schedule changes. For example, if the North Mound Spring natural refuge site is not established in 2015, it would still be the highest priority conservation action to implement the following year.

Conservation Action	Responsible Parties	Year of Implementation
1. Establish Malpais Spring Refuge Populations		
1a. North Mound Spring	WSMR, NMGF, USFWS	1 - 2015
1b. Mound Spring	WSMR, NMGF, USFWS	2 - 2017
1c. South Mound Spring	WSMR, NMGF, USFWS	2 - 2016
1d. Barrel Spring	WSMR, NMGF, USFWS	3 - 2018
2. Control Saltcedar and Monitor Treatments		
2a. Spring Sites	WSMR, NMGF, USFWS, HAFB	1 - 2015-20
2b. Salt Creek and Lost River	WSMR, NMGF, USFWS, HAFB	2 - 2017-22
3. Improve Population and Habitat Monitoring	WSMR, NMGF, USFWS, HAFB	1 - 2015-16
4. Develop Ecological Restoration Plan for Malpais Spring	WSMR, NMGF, USFWS, HAFB	2 - 2016-18
5. Refine Delineation of Aquifer Recharge Zones	WSMR	3 - 2017
6. Review Installation Activities	WSMR, HAFB	Ongoing
7. Reduce Potential for Land-Based Chemical Spills	WSMR	3 - 2015-20
8. Conduct Research in Support of Conservation	WSMR, NMGF, USFWS, HAFB	Ongoing

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Appendix A. Assessment of the Current Monitoring Program

A.1 Introduction

Ecological monitoring is a purpose-driven endeavor, and therefore requires definition of specific objectives. Prior to revision of the White Sands pupfish monitoring plan in 2008, there were no objectives for monitoring. Consequently, annual monitoring data were reported without any reference to thresholds that would trigger concern about the status of the species. The revised monitoring plan established objectives for monitoring for the first time (Blue Earth Ecological Consultants, Inc., 2009). The objectives included short-term thresholds evaluated through paired *t*-test and longer term objectives evaluated through regression analysis.

In retrospect, the short-term objectives did not provide meaningful insight into population status because of the "noise" or variability inherent in the system associated with annual climatic variation and habitat conditions, as well as variation associated with sampling. The longer term (*i.e.* three or more years) regression analysis was more informative of trends in abundance at monitoring sites. The revised monitoring plan specified a monitoring objective of the ability to detect a decline in abundance of 50 percent or more, with a significance level of 0.1 and power of 0.8, lasting three years or more, at a specific monitoring site.

Peer review of the draft conservation plan brought to light several important shortcomings regarding the analysis of trends in abundance described in the 2009 monitoring plan and the issue of catch per unit effort as an index of abundance. Accurate monitoring data are critical, as they provide the objective basis for determining the status of the species. Therefore, these two components of the monitoring program (power analysis of trend data and catch per unit effort as an index of abundance) were evaluated to determine the strengths and weaknesses of the current design, and to provide recommendations for improving monitoring to provide better information for assessing the status of the species.

The monitoring data were re-analyzed in a more appropriate manner, as described below, to assess the statistical power of the current program to achieve the 2009 plan's trend detection objective (*i.e.* detect a 50 percent decline in abundance, as indicated by catch-per-unit effort, over a three-year period with a significance level of 0.1 and power of 0.8). Finally, the use of catch per unit effort was evaluated by examining the validity of assumptions for using it as an index of absolute abundance.

A.2 Analysis of ***C***/***f*** Trend

The first step in the analysis was to sum annual catch per unit effort (***C/f***) data from all traps at a site as opposed to calculating mean catch (Freund, 1971), and subjecting these data to linear regression analysis. The group of traps at a given site were treated collectively as a single sample, analogous to total catch in an electrofishing sample of a stream segment (*e.g.* Dauwalter *et al.*, 2009). Combining the catch from all traps at a site for each sampling visit had the benefit of eliminating the effect of between-trap variance, and combination also avoided potential violation of the sample unit independence assumption associated with analysis based on individual trap catches.

The 2009 monitoring plan focused first on a paired-sample design developed to detect significant differences from year ***x*** to year ***x*+1** (Blue Earth Ecological Consultants, Inc., 2009). However, graphical analysis of mean ***C*/*f*** trend revealed substantial variation between consecutive years that was typically unrelated to longer-term patterns. Therefore, the emphasis here is redirected to trends in total ***C*/*f*** over a period of three or more years at each site. This is arguably a much better indicator of what is happening to the species at a particular site than is a paired-sample test of mean ***C*/*f*** from consecutive years.

The second step was to test the summed (*i.e.* total) ***C*/*f*** data for normality using the Shapiro-Wilk test with á= 0.05 (*i.e.* p>0.05 means the data are normally distributed; Zar, 2010: 95). In cases where the ***C*/*f*** data were not normally distributed a square-root transformation (Zar, 2010: 357) was applied and the transformed data were re-tested for normality.

Total ***C*/*f*** was regressed against time for two periods: 1) the entire monitoring data set from 1995 through 2013; and 2) the period covered by the revised monitoring plan, 2008 to 2013. Figures A1 through A4 show the results for the four monitoring sites: the Salt Springs and Range Road (RR) 316 sites on Salt Creek and the Upper Marsh and Middle Marsh sites at Malpais Spring.

The final step in the analysis was to explore variation in ***C*/*f*** with respect to environmental factors using correlation analysis and available data to determine if any patterns emerged. This was conducted to find out if monitoring was potentially tracking changes in ***C*/*f*** associated with environmental variation.

A.2.1 Salt Creek

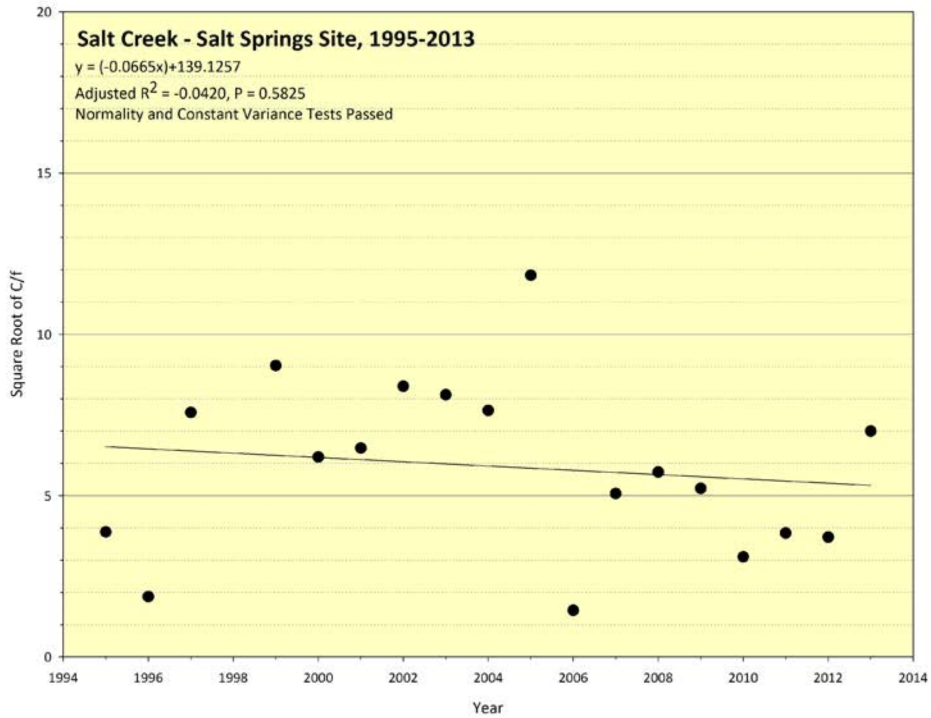
The total ***C*/*f*** monitoring data showed no trend, either declining or increasing, at the Salt Springs site over the entire monitoring period (1995-2013) or over the revised sampling design period (2008-2013; Figure A1). Both the 1995-2013 and the 2008-2013 square-root transformed data sets passed the normality and constant variance tests.

The square root transformed total ***C*/*f*** data varied considerably from year to year in the 1995-2013 regression analysis. In contrast, total ***C*/*f*** had less annual variation following implementation of the revised sampling design in 2008 (Figure A1).

At the Salt Springs site, total ***C*/*f*** was significantly correlated with several flow indices calculated from the stream gage data, and with growing and heating degree days (J. Pittenger, *in litt.*). Consequently, the monitoring data appeared to be tracking changes in abundance of pupfish that were associated with fluctuations in environmental conditions.

Similar to the Salt Springs site, the monitoring data from the Range Road 316 site did not show any significant trend for either data set (Figure A2). Both the 1995-2013 and the 2008-2013 square-root transformed data sets passed the normality and constant variance tests.

The pattern of total ***C*/*f*** in the 2008-2013 data set, which included zero ***C*/*f*** in 2011 due to complete stream drying at the site, tracked the 2008-2011 pattern in number of zero-flow days measured at the stream gage on Salt Creek (U.S. Geological Survey, 2014), which is located at the upstream end of the site. Correlation analysis found total ***C*/*f*** to be significantly correlated with the number of zero-flow days and with mean monthly flow (J. Pittenger, *in litt.*).



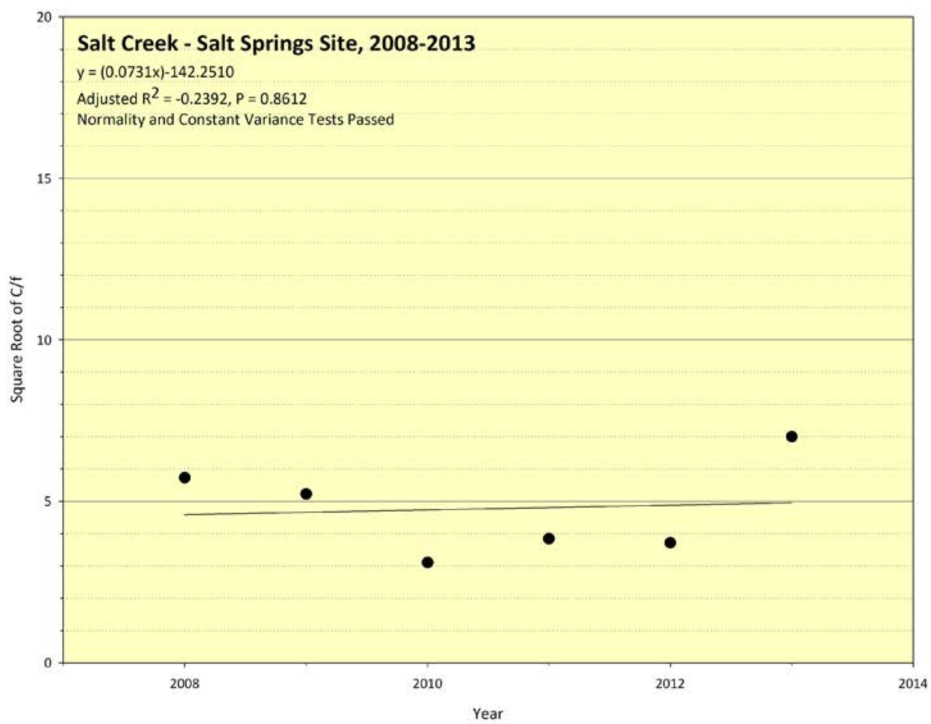


Figure A1. Regression analysis of total ***C/f*** at the Salt Springs site, 1995-2013 (top graph) and 2008-2013 (lower graph).

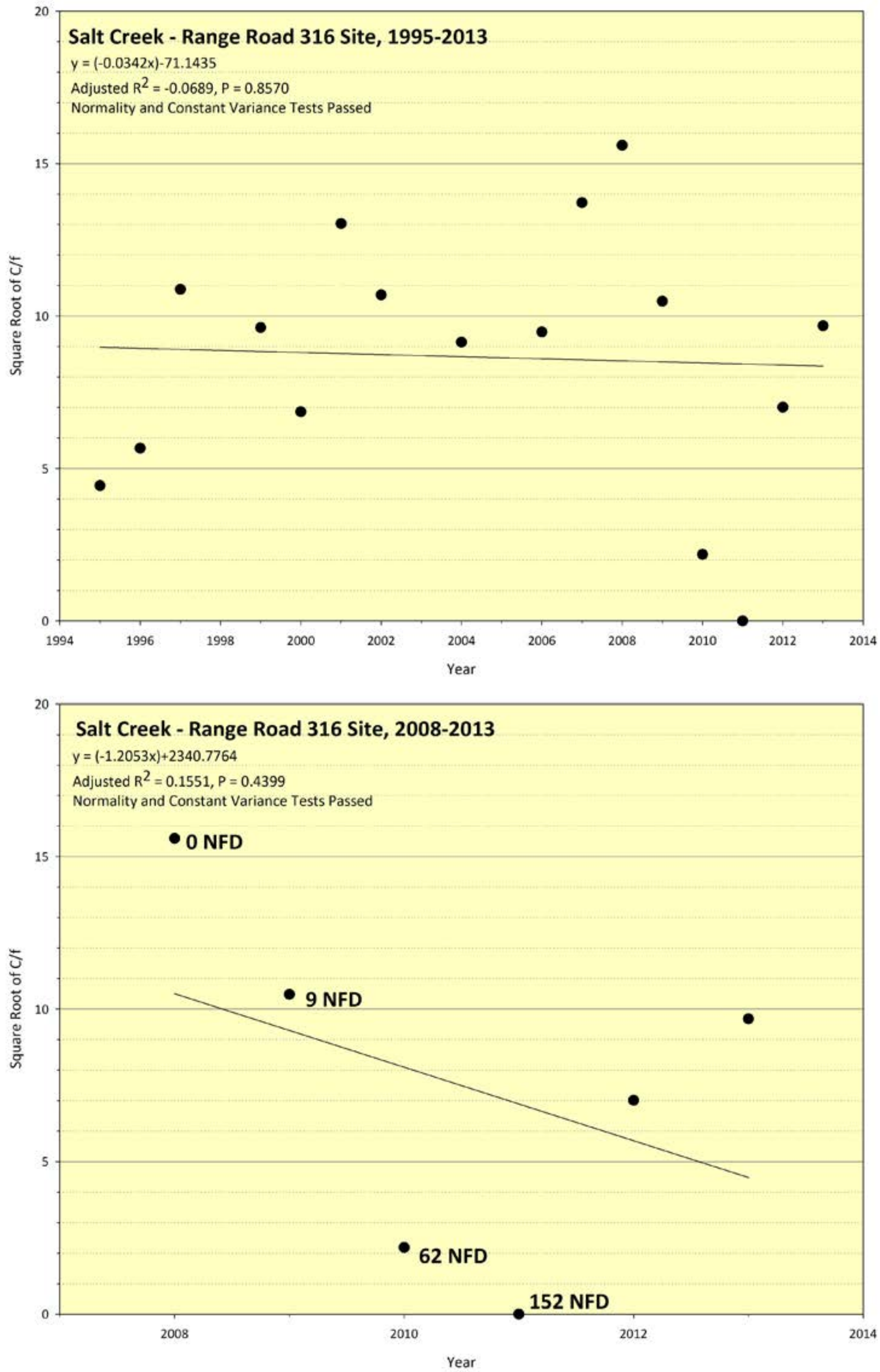


Figure A2. Regression analysis of total ***C*/*f*** at the RR 316 site, 1995-2013 (top graph) and 2008-2013 (lower graph).

The 2008-2013 regression (lower graph) also shows the number of no-flow days (NFD) for 2008 through 2011, from the U.S. Geological Survey stream discharge gage on Salt Creek near RR 316. Operation of the stream gage stopped in 2012.

A.2.2 Malpais Spring

The Upper Marsh site at Malpais Spring showed a fairly strong "V" shaped pattern in total ***C/f*** from 1995-2013 (Figure A3), but there was no significant, consistent trend of either increasing or decreasing total ***C/f*** over that time period. The 2008-2013 regression showed a marginally significant (p=0.06) increasing trend in total ***C/f*** (Figure A3). Both the 1995-2013 and the 2008-2013 square-root transformed data sets passed the normality and constant variance tests.

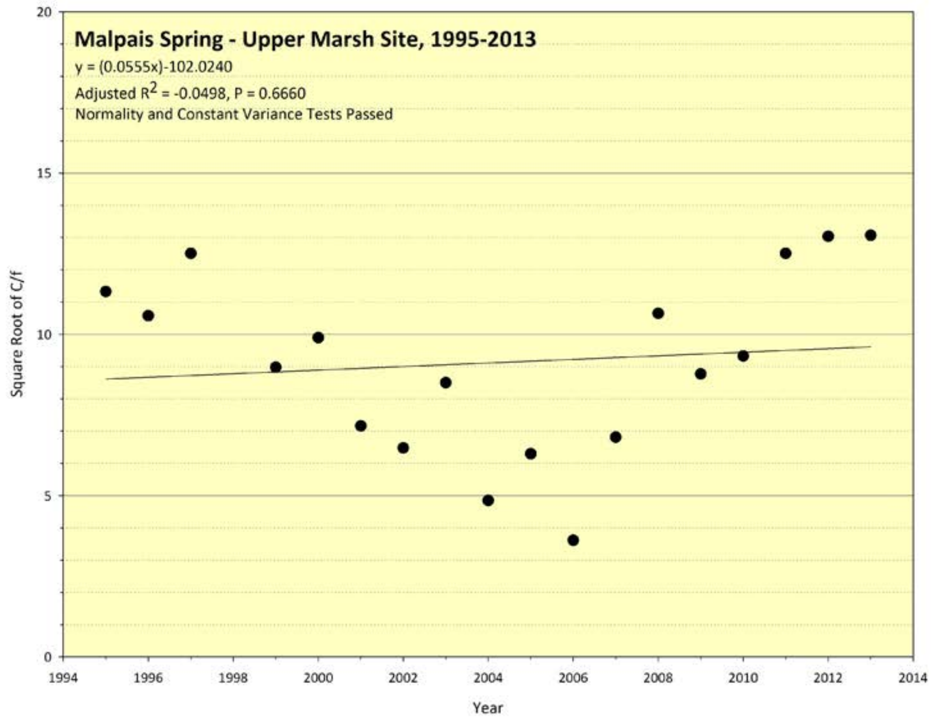
Correlation analysis did not reveal any strong associations between variation in total ***C/f*** and hydrologic or climatic variables at the Upper Marsh site. However, there was a weak negative correlation between total ***C/f*** and total annual precipitation (r=0.5, P=0.12; J. Pittenger, *in litt.*). One possible explanation for this relationship is that available habitat expanded during years of high precipitation (see section 2.6.1.3) and pupfish dispersed into the larger habitat area, resulting in lower pupfish density and, consequently, lower total ***C/f***. The strong "V" shaped pattern in total ***C/f*** also suggested that there may have been an interaction between vegetation change following removal of feral horses, which was completed in 1999, and precipitation.

The normal distribution and constant variance assumptions were not met for the 1995-2013 Middle Marsh site data set (Figure A4, upper graph). Testing of the regression hypotheses (*e.g.* slope is not significantly different from 0) requires that these assumptions be met for the tests to be valid. Violation of the constant variance assumption (homoscedasticity) results in an increased probability of a Type I error, which is rejection of the null hypothesis when in fact it is true (Zar, 2010: 337). Consequently, the conclusion of a significant declining trend at the Middle Marsh site in the 1995-2013 data set is not warranted at this time, particularly given the lack of a statistically significant declining trend in the 2008-2013 data set.

Middle Marsh total ***C/f*** was strongly correlated with several flow indices calculated from the gage data. In particular, total ***C/f*** was negatively correlated with maximum flow indices. As described above for the Upper Marsh site, this may suggest that the monitoring data tracked changes in pupfish density associated with expansion and contraction of available habitat resulting from variation in spring discharge and precipitation.

A.3 Power Analysis

Gibbs and Eden (2010) pointed out that the primary problem in trend detection is that sources of "noise" in measures obscure the "signal" associated with ongoing trends in the resource being monitored. The probability that a monitoring program will detect a trend in sample measurements when the trend is occurring, despite the "noise" in the data, is measured by statistical power. Objectives of the current monitoring program are to be able to detect a 50-percent decline in catch per un it effort over a three-year period, with a significance level of 0.10 and power of 0.80 (Blue Earth Ecological Consultants, Inc., 2009).



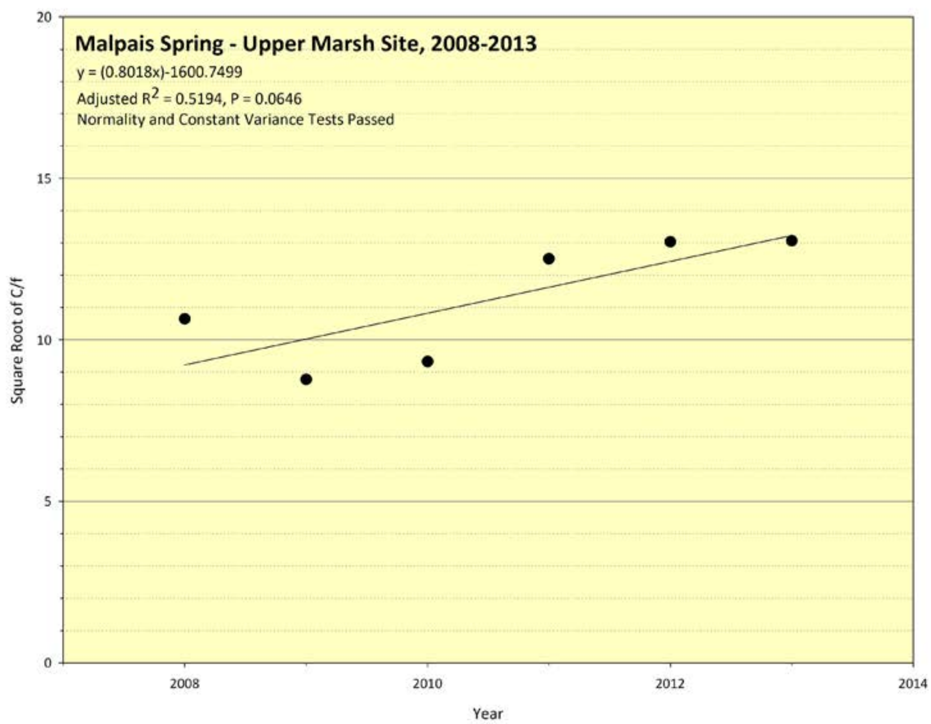
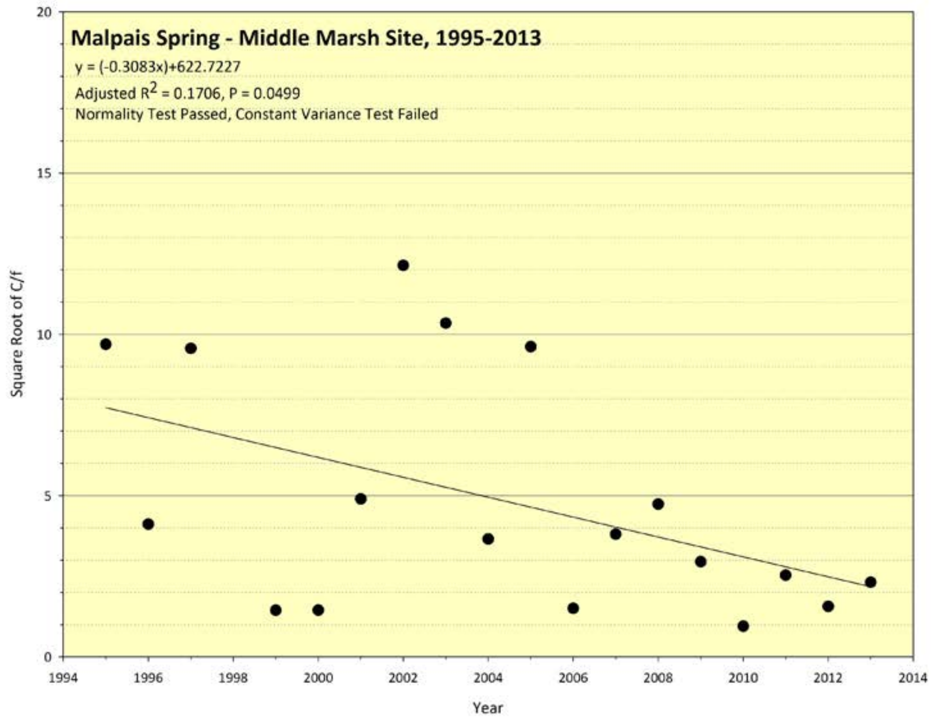


Figure A3. Regression analysis of total ***C/f*** at the Upper Marsh site, 1995-2013 (top graph) and 2008-2013 (lower graph).



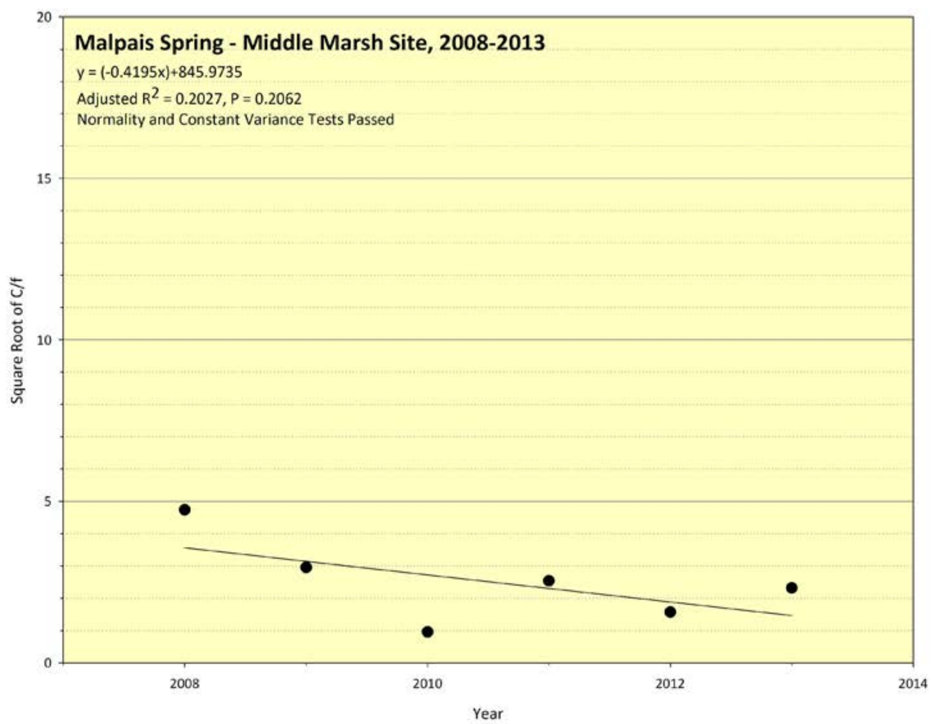


Figure A4. Regression analysis of total ***C*/*f*** at the Middle Marsh site, 1995-2013 (top graph) and 2008-2013 (lower graph).

A.3.1 Power Analysis of Linear Regressions to Assess Trends in Relative Abundance

Statistical power is defined as 1-ß, where ß is the probability of making a Type II error. A Type II error is failure to reject the null hypothesis when in fact it is false (*i.e.* concluding that no change has occurred when in fact it has).

The power of the existing monitoring program to detect a 50-percent annual change in total ***C*/*f*** was analyzed using the program TRENDS (Gerrodette, 1993), similar to procedures described by Nur and others (1999: 21-23). Four program inputs were used to solve for the fifth parameter, statistical power. The four program inputs were:

- number of samples (= number of years, ranging from three to five);
- rate of change per time step (*i.e.* year), defined as proportional increase or decline (set at 0.50 per year, based on the monitoring objective of detecting a 50-percent decline);
- measure of variation (defined as the root mean square error of the residual term in the regression analyses of 1995-2013 and 2008-2013 data sets, following Gibbs *et al*., 1998); and
- significance level = 0.10 (based on the monitoring objective).

The rate of change sign was set as negative, to be consistent with the monitoring objective of detecting a declining trend, and was expressed per time period (*i.e.* per year). A one-tailed significance test was specified to accord with a test for a declining trend. The type of change was set as linear, and coefficient of variation (CV) change was set as proportional to the inverse of the square root of abundance (Gerrodette, 1987). Equal intervals were used with no effort multiplier (*i.e.* sampling once a year every year).

The TRENDS power analysis indicated that over a three-year period, only the Upper Marsh 2008-2013 data set met the minimum power threshold of 0.8 specified in the monitoring plan to detect an annual decline of 50 percent in relative abundance (Table A1). Power of the linear regression analysis of a three-year period in all of the other data sets ranged from 0.29 to 0.50 (Table A1). The weakest regression analysis was at the Middle Marsh site, due to the relatively high variation in total ***C*/*f*** in both the 1995-2013 and 2008-2013 data sets.

The current monitoring program, represented by the 2008-2013 data sets at the four sites, met the 0.8 power threshold for detecting a 50-percent annual decline over a period of at least five years (Table A1). The longer-term data set (1995-2013) also met the monitoring objective of at least 0.8 power to detect an annual decline of 50 percent in relative abundance with $p \leq 0.10$ (Table A1).

Table A1. Power of the existing monitoring data to detect a 50-percent decline in total ***C/f*** at the four monitoring sites over time periods ranging from three to five years. The analysis was conducted using the program TRENDS (Gerrodette, 1993). Cells with bold text and green highlight indicate data sets that met the 0.8 minimum power threshold with $p \leq 0.10$.

Site	Monitoring Data Set	Power of Detecting a 50-percent Change Over Specified No. of Years		
		3 yrs	4 yrs	5 yrs
Salt Creek Salt Springs	1995-2013	0.37	0.75	**0.97**
	2008-2013	0.47	**0.90**	**1.00**
Salt Creek RR 316	1995-2013	0.42	**0.83**	**1.00**
	2008-2013	0.32	0.63	**0.91**
Malpais Spring Upper Marsh	1995-2013	0.50	**0.93**	**1.00**
	2008-2013	**0.92**	**1.00**	**1.00**
Malpais Spring Middle Marsh	1995-2013	0.29	0.55	**0.83**
	2008-2013	0.37	0.75	**0.97**

A.3.2 Statistical Power of Alternative Sampling Scenarios

The existing monitoring data sets were used to run simulations of various sampling scenarios, including the current program, to evaluate statistical power to detect a range of trends in relative abundance up to the monitoring objective of a 50 percent change. Simulations were run using the program MONITOR (Gibbs and Ene, 2010), similar to assessment of other monitoring programs (*e.g.* Galimberti, 2002). The program MONITOR uses a Monte Carlo procedure to generate many simulated sets of monitoring data based on a user-defined sampling program, and then evaluates how often that program detects trends of varying strength.

The program requires specification of the number of plots, which in the case of the White Sands pupfish monitoring program is one (*i.e.* one sample per site per year) and the start value and total variation. Two starting value-total variation sets were used in simulations for each site: the mean ***C/f*** and associated variance for the 1995-2013 period and the mean ***C/f*** and associated variance for the 2008-2013 period (the current monitoring program). In order to remove variation due to trends in abundance (Dauwalter *et al.*, 2009: 39), the root mean square error of the residual in the regression analysis was used as the variance term when computing standard deviation (Thomas and Krebs, 1997; Gibbs *et al.*, 1998; Nur *et al.*, 1999).

Parameters for the simulations were as follows.

- Intervals, which represent years, were varied to include annual sampling over three- and four-year periods and biennial sampling over a five-year period.
- The design type was specified as simple regression with log-normal deviates and total (unpartitioned) variance.
- Surveys per plot per interval was varied from 1 (a single sampling event at each site during each survey year) to 3 (three samples per sampling event during each survey year).
- Deterministic/regression type trend was specified with number of trends = 11, minimum trend = -50 percent, and maximum trend = 50 percent.
- Variance to mean relationship was modeled as constant standard deviation.
- Simulation parameters were significance = 0.10, desired power level = 0.80, number of iterations = 10,000, number of tails = two, rounding, and truncation of counts < 0.

Simulations consisting of 10,000 iterations were run for each site using start values and variance estimates from the 1995-2013 and 2008-2013 data sets. Five scenarios were analyzed for each of these two start value-variance conditions. These scenarios were defined as follows.

A The current monitoring program, which consists of one sample per year, with trend analyzed over a three-year period. This scenario is indicated by the notation **1/1/3** (sample every year/one survey per year/trend analyzed over a three-year period).

B The current monitoring program analyzed over a four-year period, indicated by the notation **1/1/4**.

C Modification of the current program to include three consecutive surveys per year, sampling every year, with trend analyzed over a three-year period. This scenario is indicated by the notation **1/3/3**.

D Sampling a site every other year with one survey per sample year and trend analyzed over a five year period (*i.e.* sample years 0, 2, and 4). This scenario is indicated by the notation **2/1/3**.

E Sampling every other year with three consecutive surveys per sampling year and trend analyzed over a five-year period (*i.e.* sample years 0,2, and 4). This scenario is indicated by the notation **2/3/3**.

<u>A.3.2.1 Salt Springs Site Power Curves</u> The five scenarios (**A** through **D**) run using the 1995-2013 mean ***C*/*f*** and associated variance (calculated as the root mean square error) are shown in Figure A5.

The black line in Figure A5 is the power curve for the current monitoring program (scenario **A**), which shows that a 50 percent annual decline in relative abundance cannot be detected with power ≥ 0.8 over a three-year period. This is consistent with the results of the regression power analysis discussed in section A.2.2. Increasing the trend-detection period to four years (scenario **B**, the red line in Figure A5) enables detection of a decline of 21 percent or larger with power ≥0.8. Increasing the number of surveys per year from one to three (scenario **C**, the green line in Figure A5), increases the detectability in relative abundance trend over a three-year period to a 14 percent or larger decline with power ≥ 0.8. Power under this scenario is near 1 for declines of 20 percent or larger over a three-year period.

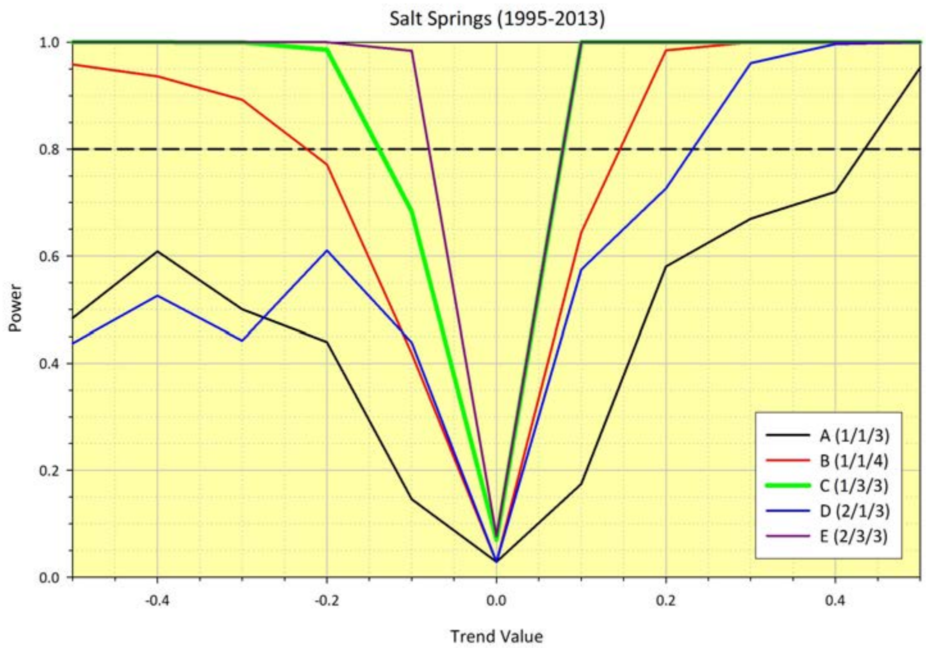


Figure A5. Power curves for the Salt Springs site, 1995-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

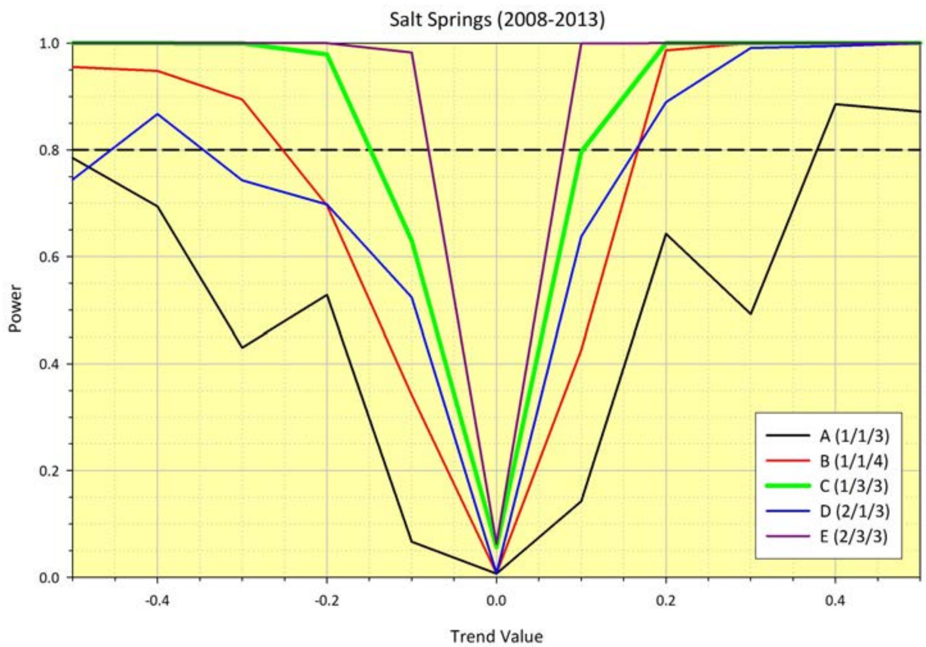


Figure A6. Power curves for the Salt Springs site, 2008-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

Changing to a biennial sampling design with a single survey per sample year (scenario **D**, the blue line in Figure A5) results in a power curve only slightly improved over the current design. However, if the biennial sampling program is modified to include three surveys per sampling year (scenario **E**, the purple line in Figure A5), power is increased considerably. This design provides the best performance (and most symmetrical power curve) of the five scenarios, with a relative abundance decline as low as seven percent over a three-year period being detected with power = 0.8.

The power curves developed using the 2008-2013 data set (*i.e.* the current monitoring program; Figure A6) show a similar pattern as the 1995-2013 data set curves. Power of the current monitoring program to detect declines in relative abundance over a three-year period is marginally better than the 1995-2013 data set, but still fails to meet the 50-percent decline threshold with a power ≥ 0.8 (scenario **A**, the black line in Figure A6). Biennial sampling with one survey per sample year (scenario **D**, the blue line in Figure A6) also has slightly higher power that the 1995-2013 data set, but it shows an abrupt drop in power at the 50-percent decline threshold. The other three scenarios show very similar power curves to those generated using the 1995-2013 data set.

A.3.2.2 RR 316 Site Power Curves Power curves for the five sampling scenarios generated using the 1995-2013 data set from the RR 316 site indicate that a 50-percent annual decline can be detected with power = 0.8095 over a three-year period (scenario **A**, the black line in Figure A7). Increasing the time-frame for trend analysis to four years (scenario **B**, the red line in Figure A7) resulted in dramatically increased power to detect smaller-magnitude trends. For example, a 10-percent annual decline (*i.e.* a net change of -27 percent over the four-year period) could be detected with power = 0.8214. Altering the design to three surveys per year with annual sampling increased change-detection power even more (scenario **C**, the green line in Figure A7). Under scenario **C**, at 10-percent annual decline (*i.e.* a net change of -19 percent over a three-year period) could be detected with power = 0.9835. The biennial sampling approach with one survey per sample year (scenario **D**, the blue line in Figure A7) provides inferior trend detection, but the biennial approach with three surveys per sample year (scenario **E**, the purple line in Figure A7) provides the greatest change-detection power of all of the scenarios. Under scenario **E**, a 10-percent annual decline (*i.e.* a net change of -34 percent over a five-year period) could be detected with power = 1.0.

Power curves generated for the five scenarios using the 2008-2013 data set (*i.e.* the current monitoring program) showed a reduced change-detection power compared to the 1995-2013 data set curves, particularly for scenarios **A** and **D** (Figure A8). The reduction in power was associated with higher variance in the 2008-2013 data set resulting from zero ***C/f*** in 2011, which was caused by stream drying at the site. The same general patterns of the power curves for the five scenarios were observed compared to the curves generated from the 1995-2013 data set.

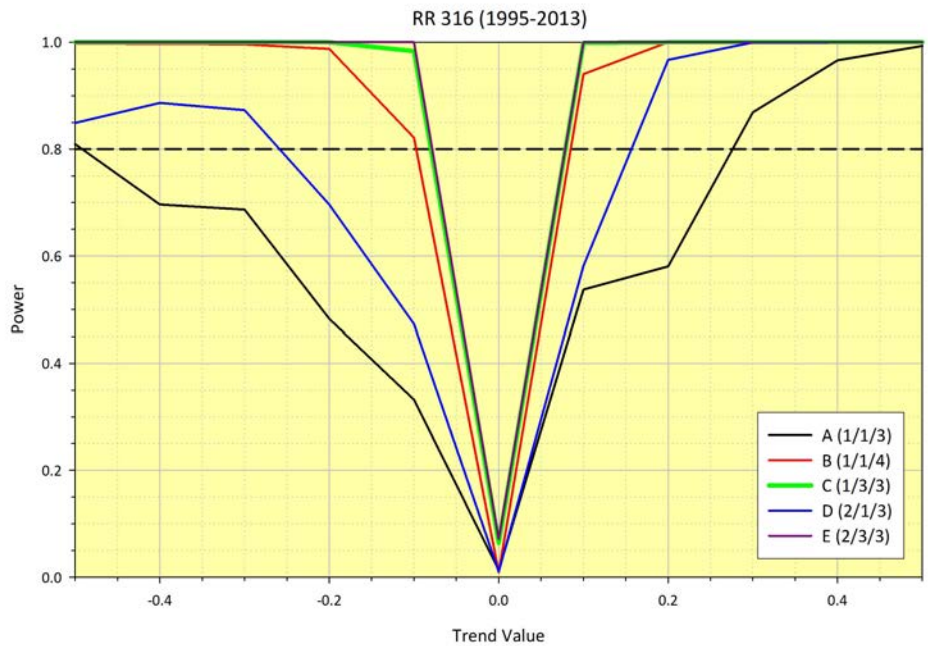


Figure A7. Power curves for the RR 316 site, 1995-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

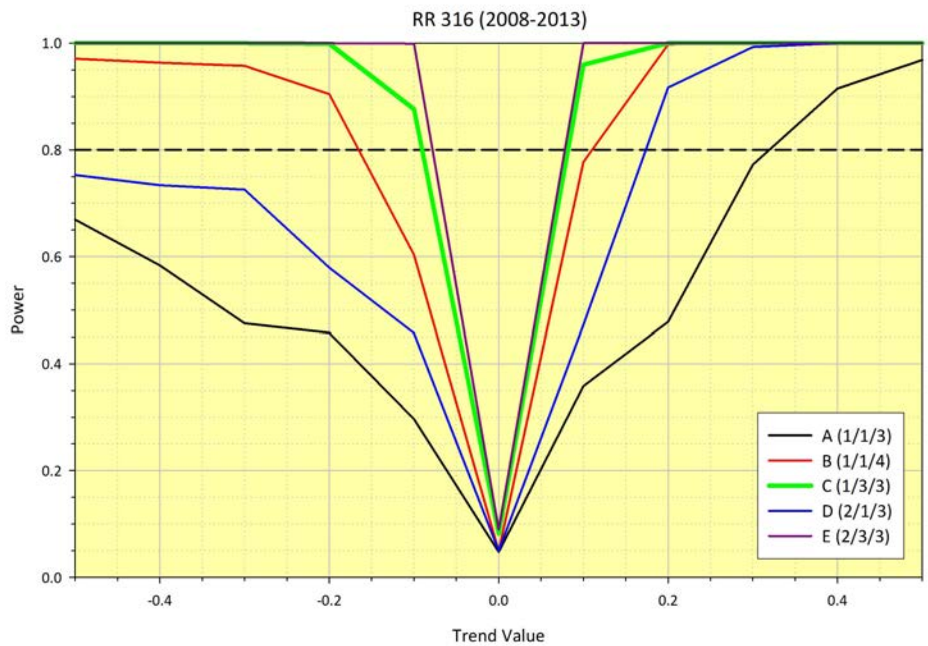


Figure A8. Power curves for the RR 316 site, 2008-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

A.3.2.3 Upper Marsh Site Power Curves All five scenarios generated using the 1995-2013 data set met the monitoring objective of detecting a 50-percent decline over a three-year period with power ≥ 0.8 and $p < 0.1$ (Figure A9). Consistent with the Salt Creek sites, the Upper Marsh site had lowest power with a single survey every year over a three-year period (scenario **A**, the black line in Figure A9), and power improved with a four-year trend analysis, increasing the number of surveys per sampling occasion, and switching to a biennial sampling program (Figure 9).

The current monitoring program showed basically identical power curves for all scenarios (Figure A10), which resulted from the low variance associated with revision of the monitoring program in 2008. A 10-percent annual decline could be detected with power near 1.0 with all scenarios, and only scenario **A** showed a slight dip in power in the 30-percent decline trend range. However, power was well above 0.8 for all scenarios at annual declines of about eight percent or greater (Figure A10).

A.3.2.4 Middle Marsh Site Power Curves Using the 1995-2013 data set, the objective of detecting a 50-percent decline over a three-year period was only met by the two scenarios that included multiple surveys per sampling occasion (scenario **C**, the green line, and scenario **E**, the purple line in Figure A11). Due to the high variance in the 1995-2013 data set for this site, none of the other three scenarios with single surveys per sampling occasion met the admittedly very large decline defined by the monitoring objective. The 2008-2013 data set produced similar curves, albeit with slightly lower power (Figure A12).

A.3.2.5 Alternative Sampling Scenarios - Conclusion Of the scenarios tested, the two with repeat surveys during each sampling occasion, whether on an annual (scenario **C**) or biennial (scenario **E**) basis, provide the greatest power to detect trends. Scenario **C** has the advantage of detecting relatively small declines over a short time period (net change of -19 percent over three years) compared to scenario **E** (net change of -34 percent over five years). However, scenario **E** would have a lower total cost because the same sampling effort would be spread over a five-year period, as opposed to a three-year period for scenario **C**. Scenarios with a single survey per sampling occasion are expected to detect changes smaller than -50 percent only when the analysis period is extended to at least four years.

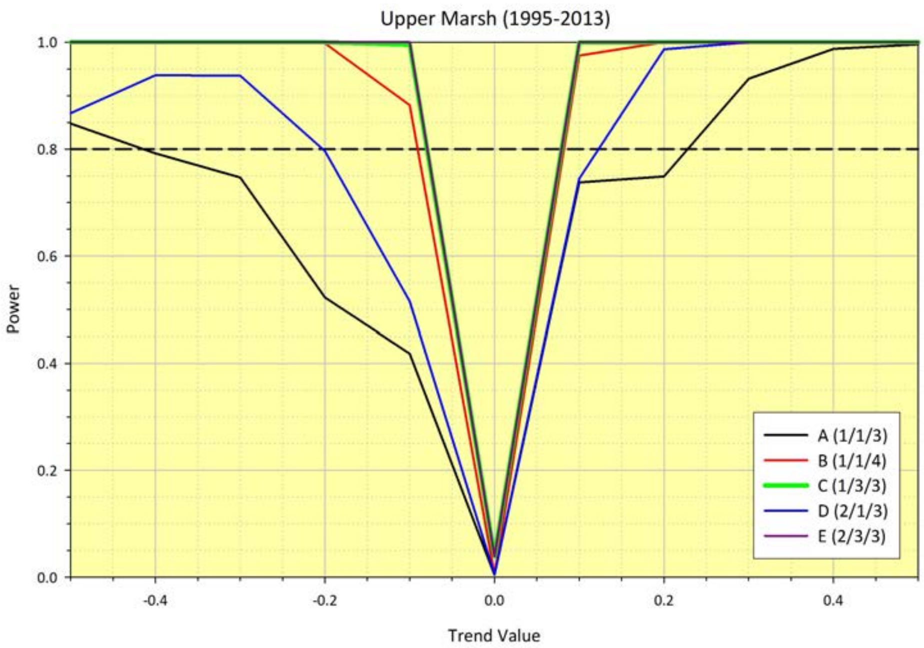


Figure A9. Power curves for the Upper Marsh site, 1995-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

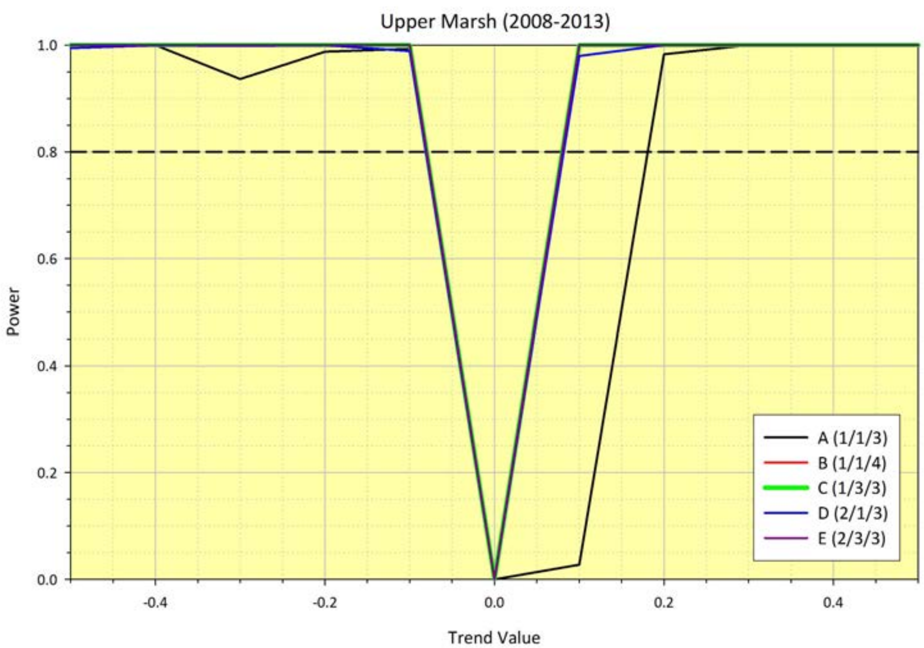


Figure A10. Power curves for the Upper Marsh site, 2008-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

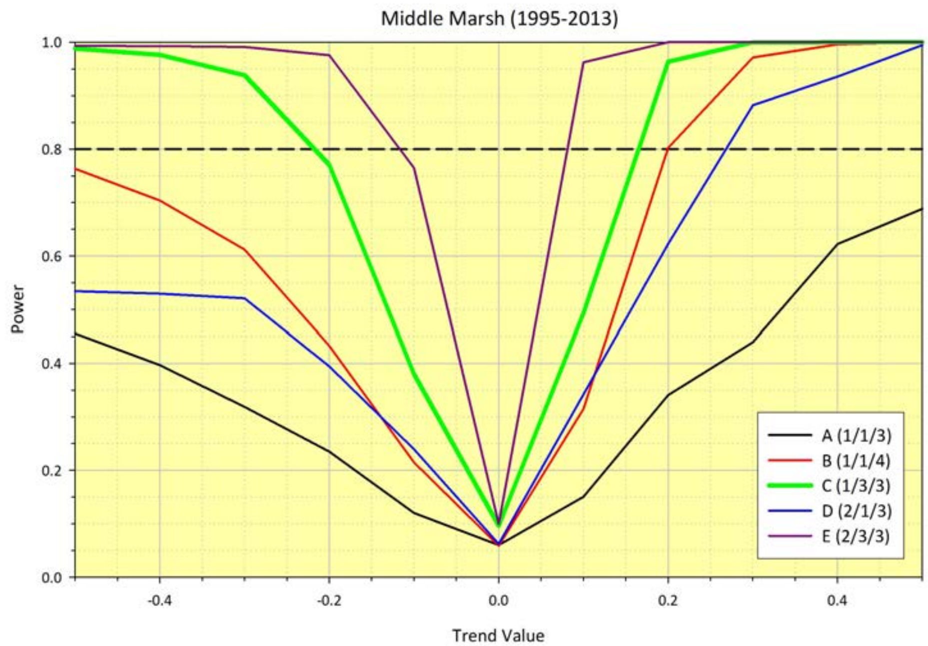


Figure A11. Power curves for the Middle Marsh site, 1995-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

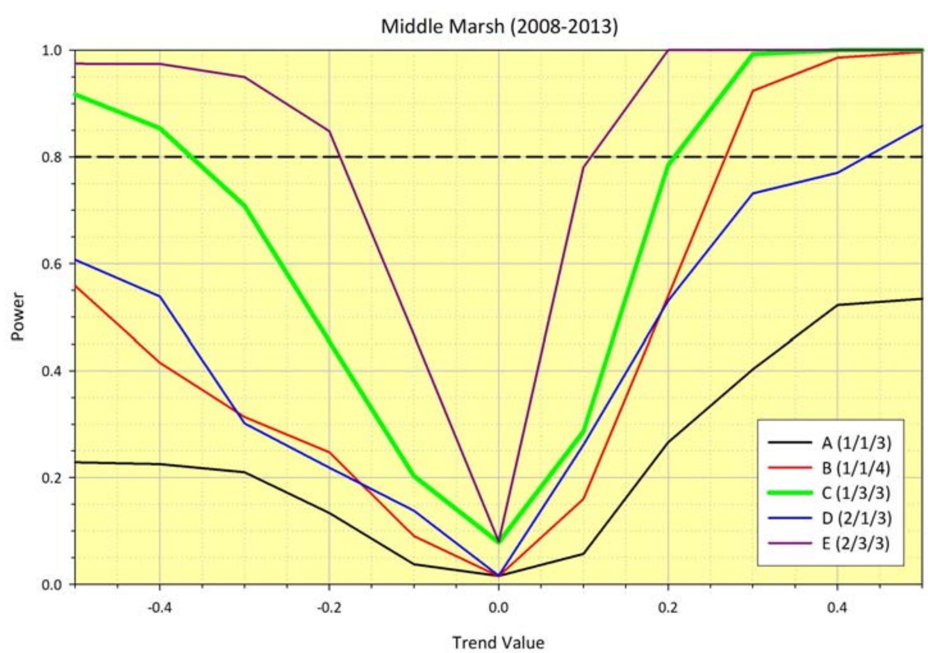


Figure A12. Power curves for the Middle Marsh site, 2008-2013. Annual trend value is shown on the X-axis and represents proportional change over the modeled period (3 or 4 years). For example, trend value = -0.2 is a 20-percent annual decline. The dashed horizontal line is the 0.8 power monitoring objective. See text for legend notation.

A.4 Catch Per Unit Effort as an Indicator of Relative Abundance

Population monitoring is defined as repeated measurement over time of an indicator, or state variable, that is informative of the status of the population (Noon, 2003). Of all the steps in design of a monitoring program, selection of the indicator, or state variable, to measure is the most important (Noon and McKelvey, 2006).

Numerical (*i.e.* absolute) abundance of fish may be estimated by mark-recapture, removal, or enumeration by direct observation of all fishes within an isolated habitat (Hayes *et al*., 2007:327). However, in many cases determination of absolute abundance is not feasible because sampling requirements can be onerous and very expensive, or it may be logistically impossible. In such cases, relative abundance may serve as an appropriate index of true abundance as long as vulnerability to the sampling method remains constant over time. Because methods to determine absolute abundance are not feasible to implement in the native habitats of White Sands pupfish, relative abundance was selected as an index of absolute abundance.

In fisheries science, the most common indices of relative abundance are determined from catch per unit effort data (Hubert and Fabrizio, 2007: 279). Catch per unit effort is defined mathematically as $\boldsymbol{C/f = qN}$, where $\boldsymbol{C}$ is the number of fish captured, $\boldsymbol{f}$ is the unit of effort expended, $\boldsymbol{q}$ is the probability of capturing a fish in one unit of effort (*i.e.* catchability), and $\boldsymbol{N}$ is the absolute abundance of fish.

Applications of $\boldsymbol{C/f}$ data include monitoring of abundance over time, evaluation of spatial distribution patterns, and comparative assessment of populations (Hubert and Fabrizio, 2007: 280-281).

A.4.1 Assumptions

Catch per unit effort must have a linear relationship with absolute abundance for it to be an accurate index. This means that as absolute abundance declines, the number of fish captured in one unit of effort declines. The proportional relationship between catch and abundance is represented by the equation $\boldsymbol{C = fq(N/A)}$; where $\boldsymbol{C}$ is catch, $\boldsymbol{f}$ is effort, $\boldsymbol{q}$ is catchability, $\boldsymbol{N}$ is absolute abundance, and $\boldsymbol{A}$ is the area in which the population occurs (Hubert and Fabrizio, 2007: 282). This equation can be rearranged into catch per unit effort as $\boldsymbol{C/f = q(N/A)}$. So, if $\boldsymbol{q}$ (catchability) is known, $\boldsymbol{C/f}$ is a measure of fish density ($\boldsymbol{N/A}$). Two important assumptions inherent in this model are constant catchability ($\boldsymbol{q}$) and constant area ($\boldsymbol{A}$).

<u>A.4.1.1 Constant Catchability Assumption</u> As Hubert and Fabrizio (2007: 282) note, the constancy of $\boldsymbol{q}$ determines how well $\boldsymbol{C/f}$ serves as an index of abundance. However, catchability varies with changes in spatial distribution of fish, and $\boldsymbol{C/f}$ may just as often indicate changes in fish distribution as it indicates changes in abundance (Hubert and Fabrizio, 2007: 283). Furthermore, distribution and abundance may change concurrently, in which case the effects are confounded in $\boldsymbol{C/f}$ measures. Therefore, $\boldsymbol{C/f}$ data must be interpreted as representing only the portion of habitat that is actually sampled. This consideration highlights the importance of adequate spatial coverage by sampling units to support conclusions that the data are representative of an entire population. Because variation in catchability decreases the accuracy of $\boldsymbol{C/f}$ as an index of abundance approaches such as stratification on factors influencing catchability,

adjustment of catchability to account for changes in capture probability, or independent estimation of catchability under various conditions should be used (Hubert and Fabrizio, 2007: 284).

A.4.1.2 Constant Area Assumption It is assumed that if abundance changes, density will also change and ***C*/*f*** will remain proportional to absolute abundance (Hubert and Fabrizio, 2007: 284). However, there are cases in which this assumption may not be met, as follows.

1) Both ***N*** and ***A*** increase, as when fish increase their spatial distribution into non-sampled areas as abundance increases, In this case, ***C*/*f*** may show no change when absolute abundance is actually increasing. Here, density is not increasing with abundance because the spatial distribution of fish is expanding.

2) ***C*/*f*** shows hyperdepletion with respect to absolute abundance. In this case, ***C*/*f*** decreases faster than absolute abundance because the most vulnerable animals are captured first.

3) ***C*/*f*** shows hyperstability. This is a case where capture of fish is efficient, effort is concentrated in areas of high density, and fish remain concentrated in these areas as absolute abundance declines (*i.e.* hyperaggregation).

A.4.2 Validity of Catchability and Constant Area Assumptions

Catchability of White Sands pupfish in minnow traps may vary with density of emergent aquatic vegetation, water depth, substrate, or other features. For example, catch rates in traps at Malpais Spring appear to be strongly influenced by vegetation cover. Variation in catchability may be reduced by altering placement of traps in locations with similar habitat conditions at a given site. The constant area assumption is best addressed by providing adequate spatial distribution of monitoring sites, which is lacking in the current sampling design.

A.4.3 Trap Saturation and Soak Time

A final consideration in assessment of the current monitoring program is the issue of trap saturation and soak time. Saturation occurs when existing catch reduces the potential for additional captures through reduction of new captures and increased escapement (Hubert and Fabrizio, 2007: 287). As soak time increases, traps may become saturated and catch per unit time will decrease. In this situation, ***C*/*f*** is not an accurate index of abundance.

Like other species of pupfish, White Sands pupfish is likely active primarily during the day and at warmer water temperatures (see section 2.5). Consequently, overnight trap sets may dilute ***C*/*f*** and limit its accuracy as an index of abundance. Daytime sets of six to eight hours may be more appropriate (*e.g.* set at 09:00, retrieve at 15:00). The relationship between soak time and catch should be investigated.

A.5 Recommendations

As discussed above, some problems with the current monitoring program include:

1) relatively low statistical power to detect small to moderate declines in relative abundance, particularly at the RR 316 and Middle Marsh sites (which limits the ability to assess status);

2) poor spatial distribution of monitoring sites (which limits the ability to make inferences about the total occupied habitat areas of Salt Creek and Malpais Spring); and

3) potential variation in catchability due to varying habitat characteristics as well as prolonged soak times and gear saturation (which limit the accuracy of ***C***/***f*** as an index of abundance).

Increasing spatial coverage or spread of monitoring sites would require increasing the number of sites, which would increase the amount of personnel and time that would have to be devoted to monitoring.

Switching to a biennial sampling plan, with one panel of sites sampled in year ***x*** and the second panel sampled in year ***x*+1** could offset increased resource requirements. For example, the number of sites could be doubled to four each at Salt Creek and Malpais Spring with no increase in required personnel and time. However, this scenario (denoted as scenario **D**) has relatively low power to detect trends in ***C***/***f***. At least three surveys per sampling occasion (scenario **E**) are required to increase the power of biennial sampling to acceptable levels.

Increasing the number of surveys per sampling occasion has the added benefit of allowing for estimation of detection and occupancy probability, which would provide information to address both spatial variation and detectability (*i.e.* catchability).

A biennial plan that has three surveys per sampling occasion (scenario **E**) provides acceptable power to detect change at all sites. This design, coupled with shorter (*e.g.* six-hour) diurnal trap sets may reduce the personnel time requirement per site enough to considerably increase the number and spatial coverage of monitoring sites. Ideally, both Salt Creek and Malpais Spring would each have at least six monitoring sites.

The location of existing and new monitoring sites may be adjusted to account for variation in habitat characteristics, with the aim of reducing variation in ***C***/***f*** and thereby increasing power to detect trends. Key habitat variables to consider would likely include emergent wetland vegetation density, water depth, and substrate.

Finally, the use of biomass rather than number of fish in ***C***/***f*** calculation should be investigated. Besides being quicker to measure in the field, it may provide a better index of population status than count data (Hayes *et al*., 2007: 357).

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Appendix B. Assessment of Vegetation Change

B.1 Malpais Spring

Anecdotal information and observations by field workers has indicated that wetland vegetation density has increased substantially at Malpais Spring since removal of feral horses was completed in 1999. In an attempt to objectively assess the magnitude of wetland vegetation change, the difference in areal extent of open-water habitat at Malpais Spring from 1985 to 2012 was evaluated using aerial imagery.

The 1985 imagery consisted of aerial photos with a resolution of 1-m^2 per pixel. In contrast, the 2012 imagery had a much finer resolution, 0.23 m^2/pixel. For analysis in ArcGIS (version 10.2.2 for Desktop; ESRI, Inc., 2014), both the 1985 and 2012 imagery were drawn as an RGB composite image with a standard deviation stretch (n = 2.5). The 1985 imagery was inverted to improve the distinction between vegetated areas and putative open-water areas. The Feature Analyst extension (version 5.1.2.1; Overwatch Systems Ltd., 2014) to ArcGIS was then used to extract and delineate putative open-water areas from the imagery. Because the 1985 imagery analysis could not be field tested, the delineation of open-water areas should be viewed cautiously as a rough estimate.

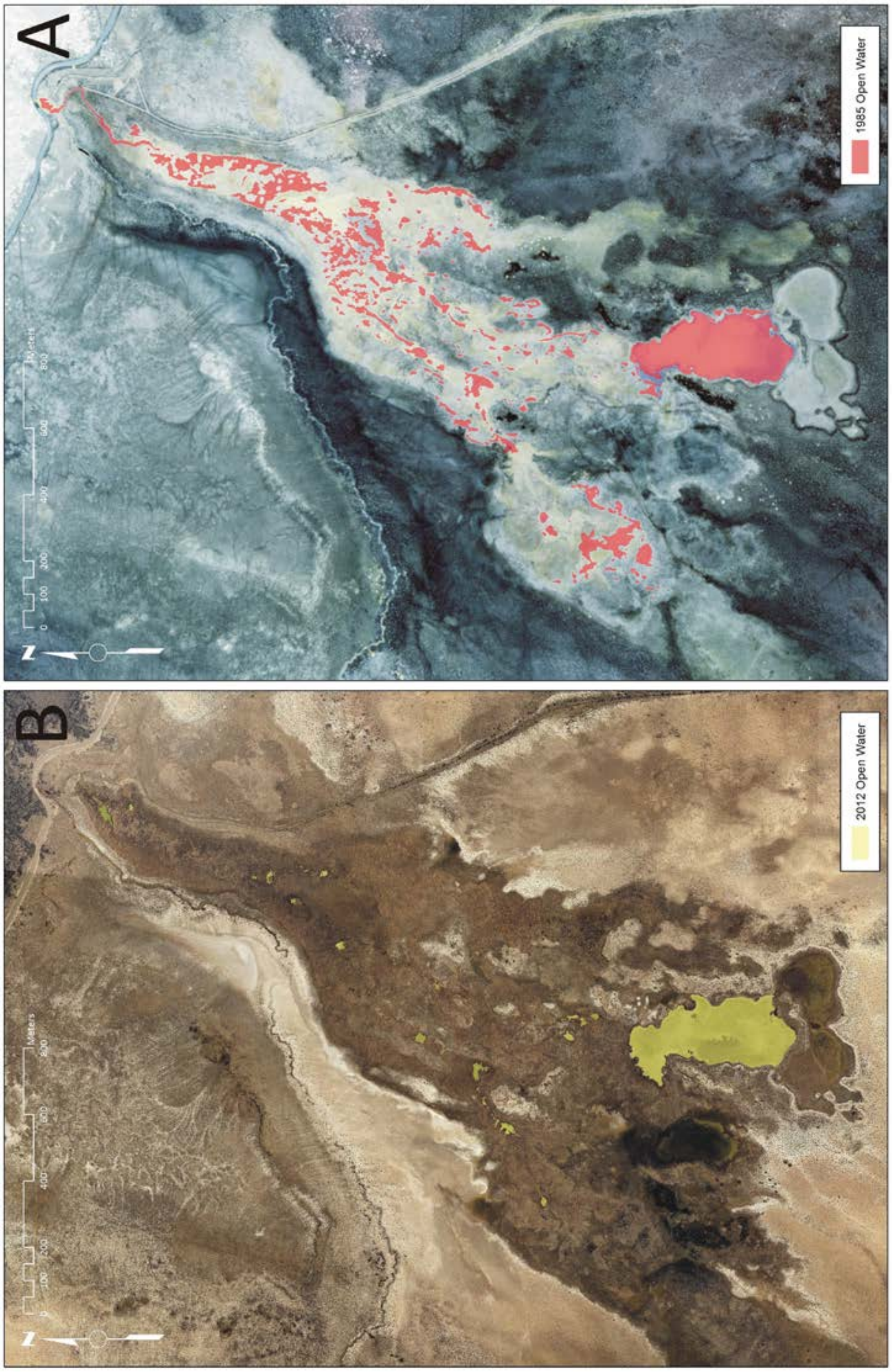
Results of the analysis of the 1985 imagery ("A" in Figure B1) were 32.3 acres of putative open-water habitat comprising 226 patches, with a mean patch size of 0.14 ac (s.d. = 0.88). The Feature Analyst delineation of Laguna Cachorrito, a clearly distinguishable, large, open-water habitat at the south end of the Malpais Spring wetland, was evaluated and found to represent the extent of that open-water body relatively accurately. Therefore, delineation of open-water habitats on the 1985 imagery was considered to be reasonably representative of the extent of open water in the wetland at that time.

Analysis of the 2012 imagery using Feature Analyst resulted in a delineation of 15.2 acres of open-water habitat ("B" in Figure B1) comprising 34 patches with a mean size of 0.45 ac (s.d. = 2.30). Again, the Feature Analyst delineation of Laguna Cachorrito was evaluated and found to be quite accurate, as was the delineation of isolated open-water pools that were mapped using GPS in the field and used as a *post hoc* test.

If it is assumed that the areal extent of Laguna Cachorrito did not change appreciably from 1985 to 2012, the difference in the size of that water body between the 1985 and 2012 delineations can be used as a measure of error in the 1985 imagery. The 1985 delineation of Laguna Cachorrito was 12.6 ac, compared to 13.7 ac in 2012, which gives a presumptive error in the 1985 open-water delineation of about eight percent. So it could be said that the open-water area at Malpais Spring in 1985 may have ranged from about 29.7 to 34.9 ac.

The imagery analysis suggests that open-water habitat at Malpais Spring declined by approximately 53 percent over the 27-year period from 1985 to 2012. Considering a typical wetland extent of 160 ac at Malpais Spring (see section 2.6.1.3), open-water habitat presumably made up about 20 percent of the wetland in 1985, compared to only about 9.5 percent in 2012.

Figure B1. Delineation of open-water habitat at Malpais Spring in 1985 and 2012.



The differences in open-water habitat from 1985 to 2012 varied with location in the wetland. The differences were lowest in the southern portion of the wetland and increased nearer to the headspring.

- The difference in delineated open-water areas from 1985 to 2012 was least at Laguna Cachorrito (Figure B2). Here, there appeared to be basically no change in the area of open water from 1985 to 2012.

- The wetland area immediately north of Laguna Cachorrito showed a modest change in open-water habitat from 1985 to 2012 (Figure B3). There are a number of open-water habitats in this area that apparently have been persistent over the 27-year period from 1985 to 2012, although some of these areas appear to have contracted over that time.

- Open-water habitats in the middle marsh area at Malpais Spring appear to have declined markedly from 1985 to 2012 (Figure B4). However, there are quite a few narrow, open-channel habitats that are present in the middle marsh that were not picked up in the 2012 delineation.

- Differences in open-water habitats in the upper marsh from 1985 to 2012 are also substantial, particularly below the spring outflow channel (Figure B5), where water begins to fan out and create a broad wetland area. However, the large pool that currently exists near the end of the discrete spring outflow channel appears to have expanded from 1985 to 2012.

Removal of feral horses was completed in 1999, so it is assumed that vegetation changes indicated by the analysis occurred over the last 13 years, rather than over the 27-year period spanned by the two sets of imagery. Caven (2014) re-sampled five Land Condition Trend Analysis (LCTA) plots at the Malpais Spring wetland that were sampled in 1999 or 2004. Increases in perennial plant cover were observed at three of the five plots, with the largest change being from 61 to 84 percent cover by perennial plant species. Additionally, Caven (2014) reported a shift in species composition from plant communities dominated by saltgrass (*Distichlis spicata*) to communities dominated by beaked spikerush (*Eleocharis rostellata*) at one of the plots.

Figure B2. Delineation of open-water habitat at Laguna Cachorrito in 1985 and 2012. Base is the 2012 imagery.

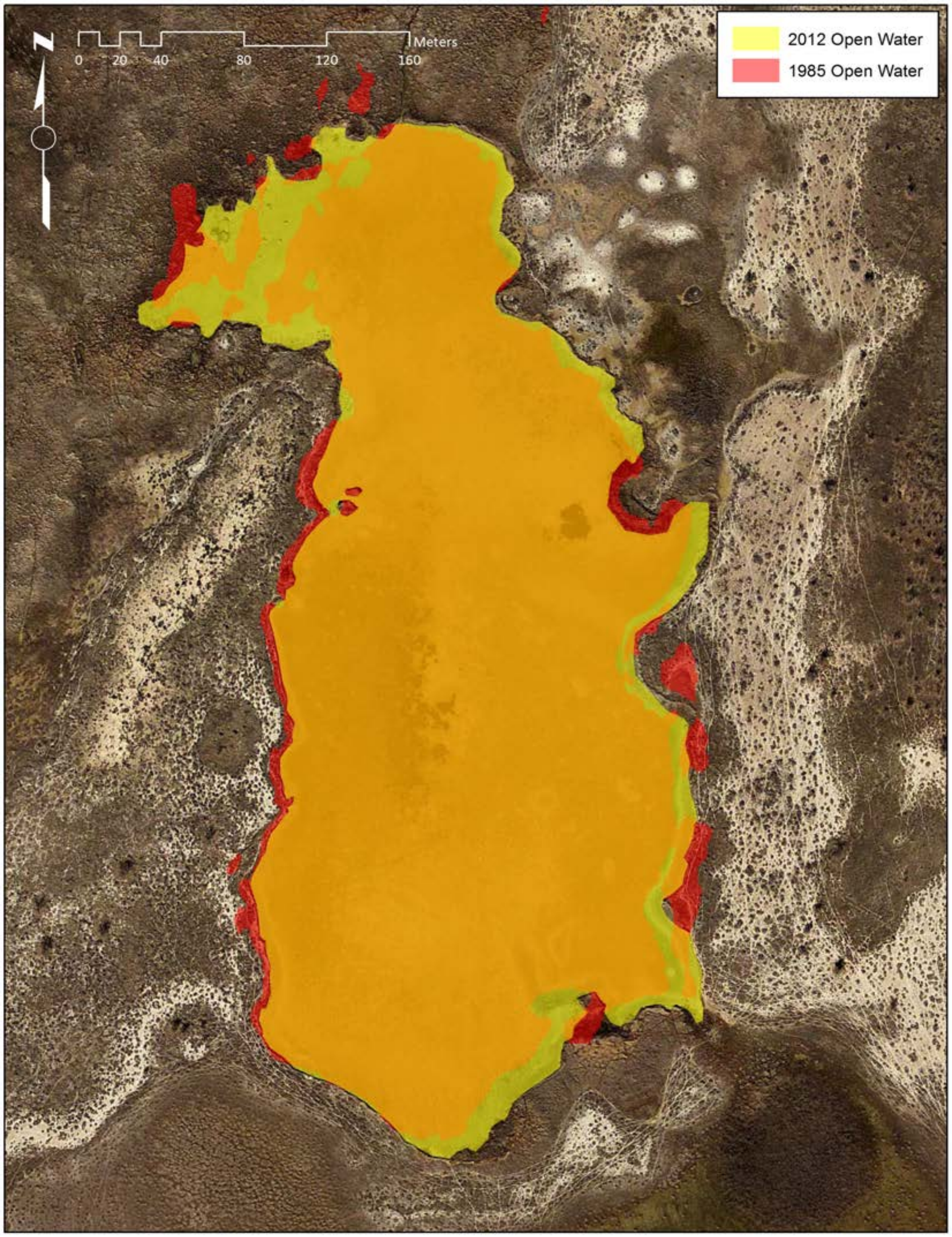


Figure B3. Delineation of open-water habitat in the lower marsh in 1985 and 2012. Base is the 2012 imagery.

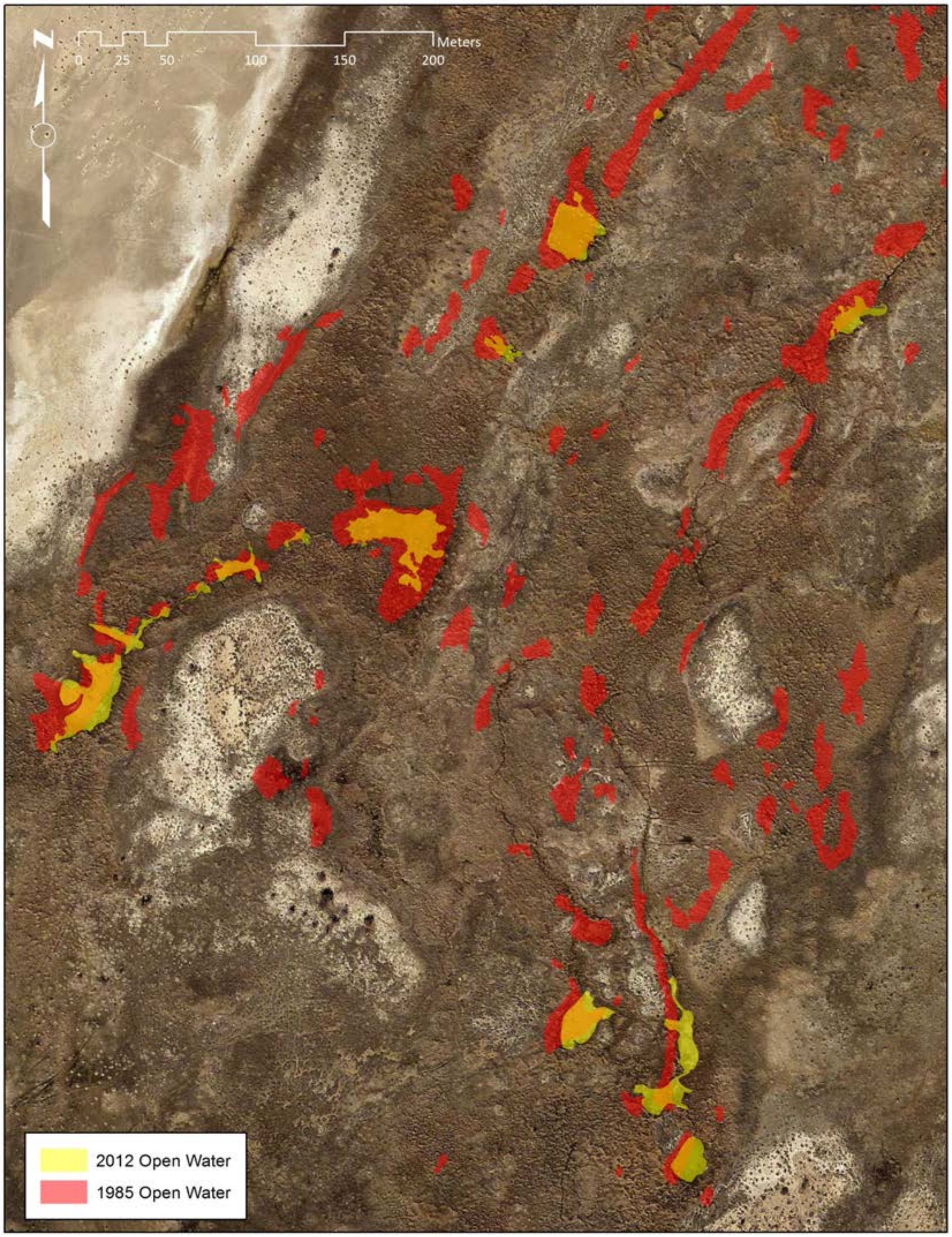
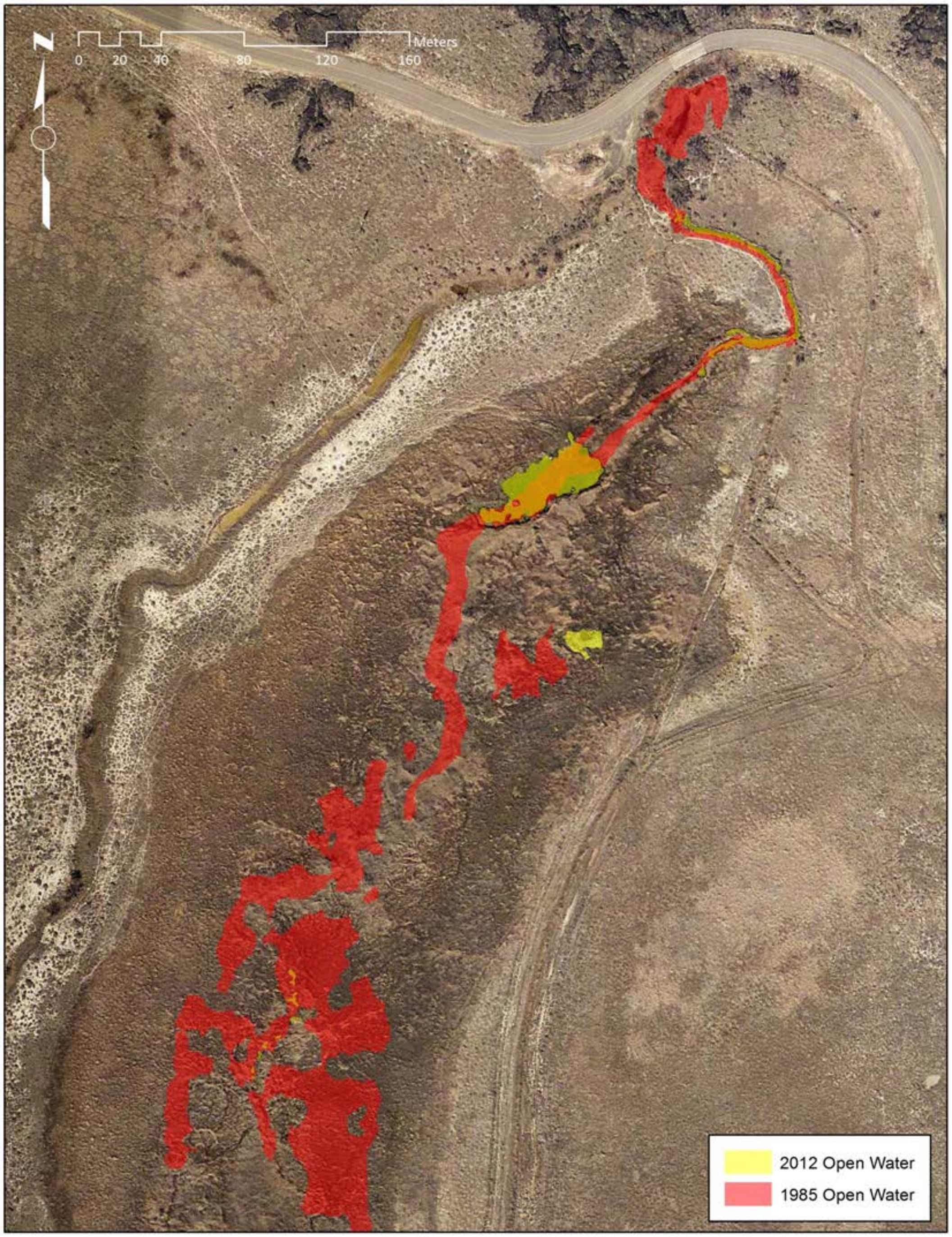


Figure B4. Delineation of open-water habitat in the middle marsh in 1985 and 2012. Base is the 2012 imagery.

Figure B5. Delineation of open-water habitat in the upper marsh in 1985 and 2012. Base is the 2012 imagery.



B.2 Salt Creek

An effort was made to delineate saltcedar coverage along selected areas of Salt Creek using the 1985 and 2012 imagery. However, the 1985 imagery did not have suitable resolution and coloration to allow for extraction of saltcedar using the Feature Analyst extension to ArcGIS. Therefore, a qualitative analysis was undertaken by comparing the 1985 and 2012 imagery rendered in grayscale for three areas: Salt Springs (Figure B7), at the Range Road 316 crossing (Figure B8), and at a site below Range Road 316 near the cable crossing site (Figure B9). The extent of saltcedar coverage at all three of these sites appears to have changed little from 1985 to 2012.

- The Salt Springs area had relatively extensive stands of saltcedar in 1985 in approximately the same locations as they were in 2012 (Figure B7).
- The spatial extent of saltcedar just upstream Range Road 316 was similar in 1985 and 2012 (Figure B8). The distribution of saltcedar farther upstream, near the confluence of Upper Basin Draw, appears to have changed in response to shifts in the braided channels of Salt Creek in this area.
- The cable crossing site had similar spatial extent and distribution of saltcedar in 1985 and 2012 (Figure B9).

This cursory analysis suggests that, in the absence of ground-disturbing activities, the spatial distribution of saltcedar along Salt Creek may be relatively static. For example, field examination of the saltcedar stand in the center-left portion of Figure B7 (2012) revealed the presence of many old, dead stems in the core of the stand and very few seedling or saplings (Figure B6).

Figure B6. Saltcedar stand at Salt Springs. View is looking downstream (E-SE) near the terminus of surface water at the spring. Photo taken on 7 November 2014 by J. S. Pittenger.

Figure B7. Spatial extent of saltcedar along Salt Creek at Salt Springs, 1985 and 2012.

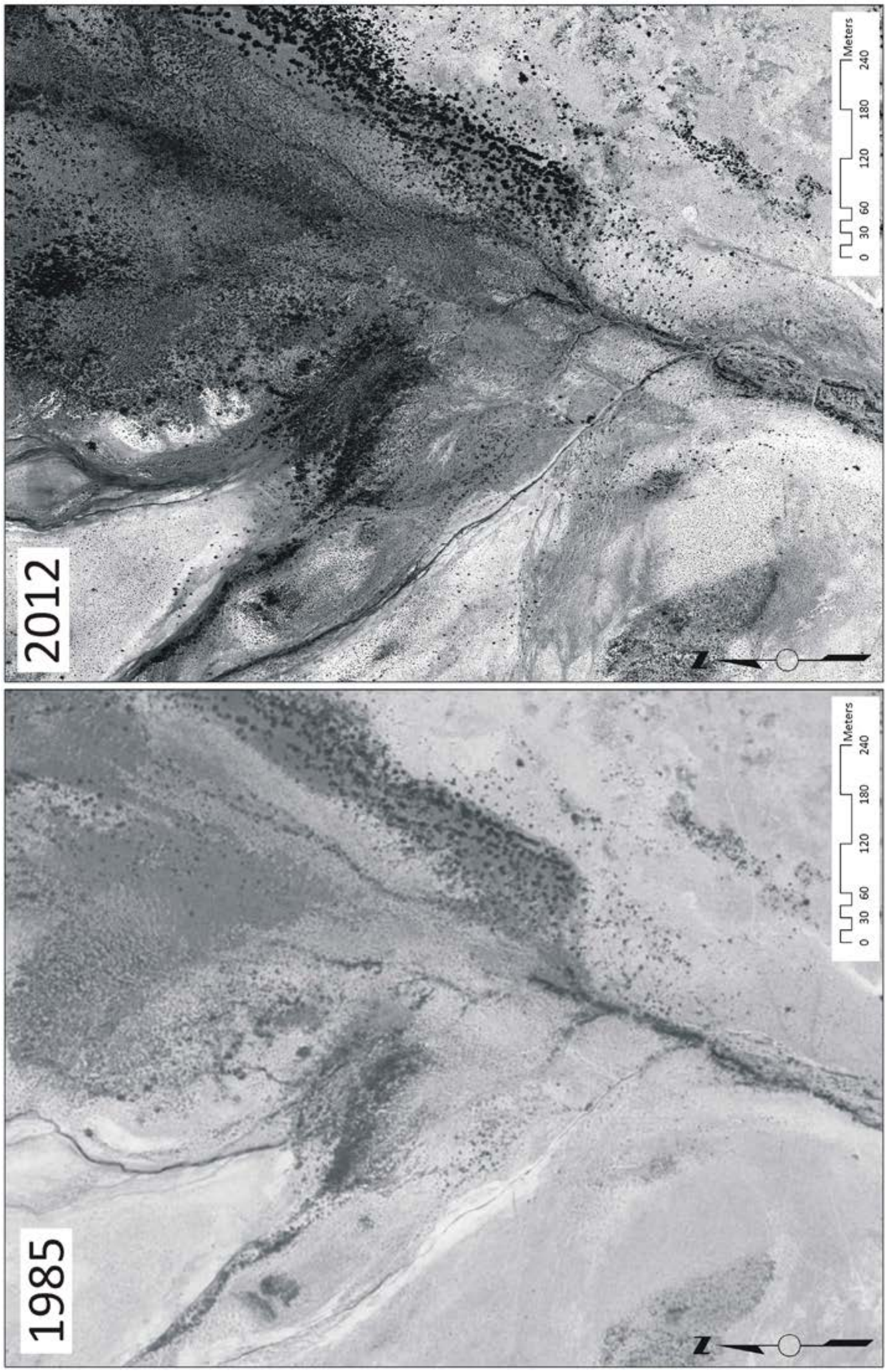


Figure B8. Spatial extent of saltcedar along Salt Creek at Range Road 316, 1985 and 2012.

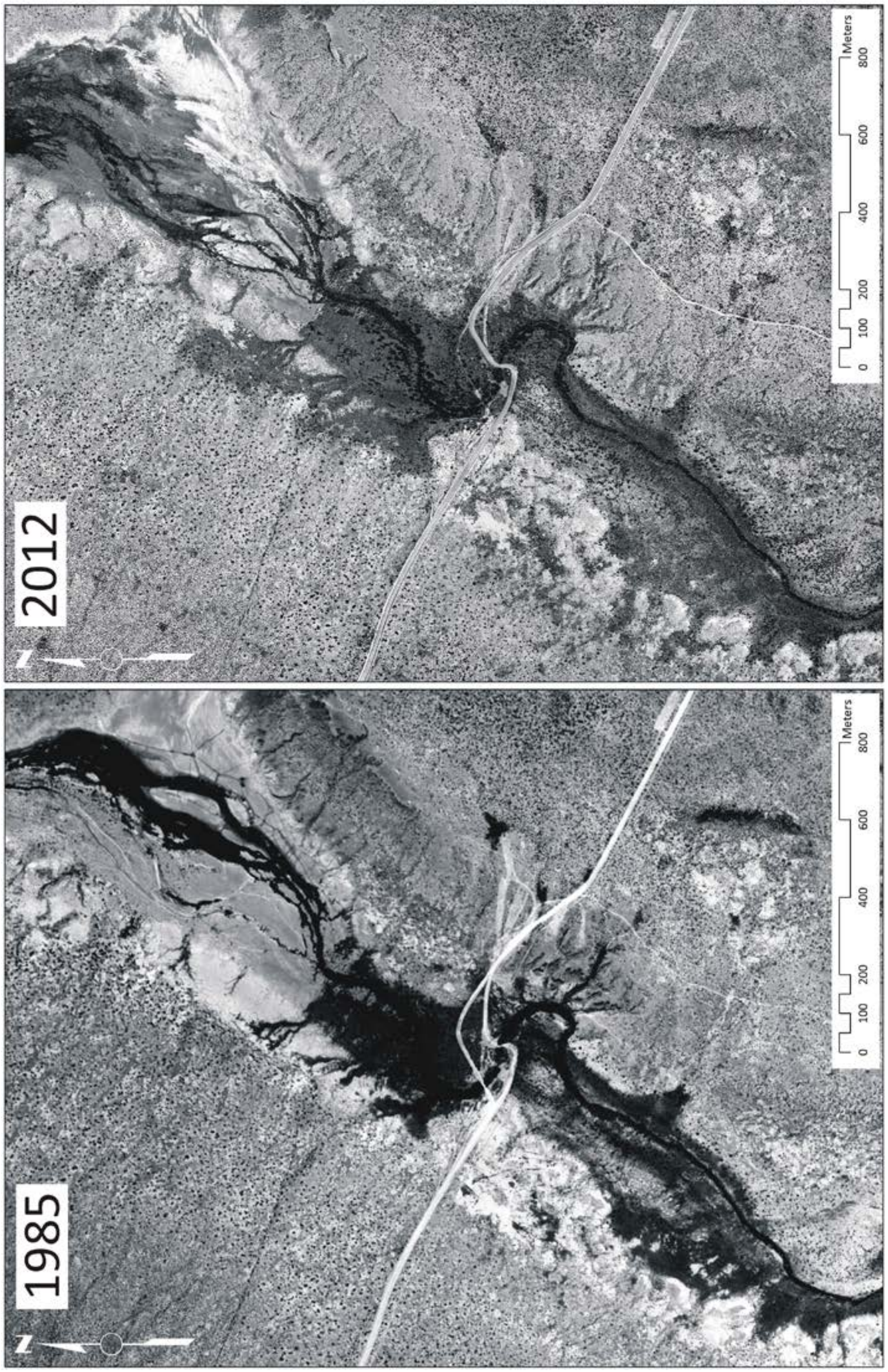
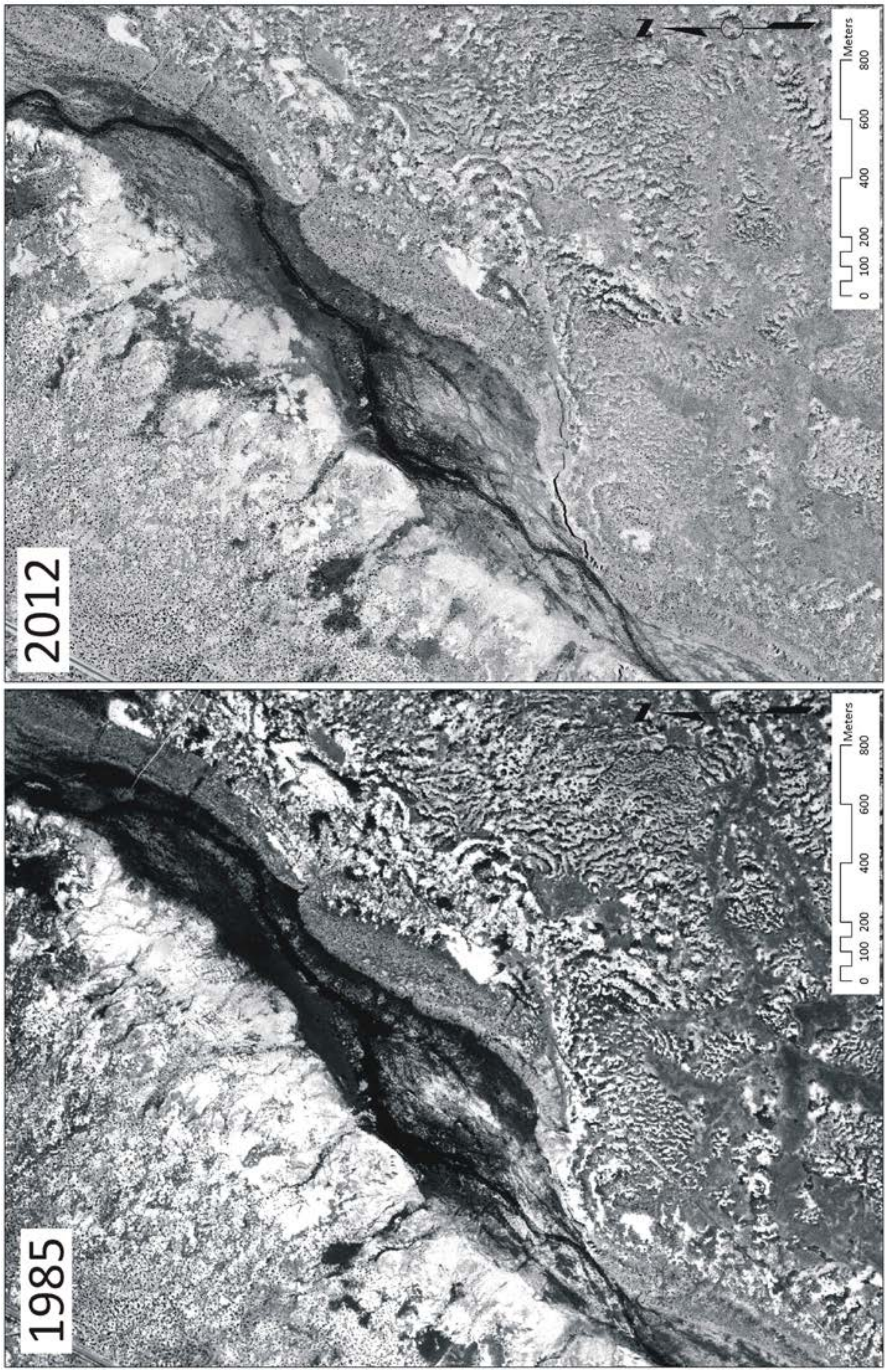


Figure B9. Spatial extent of saltcedar along Salt Creek at the cable crossing site, 1985 and 2012



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