CPS CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title
Engineering and ecological approaches reduce Pacific tree frog intrusion into leafy green agriculture

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Principal Investigator
Paul C. Davidson (October 2018–December 2019; Co-PI, January–September 2018)
University of Illinois at Urbana-Champaign
Agricultural and Biological Engineering
1304 W. Pennsylvania Avenue
Urbana, IL 61801
T: 217-300-3755
E: pdavidso@illinois.edu

Co-Principal Investigators
Michelle L. Green (PI, January–September 2018)
University of South Florida St. Petersburg
Department of Biology
140 7th Ave S
St. Petersburg, FL 33701
T: 727-873-4351
E: mlgreen1@usfsp.edu

Jonathan K. Warner
Texas Parks & Wildlife Department
Alligator Program
10 Parks and Wildlife Drive
Port Arthur, TX 77640
T: 409-736-3625
E: Jonthan.Warner@tpwd.texas.gov

Objectives
1. Test novel drift fence designs and test non-toxic deterrents to determine whether placement of chemicals or physical materials coupled with improved fence design improve frog exclusion.

2. Test optical sorting and field identification potential of thermal imager.

3. Test the efficacy of non-invasive acoustics to redirect frogs away from water sources that are near/adjacent to agricultural fields.

4. Determine the comparative risk of frog intrusion in different production environments.

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FINAL REPORT

Abstract

Frogs are a persistent nuisance in leafy green production environments, but they are considered a low-level threat compared with other wildlife, such as feral pigs. Nationwide pathogen outbreaks linked to fresh produce, however, influence growers to implement extensive food-safety practices towards all wildlife, which often leads to the removal of frogs and their habitats. While strict in-farm practices towards wildlife are understandable, they may not be necessary, especially for frogs. Previous mitigation attempts for frog intrusions have failed, mostly because frog biology was not considered in the development of exclusion protocols. Our interdisciplinary team of wildlife biologists and agricultural engineers developed an evidence-based framework to address the complex challenges associated with frog intrusions in the production of leafy greens. We studied populations of the Pacific treefrog (*Hyliola regilla*) occurring within the lettuce fields and agricultural wetlands of the Salinas Valley, Monterey County, California. From microcosm experiments, we found that fences made of aluminum siding with a 10-cm lip at the top prevented all frogs from climbing over. Aluminum fences affixed with 320-grit sandpaper were also more effective at deterring frogs than standard silt fences and fences made of window screening supported by chicken wire. We installed the most effective and most feasible fence design (aluminum siding with a lip) at a large reservoir to demonstrate that this novel frog exclusion tool can be realized at the whole-field scale. Preliminary evidence indicated that this fence prevented at least 128 frogs from entering the reservoir in just 23 days (5.5 frogs per night). The fence also required little maintenance after installation despite persistent exposure to wind speeds exceeding 20 miles per hour, indicating its durability during real field conditions. We found that a hand-held thermal imaging camera was not an effective method for detecting frogs in the field, nor was a thermal imaging camera mounted to an aerial drone. From audio attraction experiments conducted at the field scale, we found an overall net increase of frogs attributable to acoustic interventions, but results were mixed across sites and months. The results from playback experiments, while promising, were too preliminary to draw robust conclusions and the seasonal timing of experiments was not synchronized with the local frog phenology, which may have further affected our results. From 192 manual surveys of frog calling activity across 16 wetland sites, we found a pattern of low-intensity frog calling beginning in late January, which increased in synchronization with winter rainfall and peaked by late Spring. Spatial variation in rainfall and frog calling activity suggests that some regions may exhibit a delay to this general breeding pattern and that the breeding season may extend longer into the Summer at some sites. From an examination of 751 frog body sizes, we detected a pattern of adult breeding beginning in late January and extending into early June, with the potential for additional breeding bouts in late Summer if stimulated by rainfall. We inferred the timing of the egress from wetlands for dispersing juveniles to have begun in May, with major exodus events in June, July, and August. From 271 mammals at one site, we found that the presence of a fence almost doubled the total number of pitfall-trap captures, but standardizing captures per unit effort revealed species-specific differences, such that mice and shrews were captured more frequently in an unfenced portion, whereas voles were captured more frequently in a fenced portion. From 123 amphibians at the same site, we found that the presence of a fence significantly increased the total number of captures and captures per unit effort compared to a section lacking a fence. When comparing small mammals across sites, we found that mouse and mole captures per unit effort were more than two-fold higher in northern sites, whereas shrew captures were six-fold higher in central sites. For reptile captures across all sites, more than 90% occurred during April to September, indicating that adequate temperatures were reached by mid-Spring, which increased the activity of most reptile species and continued throughout the Summer and Fall. Our study provides empirical information on the vertebrate communities in the Salinas Valley and may help growers to minimize their risks of wildlife intrusion, while promoting environmental stewardship.

Background

Wildlife that use agricultural areas represent a threat to produce safety by increasing the risk of food-borne illnesses via crop contaminations (Mandrell, 2009, 2011). The zoonotic-disease outbreaks resulting from the consumption of wildlife-contaminated produce are widespread across the United States and may be increasing in their frequency (Matthews et al., 2014; Alegbeleye et al., 2018). The species of wild animals linked to food-borne epidemics are diverse (Clark, 2014; Rice, 2014; Erickson, 2016), which has made it tremendously difficult for growers to put an end to wildlife contaminations because there is no single solution to exclude all types of animals from growing fields (Jay-Russell and Doyle, 2016). As a result, farmers must rely on approaches that work for certain animal groups but not others, or "scorched earth" methods that eliminate entire habitats and their resident wildlife (Lochhead, 2009).

Food-borne epidemics frequently lead to reform in food-safety policies for the produce industry, often resulting in the widespread adoption of severe practices towards wildlife. In 2006, for example, an outbreak of *E. coli* O157:H7 from spinach contaminated by feral pigs spread across 26 states infecting more than 200 individuals and killing three (Jay et al., 2007). The 2006 outbreak led to changes in food safety with a greater emphasis on harsh in-farm requirements that conflicted with the more wildlife conscious practices previously favored (Baur et al., 2016). The rapid shift in expectations meant that growers had to adopt new food-safety standards in order to sell their products (Stuart, 2009), including practices that were detrimental to wildlife near croplands (Stuart, 2011). As these practices became more widely implemented, other wildlife groups not connected to the original outbreak, including innocuous groups such as frogs, suffered from the pervasive application of "scorched earth" methods, such as the elimination of wetlands and destruction of natural vegetation (Beretti and Stuart, 2008).

Despite their constant presence in produce production environments, frogs remain an understudied group among the wildlife that utilize agricultural areas as habitat (Gorski et al., 2013; Langholz and Jay-Russell, 2013). Destructive approaches to reduce frog intrusions into growing fields seem to be the outcome of adopting food-safety policies that conflict with empirical data. Although epidemiological sampling of amphibians is far behind that of mammals and birds, the potential for frogs to contaminate produce with human pathogens is low (Jay-Russell, 2013; Langholz and Jay-Russell, 2013). Treefrogs and toads have been found to harbor various *Salmonella* serovars from wild populations in California's agricultural fields yet at relatively low rates of infection (Gorski et al., 2013). In fact, it is unclear whether wild amphibians have ever been identified as the source of human infections via contaminated produce. Parish (1998) isolated *Salmonella* serovars from wild frogs near an orange juice facility implicated in a 1995 salmonellosis outbreak, yet the strains isolated from those amphibians were not clinically relevant to the epidemic. Other than the 1995 *Salmonella* outbreak, amphibians have not been implicated in any major food-borne illness epidemic. Furthermore, no epidemiological studies to date have isolated the deadly *E. coli* O157:H7 from amphibians in the wild (Ferens and Hovde, 2011).

Even though the evidence suggests that frogs do not pose a serious contamination risk, frogs are still problematic to the fresh produce industry because they can cause major economic losses by ending up in prepackaged produce at the hands of the consumer (Hughes et al., 2019). Frogs are also a constant presence in the reservoirs of agriculture fields and are extremely difficult to remove once a breeding population is established. Growers are constantly coming up with new ways to deal with their farm's frog problem, but with no major successes thus far. In the past, growers often installed fences to exclude frogs from sensitive agriculture areas, but these obstacles failed to inhibit frog movements, with some growers reportedly observing treefrogs climbing directly over the fences. Previous attempts at frog exclusion likely failed because treefrog biology was not considered when the designing the fences. Although studies may not have identified frogs as major vectors in food-illness outbreaks with fresh produce, the sampling of wild frogs has not been robust, geographically nor quantitatively (Gorski et al., 2013). As a result, growers cannot yet eliminate frogs as a potential source of microbial disease before much more work is done to better understand the prevalence of pathogens in wild frog populations. Because of the myriad risks that frogs pose to leafy green agriculture, there is a critical need to explore novel management practices that focus on leveraging knowledge on basic frog biology and ecological data on local populations to mitigate the frog threat, especially in the field-growing environment.

Aspects of frog populations in relation to farm operations are idiosyncratic, such that each farm possesses a unique suite of environmental and physical characteristics that contribute to a distinct frog community and their phenology. As a result, field studies across a variety of environments are needed to assess the risk of intrusion from several frog populations in relation to the variation in farm features. Site-specific data on frog activity will be vital for growers located in different areas to adopt practices based on their local conditions and to make informed decisions about where the highest risks of frog intrusions will come from. While effective management practices are critically needed, the design and implementation of practices should be cost-effective, relatively easy to install, and require little maintenance throughout the growing season and cause the least amount of disturbance to the surrounding environment.

Here, we provide comprehensive data on the effectiveness of several methods to detect, reduce, and avert intrusions of the Pacific treefrog (*Hyliola regilla*) in agricultural lands across the Salinas Valley, Monterey County, California (Fig. 1). We experimentally implemented cost-effective exclusion barriers that successfully reduced frog intrusions at multiple spatial scales. We demonstrated that thermal-based imaging technologies are not effective for detecting frogs in the field. We provided a proof of concept for the promise of using passive acoustic devices to redirect frogs away from sensitive water bodies. Finally, we rigorously collected empirical data on local vertebrate communities (amphibians and small mammals) living in agricultural areas to understand how these populations vary temporally and spatially. Our findings will enable growers to implement a nuanced and integrative system for the effective co-management of agriculture and frogs in the Salinas Valley.

Research Methods and Results

Field Sampling Methods

Permits: Animal-handling protocols were approved by the University of Illinois Urbana-Champaign's Institutional Animal Care and Use Committee (IACUC protocol 18081). Permits to conduct research in the Salinas Valley were approved by the State of California's Department of Fish and Wildlife (SC-13909 and GOID-1060196653).

Study species: We studied the Pacific treefrog (*Hyliola regilla*) because it has been reported as the most frequently encountered species by growers in our study region. This species is extremely variable in adult coloration and individual frogs can become lighter or darker and patterns can entirely disappear in response to environmental conditions, such as sunlight, but the black eye stripe does not change (Fig. 1A-H). Pacific treefrogs cover a large geographic distribution in western North America spanning from southeastern Alaska through most of California, Oregon, Washington, and Nevada, the western portions of Idaho, Montana, and Arizona, and south to Baja California (Dodd, 2013). The Pacific treefrog has been reported as the most abundant frog in California (Rorabaugh and Lanoo, 2005). In general, November–July is considered the time period when Pacific treefrogs move from the terrestrial retreats they use as overwintering sites to aquatic breeding sites, but this is variable across its range. Males (25.5–48 mm snout-vent length) and females (25–47 mm snout-vent length) are sexually mature around 1 inch in body size and could reach maturity in less than 1 year. Pacific treefrogs use a variety of habitats in their home ranges, including upland, overwintering, and aestivation sites, breeding ponds, and migratory corridors, where they are often found in moist microhabitats, such as under rocks, cracks in dry soil, and in animal burrows (Rorabaugh and Lanoo, 2005). Overwintering sites have been reported to be about 150–300 m from breeding sites, however, breeding males have made movements of up to 400 m (Schaub and Larsen, 1978). Although Pacific treefrogs can climb well, they are usually found terrestrially (Rorabaugh and Lanoo, 2005). Pacific treefrogs can occupy nearly any wetland habitat, including streams, ponds, and irrigation ditches, many of which they will also use as breeding sites (Dodd, 2013).

Study area: We studied Pacific treefrog populations in the Salinas Valley, Monterey County, California (Fig. 2). Also known as “America's salad bowl” (Anderson, 2000), the Salinas Valley is the largest growing region for leafy green vegetables in the United States, where it annually produces more than 70% of the nations' leafy green vegetables (USDA, 2014). Specifically, our research was focused on three main areas across a latitudinal gradient in the Salinas Valley: one in each of the northern, central and southern sections of the valley. Within these three areas, we selected sites in coordination with industry cooperators who directed us to locations that had some documented history of frog intrusions (Table 1; Fig. 2). Nevertheless, the documentation of frog incidents was not systematic across cooperators nor were the incidents quantitated in a way that we could use to prioritize problematic sites. As a result, we relied on anecdotal accounts of historical frog activity, sometimes based on stories that were likely not of the target species, but of some other amphibian species in the area. Furthermore, the sites we were permitted to select from were a subset of sites occupied by the Pacific treefrog within the region and thus some aspects of the environment may have been overrepresented in our sites. Inevitably, the combination of anecdotal evidence and limited site knowledge produced suboptimal site selections. Nonetheless, our selected sites did not preclude us from gathering meaningful data that will help growers combat their wildlife intrusion problems. In the Northern area, we intensively trapped frogs at a large tailwater pond (site 4) and also conducted night surveys of frog-calling activity at four other wetland sites, three of which were tailwater ponds (sites 2–3, and 5) and the other was a groundwater-well (site 1). In the Central area, we intensively trapped frogs along two straight-line transects at the bottom of an erosion-control hillside made of the coastal succulent ice plant (site 2). The hillsides are nearly contiguous but separated by a narrow road. Because this was not a wetland, we did not expect to find much evidence of frog activity, so we expanded our study to include other small vertebrates that fell in our pitfall traps, including lizards, snakes, and rodents. The cooperators that controlled this site had previously installed a series of pitfall traps (see below) to catch and kill wildlife that fell in. As a result, we leveraged the previous work at this site to test the difference between animal capture rates in pitfall traps with and without a drift fence. We also conducted night surveys of frog-calling activity at five other wetland sites in the Central area, all of which were tailwater ponds (sites 1, and 3–6). In the Southern area, we intensively trapped frogs at two reservoirs, one large (site 5) and one small (site 6) wetland, but this smaller site was decommissioned by the cooperator during our study (July 2019). We also conducted night surveys of frog-calling activity at four other wetland sites in the Southern area, all of which were large reservoirs (sites 1–4).

Frog sampling: We sampled Pacific treefrogs with both passive and active trapping methods. Drift fences, consisting of 91-cm tall silt fencing, were installed with paired pitfall traps (one on either side at the same point of the fence) around wetland sites or along an erosion-control hillside to intercept moving animals (Todd et al., 2007). Pitfall traps consisted of 5-gallon buckets embedded in the soil and were installed at approximately 15-m intervals along drift fences. A hole was cut in a series of lids to create a rim (3 in) at the top of the bucket to deter animals from climbing out (Mazerolle, 2003). A separate series of whole lids were used to cover buckets in between sampling bouts. Holes were drilled into the bottom of buckets for drainage and a sponge was placed in the bucket for moisture. During sampling bouts, the buckets were opened and checked every 24 hours. When a sampling session was over, whole lids were secured on the buckets and a portion of the fence removed to allow free passage of animals. In the Southern area, we fully encircled site 6 with a drift fence and 10 buckets, and we fenced 95% of site 5 with a drift fence and 20 buckets. Site 5 in the Southern area could not be fully encircled because it was an active reservoir with a large water pump that the cooperators needed to maintain a route to for vehicle access. In the Central area, we installed a drift fence with 50 buckets along an approximately 1000 ft-long transect at site 2, while keeping a nearby section of the same hillside fence free, but with 21 buckets. In the Northern area, we completely encircled site 4 with a drift fence and 20 buckets. We had a total of 121 pitfall traps installed across four sites. We used white polyvinyl chloride (PVC) pipes (3.81-cm diameter and 60-cm long) to construct frog retreats (Boughton et al., 2000), which were elevated off the ground by fastening them to a standalone stake or a stake along a drift fence (Myers et al., 2007). The bottom of the pipe was sealed to allow water to fill and a small hole was drilled 6 cm from the bottom of the pipe to allow drainage. We installed 25 pipes at the Southern sites (10 at site 6 and 15 at site 5), 20 pipes at Central site 2 (10 along the fence and 10 along area without the fence), and 15 pipes at Northern site 4. We also placed wooden cover boards (0.9 m x 0.9 m) flat on the ground in areas of natural travel ways around the sites. We deployed 10 boards in the Southern sites (5 at site 6, and 5 at site 5), 10 in the Central site 2 (5 along the fence and 5 along area without the fence), and 10 in Northern site 4. Passive traps, such as PVC pipes and cover boards, allow frogs to move freely in or out of them. We spaced these passive traps at even intervals at sites. We used a long-handled aquarium net to collect tadpoles from wetlands. We checked pitfall traps during 5-day intervals once per month from November 2018 to September 2019 for a total of 11 site visits, 55 days of sampling, and 6,655 total pitfall trap nights.

Night surveys: We conducted night surveys to assess seasonal trends in frog calling activity at all wetland sites (16 sites) once per month from November 2018 to September 2019 for a total of 192 site visits. Most sites were visited on the same night, but some nights only a subset of sites were visited. We standardized the time spent during night surveys across all sites (10 min/site) so that effort was equal when searching for frogs and recording calling activity. We used nocturnal visual-encounter surveys to locate frogs to detect true occupancy, which involved turning over logs, rocks, and other natural objects. Frogs detected during nocturnal searches were simply recorded, but not captured. We used the North American Amphibian Monitoring Program guidelines to generate a frog calling index score at each site during each survey (Weir et al., 2014). Scores range from 1 to 3: 1 means distinct calls of individuals that can be counted with no overlapping calls, 2 means calls of individuals that can be distinguished but some overlapping calls, and 3 is a full chorus with calls of individuals indistinguishable. We downloaded rainfall data from the University of California's Agriculture and Natural Resources Statewide Integrated Pest Management Program (http://ipm.ucanr.edu/WEATHER/SITES/monterey.html) for each weather station closest to the survey sites (Northern, Central, and Southern). We also collected environmental data from each wetland to understand what factors may influence calling activity and frog occupancy, including air temperature and relative humidity with a Kestrel 5700 weather meter, water temperature and pH with an Oakton PCTSTestr 50, and a qualitative assessment of the water quality and aquatic vegetation growth.

Frog handling: All frogs captured in traps were measured for snout-vent length and tibia-fibula length with calipers to the nearest 0.1 mm, weighed with a Pesola spring scale to the nearest 0.1 g, marked using a unique toe-clip combination (Donnelly et al., 1994), and then released at their point of capture. Some frogs were held overnight for fence trials (see below) and were marked the next morning prior to their release. Adult males were differentiated from females by the presence of a darkened vocal sac (Dodd, 2013). Gravid females were assessed by examining the presence of eggs via a transparent section of skin in the inguinal region.

Fence trials: To assess the exclusion effectiveness of different fence designs, we constructed fully enclosed terrestrial pens using PVC pipes and mesh screening (1.5 m x 1.5 m x 1.5 m) (Fig. 3). Two pens were temporarily installed adjacent to site 5 in the Southern area. Each experimental fence design was constructed as an open-air square (0.9 m x 0.9 m) that was placed in the middle of the screened-in pen for trials and then replaced when appropriate sample sizes were reached. Frogs were captured using the methods described above and held in plastic containers (22 cm x 17 cm x 10 cm) containing moist paper towels and air holes until trials. Each fence trial began at dusk and ended at dawn of the next day. To run fence trials, captured frogs were placed in the center of the experimental fence inside the pens and the top of the pen was closed. We counted the number of frogs that were able to traverse over the experimental fence and found in the outer pen in the morning. Each frog was tested only once in a fence trial and all individuals were held for less than 24 hours and released at their original site of capture after the experiment. The tops of the pens were covered with wood to provide shade and small rocks were placed in the pens for refuge for frogs during the trials. We determined whether frogs scaled the test fence by checking whether they were in the main test arena or in the center square of test fence the next morning.

Fence designs: We developed three new fence designs and one control to test their scalability by frogs. First, we used aluminum siding (0.9-m tall) with a 10-cm lip at the top (Fig. 3B) because aluminum siding can withstand exposure to harsh weather like that experienced in the Salinas Valley and we added the lip to the top because previous studies have shown that it can hinder amphibian movements (Mazerolle, 2003). Second, we used screen-porch material (1.2-m tall) supported with chicken wire (Fig. 3C) because this material is commercially available, and it showed promise as a hindrance to climbing in preliminary frog trials. Third, we tested sandpaper (320-grit size) affixed to aluminum siding (0.9-m tall) (Fig. 3D) because laboratory experiments demonstrated that this material can disrupt the adhesive properties of treefrogs and that it can induce frequent slipping during climbing (Crawford et al., 2016). Lastly, we tested the standard contractor-grade silt fence (91-cm tall) as a control because it is the most commonly installed fencing material in the Salinas Valley around water sources.

Thermal imaging: We surveyed the best available thermal imaging cameras on the market and purchased a Forward-looking infrared (FLIR) camera. We chose the FLIR E6-XT model because it is handheld, has a large screen to visualize the infrared camera output, and expanded temperature range (−20°C to 550°C) relative to other models. FLIR hand-held thermal cameras have been used successfully to image a wide variety of ectothermic species from caterpillars (Nielsen et al., 2015) to lizards (Michelangeli et al., 2018). We tested the efficacy of thermal imaging for visualizing frogs in the agricultural environment as a proof of concept as to whether this technology could be adapted by growers as a quick and reliable means to detect frogs in growing fields. We photographed frogs in a variety of field settings using the hand-held FLIR thermal imaging camera, including during the day and night. We also photographed frogs using a thermal camera attached to a drone flying at 10 m above the ground.

Acoustic playback: We selected three wetland sites in the Central area (site 1, site 3, and site 4) to conduct audio playback experiments in an attempt to attract conspecific frogs from other areas to these sites. We chose these three sites because they were the least occupied by frogs (zero or very few) among all 16 study sites during the monthly surveys from November 2018 to May 2019 (7 surveys per site). Unoccupied sites allow for a more straightforward interpretation for frogs found in these wetlands after acoustic playbacks, as they were likely attracted to them from the experiments. We used audio playback devices (FOXPRO Inc. Snow Pro Digital Game Call) consisting of a weatherproof box (10 in x 7.5 in x 4 in) that housed a rechargeable lithium battery and two large external speakers (7 in x 5 in; 10 watts, 8-ohm). We deployed one playback device per site in a position near the edge of the water to simulate where a male frog would typically call from. We played a looped 10-s recording of chorusing Pacific treefrogs (Davidson, 2014) because several frogs calling together is more attractive to conspecifics than a single frog (Buxton et al., 2018). We conducted night surveys to quantify the baseline number of frogs present at each site before audio intervention experiments, then we deployed the audio devices and set the TX433 transmitter to play continuously (volume setting 10) on loop all night. The devices were deployed shortly after sundown and retrieved the next morning. The audio playback sessions were repeated for four nights each month from June to September, except only three nights in June, for a total of 15 surveys. We examined the net change in the number of frogs from the initial survey to the last survey night.

Results

Objective 1. Test novel drift fence designs to improve frog exclusion

Fence trials: From 32 independent trials with 258 different frogs (each frog was only used once and then marked prior to their release) using four fence designs (mean = 8 frogs per trial), we found that fences made of aluminum siding with a 10-cm lip at the top were the most effective at preventing frogs from climbing over (0% escaped [0 out of 62 frogs]) (Fig. 4). Fences that incorporated 320-grit sandpaper (glued to aluminum siding) were also effective at preventing frogs from climbing over (8% escaped [5 out of 61 frogs]). Fences made of window-screen material (53% escaped [36 out of 67 frogs]) and standard silt fences (55% escaped [37 out of 67 frogs]) were not effective at preventing frogs from climbing over. Of the top two exclusion-fence designs, we determined that the aluminum siding with the lip would be the most feasible for whole field implementation because adhering sandpaper to a large fence would be a very time-consuming activity and require a lot of maintenance after installation. As a result, we installed the most effective fence design (aluminum siding with a lip at the top) at a large reservoir in the Southern area (site 5) on 27 August 2019 because this site was to be decommissioned at the end of the season. Preliminary evidence on the number of frogs found desiccated at the bottom of the fence and among the substrate collected on 19 September 2019 indicated that this scaled-up fence prevented at least 128 frogs from entering the reservoir in just 23 days (mean = 5.5 frogs per night). The fence also required little maintenance after its initial installation despite persistent exposure to wind speeds exceeding 20 miles per hour in September 2019. We recommend using wooden and metal stakes to ensure that the fence posts will not lean or break during major wind gusts and persistent exposure to high wind speeds.

Objective 2. Test potential of thermal imager for field identification of frogs

Thermal imaging: We found that thermal imaging is not an effective method to detect frogs in the field. Using a drone flying at 10 m above the ground, we could not distinguish the heat signature of two frogs relative to the background grass (Fig. 5A). These two frogs imaged by a thermal camera mounted on a drone represent two much larger species (Leopard frog [50–100 mm] and Bullfrog [100–175 mm]) than the Pacific treefrog (20–40 mm), indicating that it would be even more difficult to visualize smaller frog species using a drone. Thermal imaging using the hand-held camera taken from distances between 0.3 to 0.9 m during both day and night also were not effective for the detection of frogs in various environments (Fig. 5B–D). Thermal imaging of several Pacific treefrogs taken at night from approximately 0.9 m away did not provide strong signatures of their heat profiles that could be easily distinguished from other objects in the field of view (Fig. 5B). Images using an integrated visual field (thermal + standard camera) taken during the day at distances less than two feet away likewise did not provide frog heat profiles that could be readily distinguished from other objects in the field of view despite the unmistakable outline of the frog in the images (Fig. 5C–D). We could not rule out the possibility that a more sensitive thermal camera could improve the visualization of frogs, but that possibility seems highly unlikely given the thermal range of the model we used, and our photographs which were taken under a variety of environmental conditions.

Objective 3. Test the efficacy of non-invasive acoustics to redirect frog movements

Acoustic attraction: Preliminary occupancy data revealed that three sites in the Central area would work well for these experiments because they lacked frogs (or nearly so) and nearby sites in the region had frogs. We observed the greatest influx of frogs to sites in June, where two sites had a net positive change in the number of frogs after acoustic experiments, and only one site lost a frog (Fig. 6). In July, we found a similar overall pattern of a net increase in frogs after experiments but much more variation across sites. In August, we observed a net loss of frogs from two sites and one site had no change, whereas September showed no change in frog numbers at two sites and a net increase at one. These results suggest that non-invasive acoustics can attract conspecific frogs to a wetland site, but our data represent a small sample collected over a relatively short period of time. We found that male frogs at a wetland site were completely silent until the audio device began playing the recorded frog calls, then all the male frogs would begin calling as well. One male frog even established himself near the device over multiple nights and would vacillate between normal calls and antagonistic calls, indicating that the recordings sufficiently replicated a real frog chorus. The results from these acoustic interventions may have been profoundly influenced by the fact that these experiments were not synchronized with local phenological patterns in frog breeding, and thus even though some frogs appeared to respond positively, we have no way of knowing if the frogs would actually use these sites as breeding habitats. We suspect that these were not suitable breeding sites given that they were almost entirely unoccupied during the local reproductive season. This non-invasive technique, however, holds great potential as a tool for redirecting frogs away from sensitive growing areas when used in combination with other techniques. For example, new frog-breeding sites that are more optimally located for growers could be established if rigorous multiyear acoustic attraction protocols were combined with the creation of new wetland habitats for frogs.

Objective 4. Determine the comparative risk of frog intrusion in different environments

Seasonal and spatial variation in frog calling activity: Monthly rainfall patterns varied across sites, with those the Central and Southern areas experiencing similar timing and intensity of precipitation during 1 September 2018 to 1 October 2019 (Fig. 7). There was a general trend of peak rainfall during January to March with the peak frog calling activity shortly thereafter, with some sites in the Southern area exhibiting high levels of frog calling from after Spring rains well into late Summer. Generally, the more northern sites had less rainfall and less frog calling activity compared with the more southern sites. Of the five wetlands sites in the Northern area, only two had any recorded frog calling activity and just one of these actually reached the level of a full breeding chorus, but only during February and March surveys. In the Central area, sites 1 and 6 exhibited consistent calling activity throughout the study, but the peak in their activities were reversed, suggesting that there may different breeding peaks even at the site-specific level. Site 5 in the Central area did not exhibit any calling activity until May and then a peak in July, indicating that sites are not homogenous in calling activity at the landscape scale. All six sites in the Southern area had some level of frog calling activity throughout the study and a distinct onset of calling activity during and shortly after peak annual rainfall. Mean values for air temperature, water temperature, and pH across sites did not exhibit much variation, nor did they vary by much within a specific region (Fig. 8). The mean values for pH did not fluctuate much across regions and did not seem to have any relationship with frog calling activity. It appears that after a peak in rainfall that usually occurs by the end of March, there is an increase in both the air and waterbody temperatures, which generally coincides with an increase in frog calling activity that persists for several months thereafter.

Frog seasonal population dynamics: During all months of sampling, only 20 frogs were captured at Northern site 4 and just 67 frogs at Central site 2. As a result, we focused our results on the seasonal dynamics of frog populations on Southern sites 5 and 6 which produced the highest number of captured frogs. From an examination of 751 individual frog body sizes found in the traps from two Southern sites, we detected a clear pattern of breeding onset in late January that extended into June, with the potential for additional breeding bouts in late Summer if stimulated by appropriate rainfall (Fig. 9). By examining the number of frogs found in buckets inside the drift fence (closer to the wetland) to the number found in buckets outside of the drift fence (further from the wetland), we were able to infer the timing of the pulses of ingress to the wetlands for breeding adults and egress from the wetlands for dispersing juveniles (Fig. 10). Adult frogs capable of breeding were either already present at the wetlands or arrived during January to March. Juvenile frogs appeared in May with major exodus events from the wetlands in June, July, and August, likely stimulated by rainfall events.

Fence versus no fence for small mammals and amphibians: We captured a total of 271 small mammals at Central site 2 across all sampling months. Site 2 is split into two pitfall sampling transects (1000 m each), one with a fence and one without a fence, positioned at the base of a hillside covered in erosion control vegetation. From the transect that had a fence, we captured 186 individuals consisting of four species (112 California Voles [*Microtus californicus*], 47 California Mice [*Peromyscus californicus*], 22 Shrews [*Sorex* spp.], and 5 Broad-footed Moles [*Scapanus latimanus*]), which was more than double the amount of individuals captured at the transect that lacked a fence (85 small mammals consisting of the same four species: 42 California Mice, 21 Shrews, 20 California Voles, and 2 Broad-footed Moles) (Fig. 11). Because of the different number of buckets at each transect (50 vs 21), we standardized trapping by the number of nights the buckets were open (55 total nights). Standardized captures per unit effort (CPUE) revealed that mice (0.036 vs 0.017) and shrews (0.018 vs 0.008) were captured more frequently in the unfenced portion compared to the fenced section of site 2. In contrast, the CPUE for voles was more than double in the fenced portion (0.040) compared to the unfenced portion (0.017). The CPUE for moles were equally low between both treatments.

We captured a total of 123 amphibians at Central site 2 across all sampling months. From the transect that had a fence, we captured 107 individuals consisting of four species (69 Western Spadefoots [*Spea hammondii*], 29 Western Toads [*Anaxyrus boreas*], 7 Pacific Treefrogs, and 2 California slender salamander [*Batrachoseps attenuatus*]), which was more than six times the amount of individuals captured at the transect that lacked a fence (17 amphibians consisting of the three species: 8 Western Spadefoots, 7 Western Toads, and 1 Pacific Treefrog (Fig. 12). Standardized CPUE revealed the same general pattern, with all species captured much more frequently in the fenced portion compared to the unfenced (mean = 2.7-fold increase in CPUE).

Seasonal and spatial variation in activity for small mammals and reptiles: We captured just 11 small mammals from Southern sites 5 and 6 (7 California Mice, 3 Broad-footed Moles, and 1 Shrew). As a result, we focused our comparison on the seasonal dynamics of small mammal activity between Central and Northern populations. From Northern site 4, we captured 111 individuals consisting of four species (54 California Mice, 42 California Voles, 13 Broad-footed Moles, and 2 Shrews) from 20 pitfall traps (Fig. 13). Mouse and mole CPUE were more than two-fold higher in Northern than in Central sites, whereas shrew CPUE was six-fold higher in Central compared with Northern sites. Vole CPUE was only slightly higher in Northern compared to

Central sites. The peak in the amount of vole captures differed between the two regions by three months, with the most in March for Central sites and the most in June for Northern sites. Mice exhibited a slightly bimodal peak in captures in Northern sites (May and December), whereas a more unimodal peak was observed in Central sites (December).

We combined all reptile captures from pitfall traps and cover boards to examine spatial differences in seasonal activity and community composition (Fig. 14). Central site 2 possessed the highest species diversity of reptiles with six, including one snake (3 California Striped Racers [*Masticophis lateralis*], four lizards (67 Western Fence Lizards [*Sceloporus occidentalis*], 17 California Whiptail Lizards [*Aspidoscelis tigris*], 4 Alligator Lizards [*Elgaria multicarinata*], and 1 Side-blotched Lizard [*Uta stansburiana*]), and one skink (2 Western Skinks [*Plestiodon skiltonianus*]). Northern site 4 had the second highest species diversity with four, including two snakes (11 California Red-sided Gartersnakes [*Thamnophis sirtalis*] and 8 Pacific Gopher Snakes [*Pituophis catenifer*]), one lizard (51 Western Fence Lizards), and one turtle (1 Southwestern Pond Turtle [*Actinemys pallida*]). Southern sites had just one reptile species that dominated all captures (186 Side-blotched Lizards). Across all sites, only five reptiles were captured during January to March and just 29 were found during November and December; the remaining 317 captures all occurred from April to September, indicating that adequate temperatures beginning in mid-Spring led to increased activity of most reptile species throughout Summer and Fall, with some species not active until mid to late Summer (California Whiptail Lizard and Western Skink). Side-blotched Lizards in Southern sites were active from April to November, with a salient peak in captures during late Summer to early Fall, when most of these encounters were of newborn young.

Outcomes and Accomplishments

We successfully monitored, sampled, and inferred patterns in wild animal populations at several sites in the Salinas Valley, Monterey County, California. We were able to characterize the breeding season of Pacific treefrogs in the region and show that there is sufficient variation within and across populations to warrant additional studies in different areas. We successfully expanded the scope of our study to include all small vertebrate species found in the pitfall traps as a means to provide data for growers from a wider diversity of taxa than just a single frog species. We showed that simply installing a fence, even if it is just a standard silt fence, will significantly impact the movement of small animals in a growing area. We demonstrated that a new fence design successfully deterred frogs from climbing over the top and that this design was feasible to scale-up to enclose an entire reservoir. We showed that thermal imaging is not an effective method to visualize frogs. We demonstrated the potential that acoustic interventions hold for redirecting frogs away from sensitive wetland environments. The project successfully applied wildlife monitoring approaches in a co-management framework and integrated animal biology into exclusion tools to address intrusion risks from frogs in agriculture production environments. We developed a picture-based field guide for amphibians in the Salinas Valley. The field guide is designed for farmers to have the capability to immediately identify an amphibian when working in the field. This project supported a postdoctoral research associate, Dr. Daniel F. Hughes, who carried out the field research, conducted the analyses, created the field guide, and wrote the final report.

Summary of Findings and Recommendations

Wildlife frequently incorporate agricultural fields into their home ranges, which presents a serious threat to food safety from pathogen contamination of crops. Frogs are one such wildlife group often found in lettuce fields in the Salinas Valley of California. The intense irrigation required by leafy greens provides attractive habitat for the Pacific treefrog (*Hyliola regilla*), which is the most oft-cited species found in these sensitive production environments. Several gaps in knowledge have made it difficult for growers to sustainably control intrusions of Pacific treefrogs and our project has set out to address these gaps. The purpose of this study was to assess habitat-intrusion risks and development of a holistic framework to reduce interferences in crop fields and production environments by gaining a more complete understanding the Pacific treefrog's biology in the Salinas Valley, Monterey County, California.

- Fences made of aluminum siding with a 10-cm overhanging lip at the top prevented all frogs from climbing over. This novel fence design was realized at the whole-field scale and showed great promise for deterring frogs from entering a reservoir and for holding up to severe field conditions.
- Thermal imaging cameras are not effective for detecting frogs.
- Audio interventions hold great promise as a means to attract conspecific frogs to a wetland site. Our results, however, were based on a small sample conducted during a suboptimal time of year. Thus, even though some frogs appeared to respond positively, we have no way of knowing if the frogs would actually use these sites as breeding habitats. This non-invasive technique should be explored more thoroughly and used in combination with other methods, such as rigorous multiyear acoustic attraction protocols at newly created wetland habitats.
- Frog calling, and presumed breeding, began in late January and was closely tied with winter rainfall. Peak calling occurred shortly after the rains ceased at the end of March, but breeding likely persisted after this time. Variation in rainfall and frog activity suggests that some regions may exhibit a delay to this general breeding pattern and that the breeding season may extend longer into the Summer at some sites.
- Based on frog body sizes and sexes, we inferred that adult breeding began in late January and extended into May and perhaps June, with the potential for additional breeding bouts in late Summer if stimulated by rainfall.
- Dispersing juveniles that were born this year from eggs that were laid during February to April underwent extensive egress from wetlands for starting in May, with major exodus events in June, July, and August.
- The presence of a fence almost doubled the total number of pitfall-trap captures for small mammals, but standardizing captures revealed species-specific differences.
- The presence of a fence significantly increased the total number of captures and captures per unit effort compared to a portion without a fence for amphibians.
- Small mammal population dynamics was variable across sites, with a general increase in activity for all species shortly after winter rains.
- Reptiles were active from April to September, with two species of lizards the most frequently encountered across all sites.
- Our study provides empirical information on the vertebrate communities in the Salinas Valley and may help growers to minimize their risks of wildlife intrusion, while promoting environmental stewardship.

APPENDICES

Publications and Presentations

Publications:

Hughes, D.F., Green, M.L., Warner, J.K., and Davidson, P.C., 2019. There's a frog in my salad! A review of online media coverage for wild vertebrates found in prepackaged produce in the United States. Science of the Total Environment, 675, 1–12.

Hughes, D.F., Green, M.L., Warner, J.K., and Davidson, P.C., 2020. Field guide to color variants of the Pacific Treefrog and other amphibians of the Salinas Valley. 3 pp.

Presentations (talks):

Green, M.L., Warner, J.K., Davidson, P.C., and Hughes, D.F., 2019. Engineering and ecological approaches to reduce Pacific tree frog intrusion into leafy green agriculture. 15 min. The 10th Center for Produce Safety (CPS) Research Symposium, Austin, Texas (18 June; presented by MLG).

Green, M.L., Warner, J.K., Davidson, P.C., and Hughes, D.F., 2018. Engineering and ecological approaches to reduce Pacific tree frog intrusion into leafy green agriculture. Lightning talk (5 min). The 9th CPS Research Symposium, Charlotte, North Carolina (19 June; presented by MLG).

Presentations (poster):

Green, M.L., Warner, J.K., Davidson, P.C., and Hughes, D.F., 2018. Engineering and ecological approaches to reduce Pacific tree frog intrusion into leafy green agriculture. The 9th CPS Research Symposium, Charlotte, North Carolina. (20 June; presented by MLG).

Budget Summary

The total funds awarded to this project were $259,160. Through the end of the project period (12/31/2019), 81% of total project funds were expended. Additional funds were budgeted for travel to the CPS Symposium in June 2020 ($3,168) and publication services ($1,000) in Year 3.

Table and Figures (see below)

Table 1 and Figures 1–14

Table 1. Information for study sites in the Salinas Valley, Monterey County, California.

Area	Site Number	Sampling	Size	Site Description
Northern	1	Surveys	391 m^2	Wetland near road
	2	Surveys	286 m^2 + 184 m^2	Two tailwater ponds
	3	Surveys	420 m^2	Tailwater pond
	4	Trapping	3,983 m^2	Tailwater pond
	5	Surveys	4,670 m^2	Tailwater pond
Central	1	Surveys	162 m^2 + 1,997 m^2	Two tailwater ponds
	2	Trapping	288 m + 355 m	Two linear transects
	3	Surveys	308 m^2	Tailwater pond
	4	Surveys	355 m^2	Tailwater pond
	5	Surveys	10 m^2	Sub-pond
	6	Surveys	160 m^2	Tailwater pond
Southern	1	Surveys	4,786 m^2	Reservoir
	2	Surveys	5,750 m^2	Reservoir
	3	Surveys	4,765 m^2	Reservoir
	4	Surveys	4,636 m^2	Reservoir
	5	Trapping	4,857 m^2	Reservoir
	6	Trapping	1,242 m^2	Reservoir

Figure 1. Color pattern variation in the Pacific treefrog (*Hyliola regilla*). A: Green/brown morph, photo by Oregon Department of Fish & Wildlife. B: Green morph, photo by Marshal Hedin. C: Tan morph, photo by DFH. D: Brown morph at night, photo by DFH. E: Dark brown morph during daytime, photo by DFH. F: Brown morph without blotches, photo by US Fish & Wildlife Pacific Southwest. G: Gold morph, photo by DFH. H: Brown morph with extensive blotches, photo by DFH.

Figure 2. Map of the study sites across three regions in the Salinas Valley, Monterey County, California. Image modified from Google Earth. See Table 1 for details about the sites.

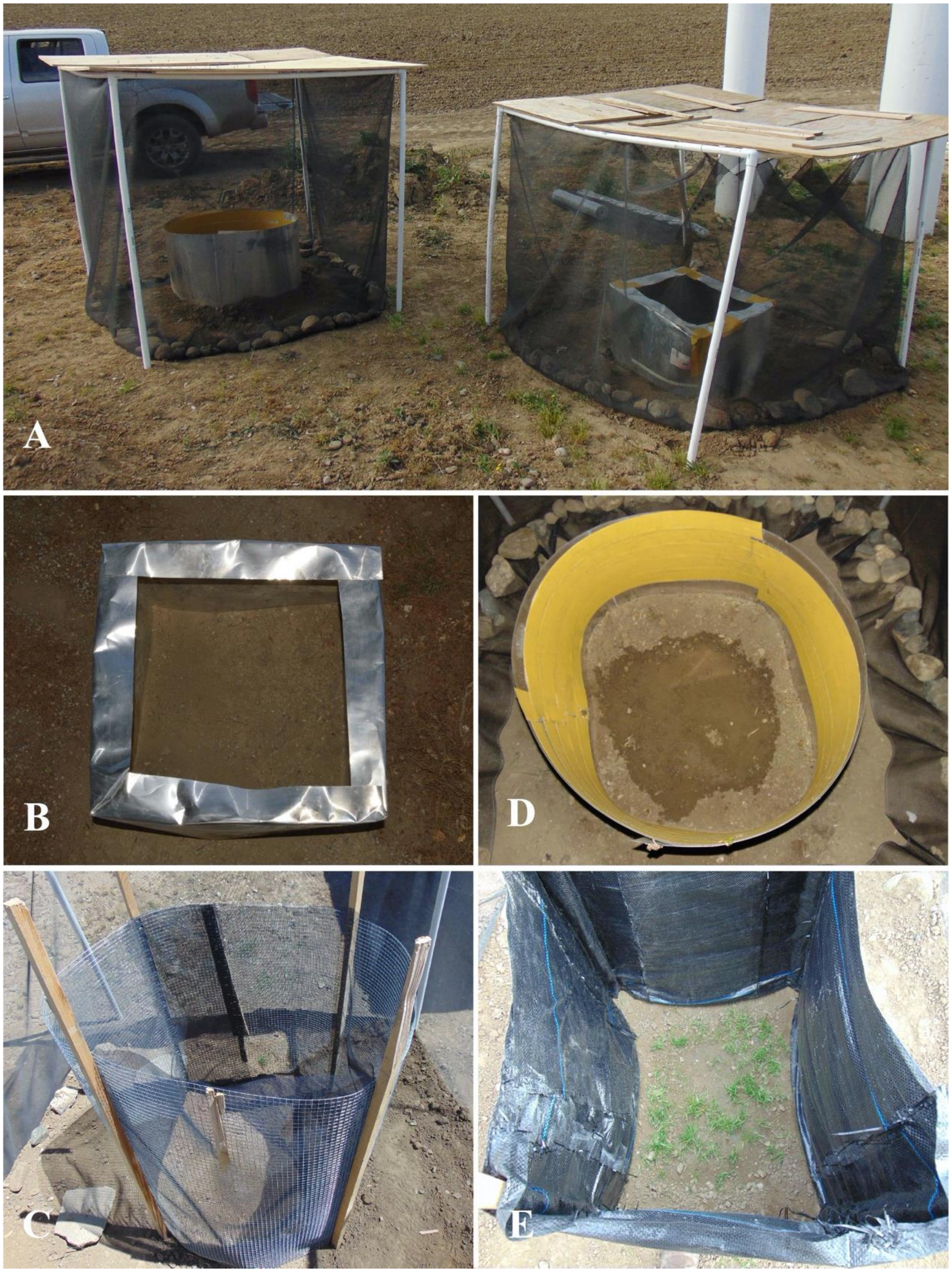

Figure 3. Experimental fence designs and outdoor pens for exclusion microcosm experiments. A: Field enclosures. B: Aluminum siding with 10-cm overhanging lip. C: Screen-porch material with chicken-wire support. D: Aluminum siding affixed with 320-grit sandpaper. E: Standard silt fence.

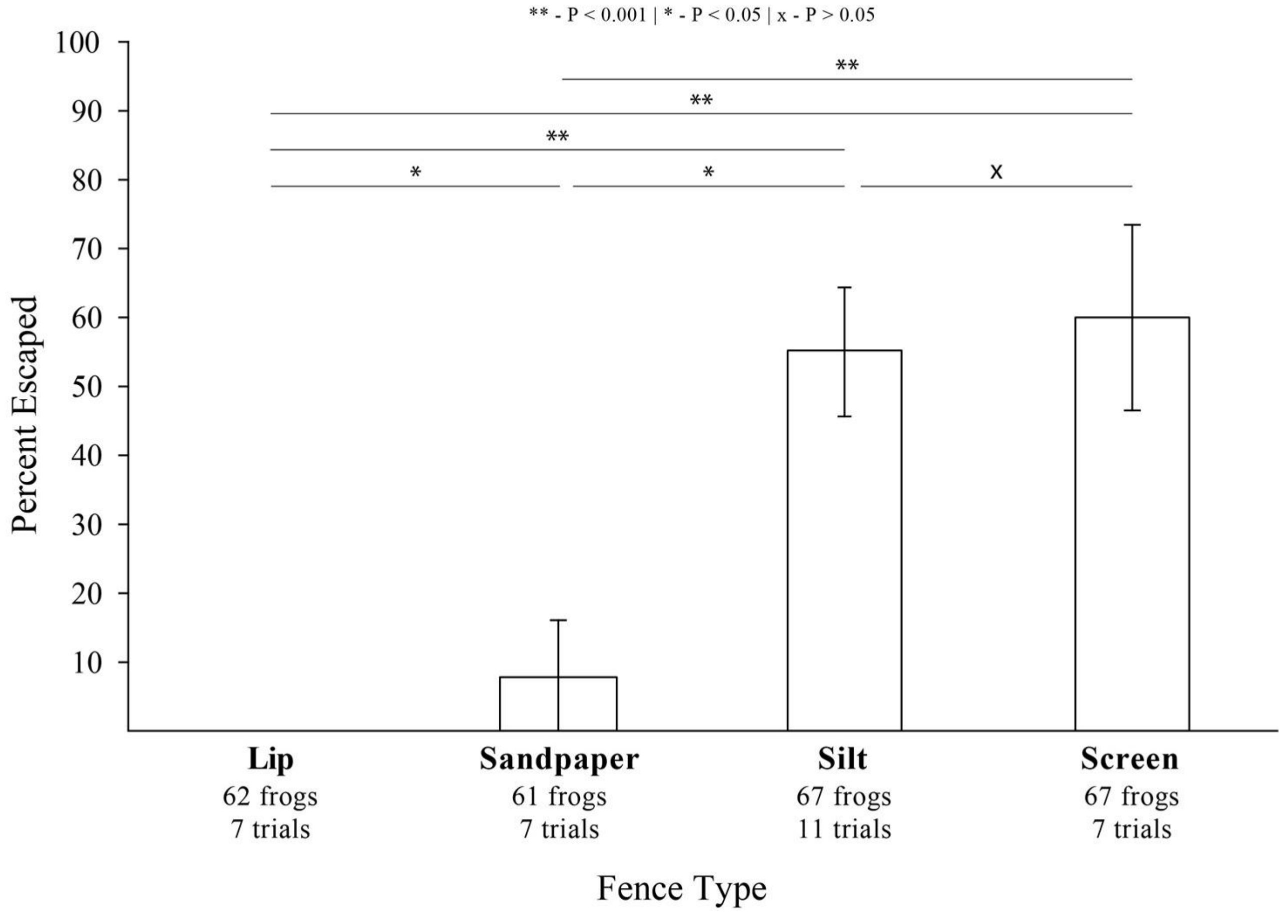

Figure 4. Results of microcosm fence exclusion experiments showing the percentage of Pacific treefrogs (*Hyliola regilla*) that escaped. Sample sizes and number of trials per fence type listed below each fence name. Lip: Aluminum siding with 10-cm overhanging lip. Sandpaper: Aluminum siding affixed with 320-grit sandpaper. Silt: Standard silt fence. Screen: Screen-porch material with chicken-wire support.

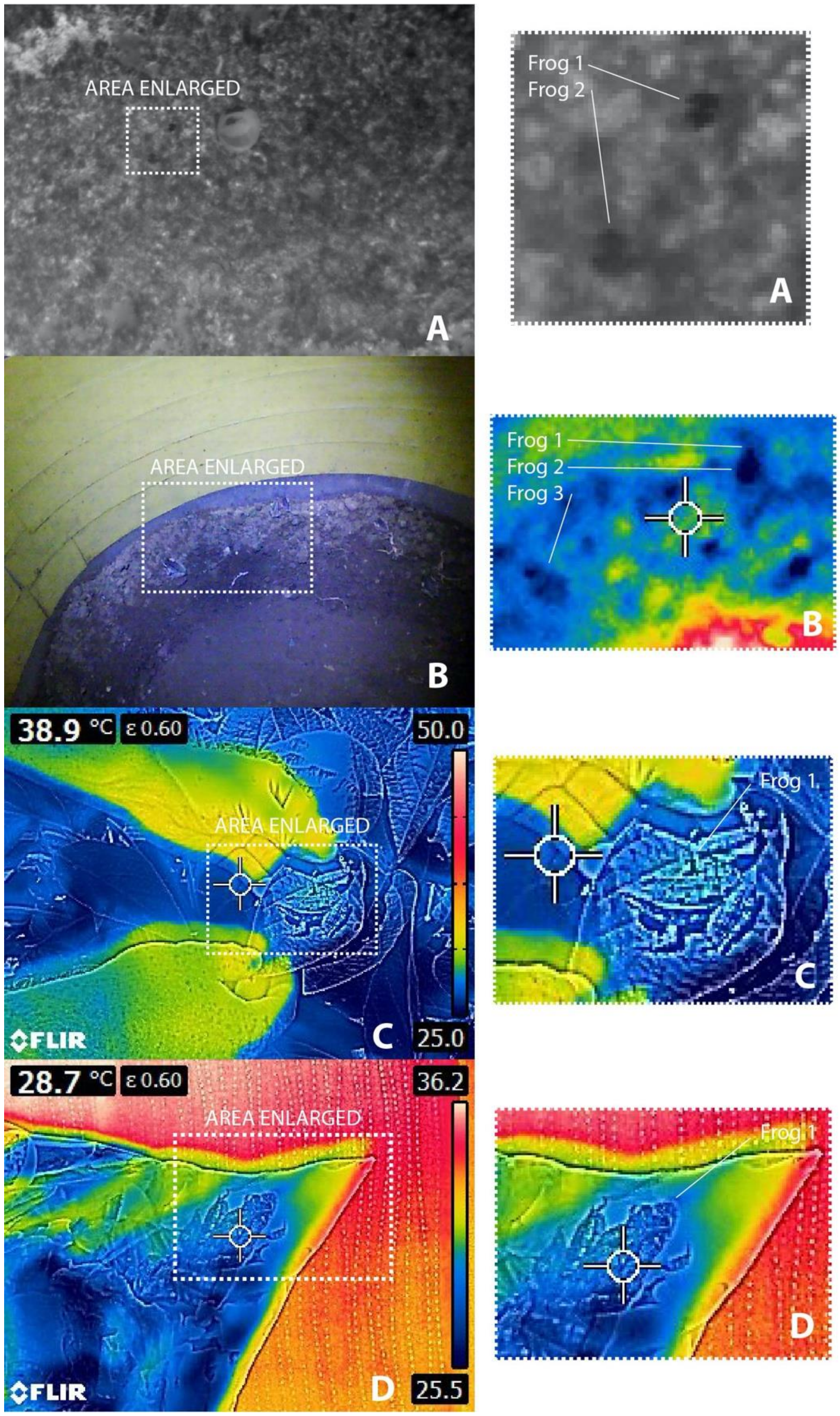

Figure 5. Thermal images of several frog species in various environments and conditions. A: Drone flying at 10 m above the ground. B: Hand-held camera from approximately 0.9 m away at night. C and D: Integrated visual field from about 0.6 m away during the daytime.

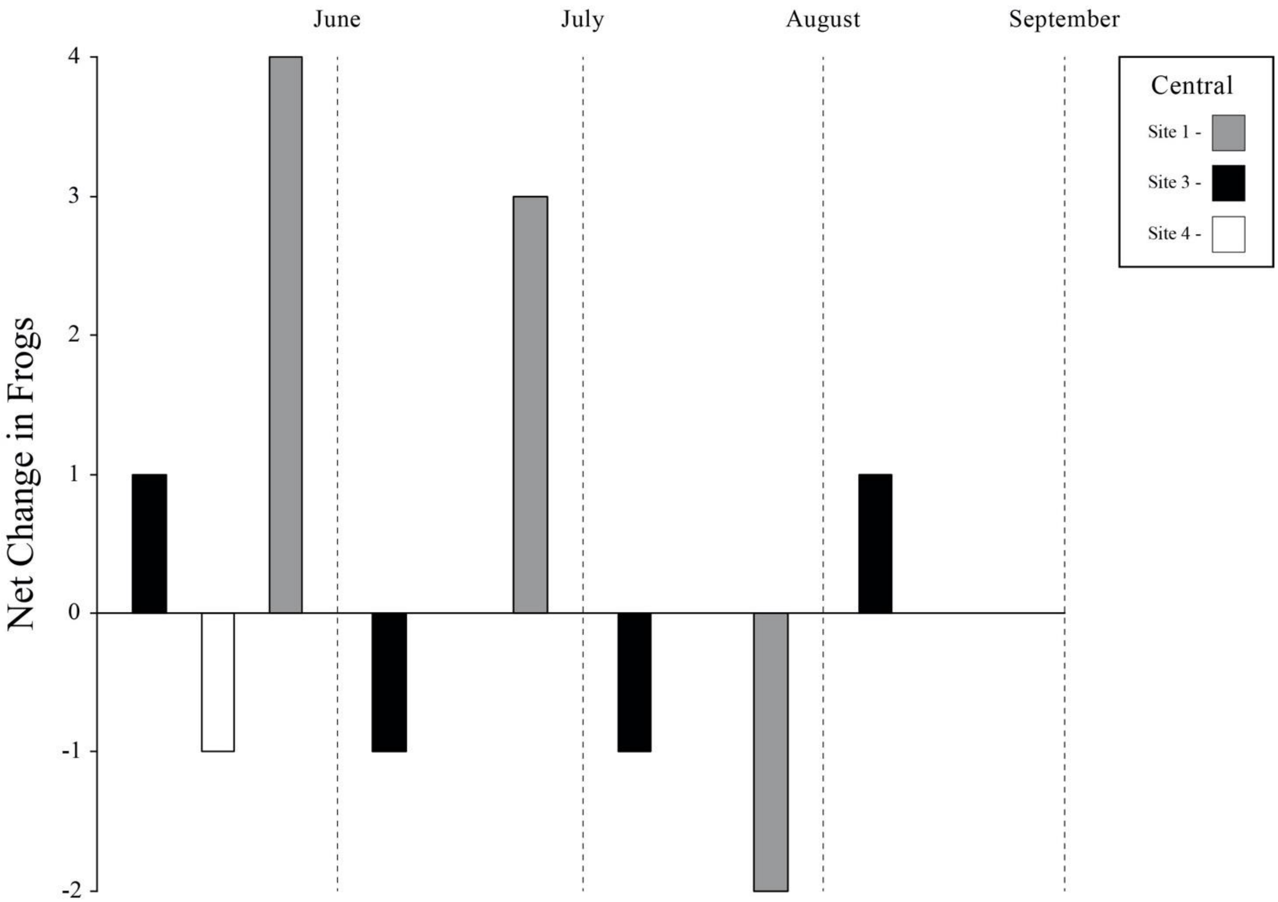

Figure 6. Results from audio playback experiments showing the net change in the number of Pacific treefrogs (*Hyliola regilla*) from the first survey night to the last survey night across four months from three Central sites.

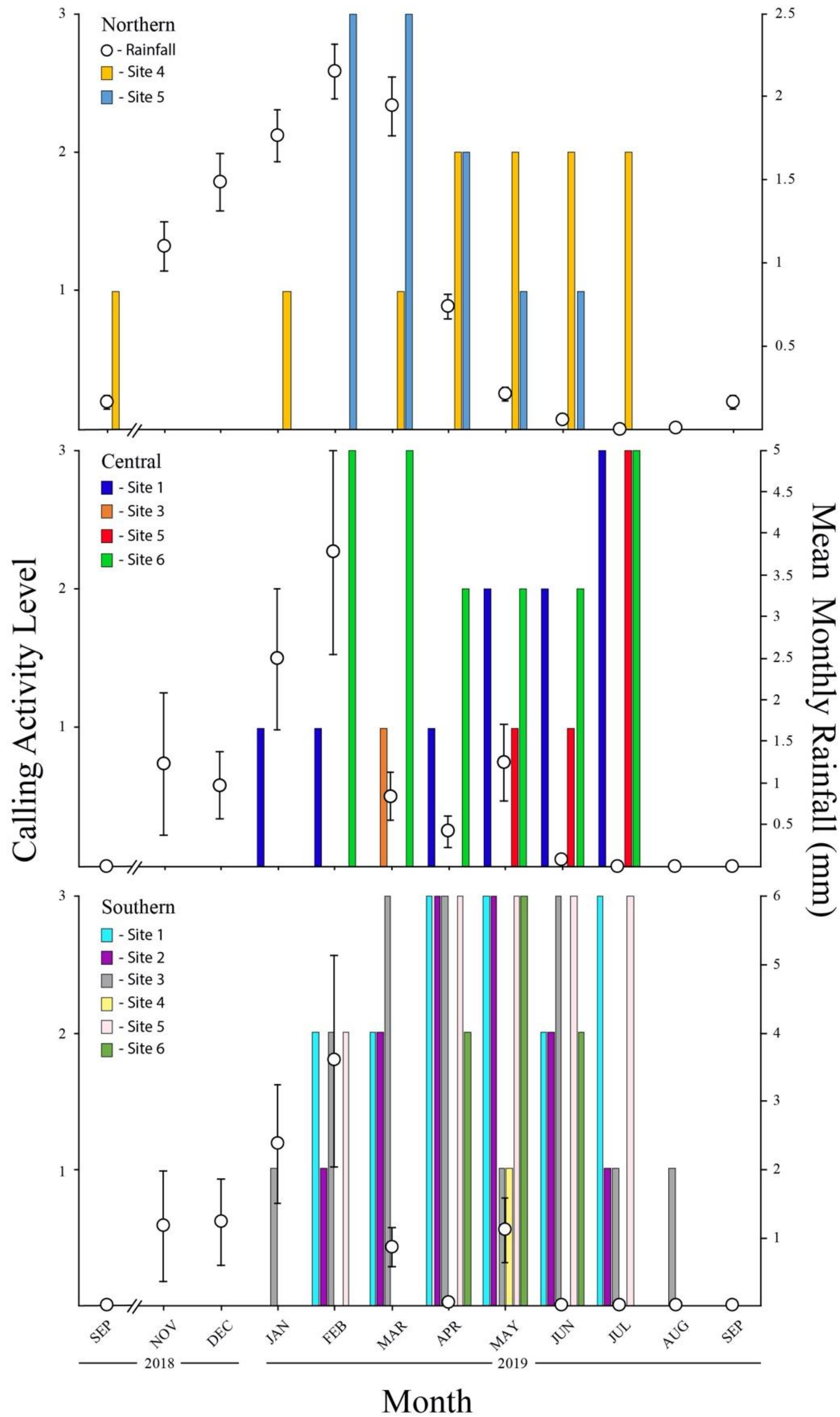

Figure 7. Site-specific Pacific treefrog (*Hyliola regilla*) calling activity on the primary axis derived from monthly surveys at all wetland sites and mean monthly rainfall on the secondary axis. Bars represent standard errors. Sites that frogs were never heard calling from are not shown.

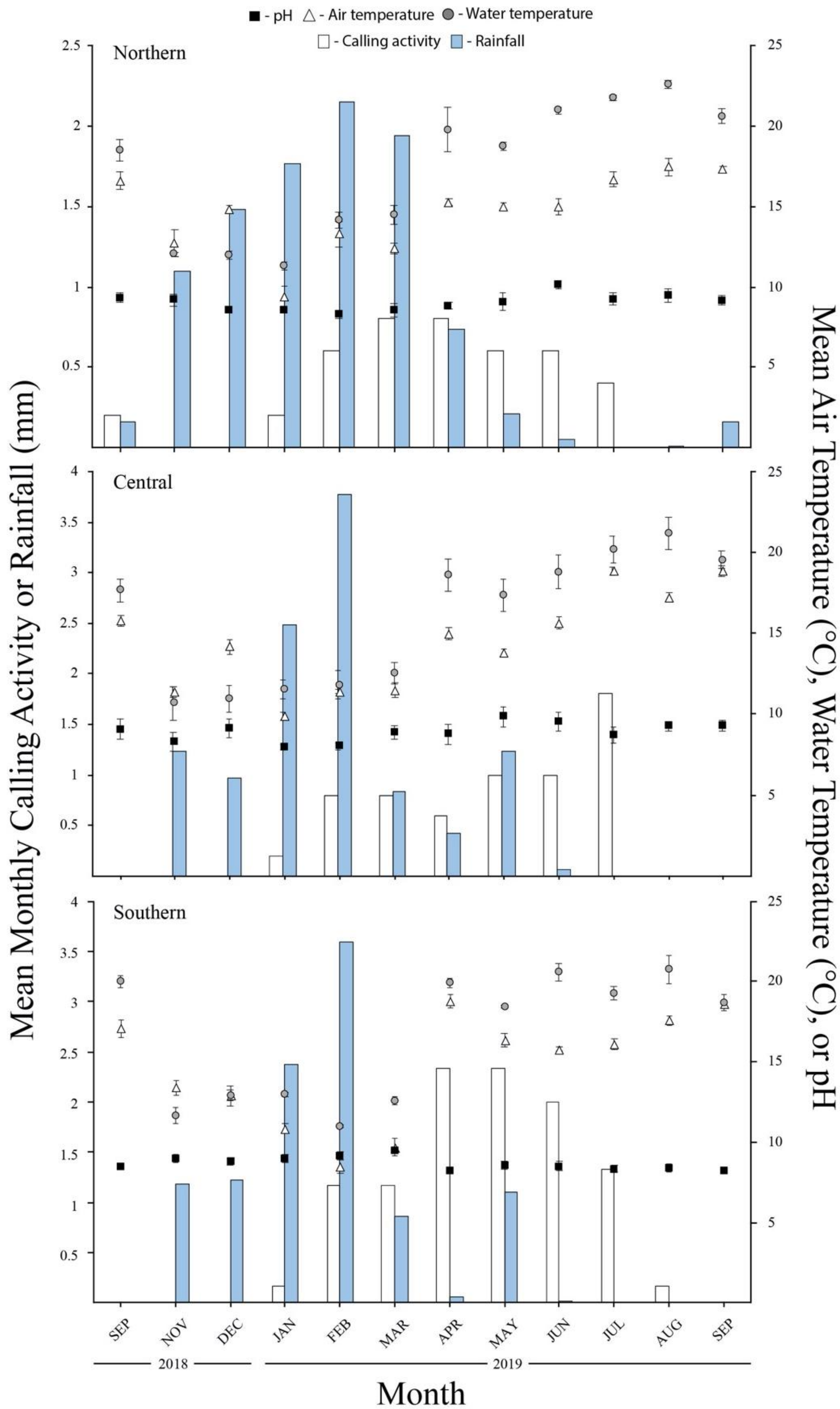

Figure 8. Regional Pacific treefrog (*Hyliola regilla*) calling activity and monthly rainfall on the primary axis. Mean water temperature, air temperature, and wetland pH on the secondary axis. Bars represent standard errors.

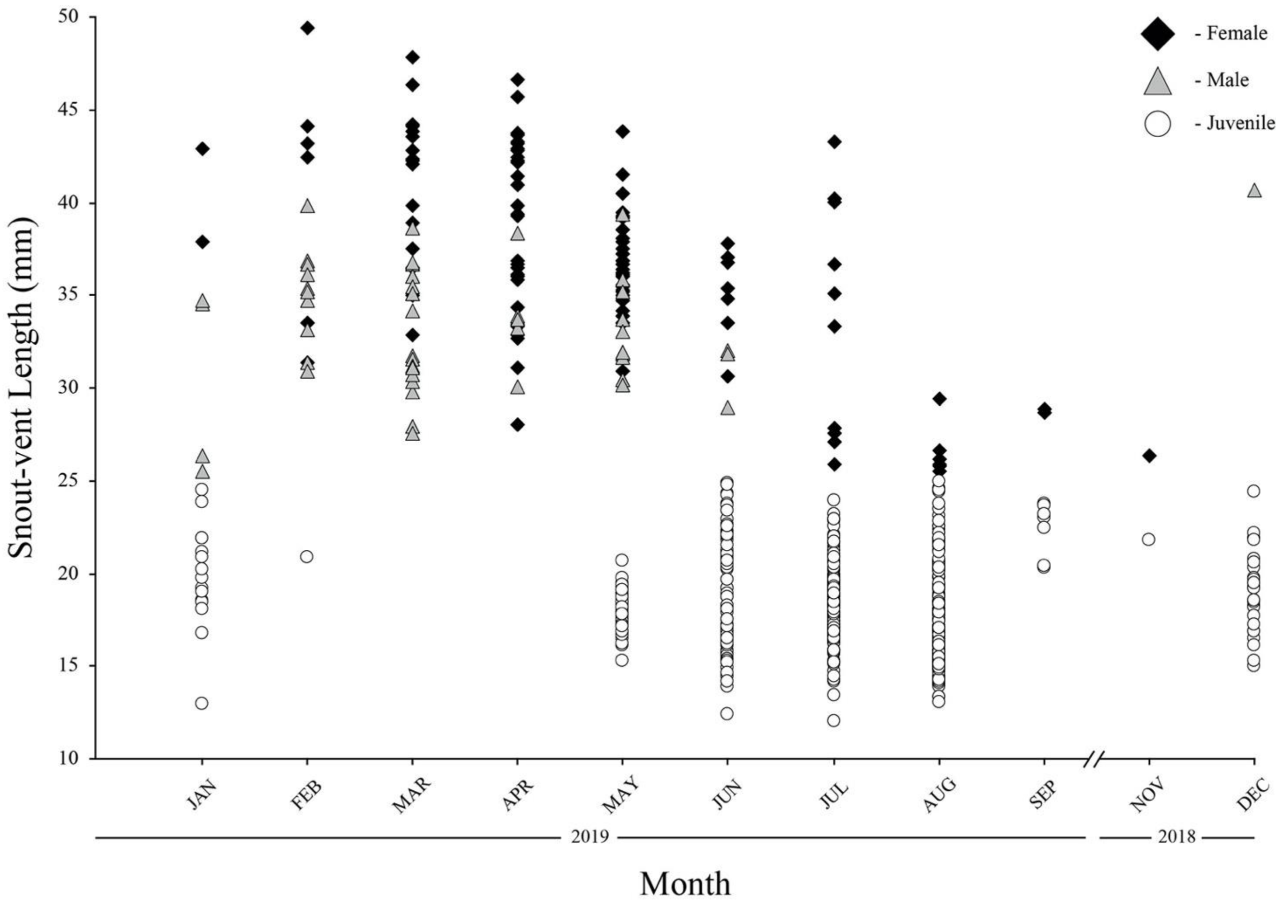

Figure 9. Monthly distribution of body sizes of the Pacific treefrog (*Hyliola regilla*) for males (n = 52), females (n = 120), and juveniles (n = 578) found in pitfall traps from sites 5 and 6 in the Southern area.

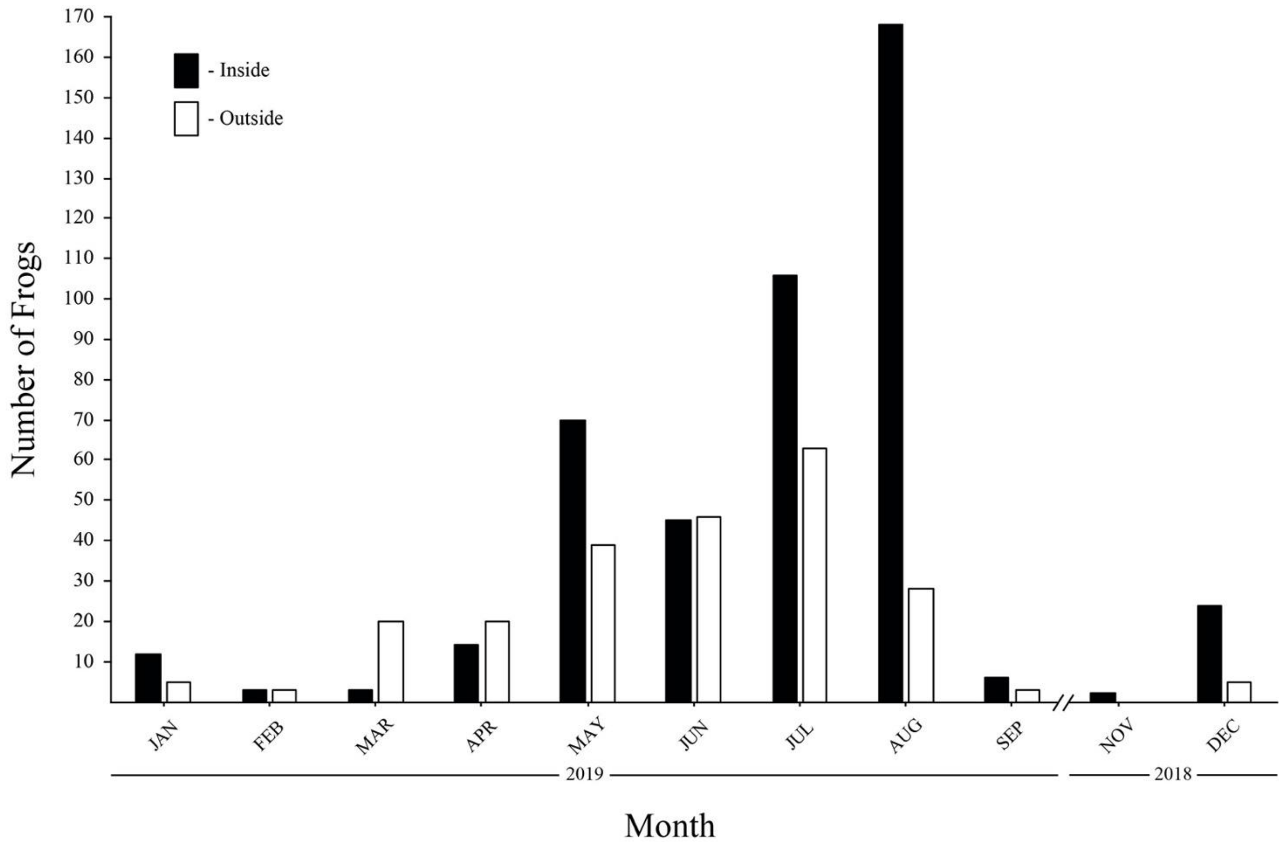

Figure 10. Monthly number of Pacific treefrogs (*Hyliola regilla*) leaving (n = 453) or entering (n = 232) the wetland reservoirs at sites 5 and 6 in the Southern area. Captures from pitfall traps only.

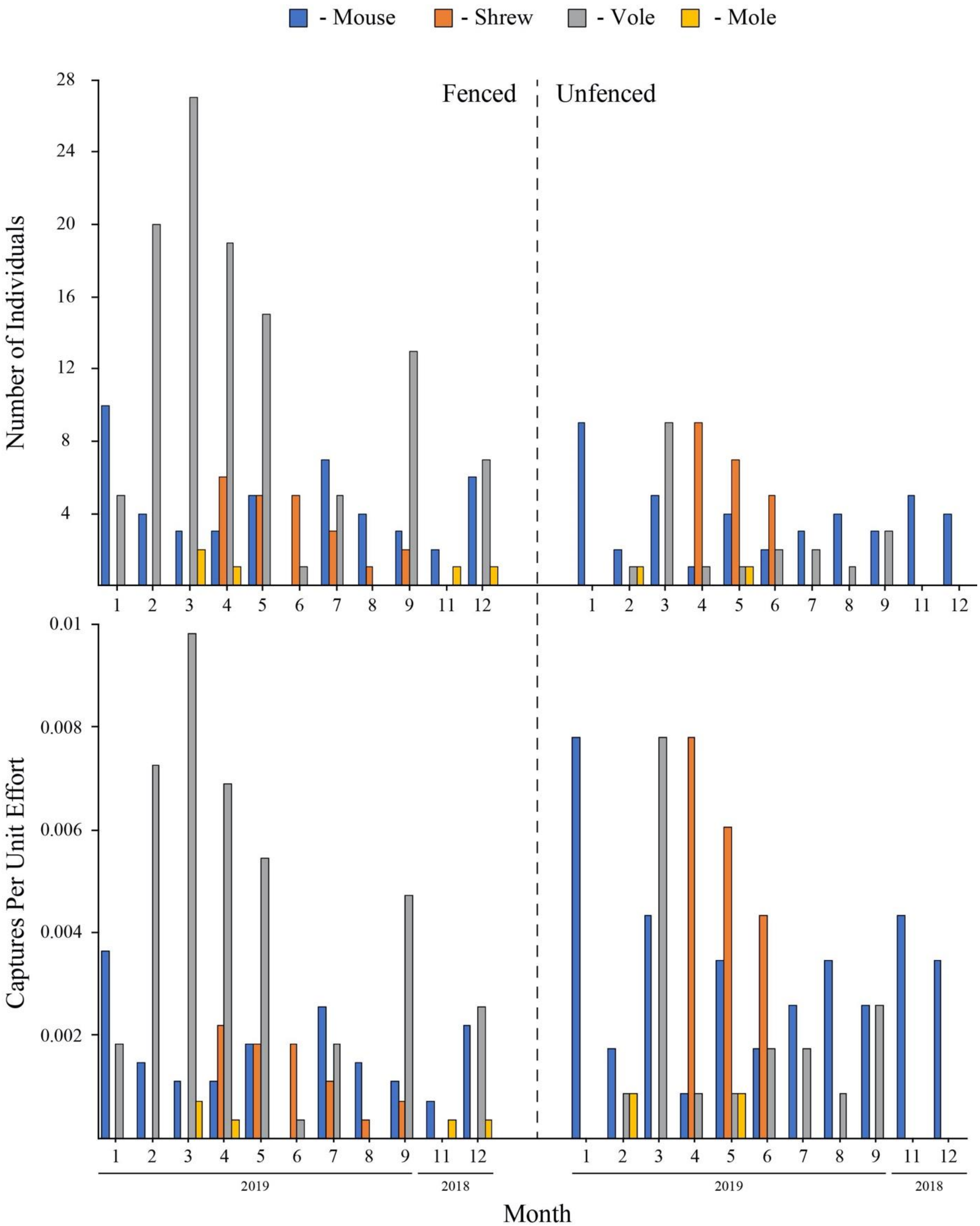

Figure 11. Monthly captures of four small mammal species between a fenced and unfenced section of site 2 in the Central area. Top: Total number of captures across treatments. Bottom: Captures were standardized by number of trap nights per site. Captures from pitfall traps only.

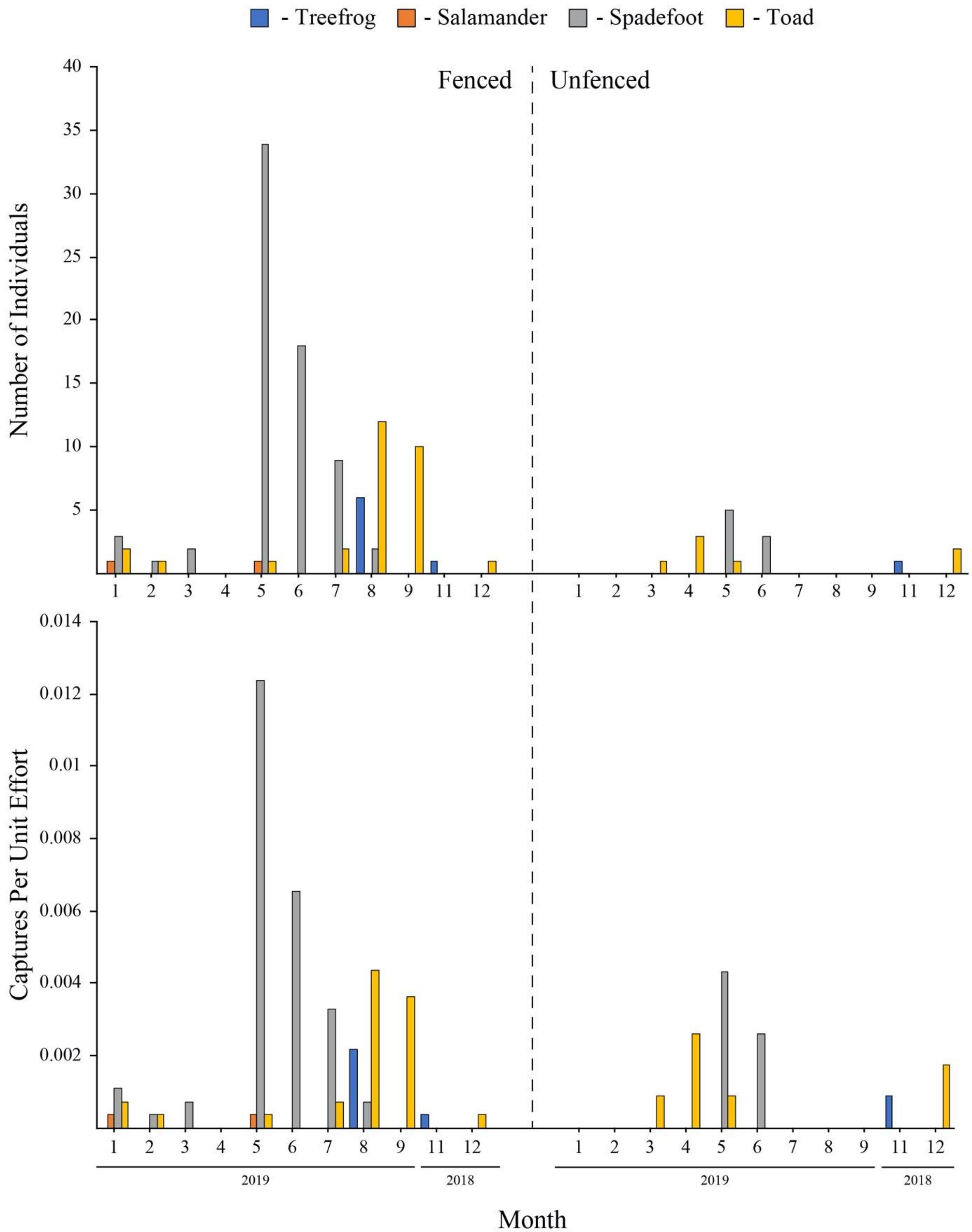

Figure 12. Monthly captures of four amphibian species between a fenced and unfenced section of site 2 in the Central area. Top: Total number of captures across treatments. Bottom: Captures were standardized by number of trap nights per site. Captures from pitfall traps only.

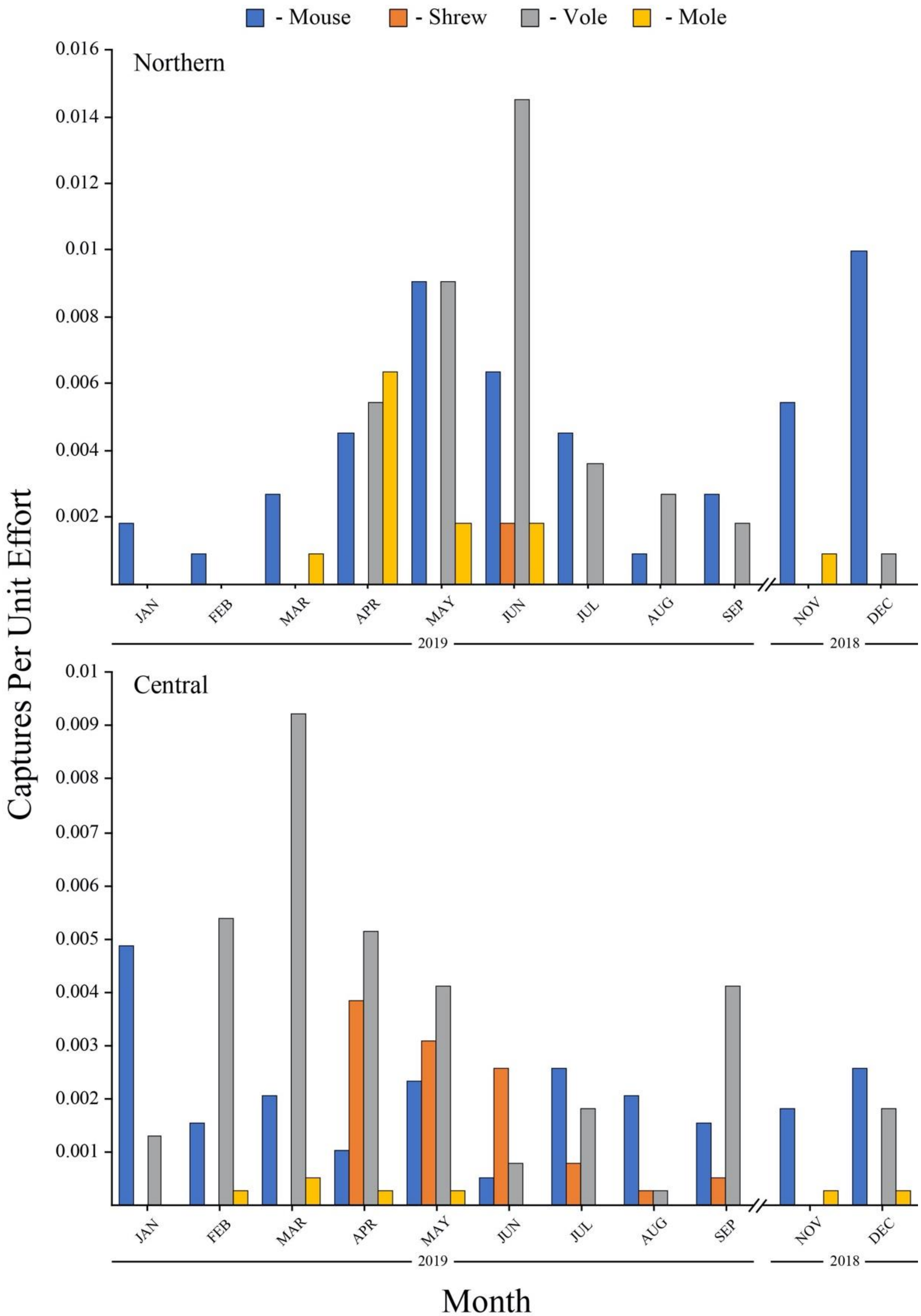

Figure 13. Spatial variation in standardized monthly captures of four small mammal species between all sites in the Northern (top) and Central (bottom) areas. Captures from pitfall traps only.

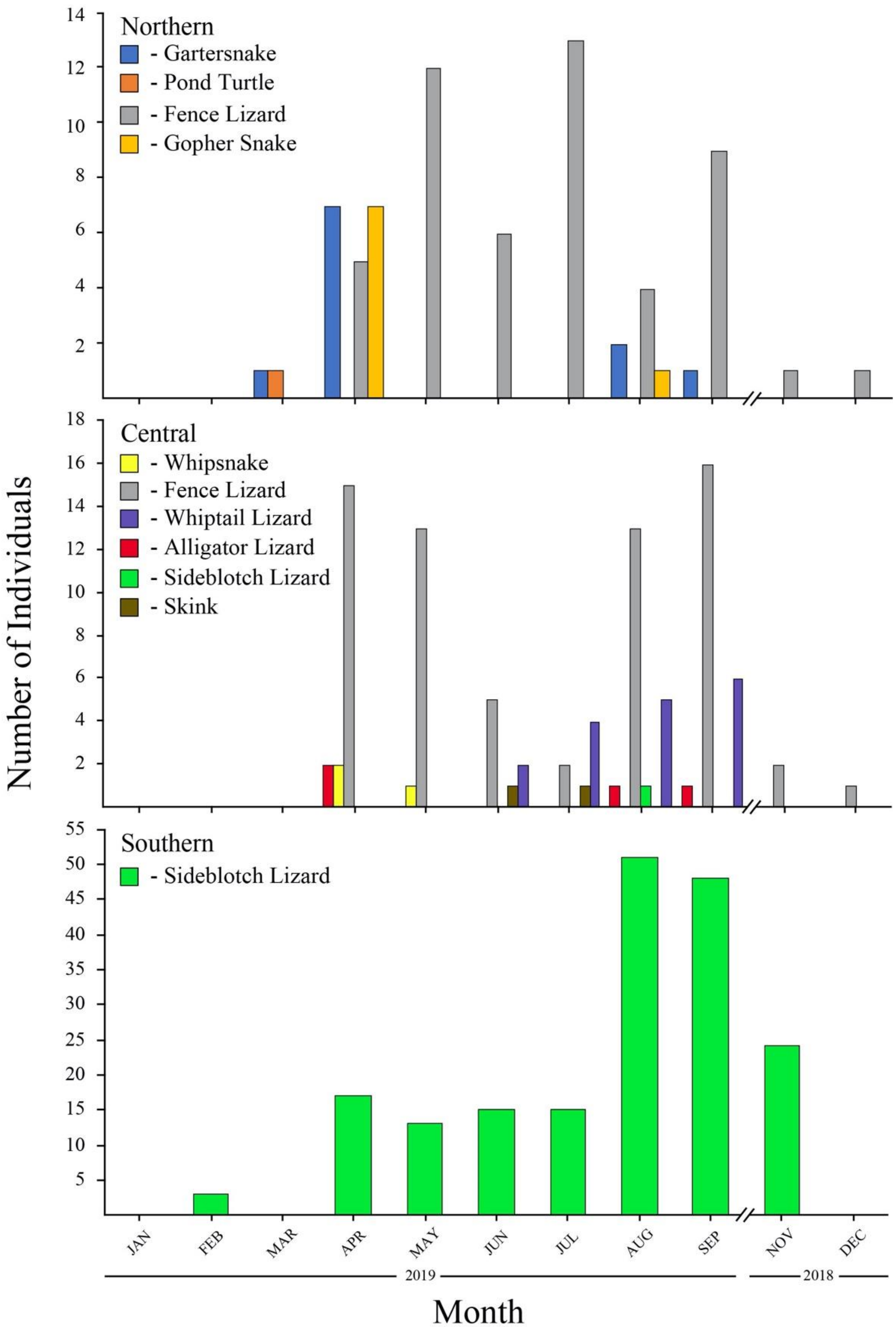

Figure 14. Spatial variation in the total monthly captures of reptiles between the Northern (top), Central (middle), and Southern (bottom) sites. All captures across cover boards and pitfall traps combined.

Suggestions to CPS

We respectfully suggest CPS reconsider providing salary support to PIs and Co-PIs. Salary support is standard practice for many funding agencies given that most academics are supported on 9-month contracts through their home institution. The responsibility of securing summer salary falls upon each individual PI. Other than that, our team fully enjoyed working with CPS.

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