INVASIVE CRAYFISH IN A DESERT SPRING SYSTEM: USING LANDSCAPE

GENETICS TO INFORM ECOLOGICAL RESTORATION

by

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Invasive crayfish in a desert spring system: Using landscape genetics to inform ecological restoration

Thesis directed by Professor Andrew P. Martin

ABSTRACT

The delimitation of dispersal routes utilized by invasive species has the potential to direct management efforts in invaded systems, and may be used to prevent the invasion of native communities. Landscape genetics provides a powerful tool to determine post-invasion movement corridors by integrating inferences of gene flow between populations with landscape connectivity metrics. This technique was used to describe dispersal patterns of invasive red swamp crayfish (*Procambarus clarkii*) through Ash Meadows, a spring system and endemic hotspot in the Mojave Desert. Red swamp crayfish have successfully invaded aquatic ecosystems across much of the globe, and cause severe ecological damage in the form of biodiversity loss and habitat destruction. In Ash Meadows, a combination of anthropogenic habitat degradation and the establishment of invasive species like *P. clarkii* has caused the extinction of an endemic poolfish, and extirpations and severe population declines of endangered pupfish. Although many Ash Meadows springs are hydrologically isolated, intermittent connectivity occurs during heavy precipitation events – allowing for dispersal of native and invasive species. We used a landscape genetics approach in combination with Geographic Information System (GIS) mapping of surface hydrology to test alternative hypotheses of *P. clarkii* invasion routes and colonization events throughout Ash Meadows. Mitochondrial DNA (COI) and microsatellite markers were used to infer colonization events and gene flow for populations at 15 sample sites. Modeled

historic outflows, in conjunction with waterway mapping based on aerial imagery and LiDAR data, reveal variable drainage routes across the flat topography of Ash Meadows. Estimates of gene flow between *P. clarkii* populations highlight the drainages utilized by crayfish to reach distal springs. Additionally, analyses of mtDNA haplotype diversity and distribution suggests that isolated springs were colonized by few individuals, and subsequent emigration has been rare. These results will inform ecological restoration in Ash Meadows by directing the placement of barriers to prevent reinvasion of distal springs after eradication of *P. clarkii* populations.

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INTRODUCTION

Invasive species are a major driver of global change and biodiversity loss (Lodge 1993, Vitousek et al. 1996, Sala et al. 2000), and can have massive economic consequences: costs of management in the United States alone are estimated to be over $120 billion per year (Pimentel et al. 2005). Understanding invasion ecology on a broad scale, as well as the specific biology of invasive species, may enable us to more effectively prevent the spread of established invaders, and reduce existing populations to manageable densities when eradication is not feasible.

Invasion ecology theory points out particular characteristics that make an organism a good invader, many of which pertain to the idea of propagule pressure, which describes the number of individuals being added to the invaded system either through reproduction, migration, or introduction by humans or other assisted transport mechanisms. A successful invasion involves the introduction of the species, subsequent colonization and establishment, and finally dispersal through the system (Sakai et al. 2001). Increased propagule pressure facilitates the success of the invasion by providing more individuals, thus increasing the likelihood of the population's persistence, and also by increasing genetic variability through the addition of new genetic material from source populations. This increased genetic variability is important for colonizing populations, native or invasive, which may be susceptible to genetic bottlenecks if the number of colonizing individuals is small. Accordingly, successful invaders often exhibit more r-selected traits (MacArthur and Wilson 1967, Pianka 1970) – fast growth rate, early reproductive maturity, large number of offspring, low parental care, small body size, and short lifespan – and are well adapted for dispersal. In addition, successful invaders may also be characterized by high genetic diversity, generalist feeding strategies, life history plasticity, and aggressive behavior

(Sakai et al. 2001).

Given this list of characteristics, it is no surprise that the red swamp crayfish (*Procambarus clarkii*, Girard 1852) has successfully invaded all continents except Australia and Antarctica (Huner 1977, Huner and Avault 1979). Native to northeastern Mexico and the southeastern United States (Hobbs 1972), *P. clarkii* has been introduced in large numbers across the globe, primarily as an aquaculture product. It is commercially favorable due to its environmental tolerance, early maturity, and high reproductive rate, which are also qualities that enable its success in colonizing and invading aquatic systems outside its native range. Combined with excellent dispersal ability, generalist and opportunistic feeding habits, and life cycle plasticity (Gutierrez-Yurrita et al. 1998, Gherardi et al. 1999, Gutierrez-Yurrita and Montes 1999), these characteristics make *P. clarkii* well adapted for invading temperate freshwater systems globally. In this chapter, I review the characteristics of *P. clarkii* that have facilitated its success as an invasive species, as well as the ecological consequences for invaded communities, and methods for eradication.

LIFE HISTORY AND ECOLOGY

Procambarus clarkii is a member of the family Cambaridae, which is made up of twelve genera, all native to North America (Figure 1). The genus *Procambarus* is distributed through eastern Mexico, the southeastern U.S., and Cuba (Figure 2), and includes more than half of the 300 cambarid species. *P. clarkii* is currently distributed on all continents except Australia and Antarctica, as it has been transported around the world for aquaculture, fish bait, and the pet trade. It is a hardy, burrowing crayfish that is adapted to seasonal, warm waters. *P. clarkii* is tolerant of low dissolved oxygen levels, as well as elevated temperatures (Huner and Lindqvist 1995, Payne 1997). As a secondary burrower, *P. clarkii* retreats to burrows for protection from
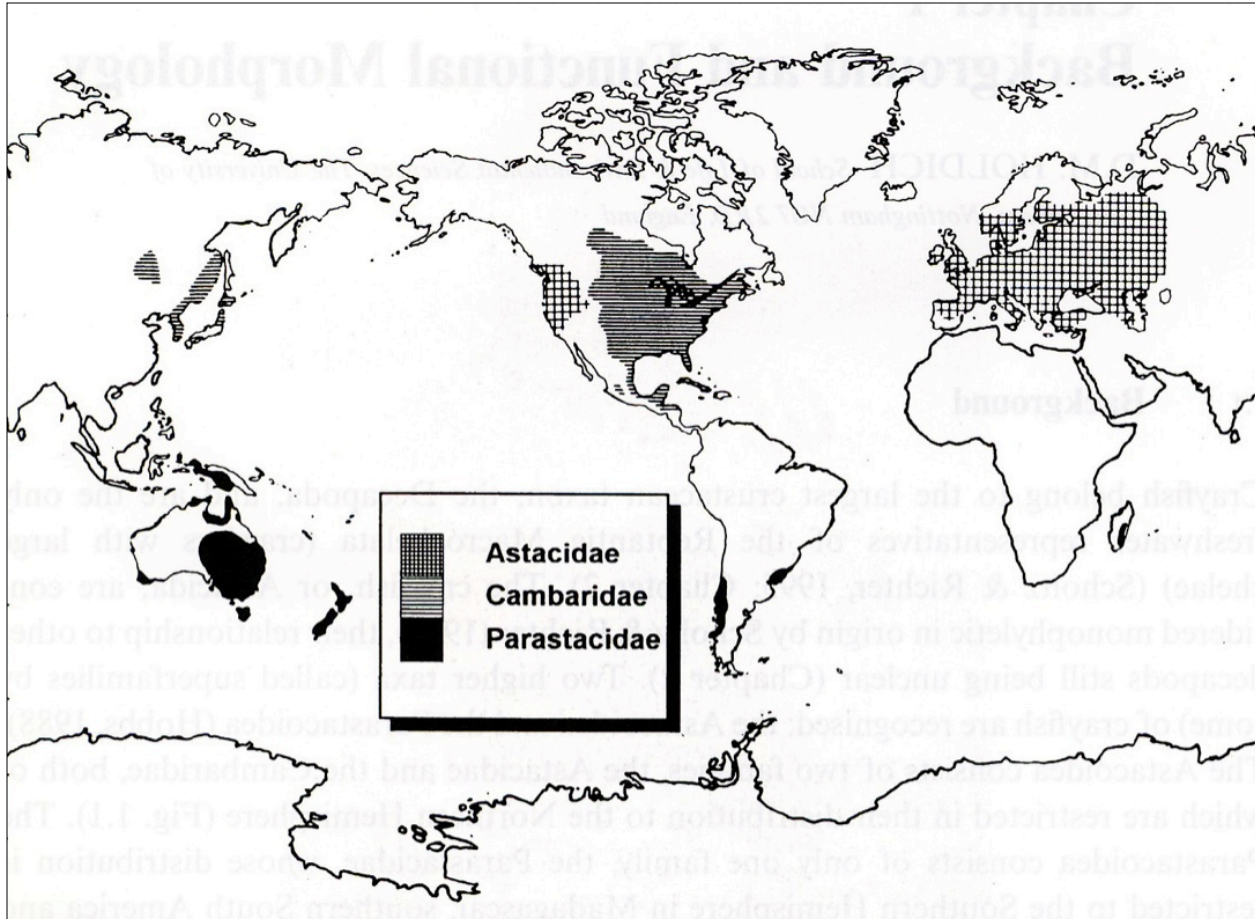
Figure 1 Distribution of the three crayfish families (Holdich 2002).

predation or relief from environmental stress, but does not rely on them constantly (Barbaresi et al. 2004). During extreme environmental conditions such as drought, excessive heat, or cold, *P. clarkii* may occupy burrows for multiple months without going into aestivation, until conditions improve (Gherardi et al. 2002, Reynolds 2002). *P. clarkii* exhibits many of the classic r-selected traits (MacArthur and Wilson 1967), which aid in its success as a colonizer. Individuals of this species have high growth rates (50g in 3-5 months, Paglianti and Gherardi 2004), reach reproductive maturity at a relatively small size (10g, Paglianti and Gherardi 2004), and have a short natural life span of 12-18 months (Huner 1988). Fecundity is high and varies with size: females may produce between 300-600 eggs, and up to 700 (Reynolds 2002). Although many crayfish species are successful colonizers, *P. clarkii* is uniquely adapted to establishing and
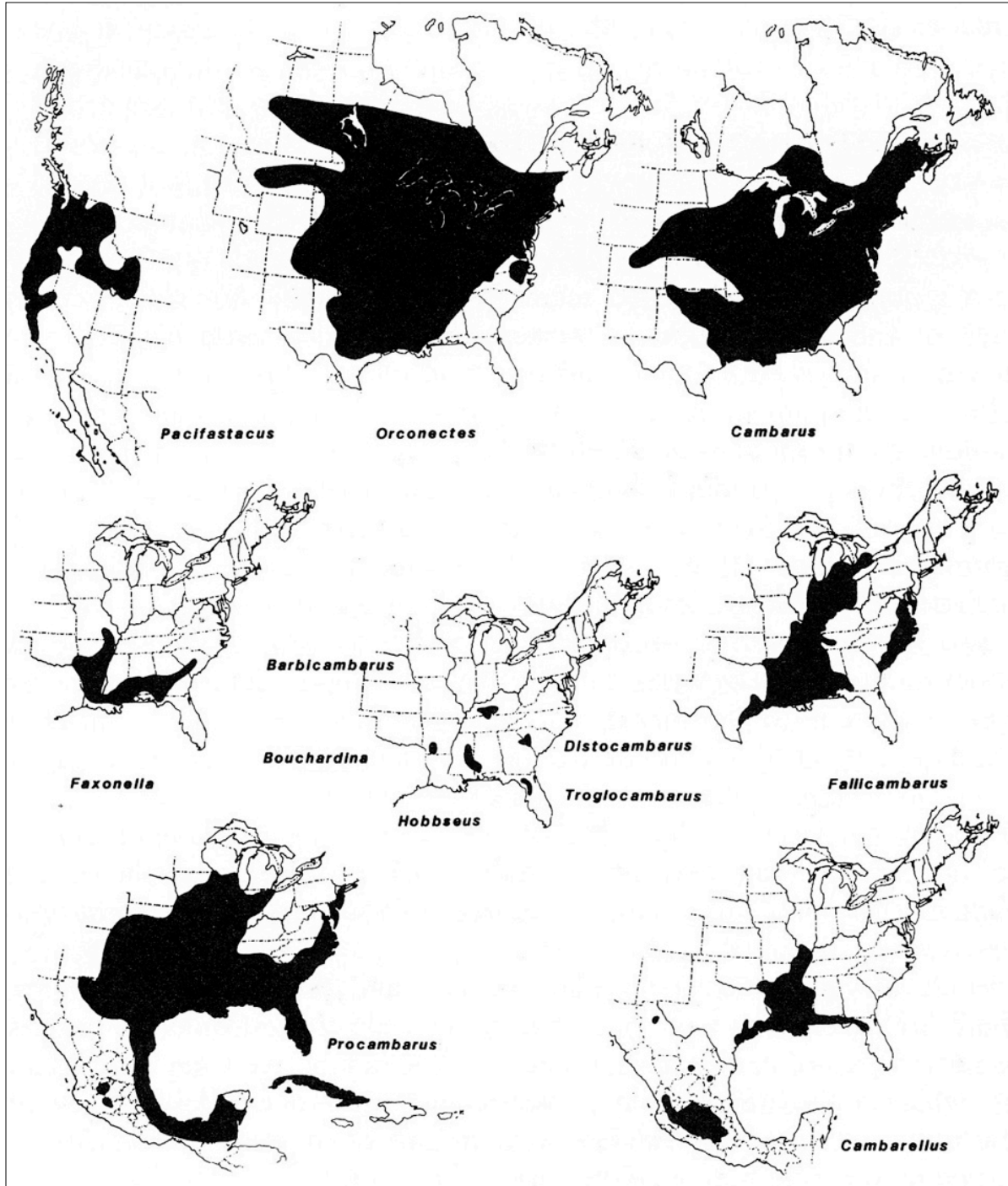
Figure 2 Distributions of nearctic crayfish genera in the families Astacidae and Cambaridae (Hobbs 1988).

maintaining populations due to the females' ability to carry young and incubate eggs at any

given time throughout the year, which enables the species to immediately capitalize on

environmental conditions suitable for the survival of offspring (Huner and Barr 1994).

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MOVEMENT

In addition to r-selected traits and tolerance of a wide range of environmental conditions, *Procambarus clarkii* also excels as an invader due to excellent dispersal ability. Studies have documented individuals moving extensively across land (Penn 1943), up and down waterways, and over barriers such as dams and streambanks (Kerby et al. 2005, Gherardi and Barbaresi 2000). Gherardi and Barbaresi (2000) used radio telemetry to track the movement of five *P. clarkii* males through rice fields near Andalucia, Spain. Over four days, the total distance of individual movement ranged from 0.41 to 16.99 km. In a mark-recapture study conducted by Kerby et al. (2005), *P. clarkii* individuals were documented moving upstream in creek sections with lower flow-rates, as well as across potential barriers (specifically, a 3 m high sloping dam). Crayfish movement was effectively impeded by high water velocity and steep vertical drops, which has been observed in other studies (Light 2003); however, individuals may be able to bypass such barriers by climbing out of the water and moving terrestrially, provided the slope of the bank is not too steep.

POPULATION GENETICS

The success of *Procambarus clarkii* as an invasive species may also be in part due to high genetic diversity of source populations, and the increase in genetic diversity of colonizing populations through repeated introductions. Early studies on the genetic diversity of *P. clarkii* populations using allozyme electrophoresis found low levels of genetic variation within native populations (H_E 0.000-0.035, Busack 1988); however, this method is known to detect only about a third of the genetic variation present (Ramshaw et al. 1979). In addition to allozyme electrophoresis, random amplified polymorphic DNA (RAPD) markers have been used to examine genetic variability of *P. clarkii* populations. Barbaresi et al. (2003) surveyed five

introduced *P. clarkii* populations in central Italy, northern Italy, and southern Portugal, and one native population in New Orleans, Louisiana. Although genetic diversity within populations was generally low (0.078 to 0.155), the results showed higher levels of heterozygosity in the introduced populations than would be expected from a simple colonization model, implying multiple introductions occurred from different sources (Barbaresi et al. 2003).

The first microsatellite markers for *P. clarkii* were described by Belfiore and May (2000). They used 23 microsatellites to examine genetic diversity of four introduced populations in California's Sacramento Valley, which showed heterozygosities ranging from 0.43 to 0.91. More recently, Barbaresi et al. (2007) used microsatellites and the mitochondrial gene region cytochrome oxidase I (COI) to examine whether invasive *P. clarkii* populations in Europe had experienced multiple introduction events, or a single introduction. This research sampled two native populations in New Orleans, Louisiana and northern Mexico, and ten introduced populations in Spain, France, Italy, Portugal, and Switzerland. The native population in New Orleans showed higher genetic diversity than the northern Mexico population (0.556 and 0.361, respectively). In contrast to previous studies, the European populations showed high levels of genetic variation based on the microsatellite data (0.319 to 0.618), indicating multiple introductions and / or high genetic diversity of the colonizing individuals (Barbaresi et al. 2007). Yue et al. (2010) used microsatellites to look at dispersal routes and genetic diversity of six populations of *P. clarkii* in China. As a whole, heterozygosity from the 365 sampled individuals was 0.73. Individual populations ranged from 0.44 to 0.72 – similar to the introduced populations sampled in Europe. Our research on *P. clarkii* populations in a desert spring system – Ash Meadows, Nevada – found similar levels of heterozygosity (see Chapter 2). 205 individuals, sampled from 15 sites, showed heterozygosities between 0.349 to 0.843 per site.

COMMUNITY ECOLOGY

As omnivorous invertebrates that often occur in dense populations, invasive crayfish affect ecological communities in varied and dramatic ways. Invasive crayfish can directly affect multiple trophic levels in ecological communities by feeding on detritus, algae, macrophytes, invertebrates, and ontogenically-suitable vertebrates (e.g., fish eggs (Soltz and Naiman 1978), tadpoles (Barbaresi and Gherardi 2000)). In addition, introduced crayfish provide a new food resource for predators (Rodriquez et al. 2005, Tablado et al. 2010), and can increase primary productivity by increasing rates of nutrient cycling (Flint and Goldman 1975). However, in general, increasing abundance of introduced crayfish yields decreased biodiversity, and decreased biomass of certain taxa (Lodge and Lorman 1987, Rodriguez et al. 2005).

The mechanisms for loss of species richness and abundance include both predation and competition, as well as physical alteration of the invaded space, which usually translates to habitat degradation for native biota. Manipulation experiments have demonstrated that increased abundance of invasive crayfish can decrease macrophyte biomass, cover, and species richness, as well as invertebrate biomass – especially herbivores and detritivores (Nystrom et al. 1996). In addition to driving community reassembly through predation, invasive crayfish also compete for food resources with other vertebrate and invertebrate herbivores by grazing on algae and periphyton, which may result in population declines or extirpation of native herbivores. Interactions between invasive crayfish and other omnivores or predators have been shown to vary depending on the ontogeny of the individuals. For example, Anastacio et al. (2011) found that *Procambarus clarkii* adults prey on *Gambusia holbrooki* (eastern mosquitofish), while the mosquitofish will prey on recently hatched *P. clarkii*. The community response to invasive crayfish does not reflect the typical cascading trophic effects usually seen with the introduction

of a new predator, due to complex interactions across multiple trophic levels (Charlebois and Lamberti 1996, Nystrom et al. 1996).

In addition to affecting community composition through predation and competition, and by providing a new food resource, invasive crayfish may also drive community reassembly and biodiversity loss through physical alteration of habitats. Crayfish can act as ecosystem engineers by constructing burrows, which can eventually lead to the collapse of banks (Barbaresi et al. 2004), and by removing macrophytes for consumption and through non-consumptive clipping (Nystrom and Strand 1996, Nystrom et al. 2001). Loss of macrophyte cover by 99% in Lake Chozas, Spain, due to introduced *P. clarkii*, resulted in increased turbidity, and decreased macroinvertebrate genera by 71%, amphibian species by 83%, and duck species by 75% (Rodriguez et al. 2003, 2005). In general, the engineering activities of crayfish act to decrease habitat complexity, which has long been predicted to decrease biodiversity (e.g., Dean and Connel 1987, Crooks 2002). In summary, the consequences of crayfish invasions for ecological communities are complex, due to food web interactions across multiple trophic levels, and may be magnified by ecosystem engineering that alters structural habitat and food resource availability for native species.

ERADICATION

Once established, the eradication of invasive crayfish has proven to be difficult due to frequent reproduction, high fecundity, and the ability to tolerate extreme environmental conditions. In addition, areas targeted for crayfish eradication usually have native species of concern that require protection from eradication efforts, making chemical control methods less desirable. Gherardi et al. (2011) reviewed the efficacy of currently available methods, including mechanical removal such as trapping and electrofishing; physically destroying crayfish habitat

by draining or diverting waterways, or erecting barriers; using pathogens or predators as biocontrols; chemical or natural biocides; and autocidal methods such as the release of x-ray sterilized males into populations, or the use of sex pheromones to prevent the location of female mates by males. Thus far, few of these methods have been successful in completely eradicating invasive crayfish populations.

Hein et al. (2007) utilized the mechanical removal method through intensive trapping of invasive rusty crayfish (*Oronectes rusticus*) in a Wisconsin lake over five summers, while restricting the removal of predatory fish (smallmouth bass and rock bass). Although catch rates declined by 95%, they failed to extirpate the population. The habitat destruction method was used with more success to eradicate crayfish populations from three isolated springs in a Mojave Desert oasis (Ash Meadows, Nevada). Springs and associated fish populations were temporarily diverted to holding tanks to dry out *Procambarus clarkii* habitat at springheads and outflows. The dry-downs lasted between one to 21 months, and successfully extirpated the crayfish populations (Weissenfluh 2008; Darrick Weissenfluh, USFWS, pers. comm.).

CONCLUSIONS

The success of *Procambarus clarkii* as an invasive species can be attributed to its life history traits – aggressive behavior, omnivory, high fecundity, tolerance for a wide range of environmental conditions – as well as the increased propagule pressure derived from human introductions and reintroductions. Recent studies have shown that *P. clarkii* may have an additional edge in invaded communities due to a high capacity for learning predator chemical cues, and an ability to differentiate between low and high risk predators (Gherardi et al. 2011). This finding supports the hypothesis that invasive crayfish have high behavioral plasticity, which has been demonstrated in previous work that showed invasive crayfish retain learned information

– such as chemical cues from predators – longer than native crayfish (Hazlett et al. 2002). All of these factors, in conjunction with the lack of successful eradication techniques, highlight the necessity for preventing the translocation of *P. clarkii* and other invasive crayfish outside their native ranges.

Once introduced, interactions between invasive crayfish and native communities are known to be complex due to omnivorous feeding across the food web, often magnified by high population densities of the invader. Effects on native communities can be magnified when invasive crayfish co-occur with other invasive species. In many systems where *P. clarkii* has been introduced, other aquatic invaders such as largemouth bass (*Micropterus salmoides)* and the American bullfrog (*Lithobates catesbeianus*) are also established (e.g., Sada 1990, Miyake and Miyashita 2011). The interactions between suites of invasive species, and the cumulative effects on the native community, should be taken into account when developing management and restoration strategies. Stable isotope analyses of four co-occuring aquatic invaders – snakehead (*Channa argus*), red-eared slider (*Trachemys scripta elegans*), American bullfrog (*L. catesbeianus*), and red swamp crayfish (*P. clarkii*) – in a lake in Japan with endangered odonates found that the snakehead fed primarily on crayfish, while the other two invasive predators showed less dependence on crayfish (Miyake and Miyashita 2011). Removal of these invasive predators – especially snakehead – could release the crayfish population, which may yield extinctions at lower trophic levels. Accordingly, understanding interactions between invaders may be a vital component in the management of invasive crayfish.

Last, the ecological consequences of invasions by *P. clarkii* are not all negative. Tablado et al. (2010) showed that invasive *P. clarkii* in southwestern Spain caused significant positive effects on communities of native predators. Compared to herbivore populations, and the same

predator species in other areas, the abundance of native species of predatory waterbirds, otters, fish, and turtles increased exponentially after the establishment of *P. clarkii.* Some of these species are considered threatened in other areas, lending support to the importance of their population growth. However, consideration for cascading trophic effects caused by an increase in top-trophic level organisms, populations declines and extirpations of organisms lower in the food web than the crayfish, as well as degradation to food and habitat resources caused by invasive *P. clarkii* populations must be taken into account when designing management plans. Although an increase in threatened native predators may be beneficial, the cumulative effects of invasive crayfish on native ecosystems may negate any positive impacts.

CHAPTER 2: *PROCAMBARUS CLARKII* IN ASH MEADOWS, NEVADA: USING LANDSCAPE GENETICS TO INFORM ECOLOGICAL RESTORATION

INTRODUCTION

Invasive species are a major driver of global change, causing loss of biodiversity (Vitousek et al. 1996), altered community assemblages (Sanders et al. 2003), and subsequent degradation to ecosystem functions and services (Pejchar and Mooney 2009). The ecological and economic damage resulting from biological invasions provide a compelling stimulus for research that informs restoration or mitigation of invaded systems, as well as the prevention of invasions. Understanding patterns and mechanisms of invasion at the landscape scale can inform management decisions in invaded systems, and may be used to prevent the spread and establishment of invasive species in native communities. Landscape genetics is a recently developed tool that uses population genetic data in conjunction with landscape connectivity metrics to infer ecological processes such as dispersal and colonization. This method is well suited for ascertaining the history of a biological invasion, as is detailed in a recent review by Estoup and Guillemaud (2010). It allows for the inference of geographical locations of source populations, introduction points, and dispersal corridors, as well as the frequency and magnitude of colonization events, reintroductions, and immigration. Landscape genetics has been used to understand invasion processes for species such as spotted knapweed (*Centaurea stoebe*) in North America (Marrs et al. 2008), common starlings (*Sturnus vulgaris*) in western Australia (Rollins et al. 2009), and cane toads (*Bufo marinus*) in northern Australia (Estoup et al. 2010).

We used a landscape genetics approach to study the invasion of a desert spring system (Ash Meadows, NV) by red swamp crayfish (*Procambarus clarkii*, Girard 1852), which are

threatening the persistence of native and endemic biota. The goal of this research was to inform ecological restoration by delimiting dispersal corridors utilized by crayfish, identifying source populations, and inferring the frequency of dispersal between springs. The invasion of Ash Meadows by red swamp crayfish provides a case study for the use of landscape genetics to understand invasion processes, and inform management, in a system that experiences rare pulses of ephemeral and geographically variable connectivity.

Covering about 23,000 acres of the arid Mojave Desert landscape in western Nevada, Ash Meadows National Wildlife Refuge (AMNWR) is comprised of over 30 springs and seeps, and associated riparian and wetland habitats. This unique desert springs ecosystem supports at least 24 endemic plant and animal species as well as 15 endemic insects and snails (Stevens and Bailowitz 2008). Included in these endemic species are four fish taxa that are currently listed as federally endangered: Devil's Hole pupfish (*Cyprinodon diabolis*), Ash Meadows Amargosa pupfish (*Cyprinodon nevadensis mionectes*), Warm Springs pupfish (*Cyprinodon nevadensis pectoralis*), and Ash Meadows speckled dace (*Rhinichthys osculus nevadensis*).

The source of the Ash Meadows outflows is a large underground aquifer, collected over an area of 11,600 km^2. The water flowing out of the springs is thought to have originated in pluvial times, between 8,000 to 12,000 years ago (Soltz and Naiman 1978). Collectively, the springs in Ash Meadows discharge 2,700 hectare-meters of water per year, or about 40,125 liters per minute (lpm) (Dudley and Larson 1976). Individual springs range in discharge rates from three lpm to 10,600 lpm. Although hydrologic connectivity is continual between some springs and reservoirs, many of the outflows from low-flow springs disappear from the land surface before connecting to other waterways (Figure 3). The Ash Meadows basin drains through the marshes of the Carson Slough to the upper Amargosa River, which flows into the southern end
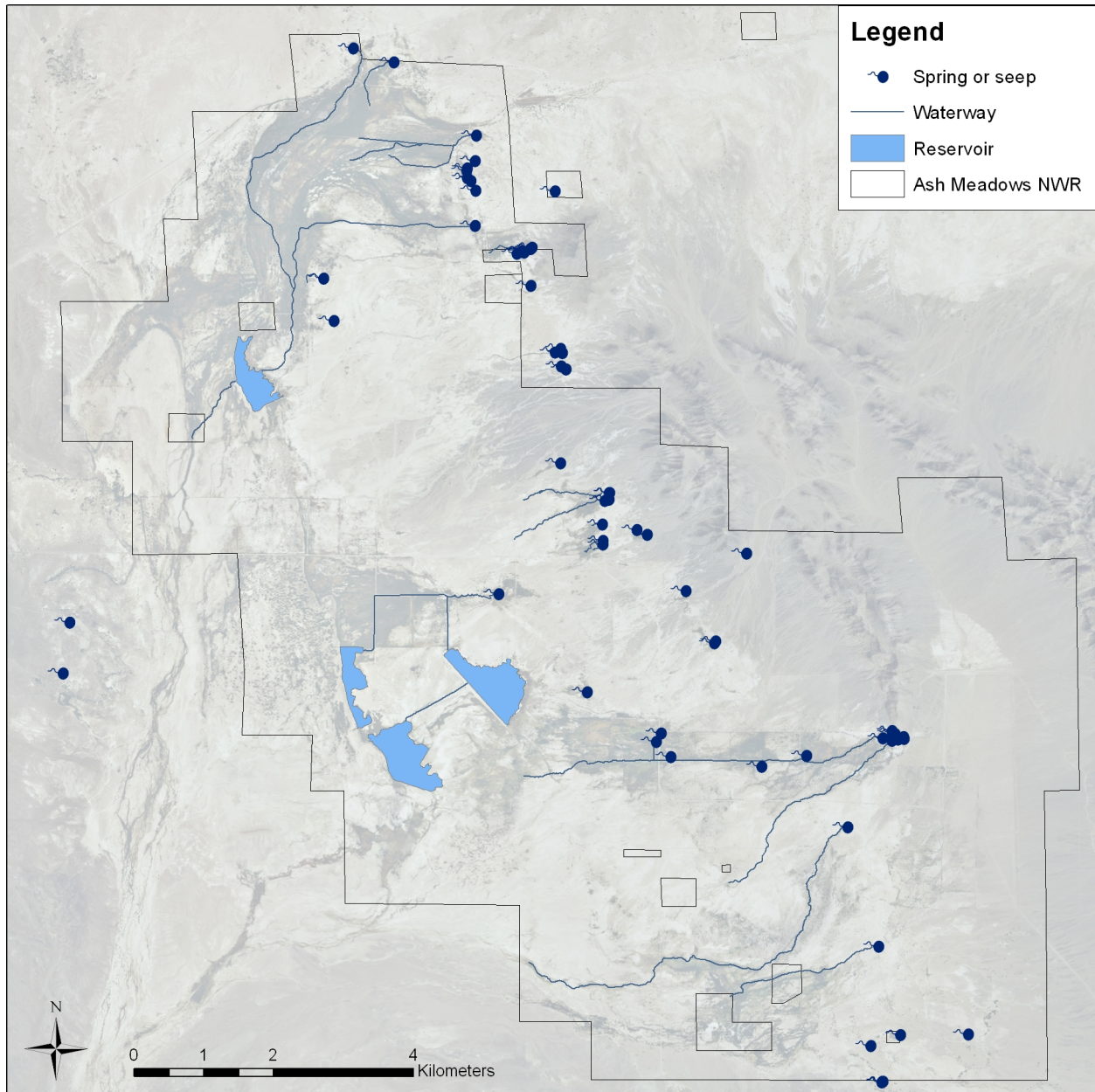
Figure 3 Springs and seeps, waterways, and reservoirs in Ash Meadows National Wildlife Refuge (AMNWR). All features were mapped by AMNWR personnel.

of Death Valley, though it is dry most of the year (Soltz and Naiman 1978). The area receives

less than 2.75 inches of rain annually, and annual evaporation is greater than 98.5 inches (Sada

1990).

As a readily available water source in the vicinity of desert population centers – Pahrump

and Las Vegas, Nevada – the springs of Ash Meadows have been utilized for agriculture,

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ranching, aquaculture, recreation, and municipal purposes, for over a century (Dudley and Larson 1976, Soltz and Naiman 1978). The surface hydrology of the system was altered extensively through enlargement of springheads, channelization of waterways, removal of streamside vegetation, and the removal of water through pumping (Dudley and Larson 1976, Soltz and Naiman 1978). The heaviest anthropogenic impacts in Ash Meadows occurred during the 1960s and 1970s. In the early 1960s, a local rancher drained the Carson Slough to mine for peat. After a few years, the land was sold to a large-scale farming corporation, which bulldozed adjacent sand dunes into the slough, and ploughed and tilled the area to be used for agriculture (Deacon and Williams 1991). Prior to its destruction, the slough habitat maintained extensive populations of Ash Meadows pupfish and speckled dace (Soltz and Naiman 1978).

At about the same time, a temporary facility was set up in Forest Spring to raise exotic aquarium fish such as green swordtail (*Xiphophorus helleri*) and arawana (*Osteoglossum bicirrhosum*) (Deacon and Williams 1991, Soltz and Naiman 1978). In 1967, land was acquired in Ash Meadows by a ranching corporation, which proposed the development of 12,000 acres for crops to feed cattle. The quantity of water pumped out of the aquifer to implement this proposed development, in combination with the removal of water from springs for irrigation below Ash Meadows, led to the lowering of the water level in the aquifer (Dudley and Larson 1976). The decline in the aquifer was observed through hydrological monitoring at Devil's Hole, which is a collapsed depression in the limestone, allowing a window into the aquifer about 50 feet below the land surface. This marked the beginning of a period of time when water removal exceeded aquifer recharge rates, resulting in the lowering of surface water levels and discharge rates from the springs (Soltz and Naiman 1978). In 1984, Ash Meadows was established as a National Wildlife Refuge to halt local water removal, alteration of streamheads, and farming.

Although water removal and hydrologic alterations ceased when Ash Meadows was inducted into the national wildlife refuge system, threats to the local biota have continued in the form of invasive plants and animals. The introduction of non-native species such as mosquitofish (*Gambusia affinis*), sailfin molly (*Poecilia latipinna*), tropical convict cichlid (*Archocentrus nigrofasciatus*), largemouth bass (*Micropterus salmoides*), green sunfish (*Lepomis cyanellus*), black bullhead (*Ameiurus melas*), American bullfrog (*Lithobates catesbeianus*), and red swamp crayfish (*Procambarus clarkii*) began in the first half of the twentieth century for aquaculture and recreation (Miller 1948, Deacon et al. 1964, Deacon and Williams 1991, Hubbs and Deacon 1964, Soltz and Naiman 1978, Sada 1990, Scoppettone et al. 2011). These aquatic invaders have caused major declines and some extirpations of endemic populations (e.g., Sada 1990, Kennedy et al. 2005), and combined with hydrological alterations, lead to the extinction of the Ash Meadows killifish (*Empetrichthys merriami*) (Soltz and Naiman 1978).

Restoration in Ash Meadows has been ongoing since its establishment as a National Wildlife Refuge. Efforts have been made to restore springheads, waterways, and marsh habitat, and to eradicate invasive species from the system. Although it may be feasible to eradicate invaders from isolated springs, it is unlikely that larger source populations can be extirpated (e.g., *Procambarus clarkii* in Peterson Reservoir). Accordingly, the next step in the restoration process is to prevent reinvasion of restored springs by dispersing individuals from source populations.

Our research focuses on *P. clarkii*, which poses an immediate threat to the persistence of the endangered Warm Springs pupfish through both predation and competition (Miller 1948, Soltz and Naiman 1978, Kennedy et al. 2005). The entire range of the Warm Springs pupfish is restricted to five springs, in the Warm Springs Complex of Ash Meadows. These springs are higher in elevation than other Ash Meadows outflows, and are hydrologically isolated from each

other and the other Ash Meadows springs under normal conditions.

The first documentation of *Procambarus clarkii* in Ash Meadows was by Robert Miller in 1942. His field notes describe surveys of native fish in the Ash Meadows springs, in addition to crayfish presence in Big Spring (now called Crystal Spring) and Fairbanks Spring (Appendix A). *P. clarkii* was first introduced into the springs of Ash Meadows for aquaculture (Deacon and Williams 1991), and was well established by the 1960s (Sada 1990). Exact dates of primary introduction are unknown, however reintroduction of new individuals occurred for decades by fishermen who used them for bait, and dumped their bait buckets into the springs and reservoirs at the end of the day. Ash Meadows presents an easy target for crayfish invasion with relatively low flow rates, flat topography, lack of competition from native crayfish, high food availability, suitable water temperatures, and good substrate for burrowing. Establishment of *P. clarkii* in Ash Meadows has likely been facilitated by high propagule pressure, through multiple introductions as well as high reproduction rates.

Although specific dates are unknown, there is some documentation of crayfish dispersal through Ash Meadows in the latter half of the twentieth century. *P. clarkii* invaded School spring, in the Warm Spring Complex, sometime after 1983 (Weissenfluh 2010); and in 1990, *P. clarkii* was documented in the following additional springs: Fairbanks, Rogers, Cold, Longstreet, Crystal Pool, Bradford #2, Forest, King's Pool, Jackrabbit, Big (Crystal Spring), and Bole (Sada 1990) (Appendix A). Comprehensive surveys of Ash Meadows springs and streams in 2007-2008 (Scoppettone et al. 2011) show a much broader distribution (Appendix A). *P. clarkii* was eradicated from School spring in 2008, and North and South Indian springs in 2011 (Weissenfluh 2008; Darrick Weissenfluh, USFWS, pers. comm.), but remains widely distributed throughout Ash Meadows (Gary Scoppettone, unpublished data) (Appendix A).

Red swamp crayfish in Ash Meadows pose a serious threat to the persistence of endemic pupfish, and other native flora and fauna. The colonization and dispersal of *P. clarkii* through the Warm Springs Complex is particularly problematic due to their impact on populations of the Warm Springs pupfish (*C. n. pectoralis*). The extirpation of the South Indian spring pupfish population is likely due to crayfish predation (Martin 2010), and other Warm Springs pupfish populations have experienced major declines after the establishment of red swamp crayfish (Andrew Martin, unpublished data). The eradication of *P. clarkii* from pupfish habitat, and preventing reinvasion of restored springs and waterways, is a vital component of the restoration of Ash Meadows. Although the Warm Springs Complex is not connected by surface flows to any other springs or reservoirs under normal conditions, intermittent connectivity occurs during extreme precipitation events, creating dispersal opportunities for both native and invasive species. Due to the flat topography of the landscape, these temporary waterways follow variable courses and may end up connecting to a variety of other outflow systems.

We used a landscape genetics approach in combination with Geographic Information System (GIS) models of historic surface hydrology to test alternative hypotheses of *P. clarkii* movement and colonization throughout Ash Meadows. Determining the movement corridors used by *P. clarkii* and the frequency of dispersal events will inform restoration efforts by directing the placement of barriers to prevent reinvasion of restored springs and their outflows.

METHODS

Study sites and sampling methods

Sample sites were designated from a subset of *P. clarkii's* range in Ash Meadows, to facilitate the testing of alternative hypotheses of movement to distal springs from all possible sources. 217 individuals were sampled from springheads, outflows, and reservoirs, at fifteen

locations (Figure 4). Two of the sampled springs – Fairbanks and Roger's – have flumes at the
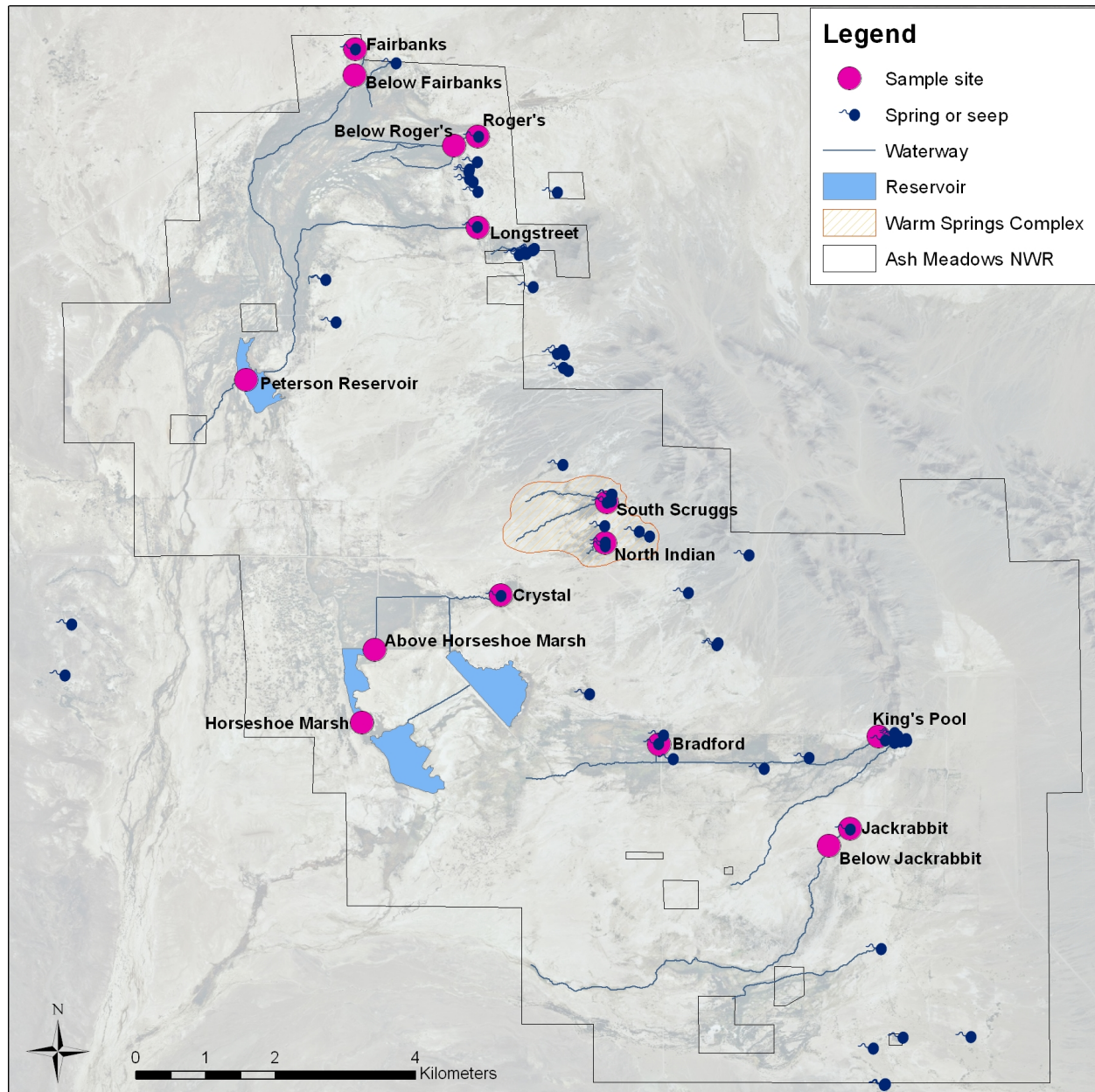
Figure 4 Location of sampling sites in Ash Meadows National Wildlife Refuge, in relation to the Warm Springs Complex. All hydrologic features and the refuge boundary were mapped by AMNWR personnel.

outflow from the spring pool. The flumes have a smooth plastic surface, and sufficiently narrow

the outflow so as to create a strong current. The combination of slippery substrate and high flow

likely impedes the movement of crayfish from the outflow into the spring pool (unless they

travel up the bank). Because these flumes may act as barriers to upstream crayfish dispersal, we

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treated the crayfish above and below the flumes as distinct populations, sampling the outflows

beyond the flumes in addition to the spring pools. Crayfish were captured using minnow traps

baited with cat food, which were submerged at sampling locations for at least three hours. All

individuals were destroyed, and tissue was collected from the cheliped or cephalothorax and

immediately placed in 100% ethanol for storage until DNA extractions were performed.

DNA processing, fragment analysis, and sequencing

DNA was extracted using QIAGEN DNeasy blood and tissue kits (QIAGEN, Valencia,

CA). Amplification and fragment analysis of nine variable microsatellite markers, described by

Belfiore and May (2000), were performed by the Nevada Genomics Center (Reno, NV). The

markers used for this study were: PclG4, PclG7, PclG15, PclG16, PclG17, PclG27, PclG28,

PclG29, and PclG48. Individuals were genotyped in GeneMapper (Applied Biosystems, Foster

City, CA). 192 individuals with the least missing microsatellite genotype data were selected for

mitochondrial DNA analyses. The mtDNA gene region cytochrome oxidase subunit I (COI) was

amplified in polymerase chain reactions (PCR) using the forward primer 5'-TTCG GGTG

GAGT TAGG TCAA - 3', and the reverse primer 5'-TACT TGCG TTCC CTGA AGAGT - 3'.

Clean-up and double-stranded sequencing of mtDNA PCR products was done by Functional

Biosciences (Madison, WI), using the same forward and reverse primers. Consensus sequences

for all individuals were trimmed and edited in Sequencher 4.0 (Gene Codes Corporation, Ann

Arbor, MI) using forward and reverse sequences aligned to a reference sequence (Taylor and

Knouft 2006).

Phylogenetics and population genetics

Phylogenies were inferred from microsatellite genotypes and mtDNA haplotypes using

PAUP (Swofford 2003). GenoDive (Meirmans and Vaan Tienderen 2004) was used to create a

matrix of squared Euclidean distances, calculated from the microsatellite data for all individuals based on the frequency of shared alleles (Smouse and Peakall 1999). The distance matrix was used to generate a neighbor-joining tree. A phylogeny of mtDNA haplotypes was inferred in a heuristic search using parsimony. Geographic distribution of haplotypes was determined from a phylogeny inferred from mtDNA sequences of all individuals.

Assignment tests and Bayesian clustering analyses were conducted in GenoDive and STRUCTURE (Pritchard et al. 2000), respectively. Individuals were assigned to populations using a log-likelihood method (Paetkau et al. 1995), based on microsatellite allele frequencies within each population. Bayesian clustering was used to infer population clusters (K) – independent from prior population designations – as well as the assignment of individuals to one or more clusters, also based on microsatellite allele frequencies. Prior values of K were assumed to be between 1 and 15 (the total number of sites sampled), and the probability of the data for each number of clusters was calculated over 500,000 Monte Carlo Markov Chain (MCMC) iterations after a 200,000 iteration burn-in period. The burn-in period runs the simulation without collecting data in order to minimize the effect of the starting configuration, and ideally begin MCMC iterations with key parameters such as F_{ST} in equilibrium (Pritchard et al. 2000).

Descriptive population genetic statistics were calculated in GenoDive, using the microsatellite data. Genetic distance (F_{ST}) between populations, genetic diversity within populations, and allele frequencies were calculated.

GIS hydrology

Possible routes of aquatic connectivity for crayfish were mapped using ArcGIS 10 (ESRI, Redlands, CA). Three layers of data were assembled to create a map of all possible waterways. Ash Meadows National Wildlife Refuge personnel mapped the first layer, which shows perennial

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waterways and major drainages in Ash Meadows. The second layer depicts historic outflows, and was modeled by Scoppettone et al. (2011) from a three-meter digital elevation model (DEM) generated from light detection and ranging (LiDAR) data, using ArcHydro Tools (ArcGIS 9.3). I mapped the third layer, which describes potential waterways and areas of inundation based on vegetation patterns and drainages observed on aerial images. Vegetation patterns in desert landscapes are an excellent indicator of waterways, as shrubs and trees tend to grow densely along washes and in areas where inundation occurs during precipitation events. The compilation of these three layers represents all possible drainages for surface water during high precipitation or flooding events, and thus describes possible movement routes for aquatic organisms.

RESULTS

GIS hydrology analysis

GIS mapping of current, historic, and potential waterways show that connectivity between northern and southern springs may increase greatly during high precipitation or flooding events (Figures 5 and 6). Outflows from the Warm Springs Complex may connect to the southern waterways by way of Crystal spring and its associated outflow. The outflow from South Scruggs spring may connect to the Crystal outflow further downstream, which connects directly to the northern waterways by way of an outflow from Peterson Reservoir. Even with high flooding events, hydrologic isolation seems to be maintained between the Warm Springs: the outflows from the Scruggs and Indian springs may extend to flow into other waterways at lower elevations, but do not connect directly with each other. Even if inundation occurred around these springs, which could enable extensive terrestrial movement by crayfish, vegetation patterns suggest that these flooded areas remain distinct between the northern and southern Warm Springs (i.e., South Scruggs and North Indian, Figure 7).
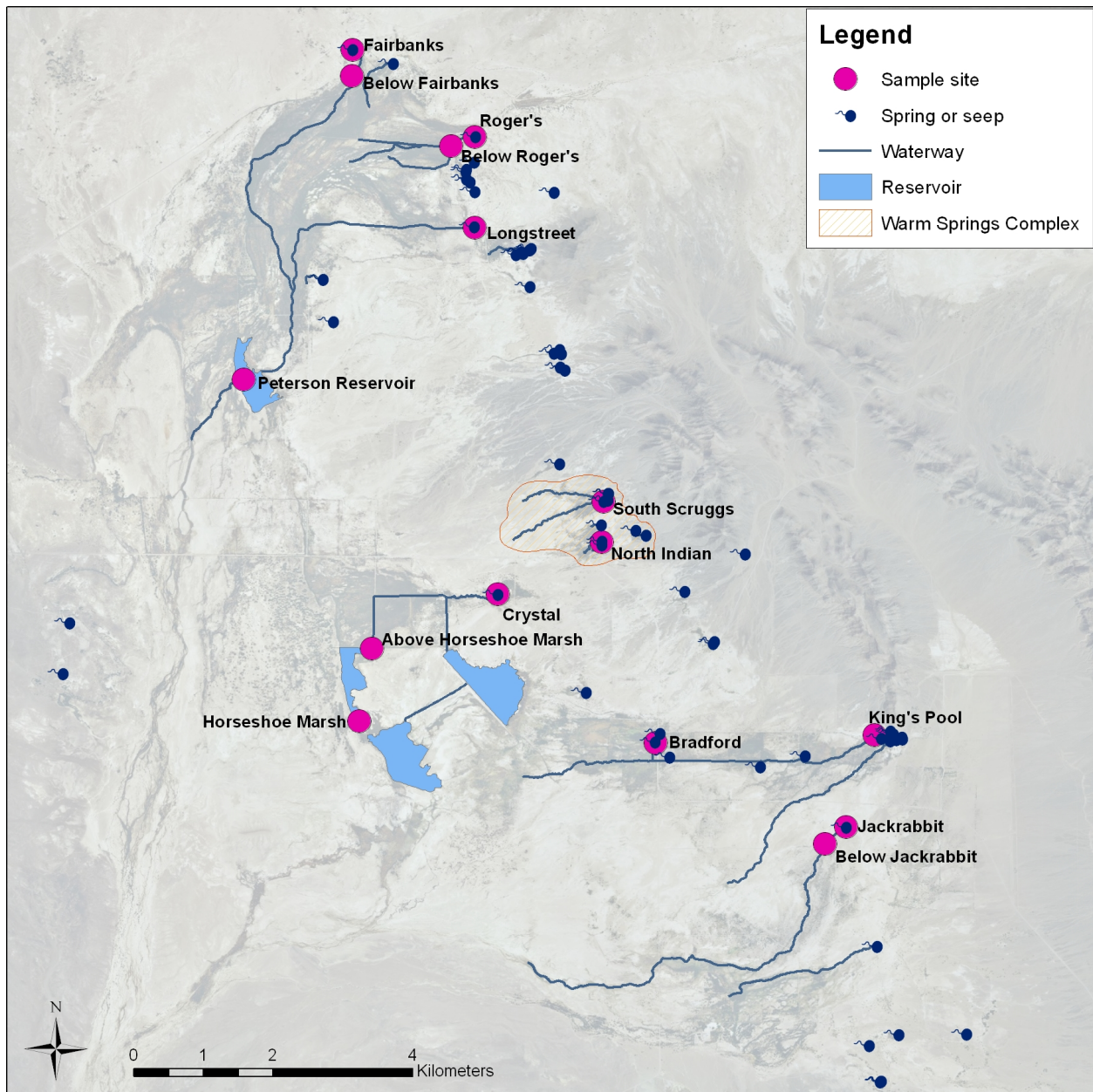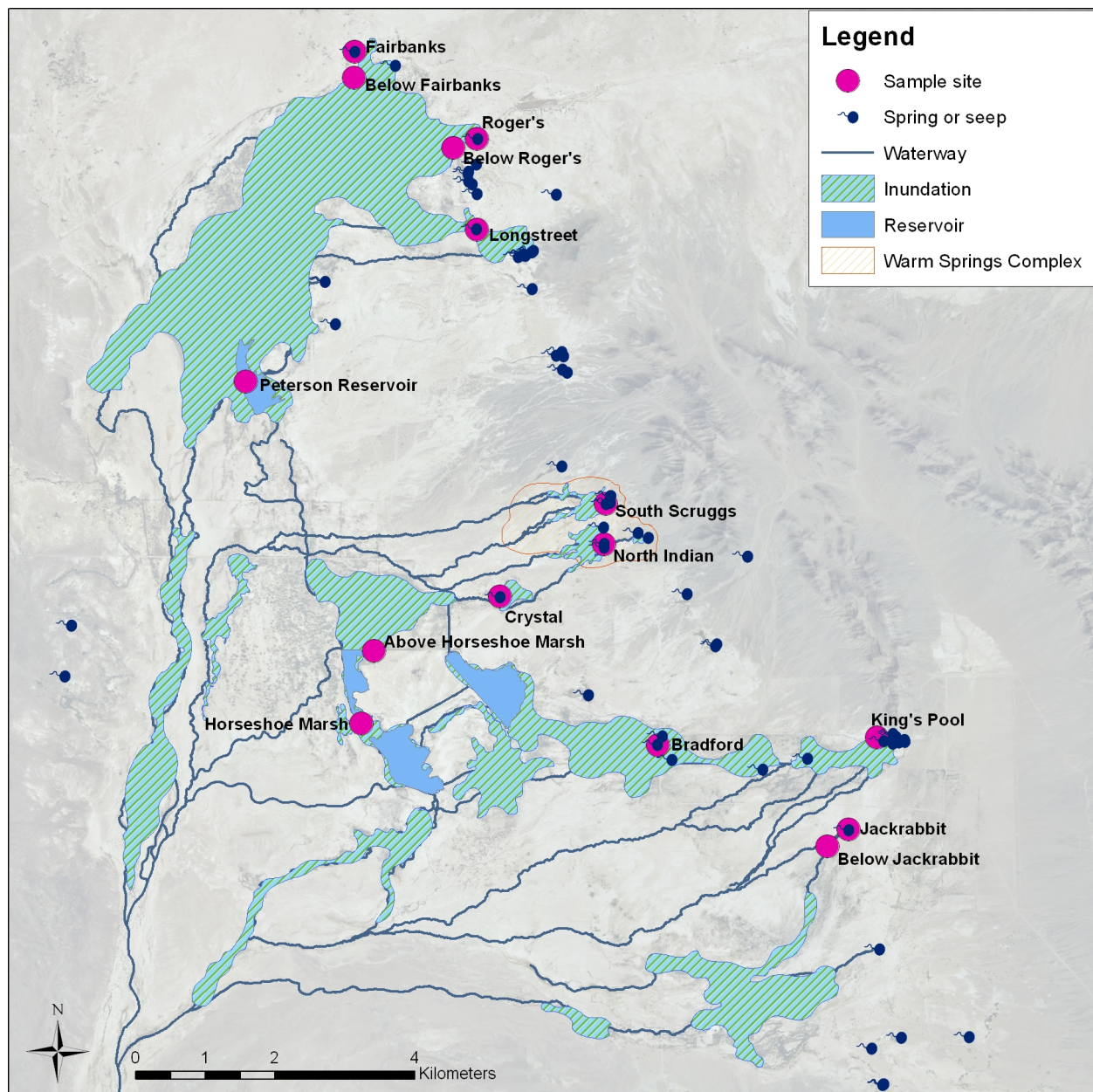
Figure 5 Sample sites, Warm Springs Complex, springs and seeps, reservoirs, and perennial waterways in Ash Meadows National Wildlife Refuge (AMNWR). All hydrologic features were mapped by AMNWR personnel.
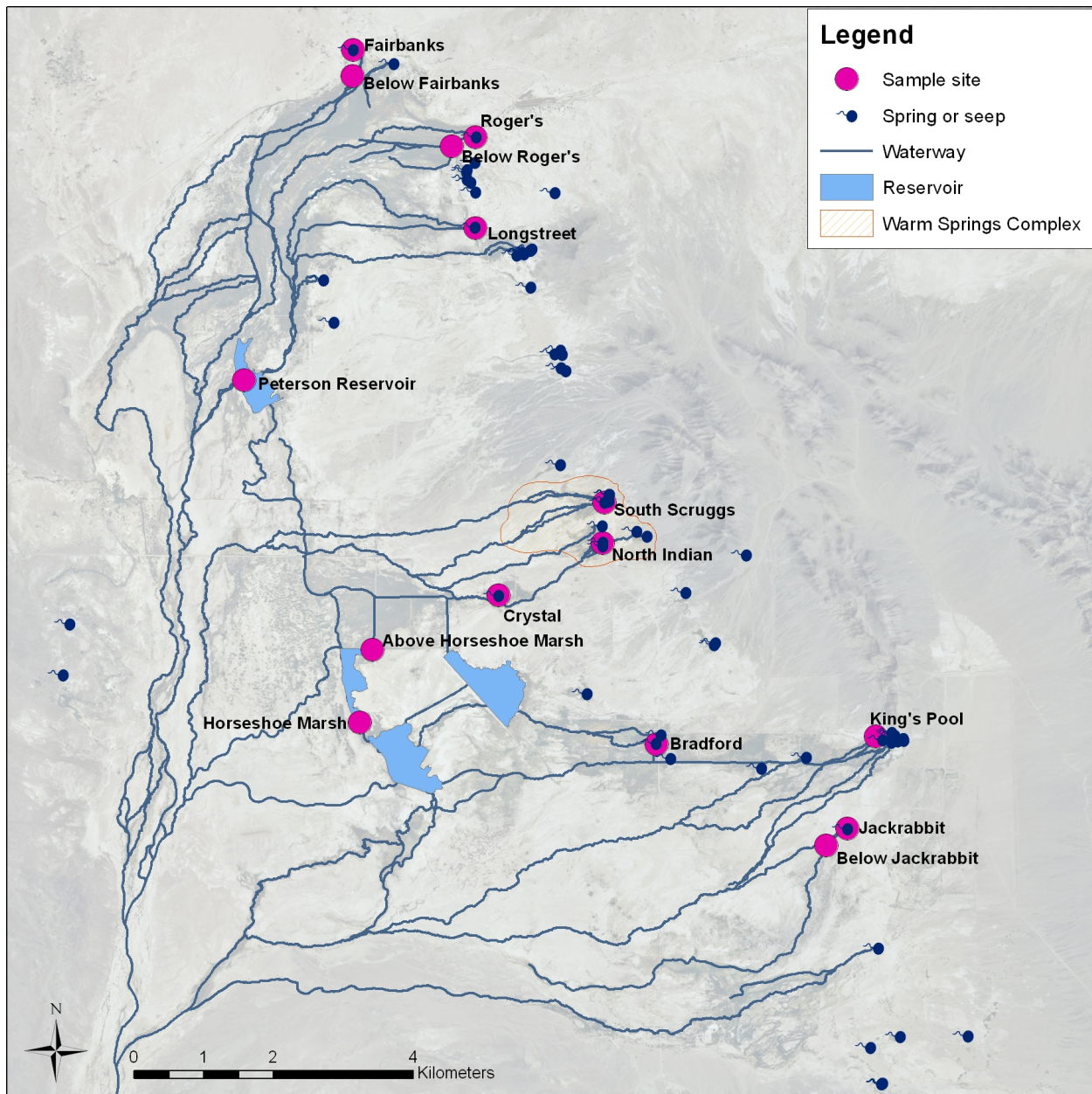
Figure 6 Sample sites, Warm Springs Complex, springs and seeps, reservoirs, and all possible waterways that may conduct surface flows during high precipitation events in Ash Meadows National Wildlife Refuge (AMNWR). Waterways represent a compilation of permanent surface flows mapped by AMNWR personnel, modeled historic flows (Scoppettone et al. 2011), and additional possible surface flows that I mapped based on vegetation patterns and drainages observed on aerial images.

Figure 7 Sample sites, Warm Springs Complex, springs and seeps, reservoirs, all possible waterways that may conduct surface flows during high precipitation events, and possible areas of inundation during flooding in Ash Meadows National Wildlife Refuge (AMNWR). Waterways represent a compilation of permanent surface flows mapped by AMNWR personnel, modeled historic flows (Scoppettone et al. 2011), and additional possible surface flows that I mapped based on vegetation patterns and drainages observed on aerial images. I used the same technique (vegetation patterns on aerial images) to map areas of inundation.

Mitochondrial DNA

Four mtDNA COI haplotypes were identified across all sampled individuals (Figure 8).
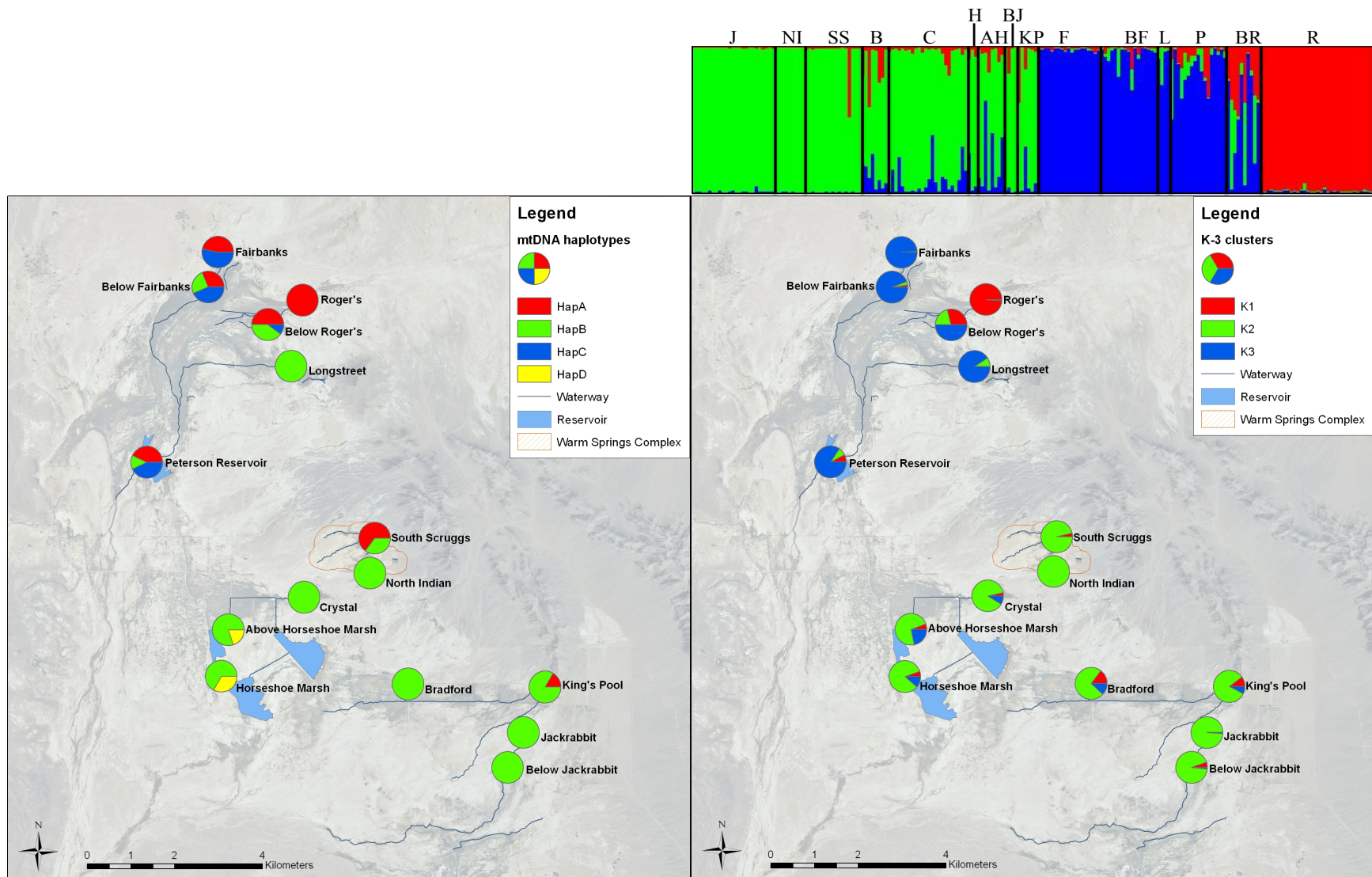
Figure 8 *Left*: Distribution of mtDNA COI haplotypes in Ash Meadows. *Right*: Assignment of individuals to population clusters (K) based on microsatellite genotypes, assuming K=3. The bar graph over the map shows assignment of individuals to one or more clusters (one bar represents one individual).

Haplotypes A and B were distributed in both the northern and southern springs, while haplotype C was only in the northern springs and D was found in two individuals in Horseshoe marsh. The populations sampled from Peterson Reservoir, and the outflows below Roger's and Fairbanks springs had haplotypes A, B, and C, while all other populations had only one or two. There were two haplotypes found in the Warm Springs Complex: both A and B occurred in the South Scruggs population, and all individuals sampled from the North Indian population had haplotype B. Figure 8 also shows the results of the Bayesian clustering analysis of the microsatellite genotypes, assuming the number of K clusters equals three. A K of three was chosen because there were three dominant mtDNA haplotypes in the sampled populations, with the fourth only being found in two individuals. The proportion of individuals within populations assigned to each cluster generally reflects the distribution of mtDNA haplotypes, with K2 dominating the southern springs, and K1 and K3 dominating the northern springs. However, the clustering analysis showed more genetic connectivity between the Crystal-Horseshoe-Bradford system and the northern springs than is represented by the mtDNA haplotypes. Also, fewer individuals from South Scruggs were assigned to the northern drainages than would be expected based on the mtDNA haplotype distribution.

A phylogeny of the Ash Meadows mtDNA haplotypes combined with those of individuals sampled from other native and invasive populations (GenBank) shows two distinct clades (Figure 9). One is comprised primarily of haplotypes sampled from the native range of *P. clarkii* in Mexico, with one haplotype from France – indicating the French population may have been colonized from individuals transported from source populations in Mexico. The second clade contains the haplotypes from Ash Meadows as well as one sampled from within the native range of *P. clarkii* in the U.S. (New Orleans). This cluster also has haplotypes from Illinois,
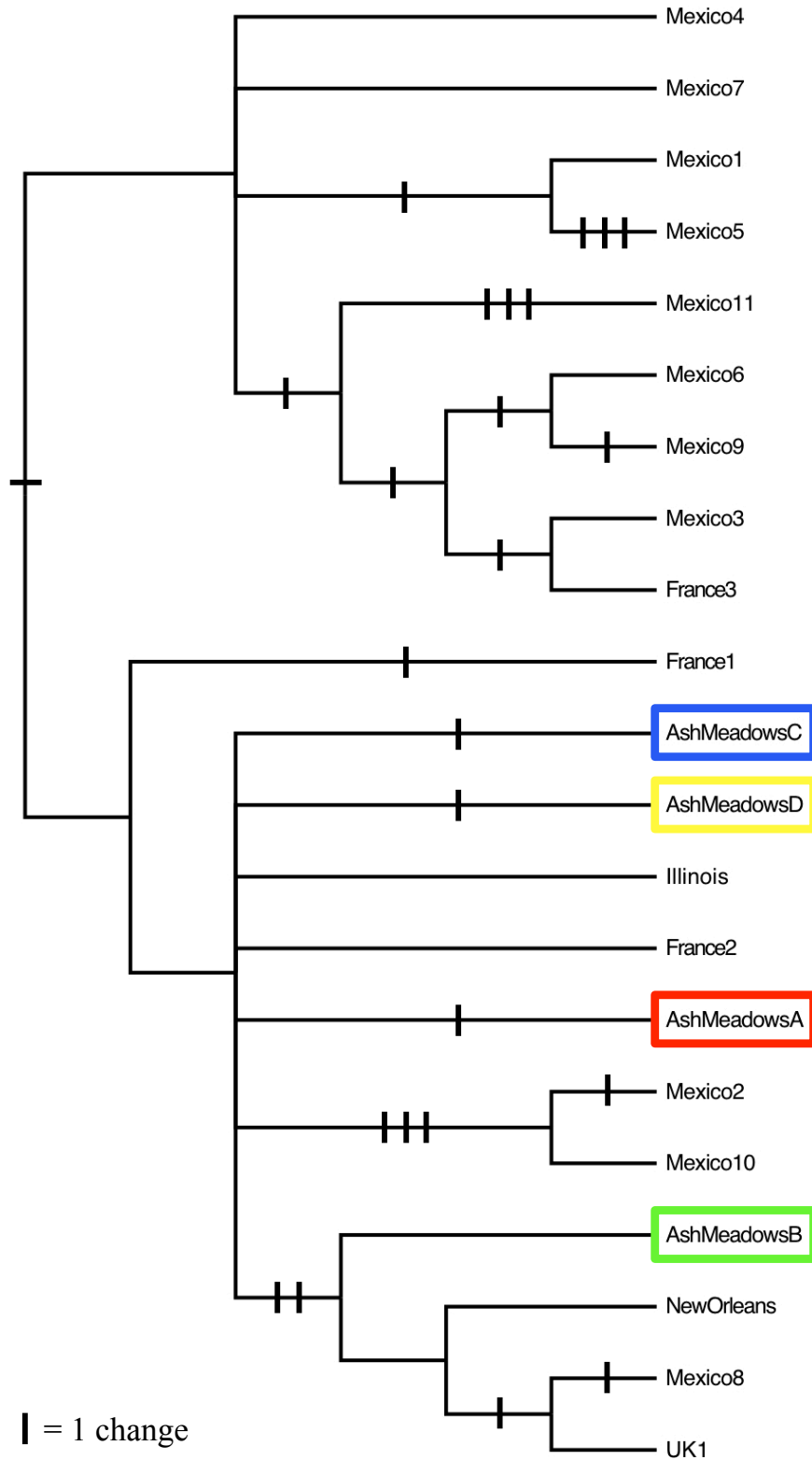
Figure 9 A phylogeny of *P. clarkii* mtDNA haplotypes, from COI sequences acquired through GenBank. Hash marks demarcate the number of nucleotide changes between sequences. Colors correspond to the haplotype distribution map in Figure 8.

France, Mexico, and the United Kingdom, which suggests that the source populations for those introduced populations in the U.S. and Europe are from the native range of *P. clarkii* in the U.S. (i.e., the Gulf Coast).

Microsatellites

A total of 97 alleles were found across nine microsatellite loci, ranging from 8 to 15 alleles per locus. The effective number of alleles per locus was between 2.05 to 2.84. The number of alleles per population was between 2.11 (North Indian) to 7.67 (Crystal), with the effective number of alleles ranging from 1.62 (South Scruggs) to 4.97 (Crystal) (Table 1).

Table 1 Genetic diversity indices for all populations: number of alleles (A), effective number of alleles (A_E), observed heterozygosity (H_O), and expected heterozygosity (H_E). N = number of individuals sampled.

Population	N	A	A_E	H_O	H_E
Bradford	8	3.667	2.498	0.519	0.644
Crystal	24	7.667	4.972	0.720	0.808
Fairbanks	19	3.667	2.137	0.415	0.478
Below Fairbanks	17	4.222	2.524	0.550	0.569
Horseshoe	3	4.000	3.441	0.741	0.843
Above Horseshoe	8	5.667	4.159	0.694	0.794
Jackrabbit	25	4.444	2.855	0.596	0.632
Below Jackrabbit	4	4.000	3.285	0.722	0.796
King's Pool	6	4.333	3.204	0.648	0.731
Longstreet	4	2.444	2.077	0.444	0.523
North Indian	9	2.111	1.663	0.420	0.349
Peterson	17	5.667	3.698	0.673	0.726
Roger's	34	3.444	2.164	0.459	0.530
Below Roger's	10	4.333	2.995	0.586	0.653
South Scruggs	17	2.333	1.618	0.340	0.356

Measures of genetic diversity (H_E) were between 0.35 and 0.84, with North Indian having the lowest diversity and Horseshoe having the highest. Populations from springs and waterways with perennial connectivity exhibited the highest heterozygosities, whereas the most hydrologically isolated springs – South Scruggs and North Indian – had the lowest.

Estimates of genetic distance between populations (F_{ST}) showed most pairs to be significantly different (Table 2). There was not significant genetic differentiation between Crystal and Horseshoe, or Crystal and Above Horseshoe. These populations are perennially connected by a slow-flowing waterway that would be easily navigable by crayfish. In addition, the population sampled from Horseshoe marsh was not significantly differentiated from the Above Horseshoe population just upstream. Although they are not directly connected by a waterway, the King's Pool crayfish were not significantly different than the population sampled from below Jackrabbit spring. Interestingly, the populations sampled from Fairbanks and below the flume, and Roger's and below the flume, showed significant – if minor – population structure, whereas the populations sampled from the pool and waterway for Jackrabbit spring are not separated by a flume, and did not exhibit significant population structuring. The greatest genetic differentiation between populations was between the pair of springs separated by the shortest Euclidean distance: South Scruggs and North Indian. As discussed previously, these springs seem to maintain hydrologic isolation from each other, even during flooding events (though they are likely to experience indirect connectivity through the outflow from Crystal spring).

The results of the assignment tests showed that individuals not assigned to their own populations were often inferred to belong to populations with high hydrologic connectivity: Peterson Reservoir, the inflow to Horseshoe Marsh (Above Horseshoe), the waterway below Fairbanks, and Crystal spring (Table 3). One individual sampled from Bradford spring was assigned to an upstream spring (King's Pool), indicating downstream movement from King's Pool to Bradford. Similarly, four individuals sampled from the waterway below Fairbanks were assigned to the Fairbanks spring pool population. However, four individuals sampled from the Fairbanks spring pool were assigned to the outflow population – potentially indicating upstream

Table 2 Pairwise genetic distances (F_{ST}) between all sample sites. Abbreviations along the top of the table begin with Bradford, and subsequently correspond to sample sites listed at the left side of the table. Values marked with NS are not significant.

| Population	BF	C	F	FB	H	HA	J	JB	KP	L	NI	P	R	RB
Crystal	0.170													
Fairbanks	0.352	0.247												
Fairbanks, Below	0.239	0.165	0.092											
Horseshoe	0.187	0.005[NS]	0.281	0.187										
Horseshoe, Above	0.146	0.012[NS]	0.241	0.134	-0.003[NS]									
Jackrabbit	0.246	0.140	0.396	0.325	0.221	0.196								
Jackrabbit, Below	0.142	0.041	0.330	0.248	0.092	0.068	0.015[NS]							
King's Pool	0.140	0.074	0.340	0.262	0.110	0.101	0.190	0.037[NS]						
Longstreet	0.248	0.164	0.284	0.200	0.164	0.096	0.309	0.208	0.232					
North Indian	0.432	0.288	0.516	0.437	0.388	0.357	0.382	0.415	0.409	0.508				
Peterson	0.155	0.081	0.149	0.045	0.097	0.057	0.231	0.126	0.141	0.127	0.360			
Roger's	0.280	0.243	0.325	0.308	0.266	0.227	0.373	0.268	0.281	0.325	0.512	0.227		
Roger's, Below	0.199	0.156	0.251	0.193	0.159	0.136	0.239	0.149	0.204	0.214	0.433	0.074	0.220	
South Scruggs	0.422	0.259	0.517	0.437	0.411	0.345	0.383	0.398	0.396	0.508	0.536	0.360	0.447	0.431

Table 3 Results of log-likelihood population assignments for Bradford, Crystal, Fairbanks, Below Fairbanks, Horseshoe, Above Horseshoe, Jackrabbit, Below Jackrabbit, King's Pool, Longstreet, North Indian, Peterson, Roger's, Below Roger's, and South Scruggs. Sampled populations are listed along the left, inferred populations are along the top. Populations with too few samples for assignment are listed as NA.

Pop	BF	C	F	FB	H	HA	J	JB	KP	L	NI	P	R	RB	SS
BF	8								1			1			
C		21				3									
F			14	4								1			
FB			4	11								2			
H					NA										
HA		8				0									
J							25								
JB								NA							
KP		3							3						
L										NA					
NI											9				
P		1		2								14			
R													34		
RB												2		8	
SS		1													16
Total	8	24	19	17	3	8	25	4	6	4	9	17	34	10	17

movement. The Warm Springs individuals mostly assigned to their original sample populations.

All individuals from North Indian were inferred to be from that population. One individual from

South Scruggs was assigned to the Crystal population, and the other 16 sampled individuals were

assigned to South Scruggs. Three populations had too few samples for assignment: Horseshoe

(n=3), Below Jackrabbit (n=4), and Longstreet (n=4).

A phylogeny of all sampled individuals inferred from the microsatellite data shows four

major clusters of branches, and two monophyletic clades: North Indian and South Scruggs

(Figure 10). The four clusters were organized generally as 1) the Warm Springs and Crystal, 2)

the Jackrabbit system and Crystal, 3) the Roger's system and Peterson, and 4) the Fairbanks

system. The nearest relatives to the South Scruggs clade were from Crystal and Horseshoe, with

North Indian as the closest sister clade.
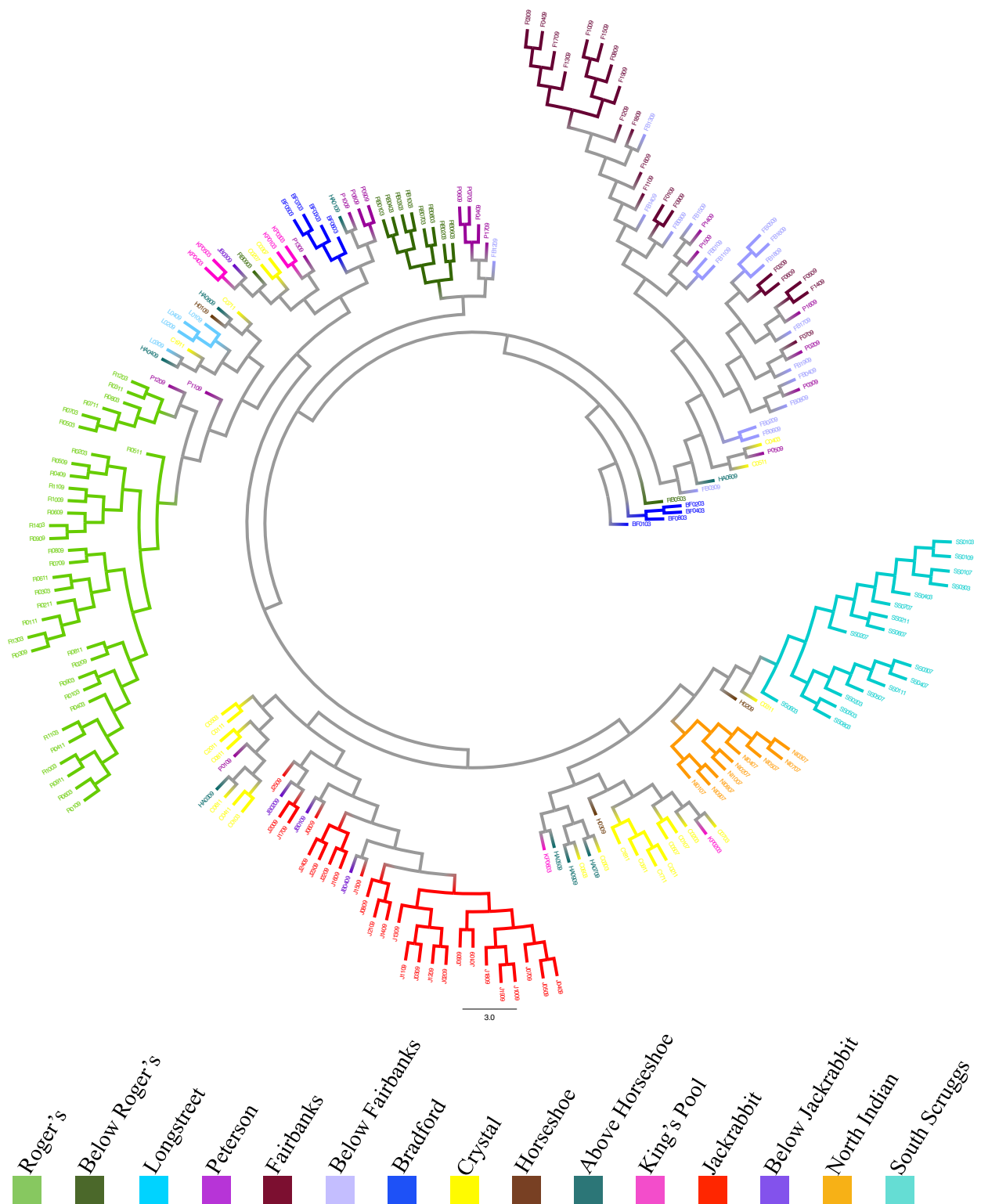
Figure 10 A neighbor-joining phylogeny inferred from the microsatellite data. Individuals are generally clustered by geographic location – northern and southern sample sites, and the Warm Springs Complex.

DISCUSSION

Invasion patterns

The results of the genetic analyses indicate that movement of *P. clarkii* between springs in Ash Meadows is congruent with hydrologic regimes. Springs and reservoirs that experience a high degree of hydrologic connectivity through perennially flowing waterways – Fairbanks, Crystal, Horseshoe, Bradford, and King's Pool – also exhibit high levels of genetic diversity and low F_{ST} values, indicating substantial gene flow. In contrast, *P. clarkii* populations sampled from North Indian and South Scruggs springs have low genetic diversity and high F_{ST} values, and these are highly isolated springs that rarely experience connectivity with the rest of the system.

The most common source of invading crayfish into the Warm Springs Complex is likely Crystal spring and outflow, based on the results of the assignment tests, the phylogenetic analyses, as well as low F_{ST} values between there and South Scruggs (.259) and North Indian (.288), in conjunction with our understanding of drainage patterns. However, the mitochondrial DNA haplotype data suggest dispersal to South Scruggs from the northern waterways as well, by way of the outflow from Peterson Reservoir. This could explain the presence of Haplotype A in South Scruggs, which is predominantly absent from the southern springs and waterways. Low genetic diversity of microsatellite genotypes in South Scruggs, and especially North Indian, suggests that these springs were colonized by a small number of individuals, and subsequent emigration from lower waterways has been rare.

Similar patterns of connectivity between springs have been demonstrated for the Warm Springs pupfish (*C. n. pectoralis*) and the Ash Meadows pupfish (*C. n. mionectes*) (Martin 2010). Evidence from microsatellite analyses of *C. n. pectoralis* suggests that connectivity within the Warm Springs (School to North Indian, South Scruggs to Marsh) has occurred at least once in

the past fifteen years, allowing movement of pupfish between springs. Based on the analysis of

potential waterways draining from the Warm Springs Complex, connectivity between South

Scruggs and Marsh springs likely happened indirectly through the outflow from Crystal spring –

which would also indicate an opportunity for crayfish dispersal from Crystal to South Scruggs at

least once in the past fifteen years. Because crayfish were first documented in the Warm Springs

Complex as early as 1983 (Weissenfluh 2010), we can estimate that that there have been at least

two dispersal events of crayfish into these isolated springs. Precipitation records from the

Community Environmental Monitoring Program (CEMP) weather station in the Amargosa

Valley indicate a handful of high precipitation events since 1999 (Figure 11). In addition,
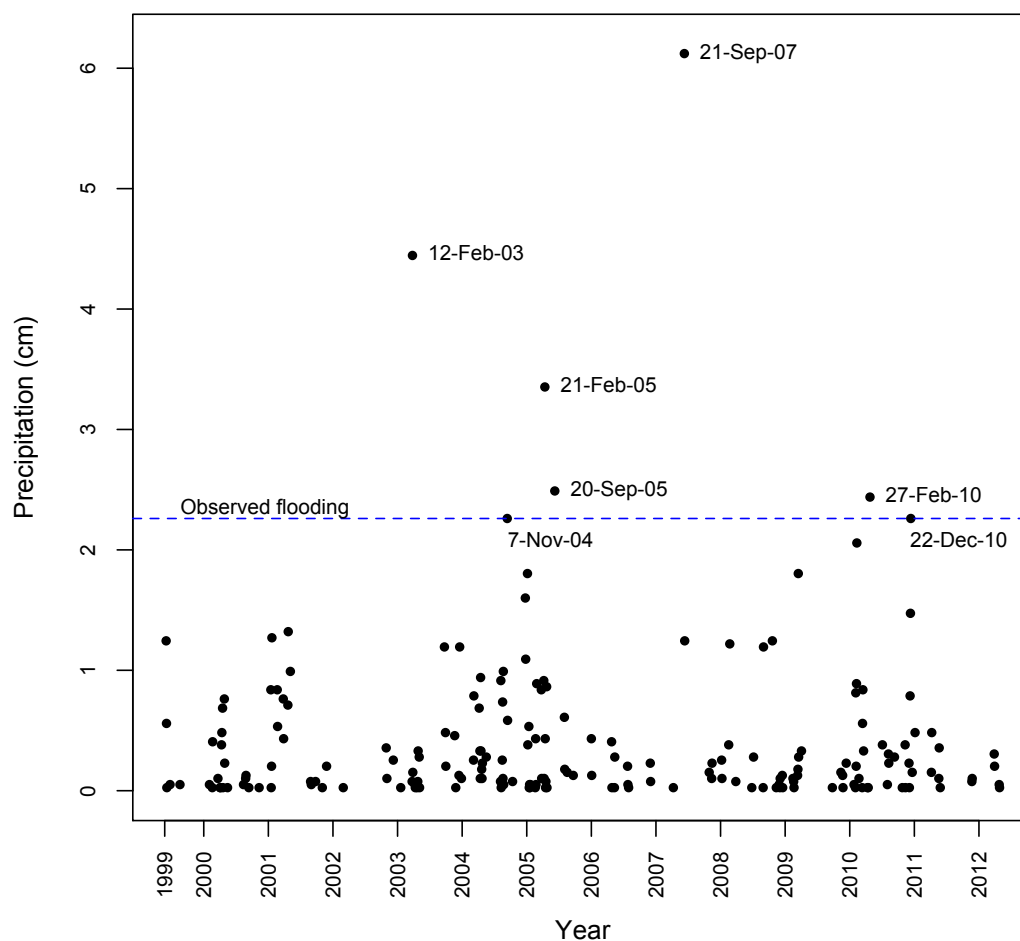
Figure 11 Precipitation in the Amargosa Valley from September 1999 through February 2012. Flooding was observed around 22 December 2010, and may have occurred an additional four times during this period with similar or greater winter precipitation events.

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flooding was documented in Ash Meadows on 22 December 2010, a day that received 0.89 inches of rain. Storms that yielded at least this much precipitation may have also caused flooding in Ash Meadows, facilitating connectivity between the Warm Springs and lower elevation springs and waterways, and providing dispersal opportunities for crayfish and other aquatic organisms. However, the height of the water table – which varies seasonally – likely influences the probability of flooding as well, with flooding being more likely during the winter months when the water table is higher.

Implications for restoration

Restoration efforts in Ash Meadows have succeeded in eradicating *P. clarkii* and other invasive aquatic species from School spring, and North and South Indian springs. These springs, and their pupfish populations, were temporarily diverted into large tanks in order to destroy crayfish habitat and eradicate their populations through desiccation. The School spring outflow was dried out for one month in the spring of 2008, and the Indian springs were desiccated for 21 months from June 2009 to March 2011. Crayfish have not been observed in the School or Indian springs since their restoration (Weissenfluh 2008; Darrick Weissenfluh, USFWS, pers. comm.). In order to prevent reinvasion of these springs, further efforts must be taken to eliminate either connectivity or source populations at lower elevations. Due to the significant cost, time, labor, and potential damage to native and endemic biota caused by the restoration process, the eradication of crayfish from all source populations in Ash Meadows does not seem feasible. Therefore, a reduction in connectivity is the best method to prevent crayfish dispersal into the Warm Springs Complex from source populations.

However, a major consideration in conserving populations of endemic fish – such as the Warm Springs pupfish and speckled dace – is the maintenance of genetic diversity through gene

flow, which requires aquatic connectivity between populations in geographically distinct springs. Accordingly, the most desirable mechanism would simultaneously prevent crayfish movement while enabling dispersal of fish. This could be achieved through directed geographical placement and the design of barriers. As was suggested by Martin (2010), one way to implement this system would be to redirect the outflows from the Warm Springs so that they connect with each other before flowing into the Crystal outflow. Based on inferences of gene flow between the northern springs and South Scruggs spring, it seems that the outflow from South Scruggs should be redirected towards the Indian Springs outflow. This would increase connectivity for the Warm Springs pupfish, while creating a single drainage from the Warm Springs Complex into the outflow from Crystal spring. A barrier could then be installed directly above the junction of the Warm Springs outflow with the Crystal outflow to prevent crayfish dispersal into the Warm Springs Complex during flooding.

CONCLUSIONS

Landscape genetics provides an informative tool that can be used to facilitate effective ecological restoration of invaded ecosystems. In this study, we demonstrated how measures of genetic distance between populations, genetic diversity within populations, as well as phylogenetic analyses can be used in conjunction with landscape connectivity metrics to infer the frequency of dispersal as well as the location of movement routes used by an invasive species. This research has direct implications for management of invasive crayfish in Ash Meadows, and also provides a case study that may be applied to other invaded systems where connectivity is rare and ephemeral. We have demonstrated how population genetic data can be used to produce *a posteriori* inferences about ecological processes such as dispersal and colonization, when such processes occur at frequencies too low for direct observational research, and when movement

corridors are temporary and geographically variable.

The importance of managing invasive species in the Great Basin and Mojave deserts is amplified by continued and accelerating anthropogenic pressures across these landscapes, in the form of water removal. To meet the demands of growing desert population centers such as Las Vegas, the Southern Nevada Water Authority and officials from satellite communities hope to increase regional water withdrawal to 2.2 billion cubic meters per year, which is estimated to exceed perennial regional aquifer yield by 250 percent (Deacon et al. 2007). This increase in pumping from wells throughout Nevada is predicted to cause a drop of nearly three meters in the aquifer that feeds the springs of Ash Meadows (Deacon et al. 2007), which in conjunction with future climate predictions of decreased precipitation in the Mojave and Great Basin deserts (Seager et al. 2007), may result in dramatic alterations to hydrologic connectivity regimes for spring systems in this area. Connectivity that is already rare and ephemeral may occur even less frequently, and for shorter durations. Although decreased hydrologic connectivity can have the positive consequence of impeding the spread of aquatic invaders, the overall effects will likely be negative: loss of aquatic, wetland, and riparian habitat, and reduced genetic connectivity for small populations of endemic biota could lead to increased rates of extirpations and eventually extinctions.

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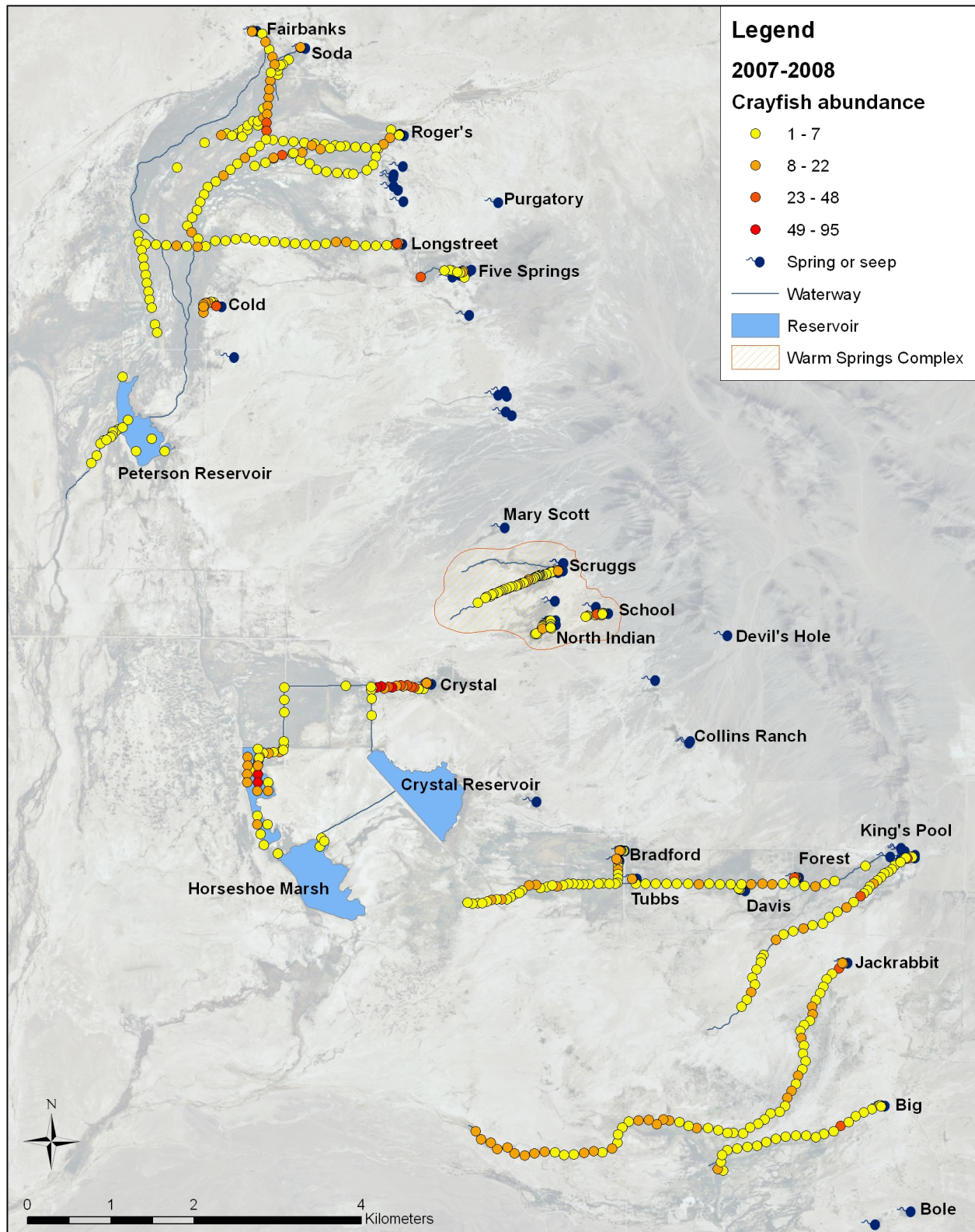**APPENDIX A: MAPS OF *PROCAMBARUS CLARKII* IN ASH MEADOWS, NEVADA**
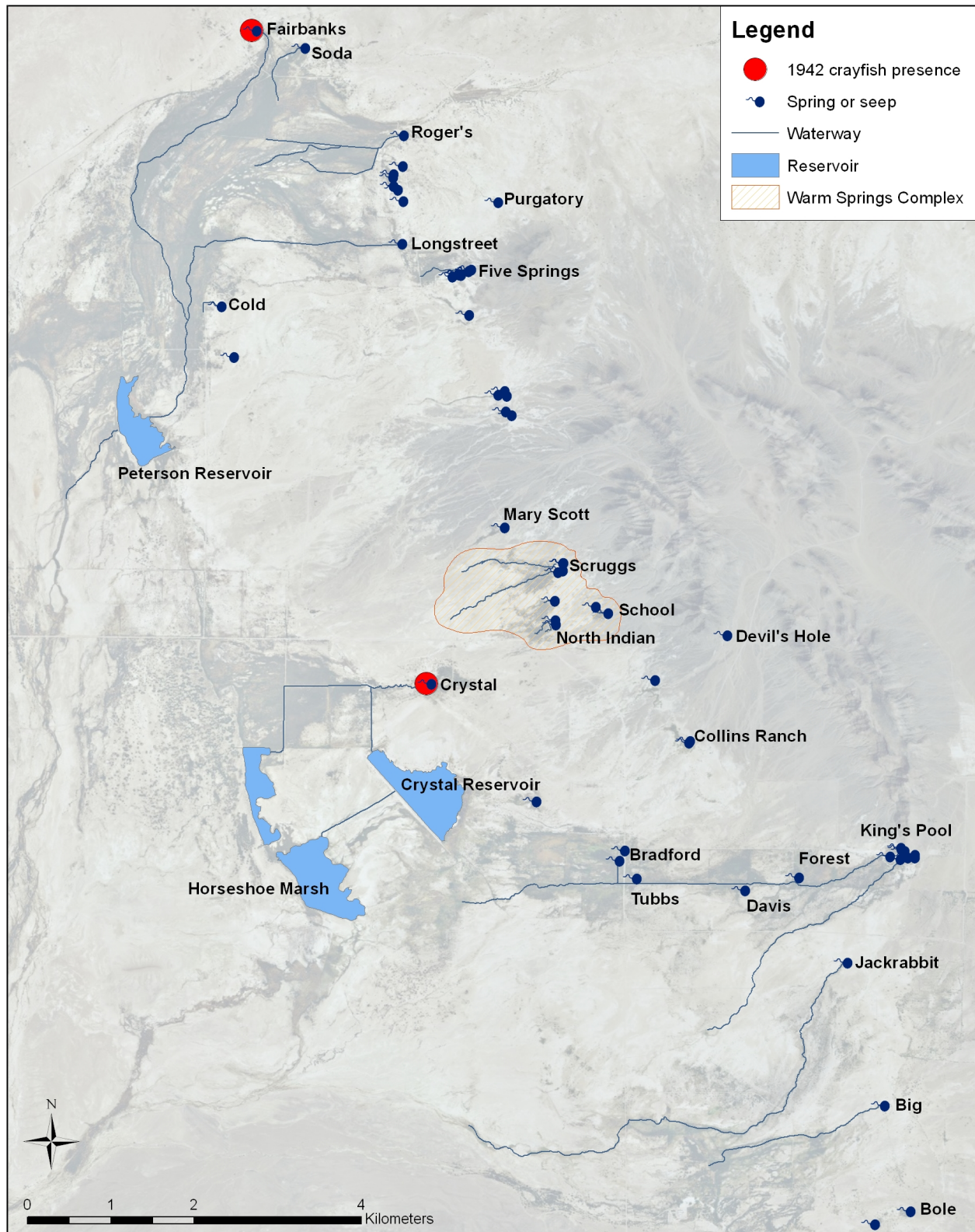
Figure 12 *P. clarkii* presence in 1942 (Robert Miller, 1942 field notes).
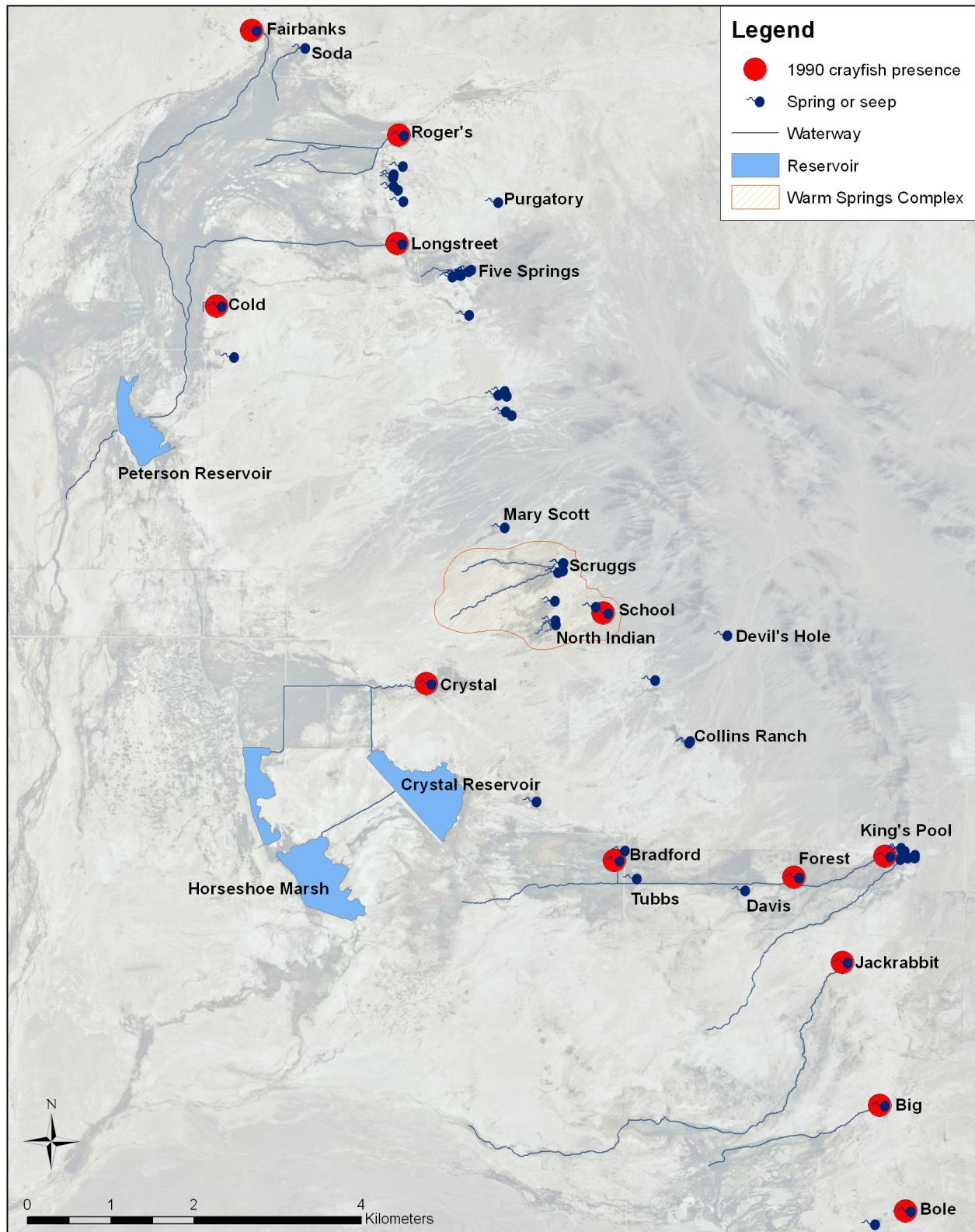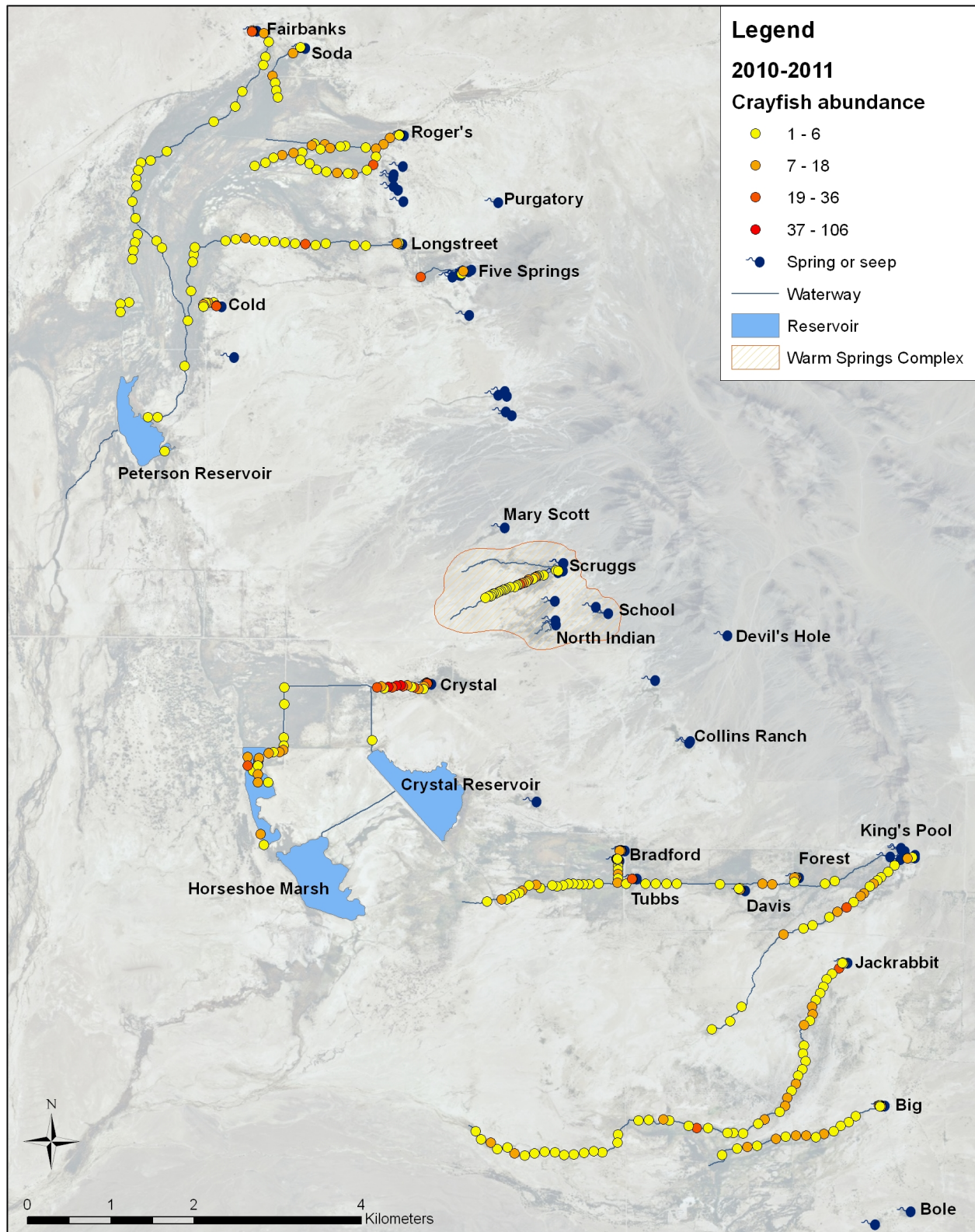
Figure 13 *P. clarkii* presence in 1990 (Sada 1990).

Figure 14 *P. clarkii* abundances in 2007-2008 (Scoppettone et al. 2011).

Figure 15 *P. clarkii* abundances in 2010-2011 (Gary Scoppettone, unpublished data).