Local Population Dynamics of the Chiricahua Leopard Frog (*Rana chiricahuensis*) a Federally Listed Frog within the Las Ciénegas National Conservation Area in Arizona.

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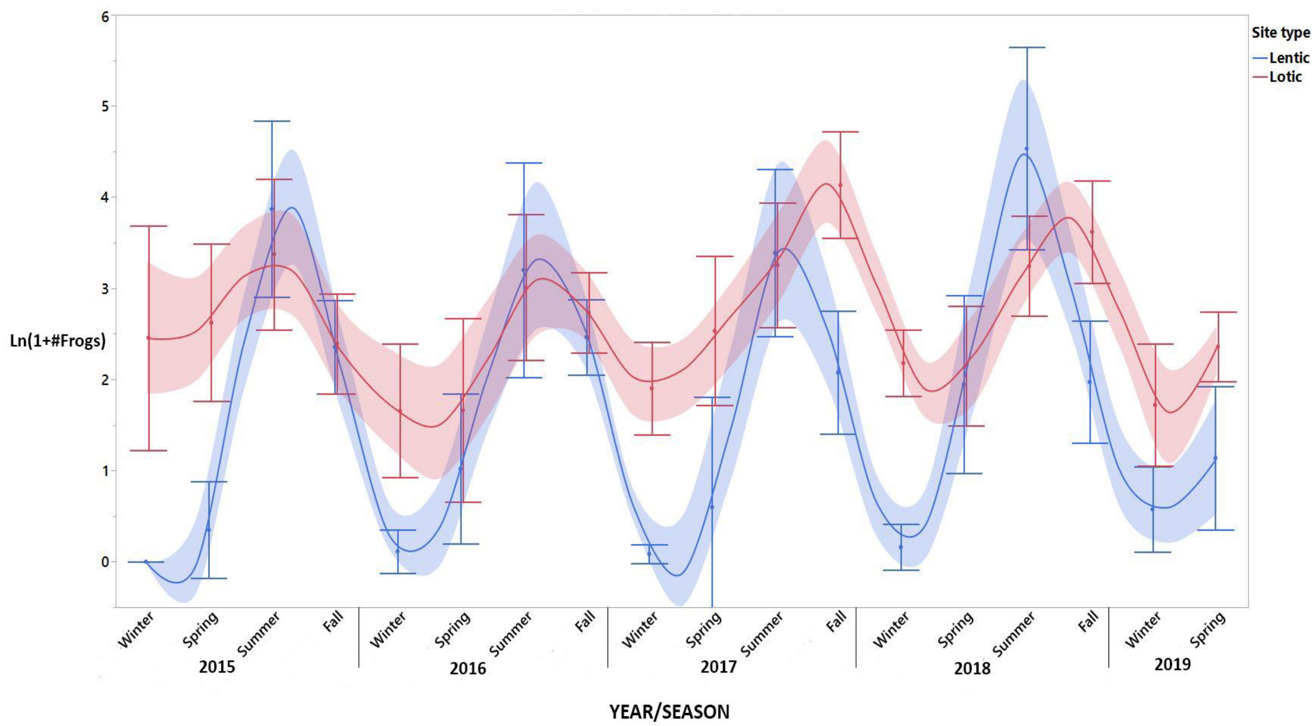

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Executive Summary

This report summarizes and analyzes the data from a five and half year study (2014-June 2019) on the status, dynamics, frog movements and response to the amphibian fungal disease, Chytridiomycosis, of the federally listed Chiricahua leopard frog populations on the Las Cienegas National Conservation Area (LCNCA) from 2014 through the spring of 2019. These populations were established through a major conservation effort begun in 2010 and ended in 2013 and has resulted in the creation of the most stable and successful conservation effort for the Chiricahua leopard frog. These efforts have resulted in the creation of a metapopulation of this frog that began with 7 breeding sites and covered 42 km2 at the end of 2013 to now consisting of 19 breeding sites and covering 84 km^2.

The major findings of this study are:

1.) Relative abundance oscillates up and down seasonally with numbers being lower in the winter months. In lentic sites frogs appear to be extirpated each winter while larvae (tadpoles) survive through the winter. Frogs overwinter in lotic sites successfully; however there is a decrease in relative abundance.

2.) Temperature differences exist between lentic and lotic sites with lotic sites fluctuating between 13 and 19°C and lentic sites fluctuate between 8 and 24°C with rapid cooling during the fall months in the lentic sites.

3.) The amphibian fungal disease chytridiomycosis is now enzootic throughout the region and every population of Chiricahua leopard frogs in the region is subjected to it. The disease is seasonal and mortality appears to be restricted to the fall and winter months. Testing for the disease should only be done during these months as during the summer detection is problematic. This disease appears to cause differential mortality depending on the flow regime of the habitat, with lentic sites suffering near to complete overwinter mortality whereas in lotic sites frog mortality is less. Temperature regime differences which may play a role in the overwinter survivorship differences between the two site types as sudden decreases in temperature have been found to reduce frog immune systems. This pattern is strong enough to encourage hypothesis testing to determine causal factors to explain this phenomenon.

4.) Chiricahua leopard frogs moved long linear-distances and colonized sites that had become extirpated due to both physical and disease related factors. Frogs were documented to move on average 7.43 km (95% CI = 6.20- 8.69 km) with a range of dispersal ranges of 1.77-15.13 km. These are the longest movements recorded for this species and have direct implications for the management and recovery of the species.

5.) A Chiricahua leopard frog metapopulation has been created on the LCNCA that has expanded in size since its inception in 2013. The initial area of 7 breeding frog sites created in 2013 covered an area of 42 km^2, as of the date of this report the number of breeding populations has grown to a grouping of populations that fulfill the following conditions: i) populations are geographically discrete; (ii) mixing of individuals between populations is less than that within them. The importance of this is that populations are able to go extinct and then be rescued by recolonization from neighboring sites, minimizing management efforts to insure Chiricahua leopard frog persistence despite serious threats such as chytridiomycosis. A metapopulation however is not resistant to bullfrog invasion.

6.) If the Chiricahua leopard frog metapopulation that has been created on the LCNCA remains on the trajectory that it has been for the past 5.5 years, as is described herein, then its future persistence in face of serious threats is optimistic. However, the metapopulation has benefitted by the intense monitoring and bullfrog removal efforts that are described in this report. These efforts end with the submission of this report with no future funding to continue. As a result if bullfrogs reinvade or a new more virulent strain of chytridiomycosis arises, more than likely, these threats will not be addressed early enough to be easily sorted out and the successes achieved will be reversed.

Introduction

In 2013 the Frog Project, a group of researchers and conservationists from the University of Arizona (UA), the Cienega Watershed Partnership (CWP), Bureau of Land Management (BLM) and private citizens finished their work to restore aquatic habitat with funding from the National Fisheries and Wildlife Foundation on the Las Ciénegas National Conservation Area (LCNCA). This project, funded by National Fisheries and Wildlife foundation, awarded to CWP that in turn funded a group of individuals from the private sector and the University of Arizona known as The Frog Conservation Project (http://frog.cienega.org/). The Frog Conservation Project was primarily focused on restoring aquatic habitat for the Chiricahua Leopard frog (*Rana* (*Lithobates*) *chiricahuensis*, CLF) by eliminating American Bullfrogs (*Rana* (*Lithobates*) *catesbeiana, RACA)* and creating permanent frog habitats throughout the LCNCA.

The invasive American bullfrog (*Rana catesbeiana*/RACA) has been a major cause of population declines in the threatened Chiricahua leopard frog (*Rana chiricahuensis*/RACH) for decades in southern Arizona. Landscape scale eradication of RACA had been accomplished in several areas in southern Arizona since 2009 (Hall 2017, Surhe, 2010). Following bullfrog eradication, RACH populations return to historically and newly occupied sites through both natural recolonization and translocation. As a result of these preliminary successes the intent of The Frog Conservation Project was to create a metapopulation (Levins, 1969, Hanski & Gilpin 1991, Baguette & Mennechez 2004) of CLF on the LCNCA. This effort began in 2010 and its success and has been described in detail within the report: *Restoring Leopard Frogs and Habitat in Sky Island Grasslands (Arizona) Final Report* (Rosen et al, 2013). This report describes how the entire upper Cienega Creek watershed was surveyed for nonnative aquatic fauna, bullfrogs were removed from the areas occupied on the LCNCA, habitats were created and frogs were translocated into these sites, and how a bullfrog buffer zone was established to protect the LCNCA from further invasions from neighboring bullfrog populations. It also described the efforts to control northern crayfish (*Orenectes virilis*) from Clyne Pond and Clyne Spring. Because of the Frog Project's efforts the number of CLF populations increased from one to eight in three years. The reader is encouraged to read the entire report as in addition to providing details regarding the efforts above the report covers the history of leopard frog distribution and nonnative predator impacts.

Realizing that the successes of the 3 year habitat restoration effort needed to be monitored to track population dynamics as well as bullfrog threats from neighboring populations, funding was provided by BLM and Sky Island Alliance to accomplish the monitoring. The major emphasis in this early period (late fall through Early Winter 2014) was to monitor the buffer zone in the Elgin area and to insure the newly cleared frog habitats (including two perennial reaches of Cienega Creek, Headwaters and Cold Spring) remained bullfrog free. The buffer zone, an area that approximately followed the length of Arizona Highway 82 from the eastern side of the community of Sonoita to a midpoint between the Mustang and Whetstone Mountains in Rain Valley, is monitored primarily twice, post monsoon. We used this time to begin to design a CLF long-term monitoring program that included bullfrog control and monitoring. This effort has evolved over time.

Around the late fall 2013 concern over the fungal disease chytridiomycosis spreading into the populations of CLF on the LCNCA came to the forefront. Large die-offs from chytridiomycosis (verified by genetic swabbing) were observed in a large population of CLF (West Tank, UTM: 522718/3516870 WGS 84) on neighboring Coronado National Forest, a population that was only 7.4Km from the nearest population on the LCNCA (Empire Wildlife Pond). Chytridiomycosis, the disease caused by the chytrid fungus *Batrachochytrium dendrobatidis (Bd)* has been implicated in global amphibian declines (Berger et al., 1998, Lips et al., 2006, Rachowicz et al., 2006, 2013, Byrne et al., 2019). In Arizona Bd has been linked with die-offs and declines in populations of CLF and has been recognized to be a major threat in the recovery of CLF (Sredl and Jennings, 2005, USFWS, 2007). Although Bd has been shown to be seasonal in its lethality (Voyles et al., 2012, Berger et al., 2004) this has never been confirmed for CLF in Arizona.

Additional funding was provided by BLM and CWP for years 2014 and 2015 to: maintain the LCNCA free of harmful exotic species, monitor and observe the recovery progress of CLF populations on the LCNCA and monitor the status, ecology and seasonality of the emerging disease chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (Bd). The results of these efforts are summarized in the report, 2014-2015 Frog Project Accomplishments at Las Ciénegas National Conservation Area, Arizona (Hall et al, 2015). This was a defining time for CLF research on the LCNCA as the long-term monitoring methodology was established, data management standardized as well as field protocols for disease monitoring, and analysis. Several wildlife ponds were established with CLF translocated into Antelope and Bald Hill wildlife ponds from a site in the Canelo Hills. Natural colonization by CLF to new sites was documented and crayfish eradication efforts in Clyne Spring and Clyne pond continued.

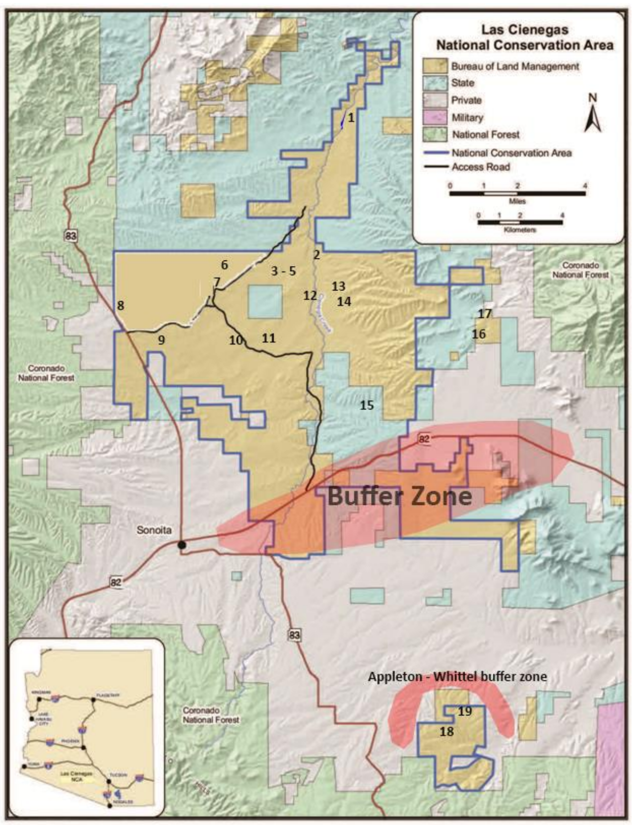
In 2015 funding was sought and obtained to continue the emphasis of monitoring the CLF populations on the LCNCA so to further increase the patterns of how these populations responded to seasonality, flow regime and disease, monitor and remove any bullfrogs in the buffer zone for 2016 through2019. In addition to describing and synthesizing the data collected during 2016 – 2019 this report will synthesize the data collected regarding population dynamics of the CLF on the LCNCA since the long-term monitoring began in 2014 through the spring of 2019.

Study Area(s)

The Las Cienegas National Conservation Area was established in 2000 and consists of approximately 170 km^2 of grasslands, riparian gallery forests, marshlands and mesquite bosques (woodlands). It was established to, "—In order to conserve, protect, and enhance for the benefit and enjoyment of present and future generations the unique and nationally important aquatic, wildlife, vegetative, archaeological, paleontological, scientific, cave, cultural, historical, recreational, educational, scenic, rangeland, and riparian resources and values of the public lands described in subsection (b) while allowing livestock grazing and recreation to continue in appropriate areas, there is hereby established the Las Cienegas National Conservation Area in the State of Arizona." (Public Law 106–5382000, Federal Registry, 2000).

There are ten major sites that were monitored from the winter of 2014 continuously until the summer of 2019. These major sites are: Cinco Wildlife Pond, Clyne Spring, Clyne Pond, Cold Spring reach of Cienega Creek, Cottonwood Wildlife Pond, Empire Gulch Spring, Empire Wildlife Pond, Gaucho Wildlife Pond, and Headwaters reach of Cienega Creek and Road

Canyon Wildlife Pond. There are other sites on the LCNCA that were monitored but because they either dried consistently and/or never housed reproducing CLF populations they were not as consistently monitored or included as major sites. These sites include: Maternity Wildlife Pond, Spring Water Wetland pond, Crescent, Egret and Heart Wetland Ponds, and Hilton Tank. Two sites, Antelope and Bald Hill were monitored and administered by the Appleton-Whittell Research Ranch of the National Audubon Society and are not included in the major analysis of this report. Site locations within the LCNCA are illustrated in Map 1. Other sites became colonized in the past few years and have become breeding sites but these sites were not monitored monthly and are not included in the detailed analysis herein. Detailed site descriptions of the major sites are given below. All Universal Transverse Mercator (UTM) are provided in datum: WGS84.



Map 1. Major site locations discussed in text: 1.) Cold Spring Reach, 2.) Spring Water Wetland Pond, 3-5.) Crescent, Egret, Heart Wetland Ponds, 6.) Karen's Tank, 7). Empire Gulch Spring, 8.) Empire Wildlife Pond, 9.) Maternity Wildlife Pond, 10.) Cottonwood Wildlife Pond, 11.) Gaucho Wildlife Pond, 12.) Headwaters Reach, 13.) Cinco Wildlife Pond, 14). Hilton Tank, 15). Road Canyon Wildlife Pond, 16) Clyne Pond, 17) Clyne Spring, 18) Bald Hill Wildlife Pond, 19) Antelope Wildlife Pond. Other breeding populations not shown are State Tank and Sue's Tank in the Clyne/Sands Ranch lands.

CINCO WILDLIFE POND (UTM: 540541 / 3516523)

This wildlife pond was constructed in 2013 and frogs were translocated into it in that year. A pipe rail fence surrounds the pond to exclude cattle. The pond is watered by a solar powered well that has been problematic since the pond was built. Often the output of the well is enhanced by the use of an electric generator to provide enough water for both the wildlife pond and watering cattle when the pasture is used for grazing. The pond is approximately 770m^2 (0.19 acres) in area and its maximum depth is 1.5 meters. Both area and depth have varied significantly over the past 6 years and the pond was close to becoming dry at least twice. Cottonwood, Willow and Ash trees have begun to grow around the perimeter so that only the north side of the pond doesn't have significant shading. Aquatic vegetation is throughout the pond with *Potamogeton foliosus*, *Chara sp.* and *Hydrocotyl sp.* covering almost the entire submerged and surface of the pond particularly in the late summer and fall. The perimeter of the pond supports dense stands of *Eleocharis sp*., grasses and forbs. This pond has never had aquatic non-natives since its construction. Desert pupfish (*Cyprinodon macularius*) where translocated into this pond and were extant throughout the study. Cinco Wildlife Pond was a major lentic site surveyed repeatedly since 2014 for this study.

CLYNE POND (UTM: 546737 / 3514461)

This is an earthen tank constructed by the Clyne family since at least the 1950's as a water source for their cattle. Local rancher "Doc" Clyne has related that the pond had CLF prior to the introduction of Largemouth bass, Bluegill sunfish, crayfish and American bullfrogs sometime in the late 1960's-70's. In 2010 when habitat restoration for the FROG Project began there were breeding populations of bullfrogs and crayfish in the pond. Bullfrogs were removed by Summer of 2010 and the pond was drained and left dry twice (November 2010 – July 2011, and Spring 2012 – August 2012) to eradicate crayfish. This effort was successful in eradicating crayfish from this pond. The pond is watered by runoff and varies in area from 323 m^2-4,532 m^2 (0.08-1.12 acres) and depth varies from 0.3 to 4.5 m. The lowest depth occurs during the summer drought from mid-June to August depending on summer rains. There is a small grove of willows at the southeastern end of the pond that is quite dense. Perimeter vegetation varies from grasses to forbs and aquatic vegetation is generally *Potamogeton sp.* and forbs, largely smartweed (*Polygonum sp*.)

Gila chub and Gila topminnow were introduced into the pond in 2016. Gila topminnow have thrived in this pond. The Gila chub however are considered extirpated from the pond possibly because the pond dried to only 13cm depth in the summer of 2018 and likely became anoxic. Clyne Pond was a major lentic site surveyed repeatedly since 2014 for this study.

CLYNE SPRING (UTM: 547043/ 3514892)

A small spring/seep that is located 480 m (0.3 miles) upstream from Clyne pond. During summer drought this spring consists of two small pools both approximately $10m^2$. Despite being so small and with intense cattle usage in the summer the springs have not dried since surveys began in 2010. The pools support *Typha* sp., *Potamogeton sp.* as well as aquatic algae (*Chara sp*.). Seep willow (*Baccharis salicifolia)*, mesquite and bunch gasses are also along the perimeter of the two pools. A population of crayfish (*Orconectes virilis*) was eradicated in 2016 after intense trapping that began in 2011. No fish species inhabit these pools. Clyne Spring was a major lotic site surveyed repeatedly since 2014 for this study.

COLD SPRING REACH (UTM start: 540305 / 3524931, end: 539661 / 3523720)

This 1,465m (0.91 mile) perennial reach of Cienega Creek begins just above the confluence of Sanford Canyon (UTM: 540287 / 3524947 and goes upstream to just below the confluence of Forty-nine wash (UTM: 539661 / 3523720). This section of Cienega Creek has perennial water for the first 900m when the stream becomes ephemeral and perennial water is found in 6 permanent pools. The last of which, below the confluence of Forty-nine wash, is the last permanent water upstream until the Headwaters reach. A large off channel spring flows into Cold Spring that gives the reach its name. In addition, the lower section of Mattie Canyon, a perennial stream, enters Cold Spring reach towards the end of the reach.

The channel is made up of several features including deep slot pools, glides, and short shallow runs. During the late spring into fall months glides and runs will be filled with emergent vegetation such as rushes and reeds and pools are often covered with a nearly complete layer of duckweed (*Lemna sp.)* that is periodically cleared by flooding. In the middle of this stream reach is a gaining area where a spring (Cold spring itself) joins Cienega Creek in a length that is 300m long.

The riparian galley forest is relatively open with Cottonwoods, Willows, Little-Leaf Ash (Fraxinus greggi), and Velvet Ash (*Fraxinus vetulina*) being common. Arizona Walnut *(Juglans major),* Netleaf Hackberry (*Celtis laevigata* var. *reticulate), Desert Elderberry (Sambucus nigra), Alligator Juniper (Juniperus deppeana)* and Seep willow (*Baccharis salicifolia)* are also present. Common aquatic vegetation includes large stands of Typha sp., Scirpus sp., Eleocharis sp.. Other dominant plants are: Floating marshpennywort, *Hydrocotyle ranunculoides* Smooth Bur Marigold, *Bidens laevis* and Potamogeton sp.. Native fish species Gila chub (*Gila intermedia)* , Gila topminnow (*Poeciliopsis occidentalis*) are found throughout the perennial stream reaches and Longfin dace (*Agosia chrysogastor*) inhabit the area particularly around the confluence with Mattie Canyon. The uppermost pools in this reach are fishless. Sonora mud turtles (*Kinosternon sonoriense*) are abundant throughout. Cold Spring Reach was a major lotic site surveyed repeatedly since 2014 for this study. Unlike the Headwaters Reach of Cienega Creek pools are limited in the Cold Spring Reach. As a result the area was not mapped as extensively as Headwaters Reach.

COTTONWOOD WILDLIFE POND (UTM: 536009 / 3514050)

One of the oldest and largest of the Wildlife Ponds on the LCNCA Cottonwood Wildlife Pond it originally was an earthen livestock tank. Solar panels were installed along with a perimeter pipe rail fence by 2012. Chiricahua leopard frog larvae that originated in Empire Gulch Spring were translocated into the pond in October 2012. Cottonwood Wildlife pond is 1,262m^2 (0.3 acres) in area and has a maximum depth of 1.8 meters. The pond has a stable water source and although its surface area does fluctuate it does not vary significantly. Overfill does flow into an earthen cattle tank that is directly beside the wildlife pond. The pipe rail fence is effective in keeping cattle out of the wildlife pond.

Canopy cover is scarce over this pond and only a single willow tree, several small mesquites and Seep willow (*Baccharis salicifolia)* shade the pond. The pond is shallow enough to allow *Potamogeton sp*., Floating marshpennywort (*Hydrocotyle ranunculoides)*, to cover the entire pond. The ponds perimeter is lined with *Eleocharis sp,* grasses and forbs including *Bidens laevis*. During early summer of 2019 the water level of the pond dropped to the lowest levels since the wildlife pond was built. This was due to pump failure and replacement during the driest time of the year. The pond never dried and flow was reestablished. Desert pupfish (*Cyprinodon macularius*) where translocated into this pond and were extant throughout the study. Cottonwood Wildlife Pond was a major lentic site surveyed repeatedly since 2014 for this study.

EMPIRE GULCH SPRING (UTM start: 534172 / 3516930, end: 534350 / 3517023)

A natural spring run that begins at a spring head located at UTM: 534095 / 3516967 and runs downstream approximately 200 m before the permanent run section begins to become shallow and dries during summer droughts. The spring run averages 2m in width. The CLF became established in the spring run sometime in the early 1990's (Jeff Simms, personal communication) and was the only reproducing CLF population on the LCNCA when CLF restoration began in 2010. Two egg masses were taken from Empire Gulch Spring in May 2011 and raised in Phoenix Zoo facilities for translocation into Wildlife Ponds being prepared on the LCNCA. In 2012 sections of 5 egg masses were taken and raised in facilities in the University of Arizona and were translocated into LCNCA sites in 2013. All of the frogs that were translocated into the wildlife ponds on the LCNCA came from these 7 egg masses originating in Empire Gulch Spring.

Empire Gulch Spring has a riparian gallery forest composed primarily of: Fremont's cottonwood (*Populus fremontii*), Goodding willow (*Salix gooddingii*), yew willow (*Salix taxifolia*), Seep willow (*Baccharis salicifolia)*and Arizona Walnut (*Juglans major*).Common plants near and within the stream bank and stream perimeter include: spikerush (*Eleocharis spp*.), rush (*Juncus sp.*), yerba mansa (*Anemopsis californica*), watercress (Nasturtium officinale), wild mint (Mentha arvensis) and duckweed (Lemna sp.).

Water flow varied from 6-45 gpm during the years 2007 – 2018 (Simms, 2018). Similar to the other lotic environments on the LCNCA the channel is made of several features including deep slot pools, glides, and short shallow runs. During the late spring into fall months glides and runs will be filled with emergent vegetation such as rushes and reeds and pools are often covered with a nearly complete layer of duckweed (*Lemna sp.)* that is periodically cleared by flooding. Empire Gulch Spring was a major lotic site surveyed repeatedly since 2014 for this study.

EMPIRE WILDLIFE POND (UTM: 530141 / 3516367)

Another of the oldest wildlife ponds on the LCNCA Empire Wildlife pond was a former cattle tank that was transformed into a wildlife pond in 2011. A pipe rail fence was constructed around the pond perimeter and cattle drinkers were constructed outside of the fence. The pond received over one thousand tadpoles in October 2012. The pond is 702m^2 (0.17 acres) making it the third largest wildlife pond after Cottonwood and Cinco Wildlife ponds. Water depth is 1.4m. Because the water supply is a line powered nearby well water levels have remained relatively stable over the years.

Canopy cover is moderate over this pond with mesquite and Goodding willow (*Salix gooddingii*) providing perimeter shade over the pond. The pond is shallow enough to allow *Potamogeton spp* to cover the entire pond. The ponds perimeter is lined with *Eleocharis spp,* grasses and forbs including *dense stands of Bidens laevis* in the late summer and fall months.

Desert pupfish (*Cyprinodon macularius*) and Gila topminnow (*Poecilliopsis occidentalis*) where translocated into this pond and both species were extant throughout the study. Northern Mexican garter snakes were translocated into this pond in 2014 and they have been observed in the pond every year since. Pump failed during the dry season of 2019 with water levels dropping dangerously shallow. Both pump and electrical infrastructure were replaced and water levels restored without significant loss of species. Empire Wildlife Pond was a major lentic site surveyed repeatedly since 2014 for this study.

GAUCHO WILDLIFE POND (UTM: 537343 / 3513951)

This wildlife pond was not built until 2013 and frogs weren't translocated into it until May 2014 when tadpoles from Empire Wildlife pond were released into the new pond. Gaucho is one of the smallest wildlife ponds at $300m^2$ (0.08 acres) in area. The pond is watered by a solar well and the pond is 2m deep. Relatively free of any canopy cover, the pond is generally covered with mats of *Potamogeton spp* and *Chara spp.*. The perimeter of the pond is covered in grasses, Eleocharis spp. as well as forbs such as *Bidens laevis*. Desert pupfish (*Cyprinodon macularius*) and Gila topminnow (*Poecilliopsis occidentalis*) where translocated into this pond and both species were extant throughout the study. Gaucho Wildlife Pond was a major lentic site surveyed repeatedly since 2014 for this study.

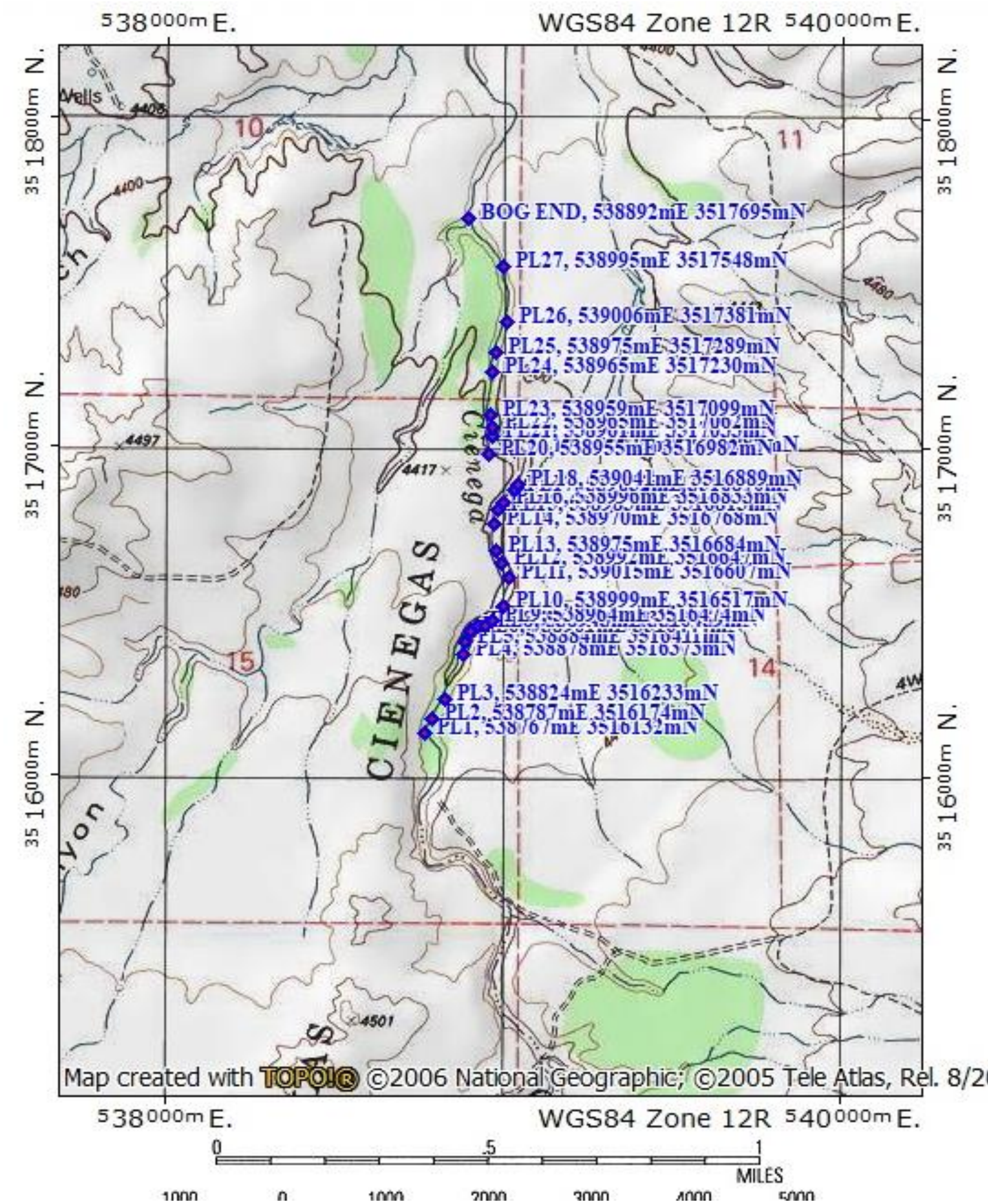
HEADWATERS REACH (UTM start: 538767 / 3516132, end: 538952 / 3517625)

The furthest upstream perennial reach of Cienega Creek, Headwaters is 1,693 m (1.05 miles) long with an average width of 2.1 meters. The channel is made of several features including deep slot pools, glides, and short shallow runs. The furthest upstream section of the reach consists of three small slot pools that usually are perennial except for years of extreme drought. The stream proper actually begins immediately after pool #4 where a surface spring begins. The stream is fed by underwater springs throughout the Headwaters but noticeably in the areas between pools 6 & 8, 11 & 12, 15 & 16, 23 &24 and 24 and 25. Map 2 illustrates the Headwaters reach and the pool locations. Because the pools are grouped closely together in some areas of the reach a table with the pool UTM's is provided in Appendix A.

Empire Gulch Spring has a dense riparian gallery forest composed primarily of: Fremont's cottonwood (*Populus fremontii*), Goodding willow (*Salix gooddingii*), Seep willow (*Baccharis salicifolia),* Netleaf Hackberry *(Celtis reticulate),* Desert Elderberry *(Sambucus nigra),* and Alligator Juniper *(Juniperus deppeana)* are also present. Common plants near and within the stream bank and stream perimeter include: cattail (*Typha spp.*) bulrush (*Scirpus spp*.) spikerush (*Eleocharis spp*.), rush (*Juncus sp.*), Yerba Mansa (*Anemopsis californica*), watercress (Nasturtium officinale), wild mint (Mentha arvensis) and duckweed (Lemna sp.). During the late spring into fall months glides and runs will be filled with emergent vegetation such as rushes and reeds and pools are often covered with a nearly complete layer of duckweed (*Lemna sp.)* that is periodically cleared by flooding.   Headwaters Reach was a major lotic site surveyed repeatedly since 2014 for this study.

ROAD CANYON WILDLIFE POND (UTM: 540853 / 3511400)

This wildlife pond has had a history of ranid frog occupancy prior to having CLF translocated into it. Prior to 2008 this site was a cattle tank that was fed by well water powered by solar panels. Bullfrogs occupied the site until 2008 (Dennis Caldwell per. com.) when the pond was dried. By the time it was first surveyed by the author in 2009 and thriving population of Lowland leopard frogs (*Rana yavapienesis*, henceforth: LLF) occupied it.   Lowland leopard frogs were salvaged by Arizona Game & Fish and the pond was dried and deepend by the livestock operator. After the deepening, the seal was lost and the pond would no longer hold water. In 2013 the Frog Project reconfigured the pond with a EPDM liner and planted with aquatic plants. A pipe rail fence already existed around the wildlife pond and the adjacent cattle pond outside of this fence was reshaped also. This pond is watered by a solar powered well and shares the water with the cattle side. CLF tadpoles were translocated into it in May 2012. The pond has no tree canopy and the perimeter vegetation consists of bunch grasses, spike rush (*Eleocharis spp.)* and Floating marshpennywort *(Hydrocotyle ranunculoides).* The pond always has a dense surface layer of *Potamogeton* spp., Chara spp. and filamentous algae. Water levels for the past 7 years have remained stable. Road Canyon Wildlife Pond was a major lentic site surveyed repeatedly since 2014 for this study.



Map 2. Headwater pools. Pool numbers are shown with associated UTM for each.

OTHER SIGNIFICANT SITES (not included in major analysis)

CRESCENT (538114/3517839), EGRET (538069/3517755) and HEART WETLAND (538051/3517853) PONDS

These three constructed ponds are within the lower Empire Gulch Cienega, a swampy marsh type habitat. All three ponds are spring and runoff fed ponds and are located 2Km upstream, in the stream course, from the confluence with Cienega Creek. The ponds were constructed in 2013 along with a large pipe-rail fence that encloses the 140, 925m2 (35 acres) of cienéga the ponds are located within. These fences exclude grazing cattle. As a result the grasses and forbs within the enclosure are often dense and reach towering heights particularly the nonnative Johnson grass (Sorghum halepense) that is common throughout the enclosed area. Sacaton (*Sporobolus wrightii*), Tobosa (*Pleuraphis mutica)* and other native and nonnative grasses are common as is spikerush (*Eleocharis spp*). Around the perimeter of each pond vegetation consists of bunch grass, spike rush, giant rush (*Scirpus spp*.), tule (*schoenoplectus americanus)* and cattails (Typha spp.). Floating vegetation is abundant throughout each of the ponds mostly consisting of Potamogeton spp. and algae. Yearly clearing of scirpus and tule have been implemented to keep ponds open and to enhance conditions for Huachuca Water Umbel (*Lilaeopsis schaffneriana* spp. *recurva)* . Gila topminnow and Desert pupfish are common in all ponds. Chiricahua leopard frogs were never translocated into these ponds.

KAREN'S TANK (534391/3517373)

Karen's Tank is an earthen livestock pond that is watered primarily by well water. Prior to 2016 the tank was only used for livestock watering. When cattle were removed from the pasture the water to the tank was shut off drying the pond. A single adult CLF was found in this tank in 2013 but the tank was allowed to stay watered only until 2016. The tank, when full, has an area of 915m2 (0.23 acres) but has been allowed to shrink to half that size before water is restored. The pond when full is surrounded by bunch grass and seep willow, and the surface of the pond is covered with Potamogeton sp.. Since 2016 this tank has been allowed to remain wetted and Chiricahua leopard frogs have colonized and bred in this tank since then.

MATERNITY WILDLIFE POND (UTM: 531704 / 3514068)

This wildlife pond was constructed in 2012 by diverting water permanently from a well-fed storage tank to an existing earth livestock pond and constructing a pipe rail fence around it to exclude cattle. Drinkers were provided for livestock use outside of the fence perimeter. As soon as the pond became watered permanently in the summer of 2012 frogs from an unknown source colonized the pond. The storage tank was used as a holding site for 200 CLF larvae that were head started at the Phoenix Zoo. The pond was shallow (< 1m depth) yet had a large breeding population of CLF inhabiting it for the two years 2013-2014. However, by the fall of 2014 the pond failed to have any depth as it had become choked with cattails, grasses and forbs and no frogs have been detected there since. The pond needs major renovation to be a viable habitat.

HILTON TANK AND WILDLIFE DRINKER (UTM: 541475 / 3514505)

This is a stock tank filled by the overflow from a fenced wildlife drinker watered by a solar well, frogs were found by Dennis Caldwell in the drinker in early 2016. The drinker and cattle tank were surveyed again in February 2016 and frogs were found in both. By April there were an estimated 50 frogs in the cattle tank and by June the pond had dried. The pond dried because of infrastructure failure. The water supply remains problematic and although frogs have colonized every year since they do not appear to reproduce or persist throughout the year. The stock tank is unfenced and heavily disturbed by livestock annually.

ANTELOP (UTM: 545962 / 3497053) AND BALD HILL (UTM: 545463/ 3496315) WILDLIFE PONDS

During 2015 surveys, we discovered a Chiricahua leopard frog population in the Canelo Hills, south of LCNCA. Stock from this population was introduced to the Audubon Society's Appleton-Whittell Research Ranch (which comprises a mix of private and BLM lands), where the species went extinct over a decade ago. The new population sites are concrete tanks supported by wells on Bald Hill, where the BLM had made renovations designed for desert pupfish and leopard frog populations. At each site 150 tadpoles were introduced early in the summer 2016 and breeding populations remain there since.

BUFFER ZONE

To insure that lands cleared of bullfrogs are protected from bullfrog sources in the Elgin/Babocomari valley, a buffer-zone was created in a swath of land from Sonoita east along Hwy 82 to the Mustang Mountains. Any bullfrogs found in the buffer zone are removed and this has proven to be an effective protective measure. Whereas biologists from the University of Arizona have been monitoring this zone since 2013, Arizona Game and Fish Department biologists along with the University of Arizona have expanded this buffer since 2015 in anticipation of the bullfrogs in the Babocomari Ranch lands being eradicated. In addition, personnel from the Appleton-Whittell Research Ranch and USFS have been removing bullfrogs from areas within the project area to protect CLF populations from bullfrogs coming from the project area. Map 1 illustrates the current extent of the buffer zone.

Methods

Count data for estimating relative abundance data was collected using the standardized method of Visual Encounter Surveys (VES) as outlined in the CLF Recovery Plan (USFWS, 2007). Typically, we carefully approached aquatic habitat scanning with binoculars or powerful flashlights, listened for calling amphibians, and walked near the edge of the water to detect frogs by either direct viewing or detecting their leaps into the water. Generally, stream banks and pond margins were thoroughly searched and rock ledges and clumps of emergent aquatic vegetation such as sedges, rushes, and bunch grasses were probed. Additional data was obtained by dip netting and seining for tadpoles as needed to determine reproduction. If there was any question as to the identity of an amphibian, we attempted to wait for the animal to resurface from the water and identified it using hand collection or visually, at times with binoculars. Surveys were done primarily at night and generally once a month at each site however in some years, sites were visited less due to weather, fire or scheduling issues. We recorded data collected in the field using either datasheets created specifically for this study and later (2016) we used the Chiricahua leopard frog VES survey sheet as is currently used in all CLF VES surveys. Data collected included: Date, Site name, UTM (WGS 84), Width (m), Length (m), ambient temperature (C°), water temperature (C°), relative humidity, water pH, survey start time, survey end time, habitat type, water source, % floating vegetation, % submerged vegetation, % perimeter vegetation, % bank vegetation, # adults, # juveniles, # larvae, # egg masses, # other aquatic reptiles or amphibians.

Any frogs larger than 60mm snout vent length were classified as adults. This had been determined and confirmed by mark/capture/recapture studies (Hall, unpublished data) on frogs within the LCNCA. Although to the inexperienced the visual determination of biphasic age class is relatively simple to the trained eye. Generally speaking however tiny frogs (metamorphs, frogs recently transformed from larvae to frogs) are easy to determine.

Universal Transverse Mercator (UTM) coordinates were recorded with Garmin GPS units using the WGS84 datum. Hanna Combo™ pH & EC meters were used to record water temperature, pH, conductivity, and total dissolved solids. A Kestrel pocket weather meter was used to record air temperature, humidity and wind speed. The CLF VES datasheet provided in the CLF recovery plan and later the revised version was used to record data for most surveys.

To determine the presence of Bd in specific sites we collected attempted to collect at least 10 individual frogs from each site both during fall and spring. These frogs were then individually swabbed using the protocol outlined in Boyle (2004). The swabs were sent to and processed by the Amphibian Disease Laboratory, San Diego Zoo Institute for Conservation Research. There the relative amount of Bd DNA for each swab was determined. Results were produced in terms of Qt a relative measure of the concentration of target in the PCR reaction (Bd). Zoospore equivalence was determined from the Qt results and used to determine relative infection load (Clare et al, 2016). Swabs were collected in summer and fall of 2014 and 2015 to determine seasonality of infection. Zoospore equivalence was used to determine quantitatively the level of Bd on the individual frog.

To determine the relative amount of both CLF and Bd environmental DNA for each sample site we used the field methodology outlined in Goldberg et al, 2011. These samples were then sent and analyzed by Dr. Goldberg's laboratory at Washington State University, Pullman WA. Samples were taken in the fall and in the winter to determine the levels of environmental DNA of both Bd and CLF as proxies for the densities of both Bd and CLF in each site by season and site type (lentic or lotic). Six lentic sites were tested (Cinco Wildlife Pond, Clyne Pond, Cottonwood Wildlife Pond, Empire Wildlife Pond, Gaucho Wildlife pond and Road Canyon Wildlife pond) and three lotic sites (Cold Spring Reach, Empire Gulch Spring and

Headwaters Reach). Water samples were collected in each site in November 2016 and February 2017. Environmental DNA samples were collected in late October of 2018 in thirteen sites: Bill's Wildlife Pond, Clyne Pond, Cottonwood Wildlife Pond, Crescent Wetland Pond, Empire Gulch Spring, Empire Wildlife Pond, Gaucho Wildlife Pond, Headwaters Reach, Hilton Tank, Karen's Tank, Oil Well Tank, Road Canyon Wildlife Pond, and Spring Water Wetland Pond. These samples were analyzed for the presence of the following bullfrog eDNA.

Temperature monitoring for each major site was performed by weighting and placing a HoboTemp™ Pro v2 data loggers (Onset Corporation, Bourne MA) at a depth of 20 cm in both lentic (n = 4, Cinco Wildlife Pond, Cottonwood Wildlife Pond, Empire Wildlife Pond, and Gaucho Wildlife Pond) and lotic (n = 3, Cold Spring reach, Empire Gulch Spring and Headwaters reach). The data loggers were downloaded at intervals of from between 6 and 14 months. Periodically the temperature loggers in lotic sites would be washed up onto the bank because of flooding. Data from these periods were easily edited out of the database as the dates when the loggers were out of the water were the result of summer monsoon flooding and the loggers recorded ambient temperature while out of the water that is dramatically warmer than water temperatures and as a result could easily be identified.

To study frog movements, frogs in a cohort were giving a site-specific mark by toe-clipping. Toe clipping has been shown to be an effective means of identifying Ranid frogs with minimum impact on the frog (Ginnan et al, 2014). Frogs were toe-clipped throughout the years 2014-2016. During these three years surveys were done in all sites wherein a sample of frogs were collected and inspected for toe clips. When a toe clipped individual was found the site-specific mark was recorded along with snout-vent length, sex and condition of frog.

Crayfish were trapped in Clyne Spring using baited minnow traps. Bait used was the dog food kibble, "Gravy Train™" as this is the bait used by AZG&FD to trap crayfish. Up to 10 traps were used in each small spring pool. Traps were used from 15 May until the onset of the summer monsoon to avoid the traps being washed out or downstream. Trapping was performed from 2011 through 2017.

All data analysis was performed using JMP™ 14.1 statistical software. All count data including Bd swab and eDNA data were first transformed using In (count +1) prior to analysis so the count data better conforms to normality. To test for normality after data transformation residuals were plotted against the quantiles of a standard normal distribution in quantile-quantile plots. Hypothesis testing used the mixed model-repeated measures platform for analysis. Data were combined in many tests based on flow regime of each site either lentic or lotic to determine if these flow regime differences could be correlated to differences in both disease and population dynamics. In order to test the effects of seasonal temperature differences, count and disease data are grouped into calendar seasons. ANOVA was used to determine differences in dispersal distances grouped by sex and size. Size is measured as snout-vent length in mm.

Results

TEMPERATURE

Result of a mixed model repeated regression analysis show that the interaction between site type and year/season was significant and differences in temperature between site type (lentic or lotic) depends on site type and year/season (C°= year/season*site type, $df = 9$, $F = 3352.45$, $P < 0.0001$). These relationships are shown in Figure 1 that demonstrates how lotic sites are more stable in terms of water temperature and the temperature fluctuates between mean temps of 13° to 19° C whereas lentic sites fluctuate between 8° to 24° C.

VES Count Surveys

A total of 558 VES surveys of the major sites were performed from 2014 through the summer of 2019. Table 1 lists number of surveys conducted by site and year. 345 surveys were conducted in lentic sites, 213 sites were conducted in lotic sites.

Table 1. Number of VES surveys per site/year.

SITE	2014	2015	2016	2017	2018	2019	TOTAL
CINCO W.P	4	12	13	12	12	6	**59**
CLYNE POND	2	4	11	12	11	6	**46**
CLYNE SPRING	2	3	9	10	11	4	**39**
COLD SPRING	4	11	11	11	11	5	**53**
COTTONWOOD W.P.	8	11	11	13	11	6	**60**
EMPIRE GULCH SPRING	3	11	13	15	18	8	**68**
EMPIRE W.P.	7	13	14	11	11	5	**61**
GAUCHO W.P.	5	11	14	12	12	5	**59**
HEADWATERS	6	11	11	11	11	5	**55**
ROAD CANYON W.P.	5	12	11	12	10	8	**58**
TOTAL	**46**	**99**	**118**	**119**	**118**	**58**	**558**

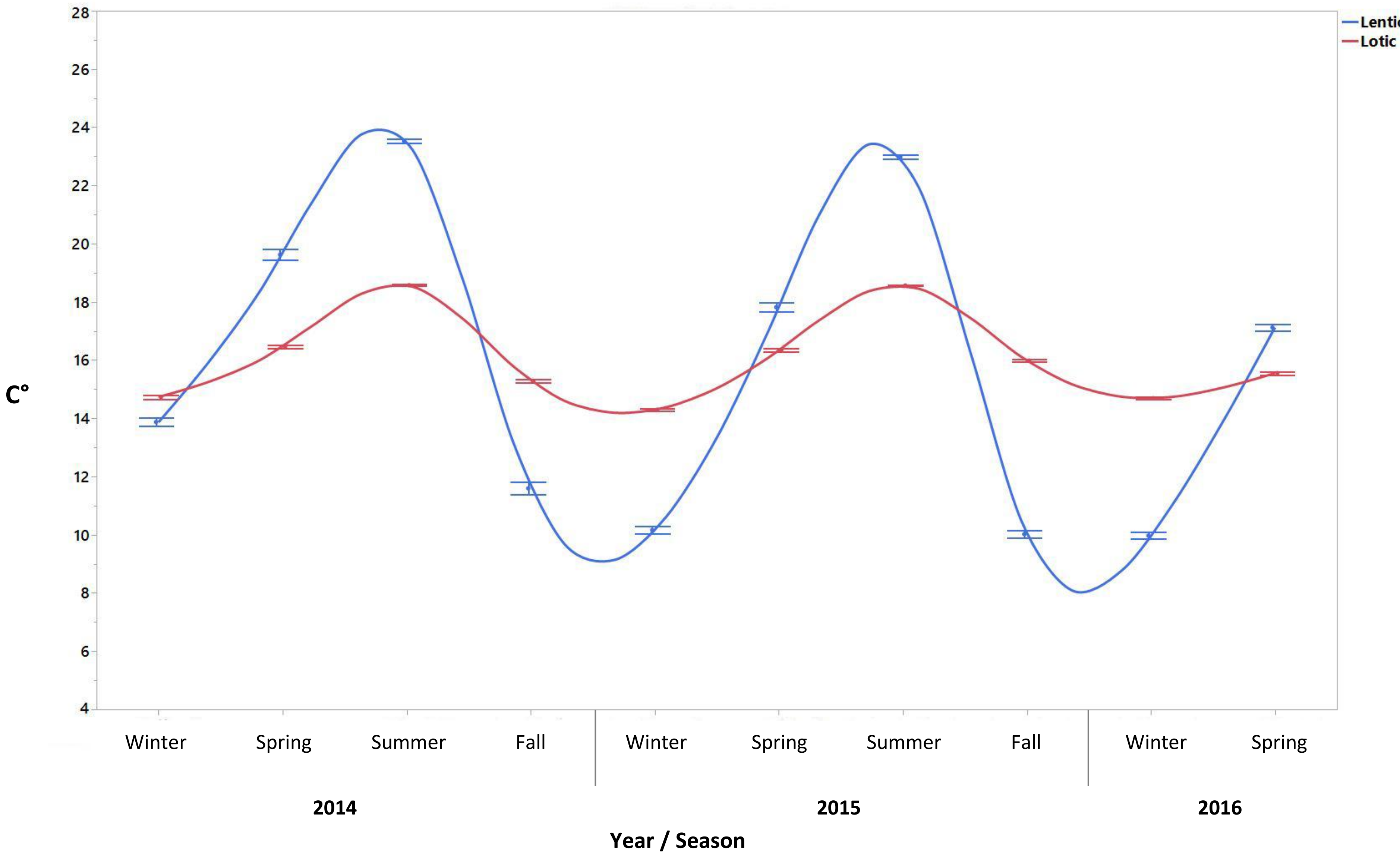


Figure 1. Results from temperature data-loggers set in both lentic sites (blue, n = 4) and lotic sites (red, n = 3) from winter 2014 through spring 2016. This was the time period that disease sampling was being performed. Dots are the mean seasonal temperature and error bars are constructed from 95% confidence intervals of the mean.

RELATIVE ABUDNANCE

Total surveys of lotic sites and lentic differed by year, primarily the number of surveys performed in 2014. As a result of this sampling difference 2014 surveys are removed from analysis because of sampling biases. 2019 survey totals differed because only two seasons of surveys were completed but these are included in the analyses because the number of surveys was consistent. Results of the full factorial mixed model whether frog count varied by season, year, site type or any interaction of the three explanatory variables are shown in Table 2. Counts of frogs are dependent on the interaction of year, site type and season. However, graphing these relationships shows that counts are generally higher in lotic sites than in lentic sites and the counts in the two site types always differed in winter and in years 2015, 2017 and 2019, in both winter and spring. The wave form of the counts over time had similar sine wave shapes in that varied with the lentic sites having greater amplitude demonstrating greater fluctuation in population number than the lotic sites. It appears that the population trends of both site types oscillate in a similar and stable manner throughout the 4.5 years of the study.

Table 2. Results of the mixed model used in determining what explanatory variables correlate to count numbers: season, site type (lentic or lotic), and year and all possible interactions of the three terms.

Fit Statistics

-2 Residual Log Likelihood	1681.17
-2 Log Likelihood	1625.90
AICc	1661.20
BIC	1731.85

Fixed Effects Tests

Source	Nparm	DFNum	DFDen	F Ratio	Prob > F
Season	3	3	492.0	87.813761	<.0001
Site Type	1	1	492.0	57.982618	<.0001
Year	1	1	492.0	6.4168383	0.0116
Year *Season	3	3	492.0	0.4604253	0.7101
Year*Site Type	1	1	492.0	0.0745351	0.7850
Season*Site Type	3	3	492.0	17.057631	<.0001
Year*Season*Site Type	3	3	492.0	5.7573873	<.0007

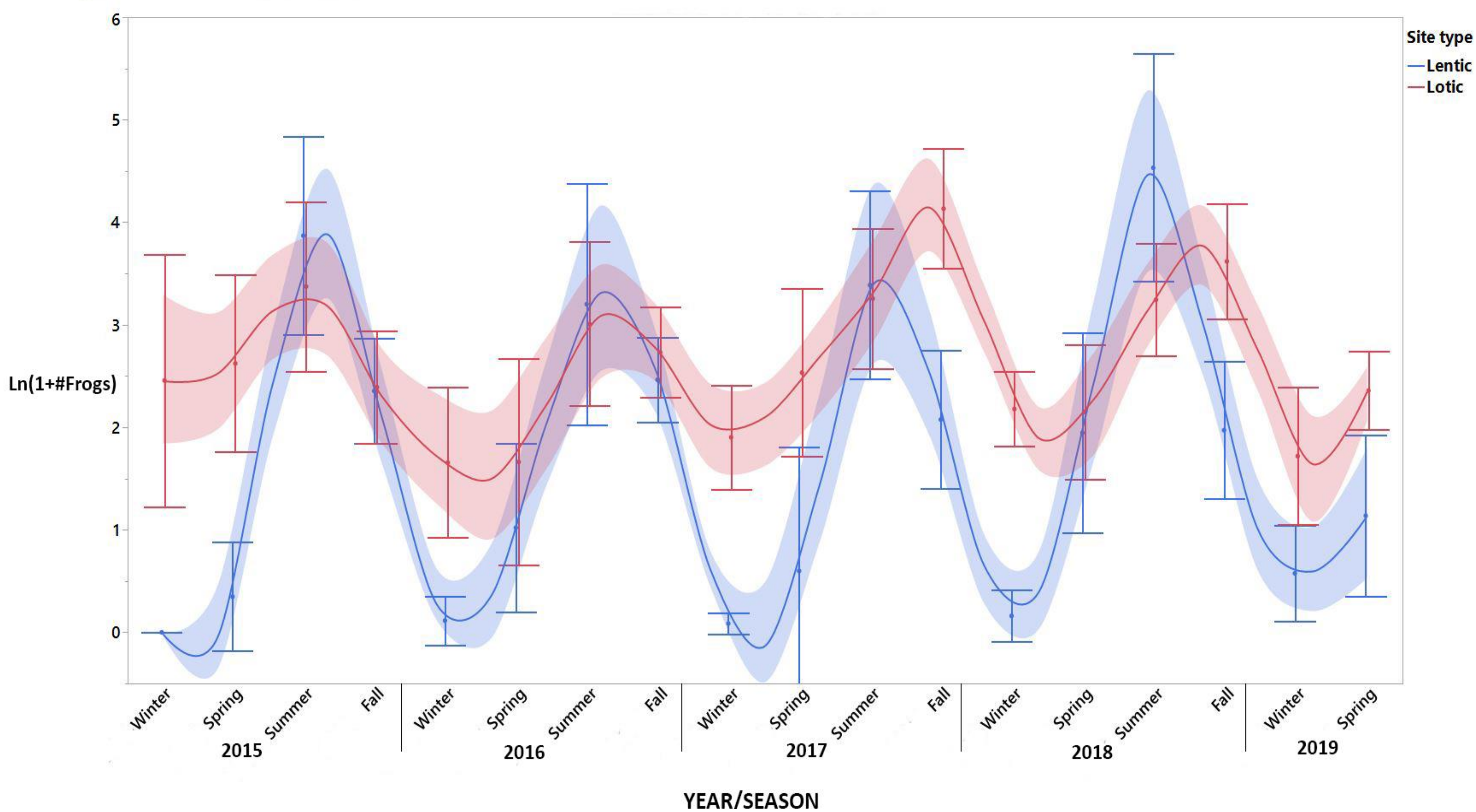


FIGURE 2. Transformed counts of frogs shown by site type, season and year. Year 2019 only includes winter and spring counts only as study concluded prior to summer and fall 2019. Dot represent means, error bars are constructed from the 95% confidence interval of the mean. Smoother curves are constructed using the cubic spline lambda function in JMP and represent the 0.99% confidence of fit area.

Cinco, Clyne, Cottonwood and Empire Wildlife ponds would experience explosive population growth during the summer months. These population irruptions occurred yearly in Cottonwood and Empire but because the well failed for several years and disease outbreaks the population in Cinco Wildlife pond failed in summer of 2017. Clyne pond also only showed irruptive growth in 2017 presumably due to drought, disease and possibly the translocation of Gila Chub, a known predator of frog tadpoles in systems with low habitat heterogeneity (Hall and Rosen, unpublished data). Frog density would begin to grow exponentially in June and continue into July as tadpoles began to metamorphose into frogs (see Appendix A. for site specific count numbers). These lentic sites would experience densities in the thousands during June and July but numbers would decline dramatically in the later monsoon presumably because of high dispersal away from the overpopulated sites. The smaller lentic sites and the three lotic sites never demonstrated these large population irruptions. All of these fluctuations can be observed by the graphs of site counts by year in Appendix A.

DISEASE (CHYTRIDIOMYCOSIS)

The first record of a chytrid outbreak in the area was a large 'die-off' of CLF occupying West Tank on the National Forest in the Santa Rita Mountains (UTM: 522725/3516899) in fall of 2013 (author, AZG&FD data base). This was a large population (>300) of CLF that is 7.4 Km (4.6 miles) directly downstream of Empire Wildlife Pond on the LCNCA. Two frog carcasses were recovered and both tested positive for Bd. No other frog die-offs were observed in the subsequent surveys of the entire area until November 2014 when CLF die-offs were observed in all of the lentic populations that occurred on the LCNCA (at that time these were: Empire Wildlife Pond, Cottonwood Wildlife Pond, Gaucho Wildlife Pond and Road Canyon Wildlife Pond). Surveys at the three lotic sites (Cold Spring, Empire Gulch Spring and Headwaters) did not observe CLF die-offs. Since the fall of 2014 die-offs have been recorded in every lentic site on the LCNCA including new sites whereas no die-offs have been observed in lotic sites.

A total of 324 swabs were taken in the summer and fall of 2014-2015. A total of 183 were from lentic sites (Cinco, Cottonwood, Clyne, Empire, Gaucho and Road Canyon) and 141 from lotic sites (Cold Spring, Empire Gulch Spring, Headwaters reach). In summer 71 swabs were taken from lentic sites and 49 taken from lotic sites, in fall 112 swabs were taken in lentic sites and 89 taken from lotic sites. Disease load on individuals was higher in the fall at both site types whereas disease load was much lower in summer at both sites. Disease (Bd) load level (zoospore equivalence) was very low in the summer months in lentic sites whereas in the lotic sites there was little difference in load levels during the summer or fall sampling (Figure 3.).

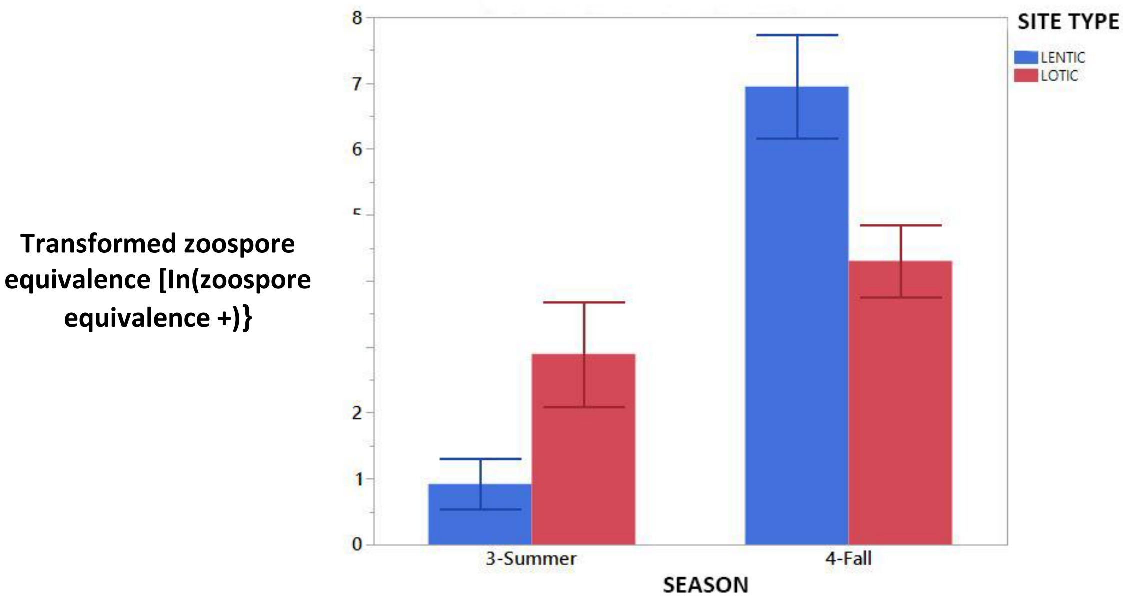


Figure 3. Disease levels from individuals sampled during summer and fall months shown by site type (lentic or lotic). Error bars constructed from 95% confidence levels of the mean.

eDNA RESULTS

Mixed model results of eDNA disease demonstrate that disease loads in the environment of each site type depend on both season and site type (Table 3.) Whereas eDNA of CLF depend only on the season sampled (Table 4.) Graphical analysis of Bd eDNA shows that while Bd eDNA differed by site and season, there was no difference in lotic sites between the seasons but were lower from lentic sites seasonally. Lentic site levels differed both seasonally with each other and with lotic site levels (Figure 4).

Table 3. Results of a full factorial mixed-model repeated measures test of season and site type on Bd eDNA zoospore equivalence.

Fit Statistics

-2 Residual Log Likelihood	135.87
-2 Log Likelihood	125.80
AICc	137.02
BIC	145.72

Fixed Effects Tests

Source	**Nparm**	**DFNum**	**DFDen**	**F Ratio**	**Prob > F**
Season	1	1	50.0	21.055519	<.0001
Site Type	1	1	50.0	0.1148762	0.7361
Season*Site Type	3	3	50.0	0.8232698	0.3686

Table 4. Results of a full factorial mixed-model repeated measures test of season and site type on CLF eDNA equivalence.

Fit Statistics

-2 Residual Log Likelihood	135.87
-2 Log Likelihood	125.80
AICc	137.02
BIC	145.72

Fixed Effects Tests

Source	**Nparm**	**DFNum**	**DFDen**	**F Ratio**	**Prob > F**
Season	1	1	50.0	21.055519	<.0001
Site Type	1	1	50.0	0.1148762	0.7361
Season*Site Type	3	3	50.0	0.8232698	0.3686

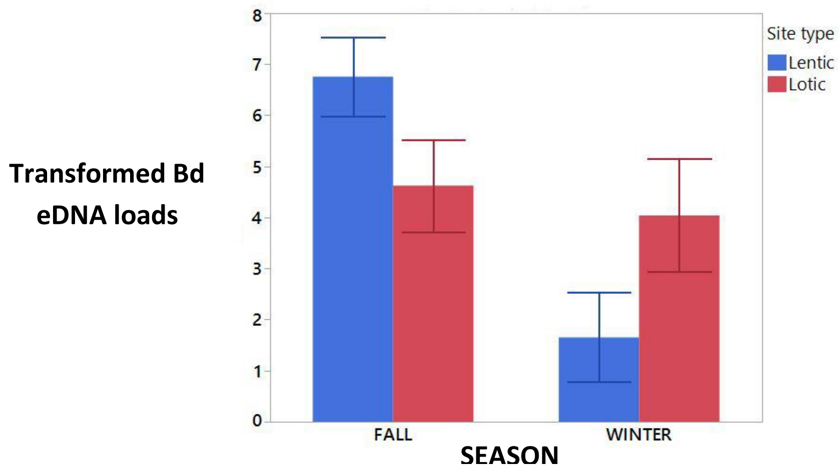


Figure 4. Transformed Bd eDNA levels [ln(zoospore equivalent =1)] by season and site type. Error bars are constructed from 95% confidence levels of the mean. Bars represent mean levels.

CLF eDNA differed by site and season. While Bd eDNA remained similar in the lotic sites they differed in the lentic sites (Figure 5).

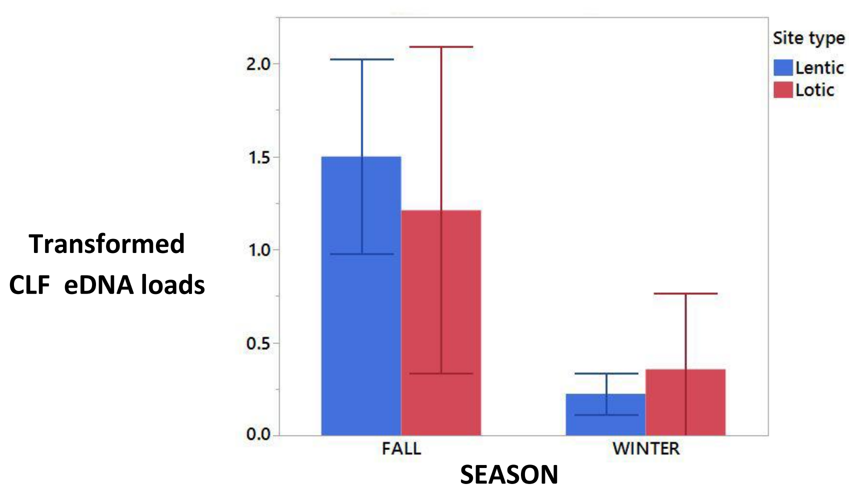


Figure 4. Transformed CLF eDNA levels [ln(CLF equivalent =1)] by season and site type. Error bars are constructed from 95% confidence levels of the mean. Bars represent mean levels.

DISPERSAL MOVEMENTS

A total of 504 frogs were given site-specific toe-clips, both adult and juvenile frogs. The number of frogs toe-clipped by year and site is given in Table 5. A total of 23 frogs were recaptured that had dispersed from their marked site (9 females, 14 males). None of the frogs recaptured were juveniles. Sex or size had no effect on linear displacement (ANOVA, Table 6). Average longest linear displacement traveled was 7.43 km (95% CI = 6.20- 8.69 km). The range of linear displacement was 1.77 – 15.13km. Of the 23 frogs that had dispersed, 14 (61%) were from sites that were documented to have dried completely or were close to drying. Maternity well dried in 2015, Cinco Wildlife Pond had a drying event in 2016, and Hilton tank dried completely in early summer of 2016. These dispersals sometimes resulted in the "rescue" of a site. During 2015 the frogs in Cinco Wildlife pond had failed to reproduce until very late in the fall. As a result of this the tadpoles from this cohort did not develop into frogs until very late in the fall of 2016. These frogs failed to reproduce before the winter die-offs and reproduction failed at this site. No frogs were observed in Cinco Wildlife pond until mid-summer of 2017 when large adults were found in the pond including one large female that had been marked in 2015 in Headwaters Reach of Cienega Creek. These frogs presumably had all dispersed into Cinco Wildlife Pond from Headwaters and possibly other population sources on the LCNCA during the particularly strong monsoon rains the area experienced in the summer of 2017. These adult frogs were able to reproduce several times in 2017 in Cinco and the pond population remains extant. This same pattern occurred throughout the same years in Road Canyon Wildlife pond but the number of frogs that appeared at this pond was much less and these frogs failed to reproduce that year. Gaucho tank has failed to reproduce twice, in 2016 and 2017, both years being rescued likely from nearby Cottonwood Wildlife Pond as it has consistently had population irruptions during the summer monsoon.

Table 5. Number of frogs that were marked, by year and site.

SITE	# MARKED 2014	# MARKED 2015	# MARKED 2016	TOTAL MARKED
Cinco Wildlife Pond	0	56	0	56
Clyne Pond	0	0	43	43
Cottonwood Wildlife Pond	0	10	0	10
Empire Wildlife Pond	20	51	0	71
Gaucho Wildlife Pond	0	13	0	13
Hilton Tank	0	0	40	40
Maternity Wildlife Pond	21	0	0	21
Road Canyon Wildlife Pond	0	11	0	11
Cold Spring Reach	0	32	7	39
Empire Gulch Spring	0	62	53	115
Headwaters Reach	12	46	27	85
TOTALS	53	281	170	504

Table 6. Results of ANOVA of dispersal distance = sex + Size (snout vent length mm).

Rsquare	0.024
No. observations	23

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.90	0.951	0.25
Error	20	76.30	3.815	**Prob >F**
C Total	22	78.20		0.7817

Parameter Estimates

Term	Estimate	Std Error	t ratio	Prob >[t]
Intercept	5.9886	4.0335	1.48	0.1533
Sex (F)	0.3950	0.5847	0..68	0.5073
SVL(mm)	-0.0156	0.0477	-0.33	0.7483

Movements of CLF particularly metamorphic (juvenile frogs that recently had meta-morphosed from larvae into frogs, generally 45-60mm in snout vent length) from neighboring sites into recently filled rain puddles was common during and after monsoon rains. Frogs of all sizes were also commonly found outside of major sites in a decreasing density from the major sites during and after major rain events. Frogs were active in every month of the year in lotic sites. During winter months frogs were primarily seen in the warmer spring gaining reaches of all three of the larger lotic sites (Cold Spring, Empire Gulch and Headwaters Reach). In Empire Gulch marked frogs had moved from the lower reaches of this spring run 200 m upstream to the spring head pools. Although warmer than the lower reach the maximum temperature difference was not that great at 1° C. In Headwaters frogs would concentrate in pools 5, 11, 16 and 24. In Cold Spring reach frogs were throughout the gaining reach just below the confluence of Cold Spring proper.

BUFFER ZONE, BULLFROG MONITORING AND BULLFROG eDNA RESULTS

Monitoring of the bullfrog buffer-zone (Map 1.) has been continuous since 2011. Several incursions into the zone have occurred during this time period. These incursions have occurred in the far eastern area of the buffer zone in the Rain Valley area. The effectiveness of the buffer zone concept can be measured by the fact that despite incursions into the buffer zone area no bullfrogs have managed to invade or colonize any habitat in the LCNCA since bullfrogs were removed from the area in 2013. Two adult male bullfrogs were discovered in the lowest reach of Empire Gulch by Gita Bodner (Nature Conservancy) in early June 2017. These bullfrogs were removed soon after. It is the belief of this author that these two bullfrogs had been overlooked by earlier surveys in 2011-2013. This area was not surveyed in the intervening years and instead of frogs dispersing into the region they were most likely “home-grown” from juveniles originating from the bottom of Headwaters reach that is less than a kilometer away from where the two bullfrogs were found. Environmental DNA samples taken from 13 sites throughout the LCNCA (for specific sites see Methods section), including Clyne Pond and Headwaters Reach (previous bullfrog population sites that had been eradicated by 2013) showed no American bullfrog eDNA was present at these 13 sites in 2018. Bullfrogs within Clyne Ranch and southeastern section of the buffer zone were removed in the summer of 2019. The buffer zone itself has been expanded and several populations of bullfrogs in the southern area of Elgin Arizona have been discovered and eradication efforts begun.

CRAYFISH MONITORING AND ERADICATION RESULTS

Crayfish were found in both Clyne Pond and Clyne Spring since at least the early 2000's and efforts such as rotenone treatment and pond draining. The crayfish in Clyne Pond were successfully eradicated by draining Clyne pond in 2013 and leaving it dry for six months. In Clyne Spring draining was not an option and physically removing the crayfish with baited minnow traps began in 2011 and continued into 2016 when the crayfish were successfully removed. Subsequent trapping in 2017 and 2018 caught no crayfish nor have crayfish been seen since the late spring of 2016 and they are now considered eradicated from the site.

Discussion

This study describes patterns of population dynamics, disease and movements of Chiricahua leopard frogs on a substantial landscape scale within the LCNCA over a 5.5 year period. The patterns described herein have never been described for CLF prior to this and are remarkable for the temporal and spatial scales they have been documented for. As a result these patterns and the implications they have for CLF conservation and recovery are important and could be used as a template for further CLF conservation efforts and planning. With the removal of bullfrogs from the LCNCA in 2013 and the set-up of CLF populations encompassing an area of 42 km^2 with 7 breeding populations the frog has dispersed so that the region contains 19 breeding populations and covers an area of 84 km^2.

One of the major goals that the FROG project set out to do in the initial 3-year (2010-2013) effort was to set up a metapopulation of CLF within the LCNCA. A metapopulation has been described as a population of populations (Levins, 1969) with other authors defining it more specifically including Levins himself in later works. Several theoreticians have developed fairly specific conditions on what defines a metapopulation (Hanski & Gilpin 1991, Baguette & Mennechez 2004). Real world application of the metapopulation concept has moved to more usable definitions such as those defined by Akçakaya et al (2007): "Our more general definition has only two requirements: (i) populations are geographically discrete; (ii) mixing of individuals between populations is less than that within them – otherwise the regional assemblage of local populations may be more aptly described as a single panmictic population.". **The results in this**

report show that the populations on the LCNCA do fulfill this definition of a metapopulation with dispersal and discrete populations and as a result a metapopulation of CLF has been created on the LCNCA.

The conservation advantage of creating a metapopulation is in a metapopulations' ability to compensate for stochastic extinction events with little or no management action in terms of translocating frogs into extinct sites. Because each population within a metapopulation is connected by dispersal, populations that flourish one year send out propagules through dispersal and "rescue" a population that has gone extinct. In this study this event has been documented to have happened at least twice. The CLF population in Cinco Wildlife pond had gone extinct due to a combination of disease die-offs, failed reproduction due to delayed frog maturity due to disease and failed infrastructure causing the pond to become nearly dry. Road Canyon Wildlife pond and Clyne pond also experienced similar events and were repopulated by dispersal. Dispersal and colonization into Clyne pond and Clyne Spring in 2015 from an unknown but highly likely from populations on the LCNCA (the nearest source population is Cinco Pond). These populations likely were the source populations for the colonization of Hospital, State and Sue's Pond (all on Pima County Sand/Clyne ranch lands) in 2019.

The extent at which a metapopulation of CLF can spread has never been documented but with continuing expansion of the Elgin bullfrog buffer-zone and the range with which CLF can move, it is likely that CLF will colonize the Elgin area much as they have the Clyne/Sands ranch lands. **Considering the results of this study it is even likely to predict that once bullfrogs are removed from the Babocomari Valley the CLF populations of the Huachuca Mountains and Canelo Hills will be connected by dispersal with the CLF metapopulation on the LCNCA and surrounding lands.** This is not only due to the movement capability of CLF but because good habitat sites are within the Elgin/Babocomari/Canelo/Huachuca area. This is known because of the work I have done outside of this study's purview. Only one published study has addressed the movements of CLF. In this study CLF were found to move on average 2.43km with a longest linear displacement of 8.5 km (Hinderer et al., 2017). In contrast this study reports on average linear movement of 7.43 km and longest linear displacement of 15.13 km.

There are several reasons why the results are different. Hinderer et al. study was performed in a single mountain canyon whereas this study was performed in the grasslands of Sonoita where elevation grades are presumably less than those in a mountain canyon. Also the frogs were radio tracked and the frogs carried a radio transmitter that weighed up to 10% of body mass and may hamper movements. The fact that CLF in grassland habitats can move long distances is essential in developing recovery plans by addressing factors such as habitat fragmentation and the ability of CLF to colonize new habitats.

The idea of habitat quality is also addressed, in part, in this study. Frog counts were consistently higher in all of the larger wildlife ponds compared to the smaller sized ponds. This is not itself surprising but it is of note when managers are considering creating CLF habitat. The larger ponds also had large enough populations (using counts as a measure of relative abundance) to counter stochastic breeding events. Smaller ponds such as Road Canyon and Gaucho Wildlife ponds both failed to reproduce twice in the 5.5 years of the study. This failure to reproduce never occurred in the larger ponds. Features such as a permanent and reliable water source are essential for insuring population persistence. Although antidotal, ponds with dense surface layers of pondweed (Potamogeton sp.) provide ideal cover and feeding sites for juvenile frogs. This vegetation allows frogs to use the entire surface of the pond for feeding and escaping predation whereas without it only the pond banks provide this. Also, spike rush (Eleocharis sp.) should be planted around the perimeter of the pond as this provides good bank cover for frogs and does not “choke-out” the pond the way larger reeds and rushes do, at the same time not allowing these larger aquatic plants to become established. **As a result of this I would suggest managers create CLF ponds that are 1.) Build the ponds to be at least 0.1 acre in area, 2.) Have permanent water sources, 3.) Seed the pond with pondweed and spike rush for cover. 4.) Build ponds with a steep and deep center that can act as a reservoir and refuge for fish and frogs against drying.**

The amphibian disease Bd is now enzootic throughout the LCNCA and neighboring regions. Remarkably this disease has less of an impact on the populations of CLF in all four lotic habitats (Clyne Spring, Cold Spring, Empire Gulch and Headwaters) than in the lentic habitats. Disease loads on individual frogs in lotic sites is lower than in lentic habitats in the fall and overwinter mortality is also lower in the lotic sites. In the warmer seasons Bd load levels almost disappear whereas in the lotic sites Bd load levels while lower in the summer are almost not significantly different than in the fall season. The pattern of frog survivorship being higher in the cooler seasons in lotic sites than lentic sites has been consistent throughout the entire study length. There is some indefinable factor or suite of factors in the lotic sites that allows frogs to be more resistant to the disease. One such factor may be due to the differences in the temperature regime between the site types. Each of the lotic sites has gaining spring reaches or arise from spring water sources that remain thermally consistent and cool regardless of the season. In contrast the lentic sites are less constant and their thermal regimes are dependent on ambient temperatures resulting in higher average temperatures in the summer and lower temperatures in the winter. Studies have shown that sudden drops in temperature have negative effects on amphibian immune systems (Maniero and Carey, 1997, Raffel et al., 2006. Butler et al., 2013). Although the enzootic chytrid infections are throughout the LCNCA metapopulation of CLF this condition although having negative effects on frog overwinter survivorship the impact on the overall survivorship of the metapopulation has been negligible. Populations that have been extirpated through disease have subsequently been recolonized ("rescued" in metapopulation terms) by dispersing frogs from populations within dispersal range.

Regarding chytrid disease and temperature, there has been a general consensus among researchers that temperature is an important factor in this disease, the mechanisms that underpin the temperature effects on chytridiomycosis have not been fully resolved (Venesky et al. 2014). What is observed within the LCNCA populations is there is a definitely different response to the disease depending on the flow regime, lentic or lotic and this correlates to the temperature differences. A probable hypothesis based on these studies observational results is that the sudden drop in temperatures seen in the lentic sites in fall and winter may stress the frog immune systems to the point the frogs cannot cope with the disease and succumb. It is also conceivable that the frogs in lentic systems are exposed to much more infective zoospores (the infective stage of the disease) as these were shown to be significantly higher in the lentic sites versus lotic sites. Indeed this speculation may be beyond the scope of this study that primarily reports the observational results. These results can however be used to create hypotheses to test in some future efforts.

In Frog tadpoles, chytrid fungus infects only the keratinized mouthparts (Chytrid fungus infects keratinized skin cells, the only part of the tadpole body containing keratin are the mouthparts). As a result tadpoles are not usually killed by Bd although it may indirectly result in mortality or lowered survival rate due to hampered feeding (Rachowicz and Vredenburg 2004; Marantelli et al. 2004, Parris 2004, Whittaker and Vrendenburg, 2011). During this study tadpole die-offs were never observed and although frog mortality was almost always 100% in the lentic sites, tadpoles remained numerous through the fall, winter and spring months. Frogs became "perennial" as a result in the lentic sites. Egg masses are generally observed in the LCNCA sites from May through October. Egg masses hatch out in 3-5 days and tadpoles transform into juvenile frogs 10 to 11 months later. Juvenile frogs generally become sexually mature in three months (Hall, unpublished data). As a result eggs laid in spring transform into juvenile frogs the following year in early to late spring and are breeding adults in summer. Eggs laid in October transform in August and September. In lentic sites frogs may not grow old enough to breed before succumbing to chytrid. This is what happened in Cinco, Gaucho and Road Canyon wildlife ponds and it resulted in CLF becoming extirpated in these ponds until dispersal from other sites recolonized the pond. The frogs that colonized the ponds in Cinco and Gaucho were adult frogs and successfully reproduced in mid-summer and fall resulting in frogs that could reproduce successfully the following years. In ponds such as Clyne, Cottonwood, and Empire frogs are able to lay several cohorts of eggs in the summer and fall resulting in adult frogs being able to reproduce every year before dying of chytrid. Lotic sites do not demonstrate this pattern of perennial frogs as frogs can survive (although there is some overwinter mortality) for years in this site as mark/recapture has shown.

Some studies on population effects of chytrid infections have shown a correlation between higher rates of chytrid survivorship and lower levels of canopy cover (Becker et al, 2012, Beyer et al, 2015). In this study I found just the opposite to be the rule. All of the lentic sites had <5% canopy cover. In contrast the lotic sites averaged >55% canopy cover. This may be due to the fact that water temperatures in the lotic sites are more dependent on the gaining spring's water temperature creating stable thermal regimes rather than solar energy inputs.

Several amphibian species are resistant to chytrid infection and some appear to have developed resistance to the disease (Fu and Waldman, 2017). Some populations of Chiricahua leopard frogs in Arizona have a genetic factor that allows the frogs with the factor to survive overwinter (Savage et al., 2018). This resistance does not seem to be developed in the CLF populations on the LCNCA despite the fact that in lotic sites overwintering survival is much higher than in lentic sites where it appears that overwinter survival is close to zero. It is unlikely that there is much difference genetically between the lotic and lentic populations as 1.) All the frogs translocated into the wildlife ponds and stream reaches in 2011-2014 came from the same population, Empire Gulch Spring, and, 2) Considering how frequently and how far CLF disperse it is unlikely that an advantageous adaptation would not spread throughout the metapopulation rapidly. No such survivorship has been seen yet in the lentic sites on the LCNCA. However, some adult survivorship was observed in Cottonwood Wildlife Pond in the spring of 2019.

Neighboring populations of invasive bullfrogs, although no longer in the LCNCA, remain a serious threat to the populations of CLF in the LCNCA. Bullfrogs would have undoubtedly spread into the LCNCA if not for the vigilance given to the buffer zones in the Rain Valley and Elgin areas. This successful bullfrog monitoring must continue but at the present time there are no funds to do so. There are still bullfrog populations in the Elgin areas and the Babocomari river valley that are within dispersal distance of bullfrogs into the buffer zone in Rain Valley and Elgin areas. These stepping-stone populations will then be in striking distance to invade the CLF populations on the LCNCA. The cost of the 2010-2013 successful bullfrog eradication was approximately $225K. Subsequent bullfrog monitoring of the buffer zone and eradications of Rain Valley invasions varied from $10-20K. Any establishment of bullfrogs on the LCNCA and subsequent eradication, particularly if bullfrogs invaded and established in Headwaters Reach would undoubtedly cost much more. **It is much more cost effective to prevent bullfrogs from invading the LCNCA by maintaining the bullfrog buffer zone and monitoring the existing CLF populations for bullfrog invasions.**

Recent interest in introducing beaver (*Castor canadensis*) into Headwaters and Cold Spring reaches of Cienega Creek are cause for concern in terms of bullfrog establishment. My strongest concern is that Chiricahua and lowland leopard frogs, and Mexican gartersnakes could be negatively affected if beaver ponds were to be colonized by bullfrogs, which generally can be expected to find larger, deeper, still, warm waters more favorable than the currently existing cienéga-stream habitat. Because of this mitigation should include bullfrog monitoring and if necessary eradication funds. In addition considering the significant correlations between habitat temperatures and overwinter survivorship , it is possible that beaver ponds might bury the spring gaining reaches in the creek that moderate seasonal fluctuations in water temperature that are associated with high adult survivorship of Chiricahua leopard frogs at the LCNCA despite the presence of *Bd*, the chytrid fungal pathogen. I am not suggesting this is necessarily a likely outcome, but that it is possible, and could lead to reduced adult frog overwinter survivorship and thus reduce desirable metapopulation dynamics.

To insure that the successes of the LCNCA CLF metapopulation continues I suggest the following monitoring program be integrated into the BLM's management program of the area:

1. Surveys of all populations, lentic and lotic should be performed as described in this report so that any finding can be directly compared with the results herein. VES should be done, ideally, once a season at each site. I suggest winter surveys be performed in February, spring surveys in May, summer surveys in August and fall surveys be performed in November. Survey results should be recorded in the field using the AZG&FD RACH/Riparian Herpetofauna VES Datasheet, April 2017, version 2.1.1.

2. All personnel should have attended the CLF Field Workshop that is given once a year by AZG&FD and USFWD. This workshop is required to apply for a USFWS permit to work with *Rana chiricahuensis* as the frog is a federally listed species. Workshop attendance is also required for an AZF&FD scientific collecting permit. Attendees of the workshop are trained in fog identification and VES procedures.

3. The bullfrog buffer zone should be monitored at least twice yearly and more if deemed necessary. Buffer zone surveys should be performed during the summer monsoon, July through September. If bullfrogs are found in the bullfrog buffer zone, surveys and eradication efforts should be financed until the bullfrog threat is removed.

4. Survey results should be compared to those provided here and analyzed by an individual familiar with the metapopulation so that an evaluation of the metapopulation can be provided to land managers with suggestions on measures to address the threats.

5. An alarm network or task force should be established between all participating land managers, AZG&FD, BLM and USFWD and others as needed to deal with any bullfrog invasions into the LCNCA CLF metapopulation occur. This would in effect serve in the absence of the author's role in this that will no longer occur as funding has not been provided.

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Appendix 1. Site Counts of combined site types and specific sites.

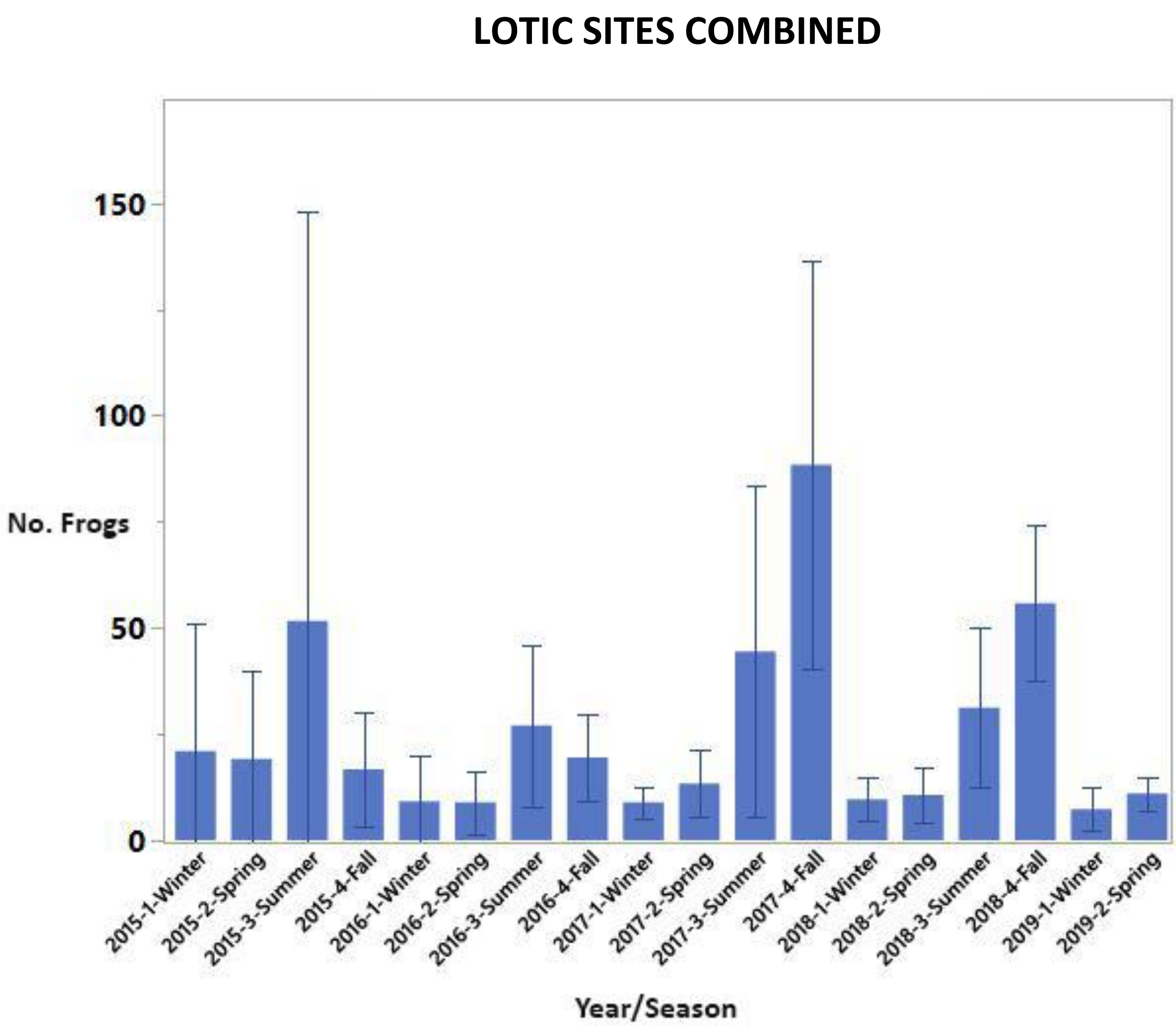

FIGURE A. Mean number of frogs counted during VES surveys by year and season in all Lotic Sites combined.

LENTIC SITES COMBINED

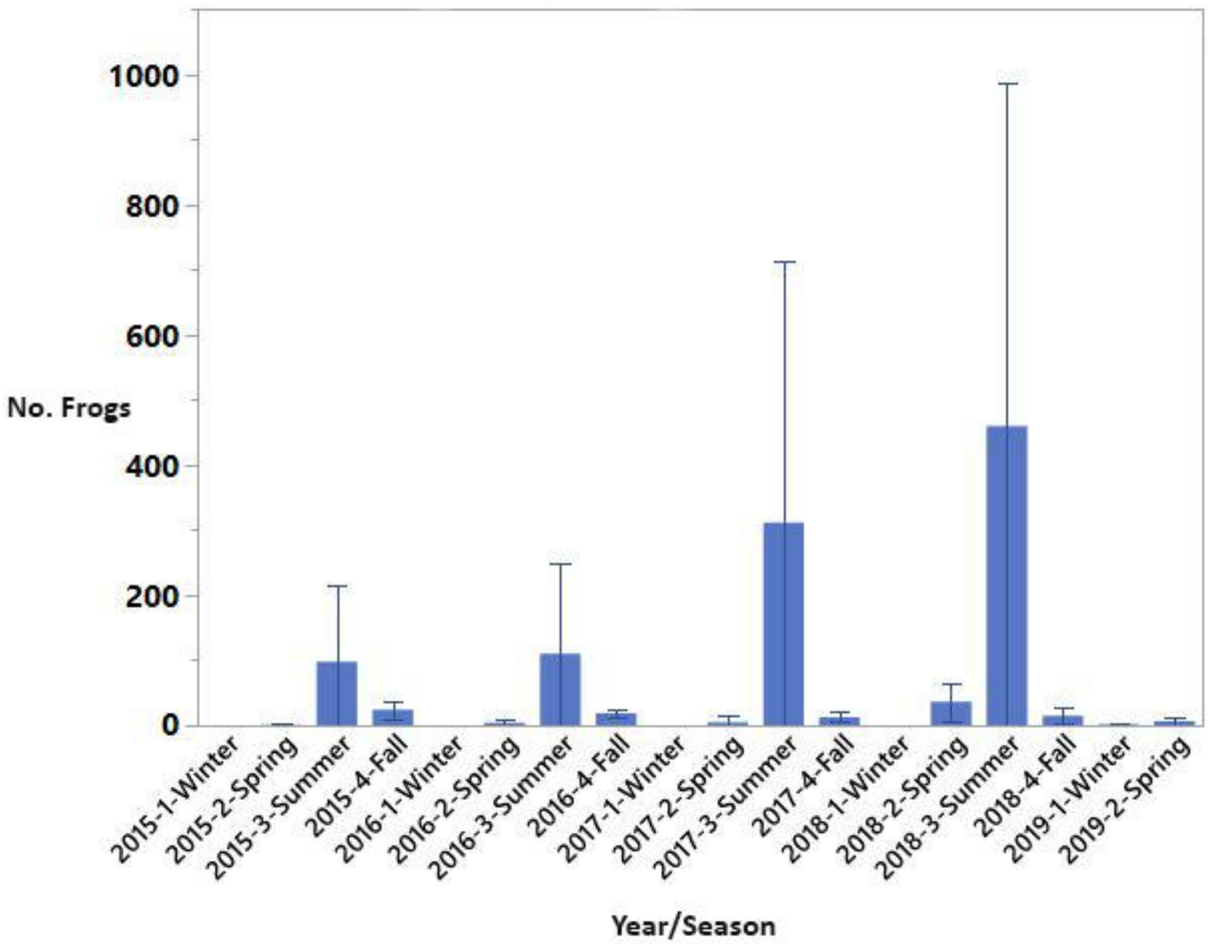

FIGURE B. Mean number of frogs counted during VES surveys by year and season in all Lentic Sites combined.

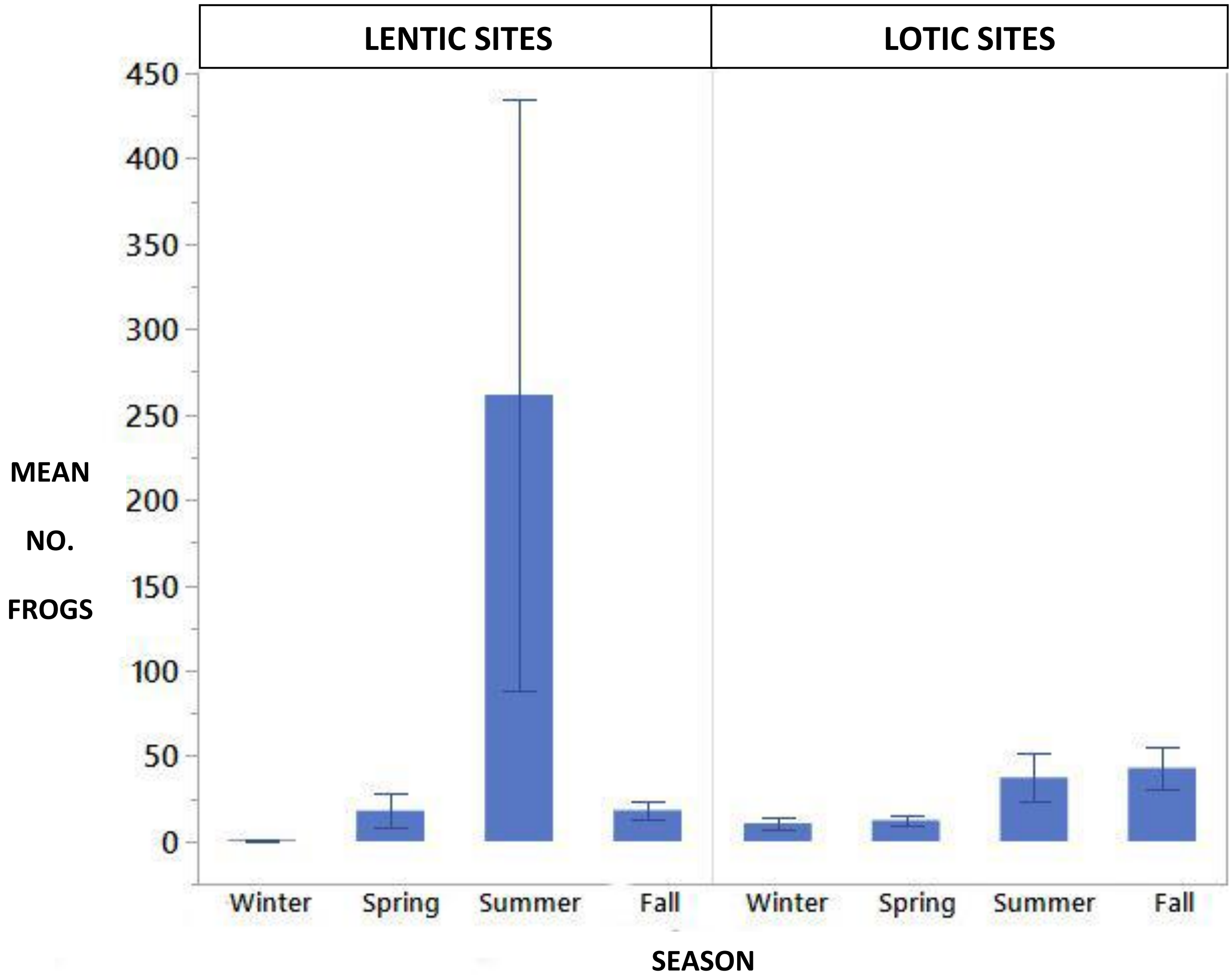

FIGURE C. Mean number of frogs counted during VES surveys by site type and season for all years data was collected. Bar represents mean number; error bars are constructed using 95% confidence intervals of the mean.

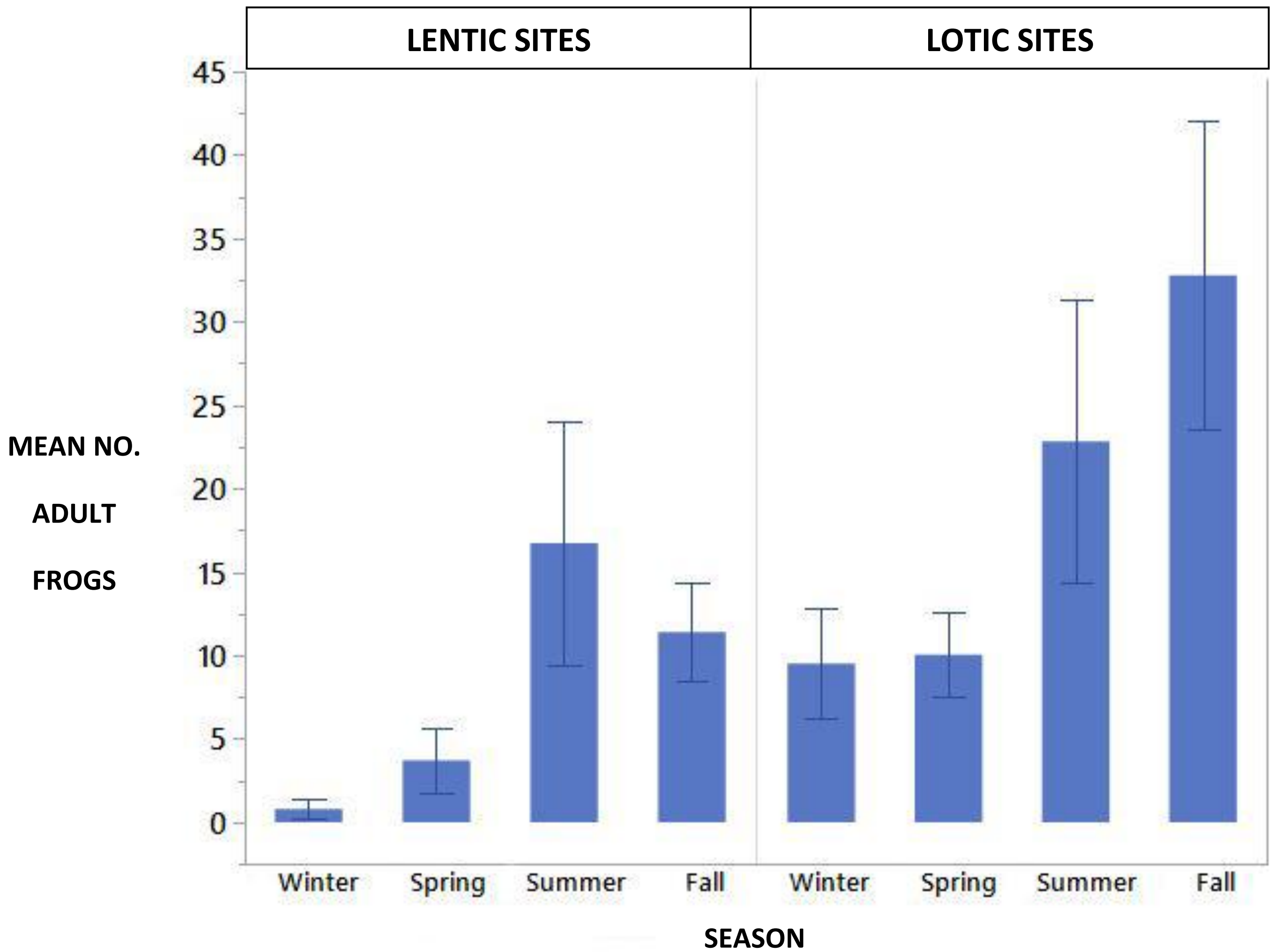

FIGURE D. Mean number of adult frogs counted during VES surveys by site type and season far all years data was collected. Bar represents mean number; error bars are constructed using 95% confidence intervals of the mean.

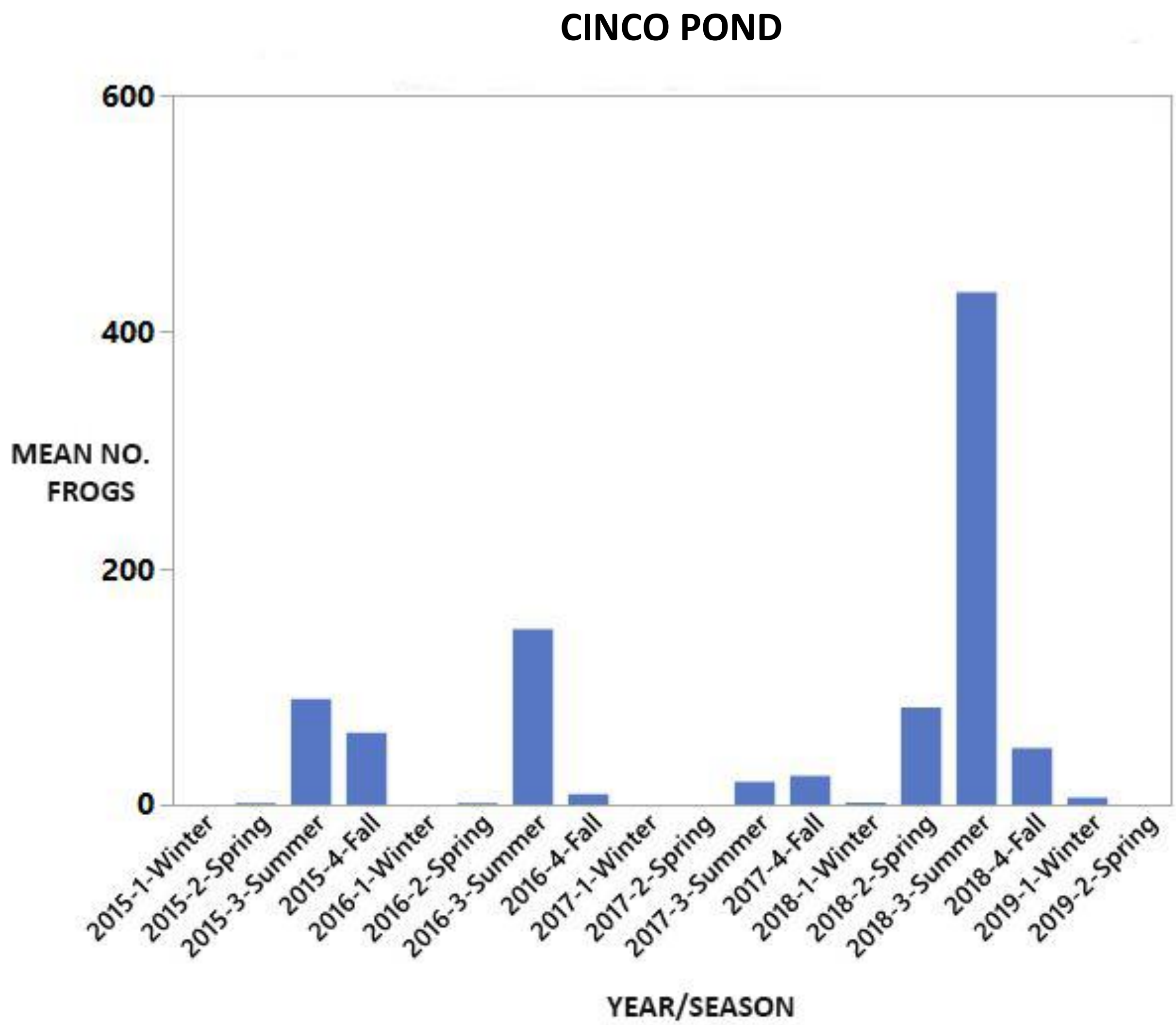

FIGURE E. Mean number of frogs counted during VES surveys by year and season in Cinco Pond.

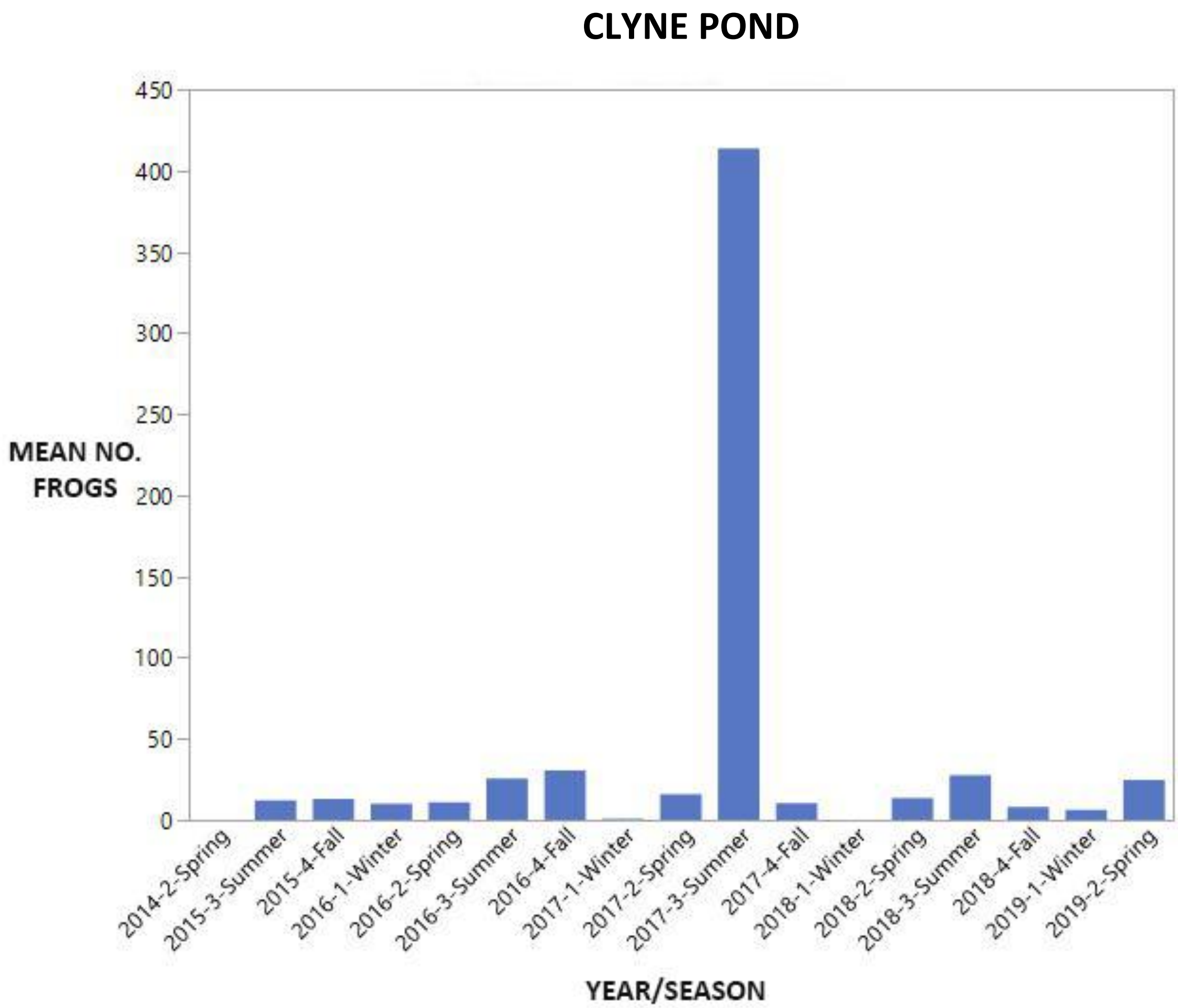

FIGURE F. Mean number of frogs counted during VES surveys by year and season in Clyne Pond.

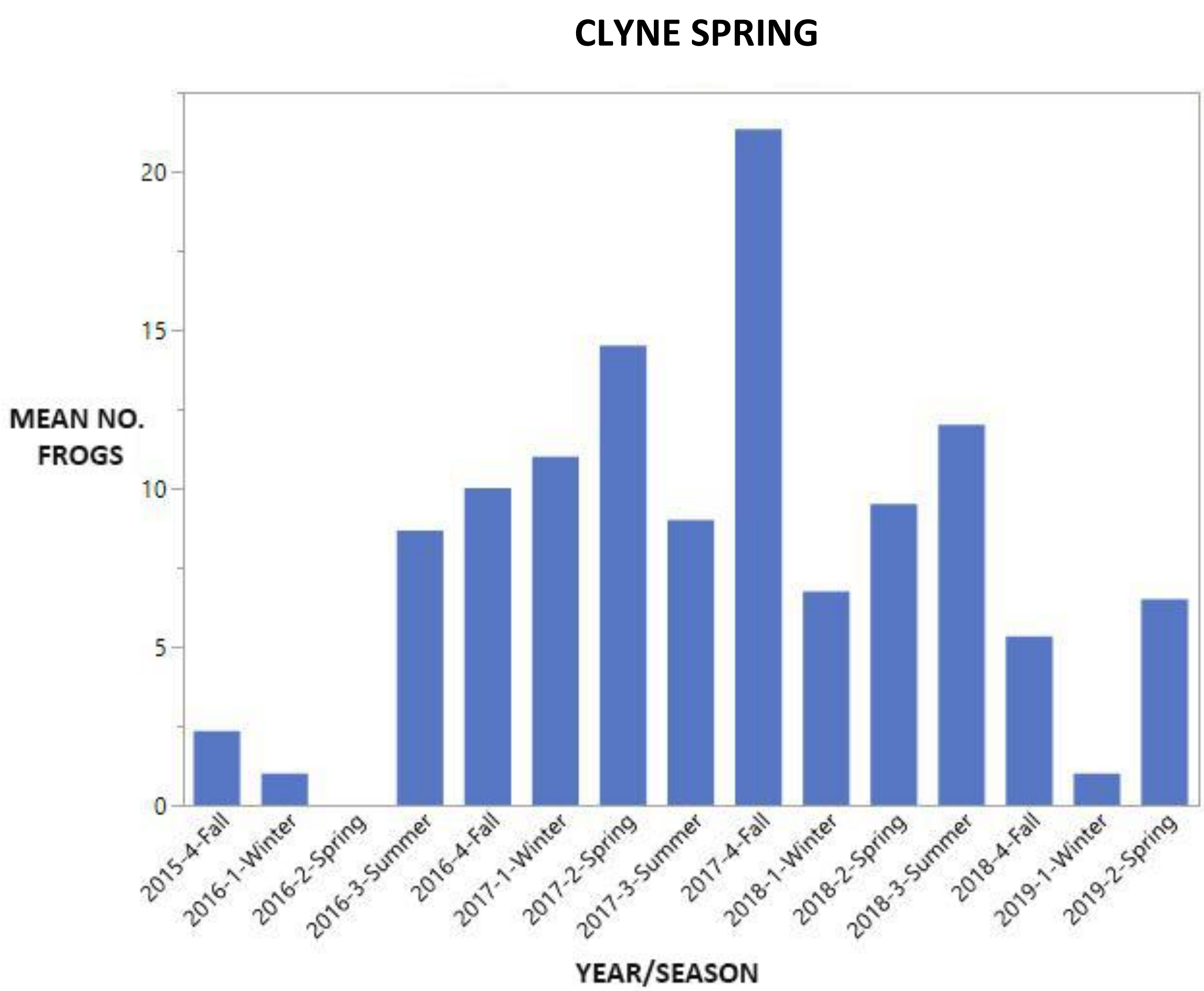

FIGURE G. Mean number of frogs counted during VES surveys by year and season in Clyne Spring.

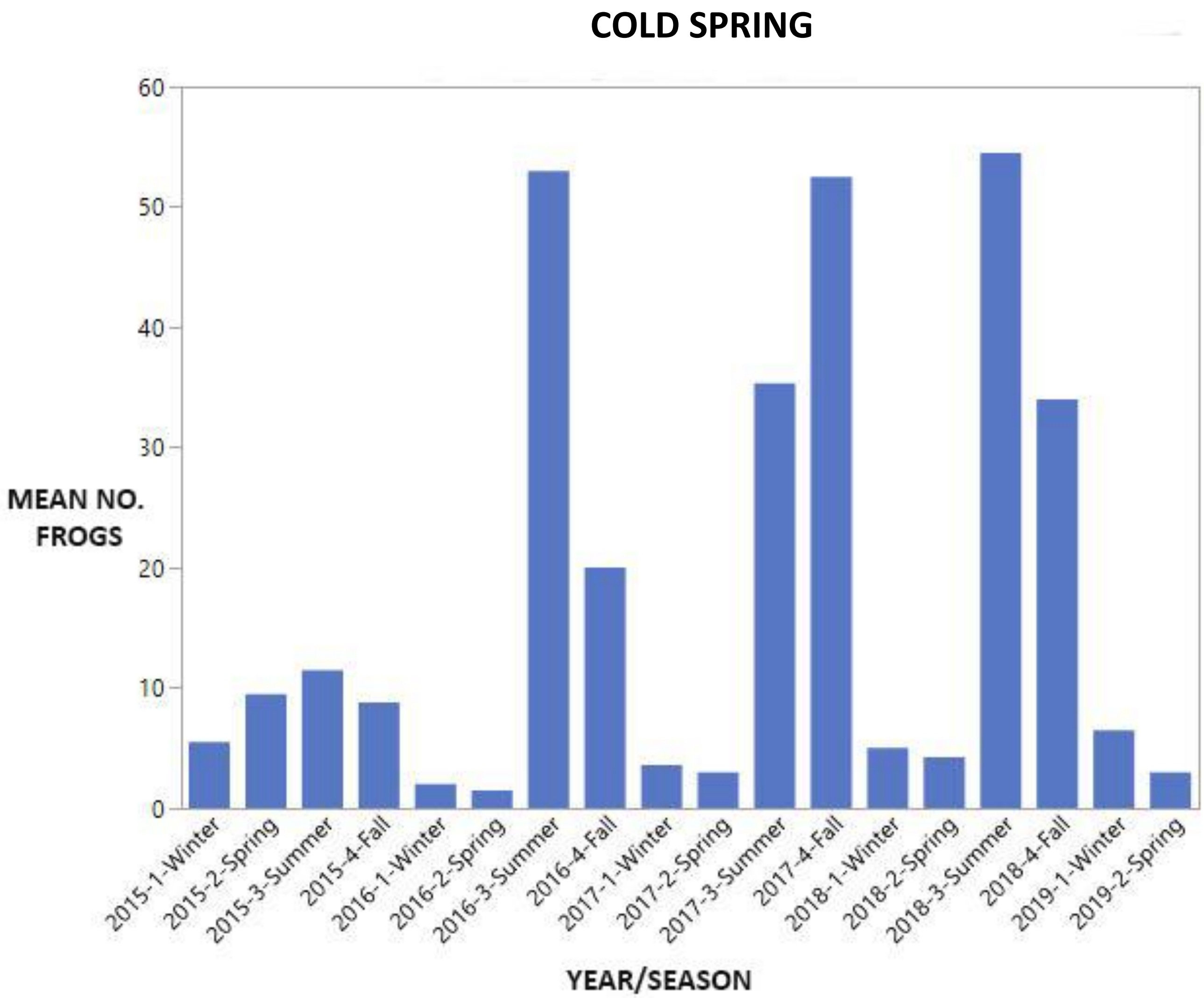

FIGURE H. Mean number of frogs counted during VES surveys by year and season in Cold Spring.

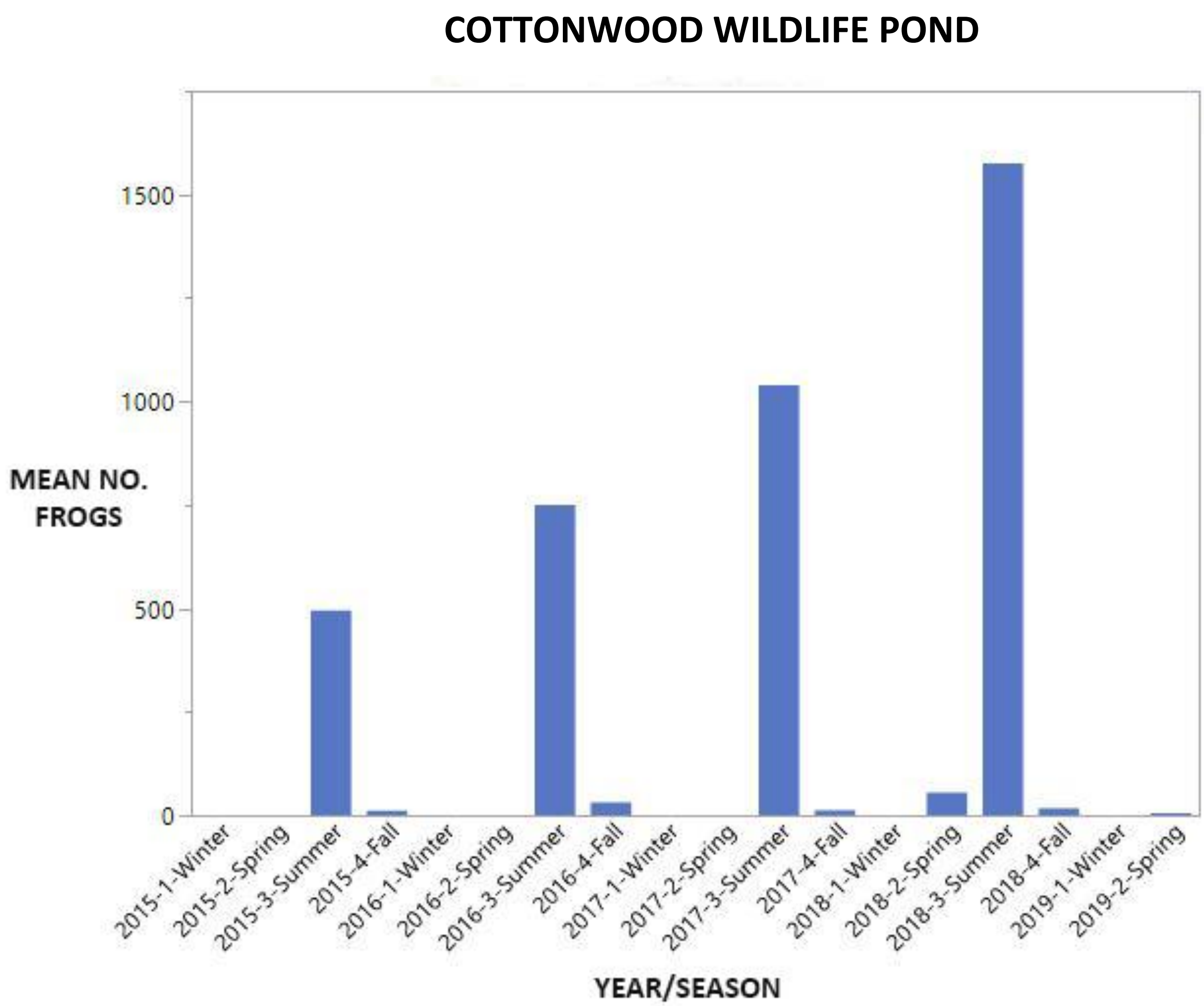

FIGURE I. Mean number of frogs counted during VES surveys by year and season in Cottonwood Wildlife Pond.

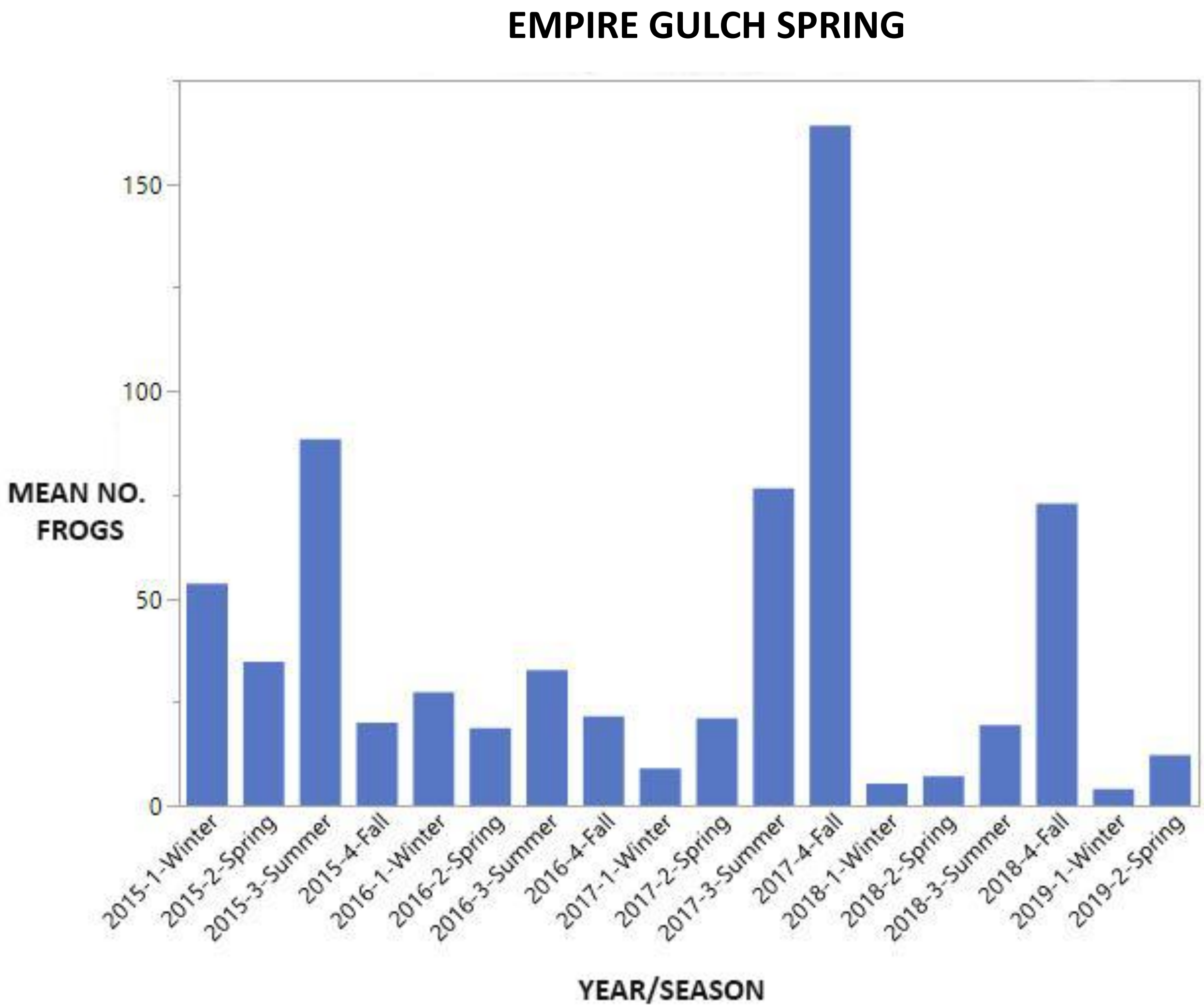

FIGURE J. Mean number of frogs counted during VES surveys by year and season in Empire Gulch Spring.

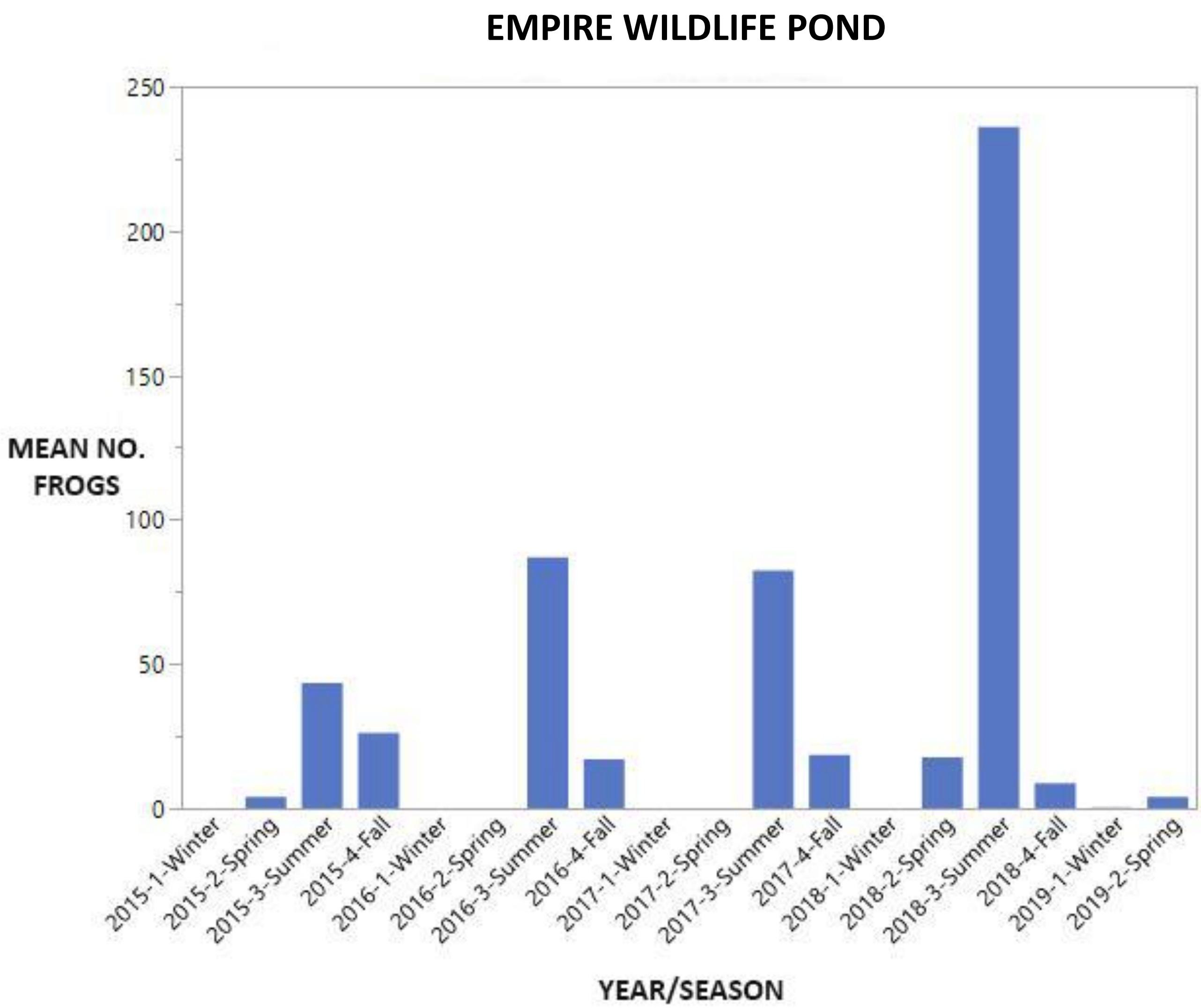

FIGURE K. Mean number of frogs counted during VES surveys by year and season in Empire Wildlife Pond.

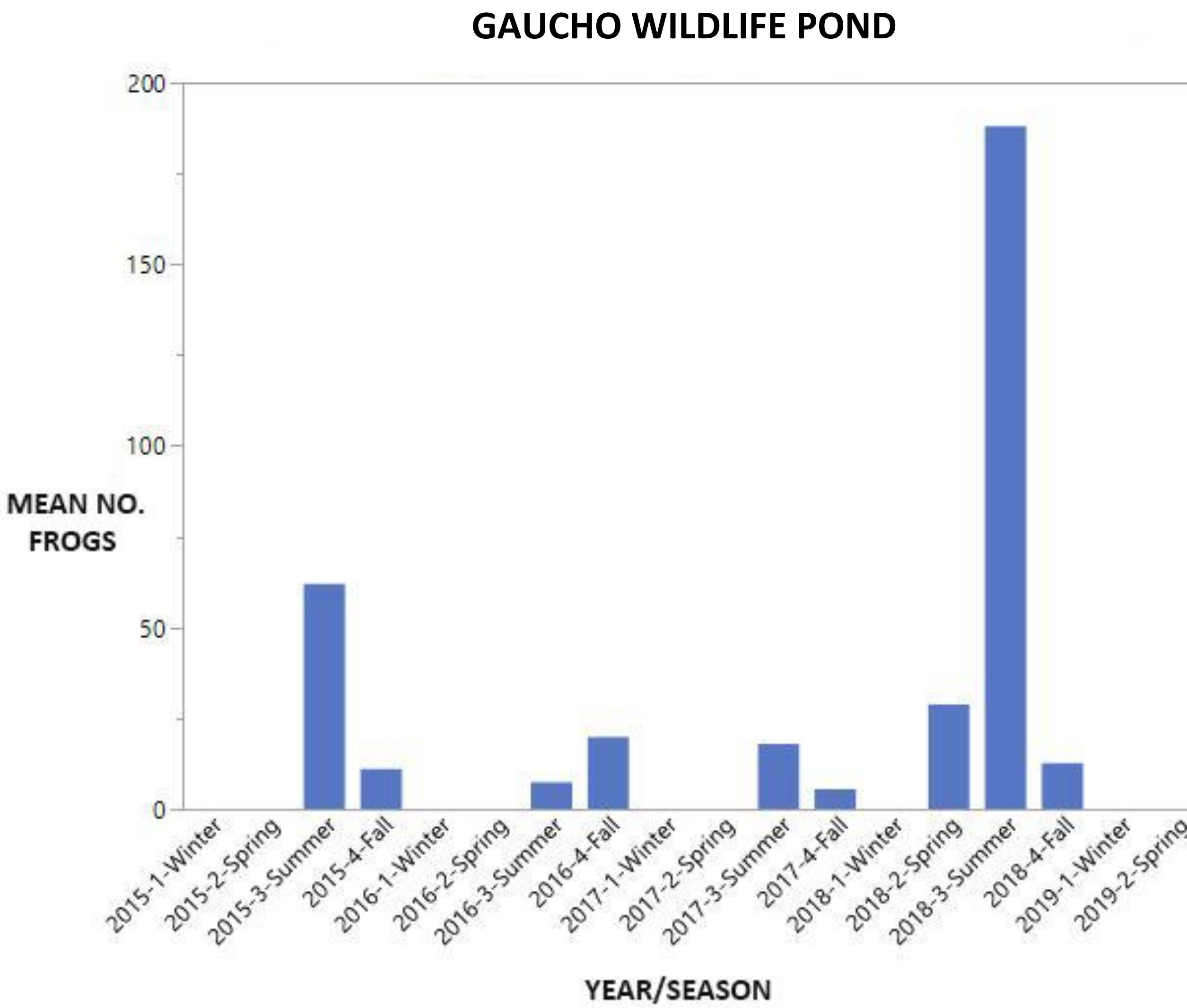

FIGURE L. Mean number of frogs counted during VES surveys by year and season in Gaucho Wildlife Pond.

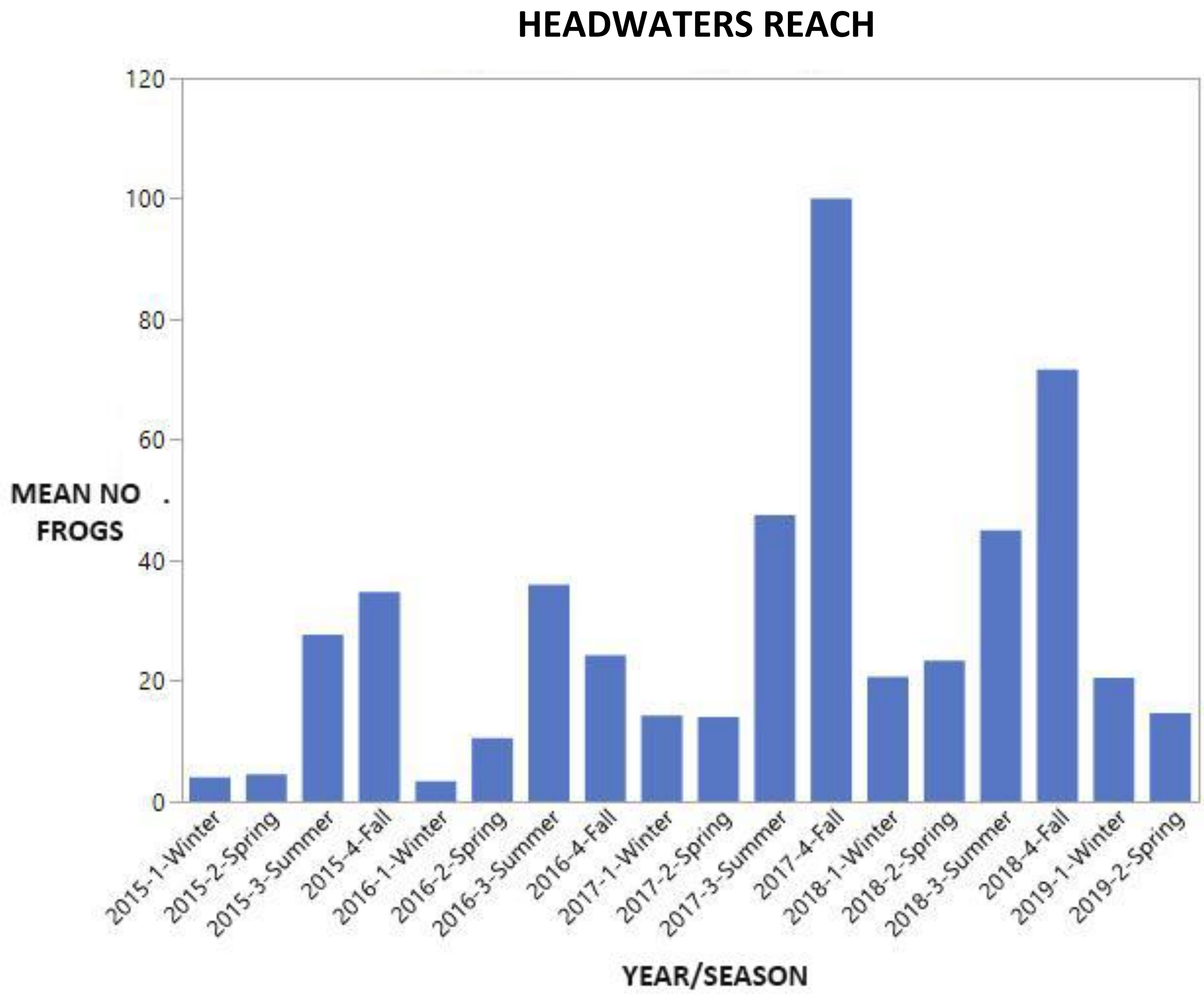

FIGURE M. Mean number of frogs counted during VES surveys by year and season in Headwaters Reach, Cienega Creek.

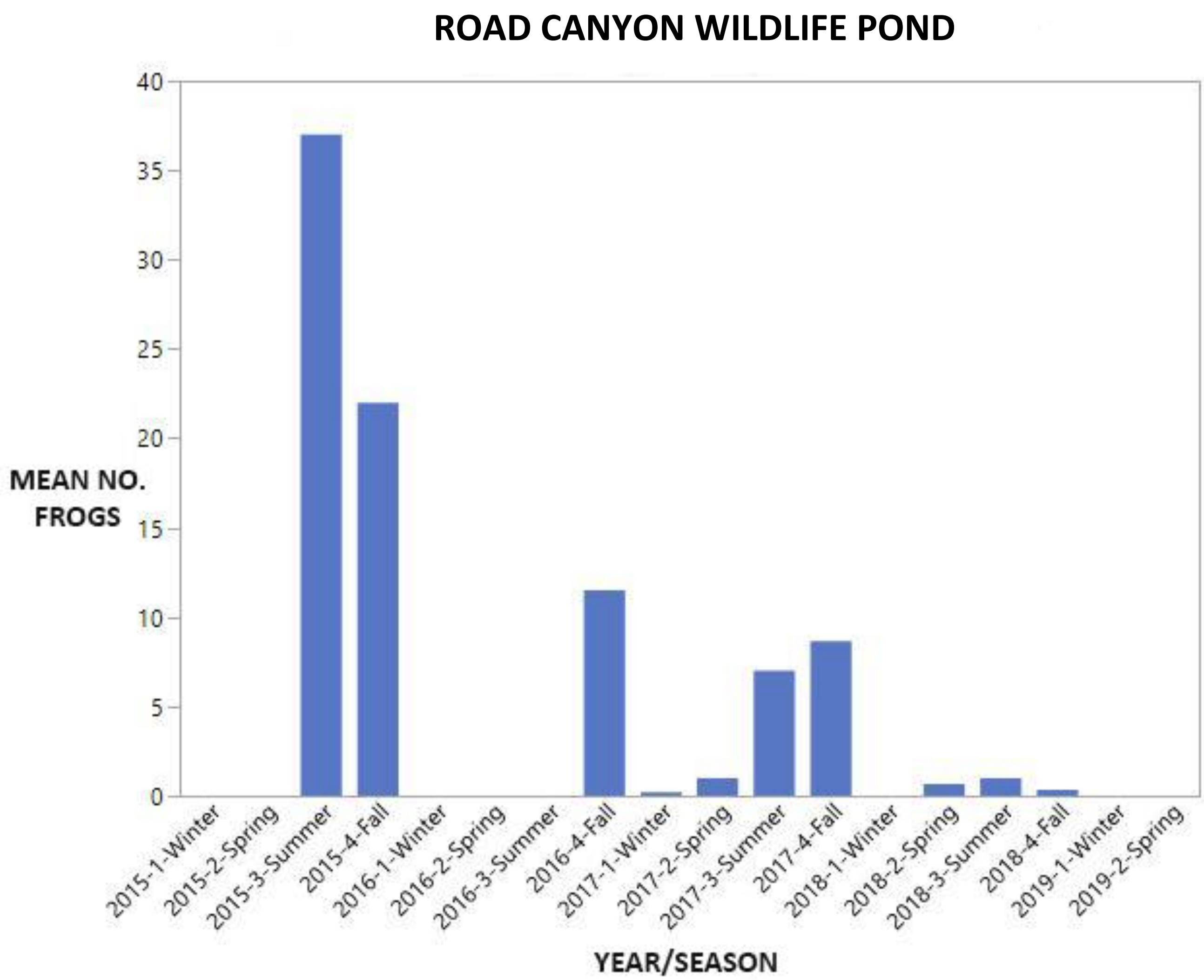

FIGURE N. Mean number of frogs counted during VES surveys by year and season in Road Canyon Wildlife Pond.