The Efficacy of Abiotic/Biotic Factors and Trap Design for Effective Capture of Invasive American Bullfrog Tadpoles (*Lithobates catesbeianus*)

by

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Photos Taken by Researcher

Contents

Executive Summary

An invasive species is defined as a "non-native to the ecosystem whose introduction causes environmental impact (USDA, 1999)." In the early 1900's, the American Bullfrog was introduced to many areas in the Western United States, exhibiting many biological characteristics that contribute to its invasiveness, such as broad temperature tolerance, the ability to adapt quickly, and a diet consisting of anything they can swallow. In this study, bullfrog tadpoles were netted from ponds within Blanca Wetlands, CO (a federally managed property with several bullfrog-infested wetlands) with the goal of investigating effective and efficient capture in a real-world setting. Both abiotic and biotic attractants were tested using a 340 L choice chamber to initially determine which baits might be effective. It was found that fish meal, algae wafers, trout, and a diving flashlight were significantly attractive in a lab setting. Field trials were conducted in a bullfrog tadpole infested geothermal pool. Findings demonstrate that the flashlight combined with the steel trap had a significant capture rate as well as the combination of Promar with trout bait. In addition, field testing revealed the capture of three different size classes of tadpoles based on histogram analysis. Tadpole length data revealed that Promar traps caught smaller tadpoles while steel traps caught larger tadpoles. This research is critical for understanding tadpole seasonal behavior, implicating that the large-scale deployment of different trap/bait combinations can be used to reduce the spread of the invasive bullfrog by removing them in high quantities before they metamorphose into adults.

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There are several people I would like to express my deepest thanks and appreciation to; first Loree' A. Harvey, thank you so much for the opportunity to volunteer and do amphibian research with you last summer and sharing your expertise with me. From teacher, to mentor, to friend, you have been my biggest supporter, pushing me to my best and for that, I'm eternally grateful; Moses Martinez my big brother, you have supported me doing Science Fair since seventh grade – you're always interested in the work I am conducting and I know I can count on you to give me honest feedback; Sandra and Ernie Martinez my grandparents, you both have supported me during each experimental project I have ever conducted and I'm eternally grateful for all the time you guys allow me to work on my projects; my friends - you guys have been supportive from day one and you have made all these Science Fair years and experience and a pleasure - from late nights to coffee drinking, I'm glad we have grown closer through science; and finally, the Monte Vista School District, thank you for supporting Science Fair and understanding the importance of student research and for supporting me all these years.

Introduction

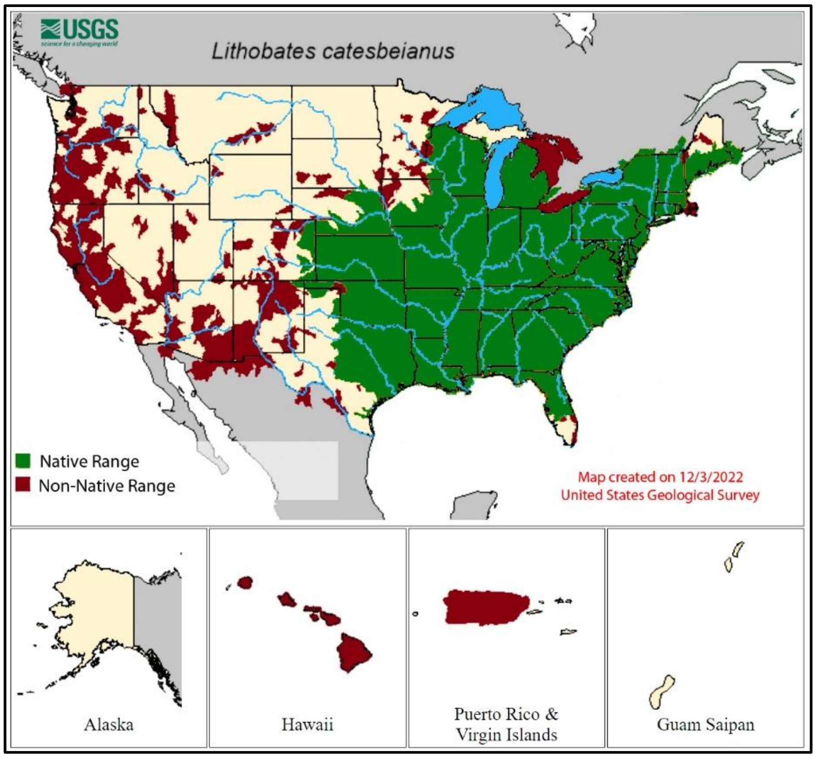
Rationale

What is an Invasive Species?

An invasive species is defined as a “non-native to the ecosystem whose introduction causes or is likely to cause economic or environmental impact (USDA, 1999).” This definition implies that an invasive species can 1) enter an ecosystem, 2) establish a population, and 3) spread (Oregon State University, 2014). Invasive species tend to spread primarily due to human activities such as travel, trade, tourism, illegal stocking, etc. Not only are animals’ invasive species but so are plants. Plants tend to become invasive due to higher average temperatures enabling warm weather adapted plants to move into new areas. In addition, invasive species are a big threat to native species due to the natives not having the characteristic of evolving defensives against the invasive species. Direct threats include preying on natives, outcompeting native species for food, carrying or spreading diseases, and preventing natives from reproducing or killing native species offspring (National Wildlife Federation, 2022).

For example, the American Bullfrog exhibits many biological characteristics that contribute to its invasiveness. The bullfrog has a broad temperature tolerance, an ability to adapt quickly, a diet that consists of anything it can swallow (invertebrates and small vertebrates), an effective predator defense, high fecundity, and can aggressively disperse locally and occupy new habitat (Oregon State University, 2014).

Map 1.



The American Bullfrog *(Lithobates catesbeianus,* formally known as *Rana catesbeiana)* is native to the central and eastern United States. However, in the early 1900’s, this species was introduced to many areas in the western United states (North America, Europe, South America, Asia, Caribbean Islands, Hawaii, Oregon, Washington, and California) (Oregon State University, 2014).

Map 1 (created by USGS, 2022) is the distribution (native and non-native) of the American Bullfrog across the United States.

Environmental Factors & Invasive Strategies of the American Bullfrog

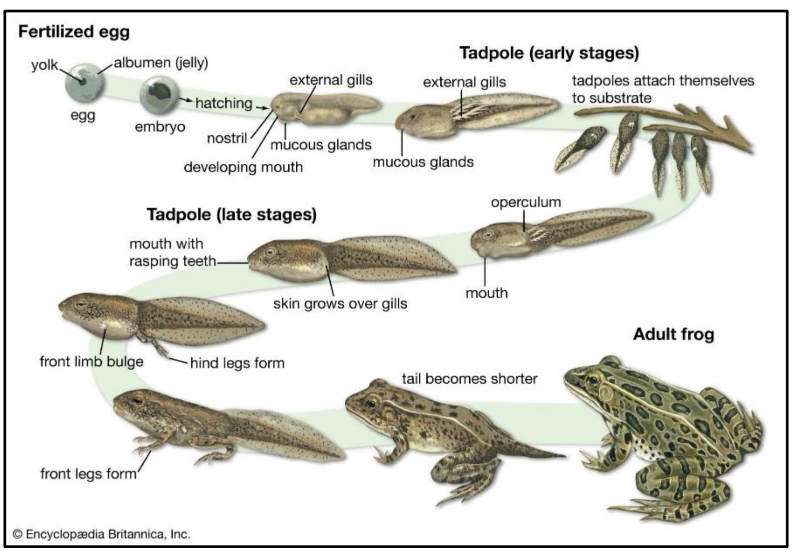
The American Bullfrog thrives in habitat that is characterized with thick vegetation, warm temperatures, and stagnant water, including permanent wetlands, canals, marshes, swamps, and ponds. Because of this, this species can establish quickly due to its "hardy biological character" and its attributable adaptability to these specific areas. In addition, climate change has been one of the biggest factors involving the spread of the bullfrog. As global temperatures continue to rise, this allows the bullfrog to establish in higher altitude locations, making the western states more vulnerable to invasion. Furthermore, the bullfrog has a high reproduction rate (an adult female can lay over 20,000 eggs) and in turn, is not very palatable to many predators due to a toxic skin secretion found in both adults and tadpoles (Oregon State University, 2014).

Reasons for introduction include the improvement to the aesthetics of habitat, becoming a harvestable game animal (due to the demand for frog legs), release and escape via pet trade, and the decrease of agricultural pest control. Since introduction, the bullfrog has been the cause of many ecological impacts.

In introduced ecosystem and wetlands, the American Bullfrog has been responsible for the decline of native anuran species as well as many other vertebrate groups. The bullfrog can consume high amounts of food relative to native amphibian species, egg masses, snakes, birds, rodents, and other invertebrates. Furthermore, in addition to lowering biodiversity levels, American Bullfrogs carry the spread of Ranavirosis. This severe virus can cause skin ulcers and internal bleeding, once again, affecting native frog species internationally. Bullfrogs can also carry the Chytrid Fungus which attacks keratin, making it difficult for the respiration in skin to work properly (Daughterty, 2022). This disease can be transferred to many amphibians, contributing to dwindling anuran populations around the world.

American Bullfrog Life Cycle, Development, Activity, & Diet

The American Bullfrog Life Cycle



Graphic obtained from Britannica.com

The life cycle of the American Bullfrog is complex and unique. As the eggs spawn, the male bullfrog fertilizes the clutch and after about 6-21 days, the eggs hatch. As tadpoles the bullfrogs develop tiny teeth that aid in chewing algae. After about nine weeks, tadpoles will sprout legs and the body will start to elongate. By 12 weeks, the tadpoles will have only a tail stub and will soon leave the water to start this process over again (between 12-16 weeks). This process is somewhat standard for many amphibians however, the American Bullfrog is one of two species that overwinter as a tadpole (the other being the Green Frog) and will transform into a young frog the following summer.

Gosner Stages

In many bullfrog tadpole studies, researchers have separated the tadpoles into Gosner Stages to see if there is a different outcome between the age groups. Goner Stage is a generalized system for categorizing the embryo and larval development in anuran species. The Gosner Stage chart consists of 46 stages, 1-19 being an embryo, 20-25 being a hatchling, 26-41 is a tadpole, and 42-46 is a metamorph (Gosner, 1960).

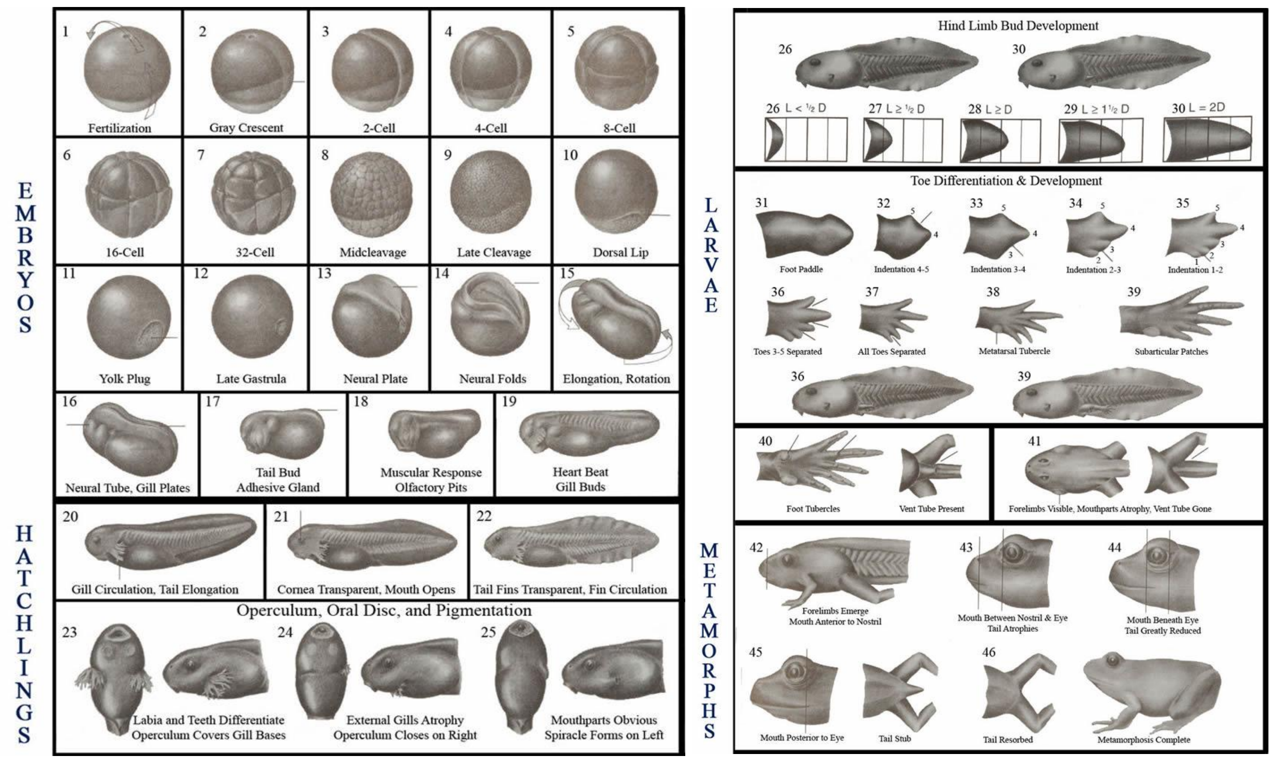


Image obtained from https://www.virginiaherpetologicalsociety.com/amphibians/amphibian-development/amphibian-development.htm

Summer Activity

While anurans undergo the developmental stage, their habitat selection does not remain constant. Anura's will move based on their needs, including diet, temperature, and thermoregulation (Wollmuth & Crashaw, 1988). Specifically, bullfrog tadpoles that are in the early stages of metamorphosis will select areas with high temperatures. In addition, it is found that bullfrog tadpoles will select lower temperatures near the end of their metamorphosis. During the summertime, bullfrogs will co-exist with predatory game fish. This is extremely uncommon but due to the bullfrogs toxic nature, these fish pose no threat. The bullfrogs will often sit in the sun or on thick mats of algae. Most of the bullfrog's activity occurs between March and October. Additionally, the smaller bullfrogs tend to be more active in early spring than mature adults. Bullfrogs' activity tends to be bimodal, peaking when sufficient sun is available but dropping when temperatures become too cold.

When scattered and exposed along shorelines, bullfrogs will emit a squeak or squeal as they leap into water, often as an alarm response. However, bullfrogs produce several types of vocalizations during this seasonal period, specifically during the evening and most vigorous during the breeding months in June and July. In terms of reproduction, bullfrogs will breed in permanent bodies of water that contain thick amounts of algae and have a soft mud bottom. Precise dates of breeding in Colorado are not well known but the breeding calls are typically heard from May through August or early September.

Bullfrogs will also express aggressive territory qualities, specifically seen in male bullfrogs. Mature males will often defend territories along a shoreline until they become sexually receptive. The males will then initiate amplexus between midnight and dawn within the male territory. Male adults may also push and wrestle with one another and attempt to intercept approaching females. Once an egg mass is layed, eggs will hatch between 2-5 days depending on the temperature. Bullfrogs have one of the longest larval periods than any other anuran in Colorado (Hammerson, 1999).

Winter Activity

Currently, there is little known about bullfrog overwintering behavior or habitat due to low visual encounters. Although bullfrogs are a warm-adapted species, they do establish in areas that have ice cover during the winter months with little to no developmental growth. Because of this, bullfrogs require permanent waters, suggesting their vulnerability to control efforts during this season. For example, some studies have indicated that adult bullfrogs will overwinter in areas with greater dissolved oxygen. However, the most important fact that has been discovered it that bullfrog tadpole overwintering is communal, this means that the tadpoles will co-habitat in a small area. If the overwintering habitats preferred by bullfrogs were better understood, this could "provide opportunities for effective control efforts, especially if individuals congregate in areas where the preferred microhabitat requirements are met (Sepulveda & Layhee, 2015)." A study was conducted in the Yellowstone River corridor to try and better understand fall and early-winter movements and habitat association with juvenile bullfrogs. The bullfrogs were radio-tagged (attached to a radio transmitter) at the beginning of the study in early fall and distributed across 15,384 m however, at the end of the study (winter), they were clustered in an area spanning 130 m. In terms of habitat, bullfrogs were located 6 m off the water's edge in early fall. Later in the season, they were located 15 m from the edge indicating that they moved to deeper water with submerged vegetation. Researchers found that during the later season, bullfrogs were located 18-53 cm below the ice, containing mud and silt substrate. Despite them being under the ice in colder temperatures, they were not torpid.

The differences between the fall and winter results for bullfrog movement are significant, suggesting that communal overwintering may be a common attribute for all bullfrogs alike, including bullfrog tadpoles. This research suggests that cold winters can work as an advantage in specific regions for bullfrog removal due to the congregated distribution (Sepulveda & Layhee, 2015). Further work must be conducted to identify techniques for effective capture and direct removal during the winter months as well as gauging specific habitat characteristics for overwintering in the spot bullfrog tadpoles will be most concentrated. Because of the dropping temperatures and ice freeze, access to open water can be difficult so techniques and machines such as augers may be required.

Bullfrog Diet

Bullfrog tadpoles mainly consume algae, egg masses, invertebrates, and aquatic plant material (CDFW, 2023). At the early stages of development, bullfrogs are usually herbivorous however, as the tadpoles transition into adults, they become carnivorous preying on worms, insects, crayfish, small mammals, other frogs/tadpoles, and snakes. Bullfrogs will pounce on any approaching animal that is small enough to be captured and swallowed. In Colorado there are few predators recorded that will consume bullfrogs, including the Great Blue Heron and the Swainson's Hawk (Hammerson, 1999).

In captivity, the nutritional composition and requirements for bullfrog tadpoles remain unknown, generally administering a diet typically given to carnivorous fish. A study was conducted to find the most effective and nutritional diet for bullfrog tadpoles by exposing the tadpoles to a commercial and experimental diet. The experimental diet consisted of fish meal, soybean meal, poultry by product meal, wheat meal, corn meal, and mineral/vitamin premix. The commercial diet was analyzed and included ground whole corn, soybean meal, meat and bone meal, hydrolyzed feather meal, blood meal, vegetable fat, sodium chloride, and limestone. The animals were separated into groups and fed one of these meals three times a day. The researchers utilized the Gompertz equation which estimates weight, length, food intake, protein intake, and body composition. They concluded that the tadpoles fed the experimental diet reached a higher final weight, presented better feed conversion, and the protein was better utilized by the animals, suggesting good quality protein and better nutritional composition (Mansano et al., 2014).

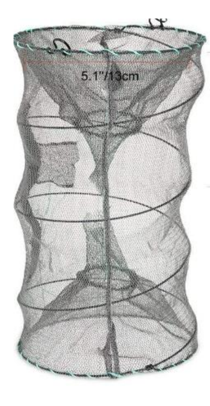
Both diets demonstrated composition with a balance of carbohydrates and protein, therefore, in this study, to ensure beneficial diet, the tadpoles were fed commercial Aquatic Tadpole and Frog Food containing fish meal, wheat flower, fermented soy product, fish oil, corn protein, dried yeast, wheat gluten, vitamin e supplement, etc (Zoo Med, Inc.) as well as baby spinach, containing a variety of vitamins and minerals.

Trap Types

Trap design is an essential component of catching desired target species when trying to limit the capture rate of non-target species. A study was conducted to examine 12 trap designs to capture the invasive red swamp crayfish. The traps tested are commercially available with additional modifications such as color and size. After Chi Square analysis, researchers determined that the most effective trap for catching crayfish was the Promar Mesh traps however, the Gee Minnow traps caught the most bycatch, including tadpoles. The results of this study indicated that

Trap Images obtained from Amazon.com

Promar Mesh



Steel Gee Minnow

the combination of trap types maximize the catch efficiency of crayfish (De Palma-Dow et al., 2020). This outcome can be possible for other aquatic species such as bullfrog tadpoles.

In this study, black Promar Mesh traps and steel silver Gee Minnow traps were used (unbaited and baited) to effectively capture bullfrog tadpoles (commercially sold on Amazon.com).

Connectivity Among Wetlands for Amphibian Populations

Habitat connectivity is considered to be one of the most important factors that can lead to the persistence of anuran populations, especially for species that have conservation concern. Because many amphibians require aquatic habitat for breeding, larval development, and upland terrestrial habitat for adult life stages, they are sensitive to loss or alteration of both habitat types. Specifically, wetland loss can impact anurans directly by removing habitat but also indirectly by increasing the distance among the wetlands. Amphibian dispersal relies heavily on wetland distribution within surrounding habitat. If wetlandscapes are too far apart, anuran specie reproduction is much harder to achieve (Zamberletti et al., 2018).

Although wetland connectivity can provide sustaining wetland biodiversity, it can also impose negative effects on native species due to the spread of invasive species. A study was conducted in Japan where researchers examined a farm pond system to demonstrate how habitat connectivity and a shared predator can affect native species through the spread of invasive species. It was concluded that with increasing pond connectivity, bullfrog densities increased. This finding suggests that high pond connectivity may promote migration of bullfrogs and enhance local population size. The depression of connectivity can be an effective strategy for controlling invasive species (Atobe et al., 2014). Another study modeled wetland removal and restoration which involved the introduction of native anuran species. Researchers found that wetland removal was a cascading issue because it reduced the number of species and increased the dispersal distance. On the other hand, wetland restoration reduced individual breeding. Both strategies have different impacts on anuran species, demonstrating that wetland removal can result in population loss which cannot be compensated by wetland restoration (Allen et al., 2020).

Many studies have highlighted that wetlands and their connectivity can decrease the availability of breeding sites and degrade the connectivity networks across a hybrid landscape. Maintaining a functionally connected wetland could become critical for safeguarding amphibians, especially with the changing climate. Although connectivity among wetlands is negative in the way it can spread invasive species, it is critical for native species to thrive. Habitat loss is significantly detrimental to native species and outweighs the odds of the spread of invasives.

American Bullfrog Baits & Attractants

There have been very few studies conducted on what type of attractants can effectively capture or lure bullfrog tadpoles. Many management plans try to remove bullfrog populations by capturing them as adults. However, one adult bullfrog is incomparable to the thousands of eggs that can be laid. Effectively capturing bullfrogs before they become terrestrial (as tadpoles) can provide effective control efforts to remove bullfrogs in larger quantities, reducing overall population numbers before they reach metamorphosis. Due of the lack of effective protocols specific to bullfrog tadpoles, this study is informed by research that has been conducted on other anuran and aquatic species for possible bait usage. A study was conducted to examine the capture success of baited and un-baited funnel traps. According to Grayson and Roe (2007), funnel traps have been used to capture adult and larval amphibians with baits such as shrimp or salmon eggs. In studies of fish trapping, light is a common attractant type that is used to increase the capture rates of aquatic amphibians. In Grayson and Roe's study, commercially available funnel traps were baited with glow sticks that had a 6-8 hour glow time and then deployed during the night. This study was conducted in a pond with various frog types, however, dominated with bullfrog tadpoles. On average, more bullfrog tadpoles were captured in traps with glowstick than without, capturing about 14 tadpoles in a single trap (Grayson & Roe, 2007).

In addition, another study was conducted on temperature selection by bullfrog tadpoles. Researchers set up a concentration gradient in a pond where warm water would flow through pipes. In the summertime, the tadpoles selected temperatures around 28.9 °C and in the wintertime, selected temperatures around 20.0 °C. The results of this study provided distributed patterns, suggesting that preferred temperatures of bullfrog tadpoles and time of year have a strong correlation (Nie et al., 1999). This information is important, indicating when temperature or heat can be used as an attractant for bullfrog tadpoles.

In many tropical countries, the invasive Cane Toad has been released. Like the bullfrog, this species is harming native populations via poison and competition for resources. A study was conducted to try to control this species with their own defense mechanism. Because Cane Toad tadpoles are cannibals, when they detect a newly laid clutch of cane toad eggs, they attack it. In this study, the poison was squeezed out of dead Cane Toads and used as bait. The traps that were baited with the poison caught over 40,000 toad tadpoles in less than a week (CaneToadsinOz, 2012). This information and test has not yet been replicated with bullfrog tadpoles. Cane Toad poison could be a potential attractant or a bullfrog egg mass itself. This topic has yet to be demonstrated; however, is achievable if planned correctly.

Another potential bait type is different proteins, commonly studied for bullfrog diet. Filho et al. (2010) examined the influences of 28% crude protein on bullfrog tadpole enzymatic activity. Results demonstrated that the commercial feed provided good performance for the health of the tadpoles. The study concluded that the tadpoles fed the commercial protein had the largest growth rate, the largest weight gain, and the greatest activity. Although not many researchers have used protein as a bait type, an image demonstrates that bullfrog tadpoles become more carnivorous as they reach tadpole maturity. Chris Rombough captured mature bullfrog tadpoles feeding on a dead carp in an Oregon pond (2023). This suggests that protein could be effective as a bait type in the later stages of bullfrog tadpole development.

In turn, plant-based items could be a feasible attractant for bullfrog tadpoles. Like protein, plant-based items have not been used to attract species of anuran tadpoles. As mentioned in the **Bullfrog Diet** section, most anuran tadpoles feed on plant material particularly when they are in the early stages of development. When considering different types of plant-based items to test, they should contain more than pure algae to outcompete the natural occurrences of algae in a pond, as well as to offer a more balanced diet.

Biotic & Abiotic Attractants Tested in This Study

Table 1: List of Baits Tested in Laboratory *(Baits in Red also Tested in Field)*

Bait Category	Bait Name	Bait Description	Rational & Citation
Biotic – Protein Based	Shrimp Pellets	Sinking Pellets Made of Fish Meal, Wheat Flour, Fish Meal, etc. (36% Crude Protein, 6% Crude Fat, 8% Crude Fiber, & 10% Moisture)	As bullfrog tadpoles reach later developmental stages, their diet preferences become carnivorous (Hammerson, 1999).
	Freeze Dried Minnows	100% All Natural Minnows (60% Crude Protein, 4% Crude Fat, 10% Crude Fiber & Moister)	
	Fish Meal	Menhaden Powder (62% Protein, Containing a Variety of Fats, Minerals, & Vitamins)	
	Freeze Dried Beef Liver	Grass Fed Beef Liver (Grain & Gluten Free)	
	Freeze Dried Daphnia	Daphnia (Vitamin E, C, B1, B2, & B12, 67% Crude Protein, 9.5% Crude Fat, 4.6% Crude Fiber, 8.8% Crude Ash, & 6.9% Moisture)	
	Trout	Frozen Store-Bought Rainbow Trout (16 g Protein, 4.9 g Fat, & 40 mg Sodium)	
Biotic – Plant Based	Algae Wafers	Round Spirulina, Algae, & Veggie Sinking Wafers (30% Protein, 7.5% Fat, 4% Fiber, & 10% Moister)	Tadpoles in the early stages of development typically eat a plant-based diet (CDFW, 2023).
	Dried Spinach	Leaf Spinach (9 g Protein, 1% Fat, 11% Sodium, with Vitamin D & Calcium)	
	Plant Protein	Pea & Quinoa Powder (16 g of Protein & 10% Iron)	
Biotic – Commercial Based	Cheese Power Bait	Floating Original Scent Power Bait for Trout	This bait has a strong odor to aquatic animals (Holliday, 2008).
	Salmon Eggs	Red Salmon Eggs	Bullfrog tadpoles have consumed egg masses & Cane Toad toxin has attracted tadpoles before (CDFW, 2023 & CaneToadsinOz, 2012)
	Super Scent	UV Anise Oil Based Artificial Bait	This bait has a strong odor to aquatic animals (Holliday, 2008).
	Magic Bait	Dough of Crawfish & Chicken Blood	
Abiotic Baits	Oyster Shell	Crushed Oyster Shell & Coral Calcium (38% Calcium)	Calcium is an important mineral for tadpoles because as they metamorphose, they begin to ossify and need a large demand for calcium (Lassiter et al., 2020).
	Diving Flashlight	Underwater Flashlight (Max Range is 100 m, Submersible up to 80 ft, Three Levels of Brightness, & 1100 Lumens)	Light (glowsticks) has been tested and has captured anuran species (Grayson & Roe, 2007).

Management Plans & Control Measures

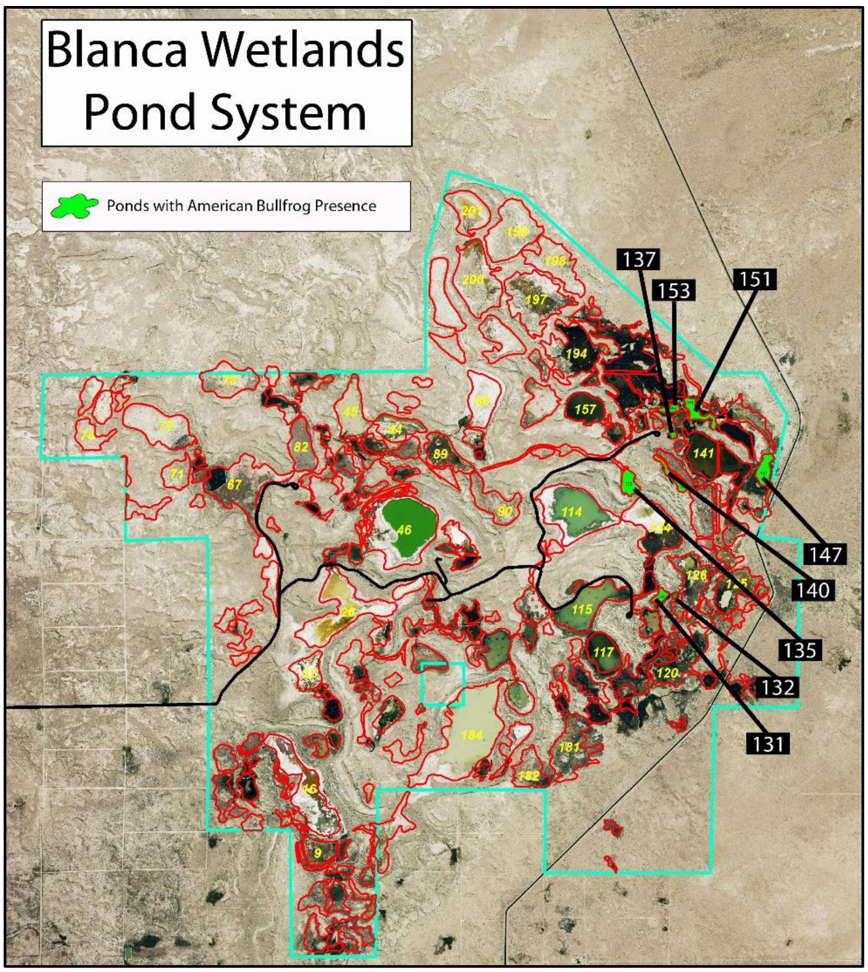
Several management plans for the American Bullfrog have been developed, including removing adult females once they emerge from hibernation and advising the public to trap and remove them with methods such as hand-netting, spearing, and destroying egg masses. Despite having these plans in place, there is still infestation leading to catastrophic problems worldwide.

Some control measures that have been used in the past and are currently being used to reduce the American Bullfrog population include the reduction of habitat, grading, visual inspection, lethal control, chemical treatment, salt, and electroshocking (Roy et al., 2015). However, these methods negatively affect other species within the area.

Blanca Wetlands Area, Colorado

A localized example of this global problem is present in the San Luis Valley, Colorado at the Blanca Wetlands Area. This area is classified as an Area of Critical Environmental Concern (ACEC). Historically, the area would receive snowmelt runoff and groundwater discharge from surrounding mountains. However, currently the wetlands receive the majority of water as well water from a multitude of sources as part of an effort to restore the wetlands system. This 10,000-acre area supports high populations of birds (including waterfowl, shorebirds, and snowy plover), fish, macroinvertebrates, many different species of plants as well as a number of amphibians. This includes many species such as the Great Plains Toads, Western Chorus Frogs, Northern Leopard Frogs, and the American Bullfrog. There are several sites on the area where water is applied and is channeled via a ditch network to several outlining playa basins. Because these wells are used annually to push water to over 1,200 acres of playa habitat, the wetlands near the source of the wells are extremely fresh and semi-permanent to permanent, perfect conditions for the American Bullfrog (Bureau of Land Management, 2002).

Map 2.



Map Made by Researcher, Background Image obtained from https://datagateway.nrcs.usda.gov/

Map 2 shows the playa system found within the Blanca Wetlands Area. Historic data and data collected in the summer of 2022 confirm the presence of American Bullfrog adults and or tadpoles in several sites on the eastern portion of the property (ponds with green fill color).

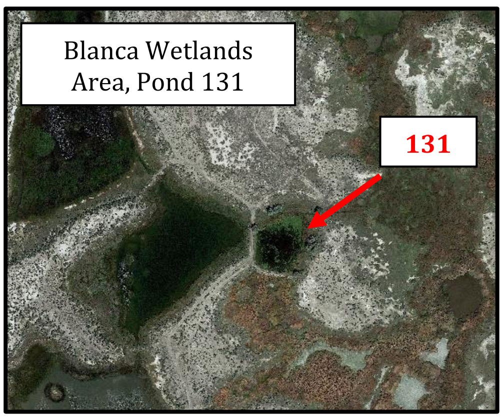
Because of the water transfer system within the Blanca Wetlands Area, it is possible that American Bullfrogs are traveling through the connected channels to reach other ponds, leading to more production of egg masses and infestation.

Focus of This Study

In this study, select American Bullfrogs tadpoles were netted from ponds within Blanca Wetlands, CO (BLM managed, a series of wetlands infested with Bullfrogs) and were tested using a choice chamber. Both abiotic and biotic attractants were used to determine which factors would allow for effective and efficient capture in a real-world setting. This research is critical for understanding tadpole behavior, which may be used to reduce the spread of the invasive bullfrog by removing them in high quantities before they metamorphose into adults.

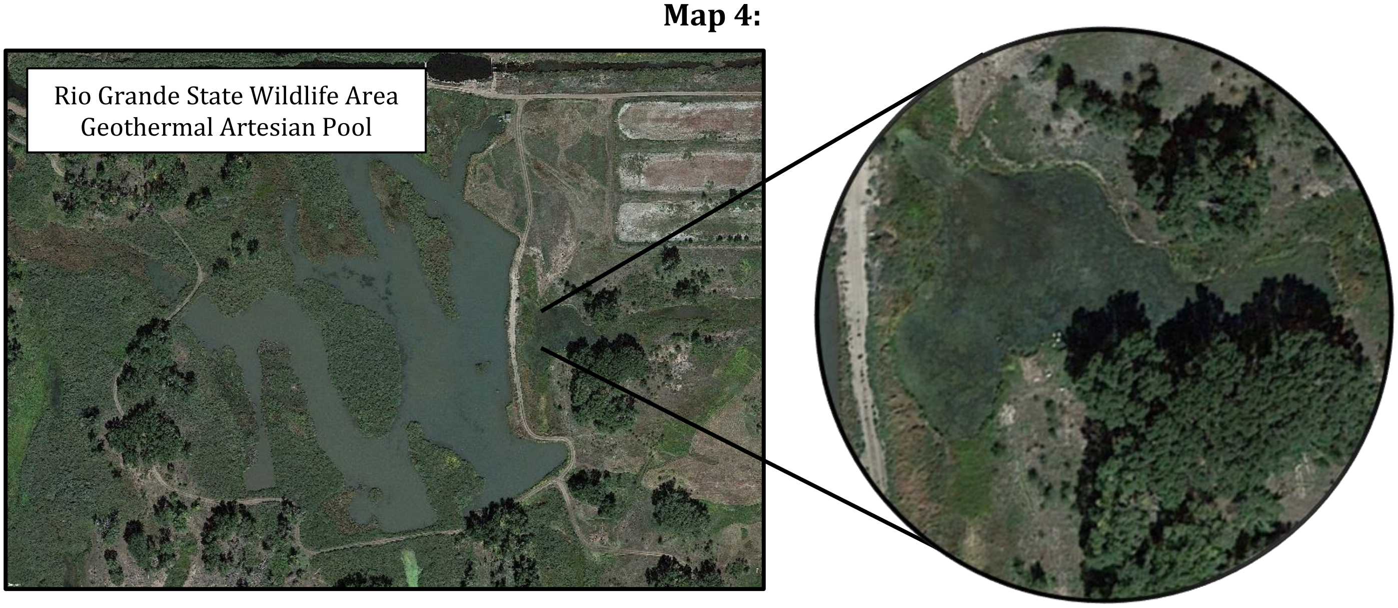
Study Sites

Map 3:



Map 3 depicts an aerial image of pond 131 at Blanca Wetlands, where bullfrog tadpoles were hand netted and used for laboratory choice chamber testing.

Map 4:



Map 4 illustrates the location where the bait and traps in this study were deployed. Initial pilot testing involved deploying traps with and without attractants on the main artesian pool however, no bullfrog tadpoles were captured. It is assumed that the main pond is devoid of bullfrog tadpoles or they are distributed in an inaccessible part of the pond. Trapping and deployment occurred in a shallow outflow slough (blowup) immediately east of the main pond. The water was warm enough to provide access in cold weather.

Scientific Components of Study

Null & Alternative Hypotheses

Choice Chamber Null Hypotheses:
There is no difference in the mean capture rate of American Bullfrog tadpoles when offered abiotic and biotic attractants.

Choice Chamber Alternative Hypotheses:
1. There is a greater capture rate when American Bullfrog tadpoles are offered biotic attractants (fish meal, freeze died minnows, shrimp pellets, commercial baits, etc.)
2. There is a greater capture rate when American Bullfrog tadpoles are offered abiotic attractants (light, temperature, minerals etc.)

Field Testing Null Hypothesis:
There is no difference in the mean capture rate of American Bullfrog tadpoles with any combination of attractant and trap type.

Field Testing Alternative Hypotheses:
1. There is greater capture rate of American Bullfrog tadpoles when biotic attractants are combined with the Promar Mesh trap type.
2. There is a greater capture rate of American Bullfrog tadpoles when abiotic attractants are combined with the Promar Mesh trap type.
3. There is greater capture rate of American Bullfrog tadpoles when biotic attractants are combined with the Gee Minnow trap type.
4. There is a greater capture rate of American Bullfrog tadpoles when abiotic attractants are combined with the Gee Minnow trap type.

Constants & Controls

Attractant Type Constants
- Placement/location of the attractant in choice chamber (left to right switch for all attractants).
- Amount of attractant for each bait type (in the lab, double it in the field).

Lab Testing Constants
- Same choice chamber for all tadpole testing.
- Acclamation period of two minutes.
- Amount of time each trial was conducted (10 minutes).

Home Tank Constants
- Home tank tadpoles were grouped into similar Gosner Stages.
- Each home tank received 3 g of Aquatic Frog & Tadpole Food daily and boiled baby spinach once a week.
- Amount of water in each tank (aged tap water).
- Water changes in each home tank (every three days).

Field Testing Constants

- Control traps were deployed with each field trial.
- Same amount of attractant type per trial.
- Traps were deployed up to 40 hours.

Lab Controls

- Choice chamber with no attractant on either side.
- Choice chamber with no attractant on one side for all experimental trials.

Field Controls

- Each trap type with no bait.

Methods

Capturing Bullfrog Tadpoles in the Field

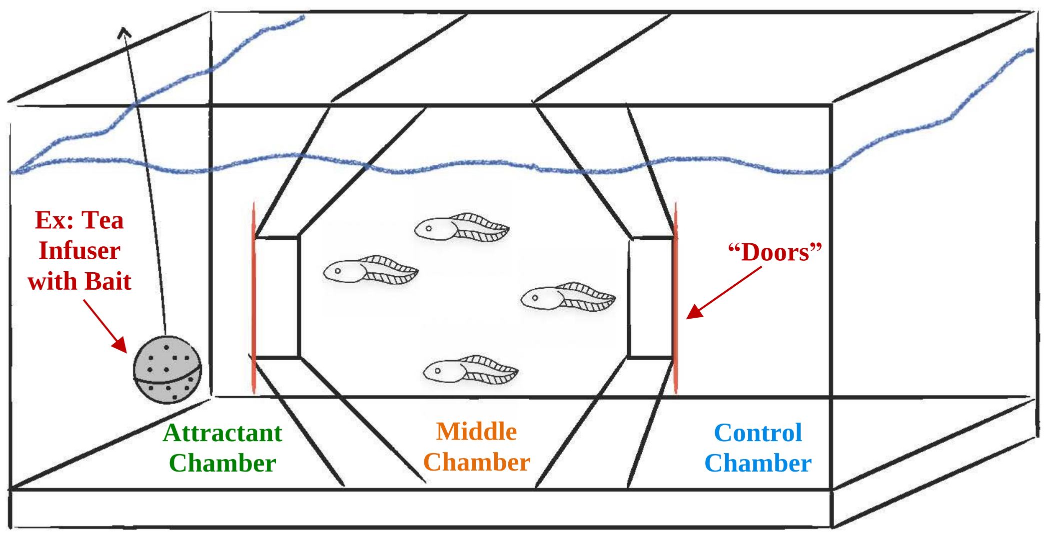
Captured American Bullfrog tadpoles were obtained from Blanca Wetlands at an infested pond (#131). Supplies were gathered (19-liter buckets, polarized sunglasses, and long-handled fishing nets) and waders were put on. The 19-liter buckets were filled with native pond water and set on the shore bank. Then, the long-handled fishing nets were used to capture tadpoles. Once obtained, the tadpoles were put into the buckets and sweeping continued until 143 tadpoles were captured (tadpoles were evenly distributed between the buckets). The 3.8 liter water jugs were then filled with native pond water to help transition the tadpoles to their captive home tanks. Once finished, all field gear was soaked in a 10% Bleach solution for 10 minutes to avoid the spread of Chytrid Fungus.

Home Tank Setup

To set up tadpole home tanks (11) in the lab setting (indoor greenhouse), each one was filled with five of the 3.8 liter jugs containing a mixture of native pond water and aged tap water. Once native water ran out, only aged water was used for water changes. Aerator lines were then assembled with tubing and aerator stones, and a single aerator line was placed into each tank. Using the Gosner stage chart, hand lens and the aquarium net, the tadpoles were sorted into their developmental stage and put into their designated home tank (15-20 tadpoles per home tank). Furthermore, the tadpoles were fed 3 grams of Aquatic Frog & Tadpole Food each day, and boiled baby spinach once a week.

Design of Choice Chamber

The choice chamber was created with an aquarium tank (189 liters). The interior length, width, and height were measured. Using the measurements, four cardboard trapezoids were created using a sharpie and scissors. The cardboard pieces were taped together and inserted within the tank to ensure fit from edge to edge. Once measurements were correct, each cardboard side was traced onto a 91x122x0.3 cm sheet of plexiglass. This process was repeated twice, having eight pieces in total. Once traced, the outline edges were lined up against a metal ruler. Then, using a boxcutter, the plexi pieces were scored about 12 times along the lines, pinned against a hard edge (metal edge of a stair in the hallway), and snapped along the scored lines. This process was repeated on all four side of each trapezoid piece. If the snap didn't occur "cleanly" leaving a nub, a hammer and a small wooden block were used to snap them off. Once all individual pieces were snapped, a hand-held belt sander was used to smooth out each edge. Finally, the pyramid shape was created in the aquarium tank. Pieces were held in place with silicone glue in the center of the tank, evenly distributed for three chambers. After silicon glue had dried, all internal surfaces of the choice chamber were sterilized with a 10% Bleach solution.



Drawing of Choice Chamber Designed by Researcher

Tadpole Trials

The choice chamber aquarium tank was filled with aged tap water. The attractant type was then placed on one side of the choice chamber, leaving the other side empty. Doors were then placed in front of the plexiglass opening of each side. After acquiring lab materials (lab notebook, timer, aquarium net), four random tadpoles were netted from one Gosner Stage home tank (26-27) and placed into the center of the choice chamber (middle). An acclamation period of two minutes was given and then the door were pulled away. A trial for 10 minutes began (using a stopwatch with four running chronometers) and were timed individually until each tadpole made a choice. Observations within the time and chosen side were written down in a lab notebook. Once the trial ended, this process was repeated for each home tank (11) for the bait selected.

At the beginning of the study, individual trials with one tadpole were run. However, there was little to no activity and the tadpoles would stay in the middle for the duration of the ten minutes. Through research, it was determined that tadpoles perform better in groups than individually. In addition, being that bullfrog tadpoles specifically congregate in the winter, there is strong rationale to test them in groups as they wouldn't be alone in a real-world setting.

In this study, bullfrog tadpoles were not tested based on Gosner Stage. Research suggests that bullfrog tadpoles do not perform differently between Gosner Stage groups.

Traps

Four standard steel Gee Minnow funnel traps (42x19 cm) and four collapsible Promar Minnow Mesh traps (32x53 cm) were obtained from Amazon.com for field testing purposes.

Deployment of Trap and Bait in the Field

Based on data and statistical analysis, only baits with significant performance in lab testing were used in the field. These included: fish meal, algae wafers, diving flashlight, and trout. Before heading to the field, traps and bait were prepared for deployment (attractants put into bait bags, maps created, and labels attached). The researcher drove to the Rio Grande State Wildlife Area for deployment. Once there, attractants and controls were deployed on an east side slough for up to a 40-hour time period. After the period, the researcher went to the study area again to record capture rate. This included counting and recording number of tadpoles, fish, macros, and other organisms captured in a field notebook. If bullfrog tadpoles were captured, they were put into plastic beakers with a labeling system and transported to lab. All fish and macroinvertebrates were released back into the water. After retrieving all gear, it was soaked in a 10% Bleach solution for 10 minutes. This series of steps was repeated for each deployment and retrieval date.

Tadpole Measurements Obtained From the Field

All captured tadpoles were measured for body and total length (body length was from "nose" to the vent area, and total length was the "nose" to tail tip). Measurements were taken using a v-shaped device (modeled after a fish measuring board) constructed from a piece of Styrofoam and 12 in ruler. Tadpoles were placed into the measuring device and lengths were recorded.

Statistical Analysis

Data was analyzed by using Chi Square goodness of fit test for lab and field testing. In addition, t-tests were used to compare variance and average between bait and trap treatments. Additionally, a correlation was used to understand the relationship between body length and total length of field captured bullfrog tadpoles.

Lab Results

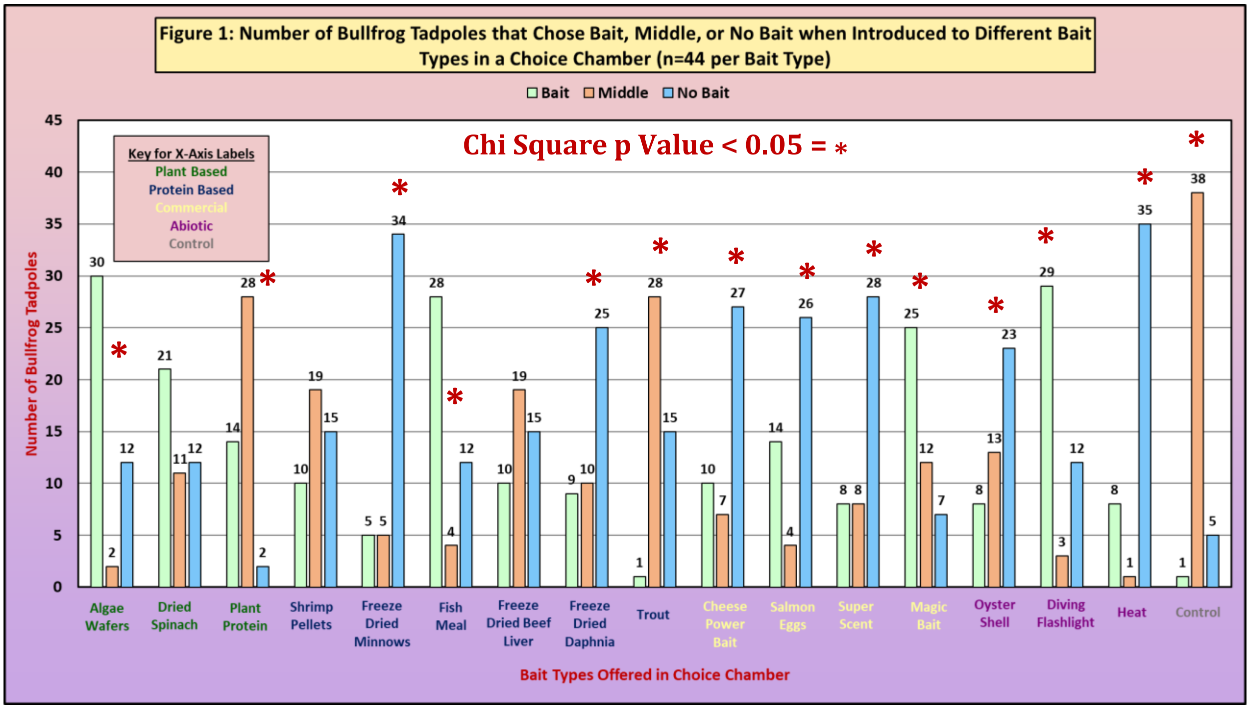


Figure 1: Number of Bullfrog Tadpoles that Chose Bait, Middle, or No Bait when Introduced to Different Bait Types in a Choice Chamber (n=44 per Bait Type)

Figure 1 depicts the distribution of bullfrog tadpole choice when offered different bait types in a lab setting. Unexpectedly, the tadpoles had the highest count of no bait when exposed to heat. In addition, the highest number of tadpoles staying in the middle occurred within the control trials of choice chamber testing. Overall, three attractants were concluded to effectively draw bullfrog tadpoles (algae wafers, fish meal, diving flashlight, and the magic bait).

Table 2: Choice Chamber Chi Square p-Values to Determine which Baits were Attractive to Bullfrog Tadpoles

Categories of Bait Types	Bait Type (vs No Bait)	Chi Square p-Values	Significance For No Bait, Bait or Middle Chamber? (n=44)
Biotic – Protein Based	Shrimp Pellets	0.248	Not Significant
	Freeze Dried Minnows	0.000	No Bait
	Fish Meal	**0.000**	**Bait**
	Freeze Dried Beef Liver	0.248	Not Significant
	Freeze Dried Daphnia	0.004	No Bait
	Trout	0.000	No Bait
Biotic – Plant Based	**Algae Wafers**	**0.000**	**Bait**
	Dried Spinach	0.125	Not Significant
	Plant Protein	0.000	Middle
Biotic – Commercial Based	Cheese Power Bait	0.000	No Bait
	Salmon Eggs	0.000	No Bait
	Super Scent	0.000	No Bait
	Magic Bait	**0.003**	**Bait**
Abiotic Baits	Oyster Shell	0.018	No Bait
	Diving Flashlight	**0.000**	**Bait**
	Heat	0.000	No Bait
Control	**No Bait**	**0.000**	**Middle**

Table 2 includes the Chi Square analysis (p values) for each attractant in the choice chamber lab testing. Through this statistical analysis, baits that were significant in attracting bullfrog tadpoles were highlighted in yellow (fish meal, algae wafers, magic bait, and the control). The control was significant for the middle chamber when no bait was offered on either side, suggesting that bullfrog tadpoles actually make decisions based on the presence of bait.

Table 3: Comparison Between Bullfrog Tadpole First and Second Decision for Choice Chamber Trials	
Bait Type	**Choice of 1st vs Choice of 2nd (# Different, out of 11 Groups)**
Shrimp Pellets	8/11
Freeze Dried Minnows	3/11
Fish Meal	5/11
Freeze Dried Beef Liver	6/11
Freeze Dried Daphnia	4/11
Trout	6/11
Algae Wafers	4/11
Dried Spinach	8/11
Plant Protein	5/11
Cheese Power Bait	6/11
Salmon Eggs	5/11
Super Scent	1/11
Magic Bait	4/11
Oyster Shell	7/11
Diving Flashlight	5/11
Heat	5/11
Control	4/11

Table 3 shows the comparison between the first and second tadpole's choice, with the goal of determining if the choice of the first tadpole influences the choice of the second tadpole when tested in groups. For example, in the shrimp pellet trials, 8 out of 11 "second" tadpoles made a different choice than the first tadpole that chose out of that specific group trial. This data suggests that the first tadpole to choose out of each trial did not influence the second tadpole's choice.

Figures 2, 3, 4, and 5 demonstrate time analysis of the choice that the tadpoles made in **Figure 1**. Tadpole choices were averaged for a specific bait type and separated into their corresponding bait category (which included protein based, plant based, commercial based, and abiotic).

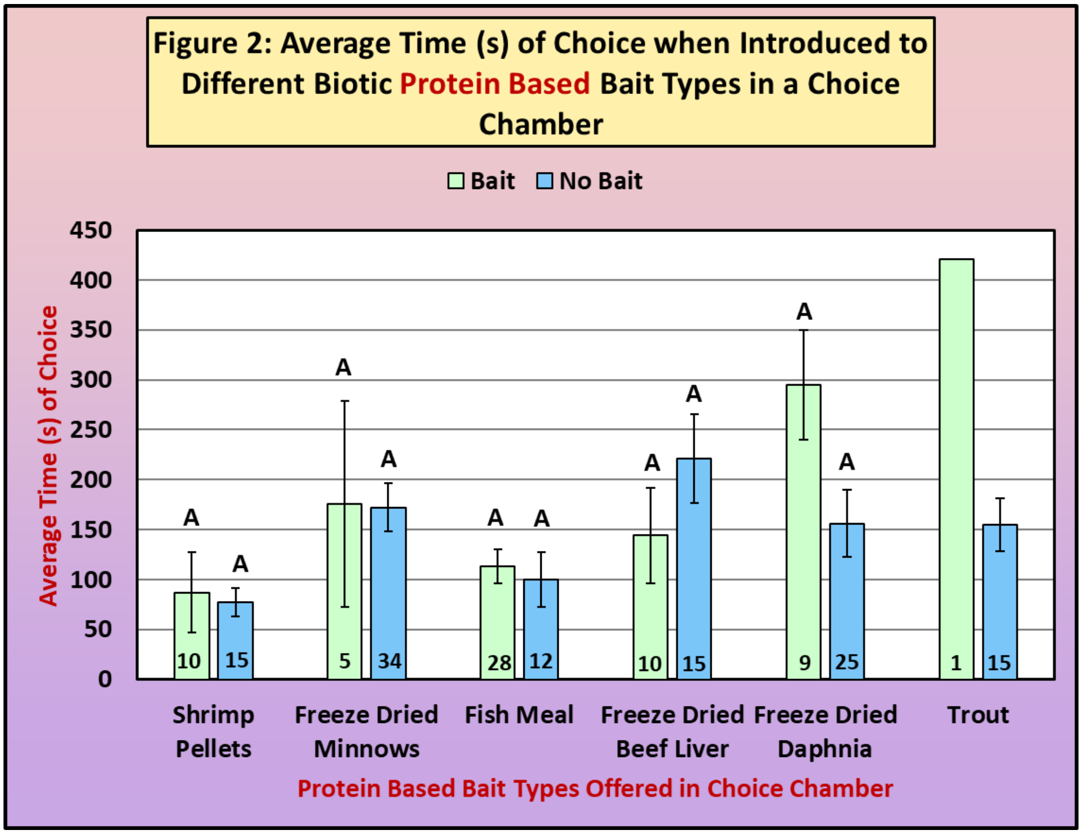


Figure 2 illustrates the average time (s) that tadpoles took to choose the bait or no bait side for protein-based baits which included shrimp pellets, fish meal, freeze dried beef liver, freeze dried daphnia, and store bough frozen trout fillets. When time was compared between the bait and no bait for all the attractant types, there was no significance between choosing faster or slower. In addition, bullfrog tadpoles were quickest to make a choice during the shrimp pellet trials but slowest to make a choice during the trout trials.

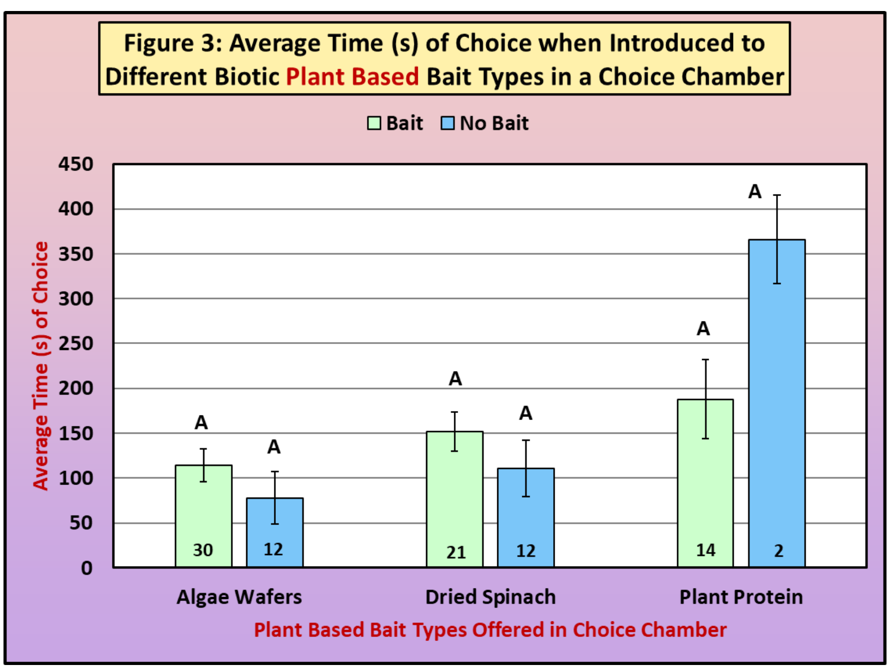


Figure 3 depicts the average time (s) that bullfrog tadpoles took to choose either bait or no bait for plant-based attractant types. When comparing average time for one attractant type, there is no significant difference between choosing bait or no bait. Despite this, on average tadpoles chose quicker when introduced the algae wafers but chose slower when introduced to plant protein.

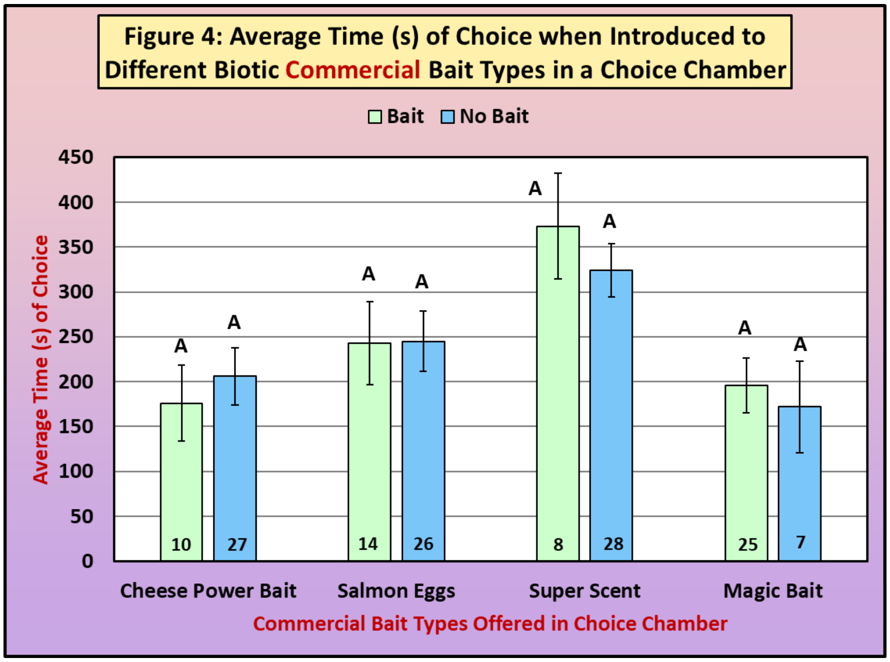


Figure 4 displays the average time (s) that bullfrog tadpoles took to choose bait or no bait for commercial attractant types. Notably, on average, bullfrog tadpoles took longest to make a choice for all commercial baits when visually compared to protein based, plant based, or abiotic attractants. However, tadpoles took the most time to choose the super scent bait (which smelt of anise). On the other hand, cheese power bait and the magic bait demonstrated similar choice time which happens to be the fastest out of all the commercial attractant types tested.

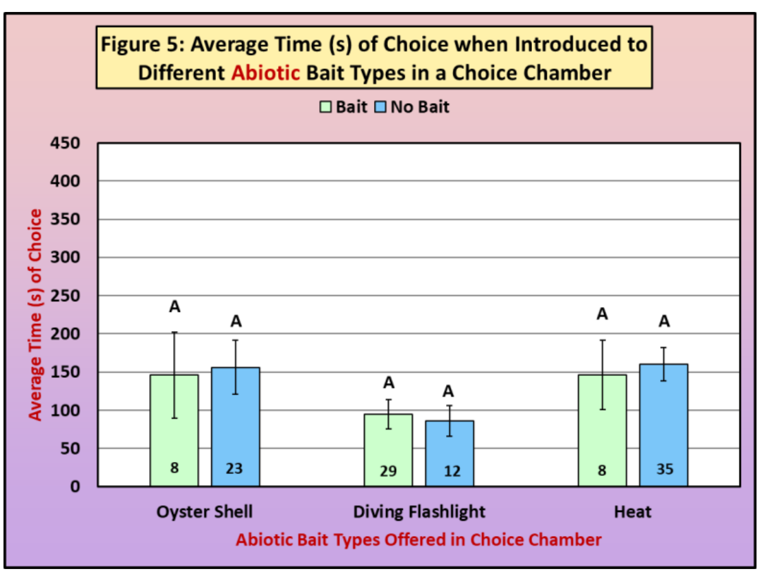


Figure 5 exhibits the average time (s) bullfrog tadpoles took in the choice chamber to choose either bait or no bait for different abiotic attractant types. Overall, bullfrog tadpoles chose the diving flashlight the fastest for both bait and no bait. In addition, oyster shell and heat relatively had the same time of decision in the choice chamber.

Table 4: Choice Chamber T Test p Values for Duration of Choice for Each Bait Type

Categories of Bait Types	Bait Type vs No Bait	T-Test p-Values	Significance For No Bait or Bait? (n=44)
Biotic – Protein Based	Shrimp Pellets	0.813	Not Significant
	Freeze Dried Minnows	0.937	Not Significant
	Fish Meal	0.676	Not Significant
	Freeze Dried Beef Liver	0.246	Not Significant
	Freeze Dried Daphnia	0.048	No Bait
	Trout	Inadequate n	Inadequate n
Biotic – Plant Based	Algae Wafers	0.310	Not Significant
	Dried Spinach	0.295	Not Significant
	Plant Protein	0.073	Not Significant
Biotic – Commercial Based	Cheese Power Bait	0.569	Not Significant
	Salmon Eggs	0.981	Not Significant
	Super Scent	0.478	Not Significant
	Magic Bait	0.696	Not Significant
Abiotic Baits	Oyster Shell	0.887	Not Significant
	Diving Flashlight	0.764	Not Significant
	Heat	0.786	Not Significant

Table 4 depicts the t-Test p-values for bullfrog tadpole duration of choice for each bait type in the lab setting (data for **Figures 2, 3, 4, and 5**). Overall, there was no significance in the duration of time involving tadpole choice. In addition, the baits that resulted with significant attractiveness did not include faster choice time and vise versa.

Field Results

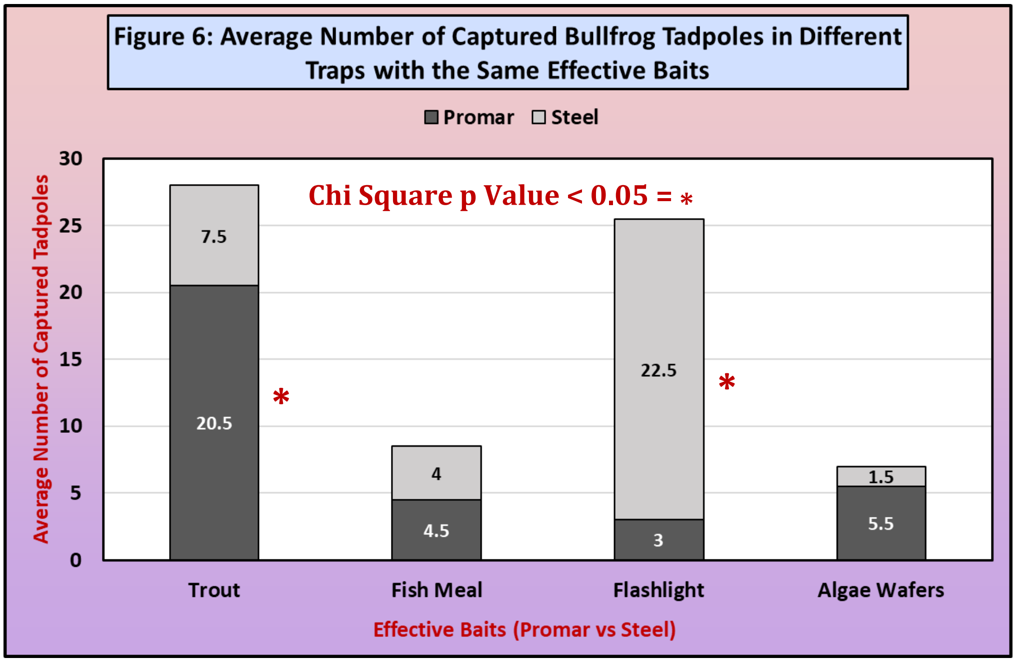


Figure 6 demonstrates the average number of bullfrog tadpoles captured between trap types with different attractants. The stack bar shows that on average, the Promar trap with trout caught a high abundance of bullfrog tadpoles. On the other hand, the Promar trap with the flashlight caught the least amount of tadpoles. In addition, the steel traps caught the most tadpoles when combined with the flashlight.

Table 5: Chi Square p-Values for Trap vs Trap Comparisons with Various Bait Types in the Field

Bait Type	Comparison (Trap vs Trap)	Chi square p-Values	Significance for Trap Type?
Trout	Promar vs Steel	**0.014**	**Promar**
Fish Meal	Promar vs Steel	0.864	Not Significant
Flashlight	Promar vs Steel	**0.000**	**Steel**
Algae Wafers	Promar vs Steel	0.131	Not Significant

Table 5 is the Chi Square analysis that corresponds with **Figure 6.** The p-values indicate that when both Promar and steel were baited with trout, the Promar trap caught more bullfrog tadpoles. In addition, when the Promar and steel were both baited with the flashlight, the steel trap caught more tadpoles.

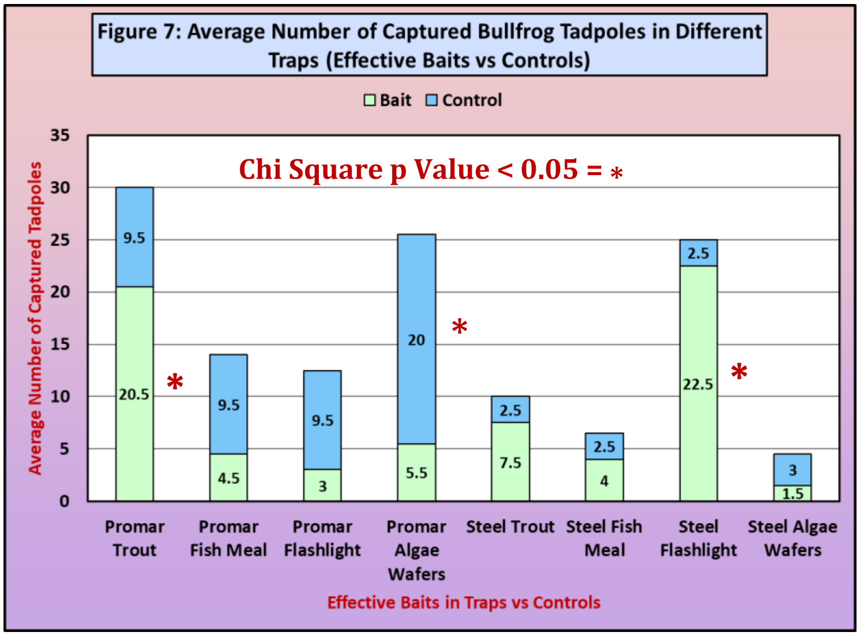


Figure 7 demonstrates the average number of captured bullfrog tadpoles in different trap types paired with baits, compared to the control. The Promar trap combined with algae wafers caught the least amount of tadpoles. Further, when the steel trap was combined with the flashlight, it had the highest capture rate and when the Promar was combined with the trout, it had the highest capture rate. Overall, the Promar control traps captured the most tadpoles.

Table 6: Chi Square p-Values for Trap and Bait Type vs Control in the Field			
Trap Type	**Comparison (Bait Type vs Control)**	**Chi Square p-Values**	**Significance for Bait Type or Control?**
Promar	Trout vs Control	**0.045**	**Trout**
Promar	Fish Meal vs Control	0.181	Not Significant
Promar	Flashlight vs Control	0.066	Not Significant
Promar	Algae Wafers vs Control	**0.004**	**Control**
Steel	Trout Vs Control	0.114	Not Significant
Steel	Fish Meal vs Control	0.556	Not Significant
Steel	Flashlight vs Control	**0.000**	**Flashlight**
Steel	Algae Wafers vs Control	0.480	Not Significant

Table 6 is the Chi Square analysis that corresponds with **Figure 7**. As a result, when the Promar was combined with the trout and compared to the control, it was significant. In addition, when the steel was compared to the flashlight, it was extremely significant. However, when the Promar was combined with the algae wafers, the control outperformed this specific combination.

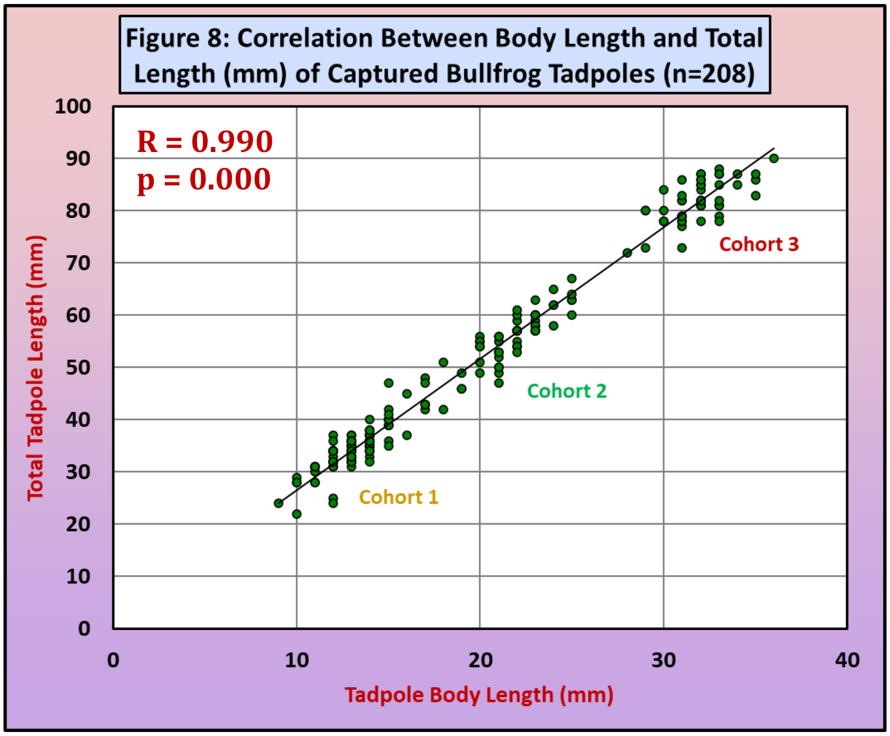


Figure 8 represents the correlation between body length (nose to vent) and total length (nose to tail tip) of bullfrog tadpoles captured in the field regardless of trap and attractant type. This correlation revealed that three sizes of bullfrog tadpoles were captured in the field, suggesting that three different cohorts (multiple hatch years) were represented in the pond.

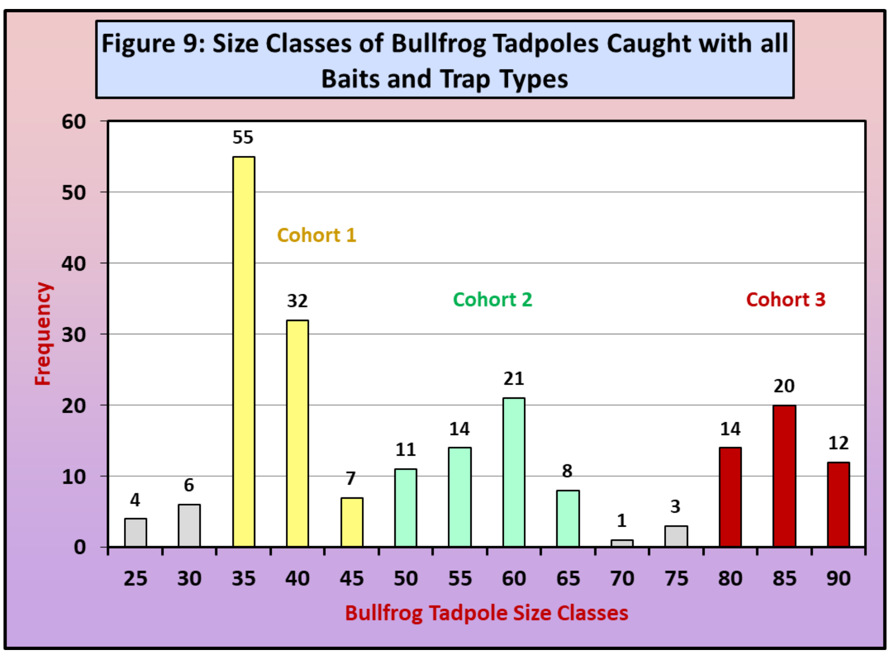


Figure 9 represents the frequency and size classes of bullfrog tadpoles captured in the field. **Cohort 1** is total tadpole lengths mode from **31-45**, **Cohort 2** is the total tadpole length mode from **46-65** and **Cohort 3** is the total tadpole length mode from **76-90**.

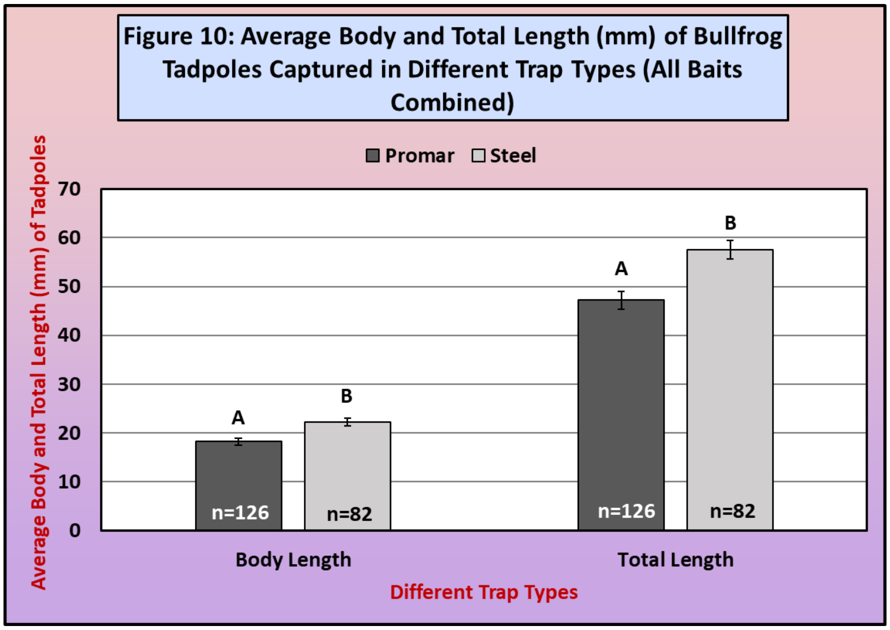


Figure 10 illustrates the average body and total length of captured bullfrog tadpoles regardless of attractant type. Notably, the Promar trap caught smaller tadpoles while the steel trap caught larger tadpoles.

Table 7: T-Test p-Values for Average Body and Total Length of Captured Tadpoles in Different Trap Types		
Trap Type	Comparison (Trap vs Trap)	T-Test p-Values
Body Length	Promar vs Steel	0.000
Total Length	Promar vs Steel	0.000

Table 7 corresponds with **Figure 10**, demonstrating the significance of body and total length when compared for both trap types.

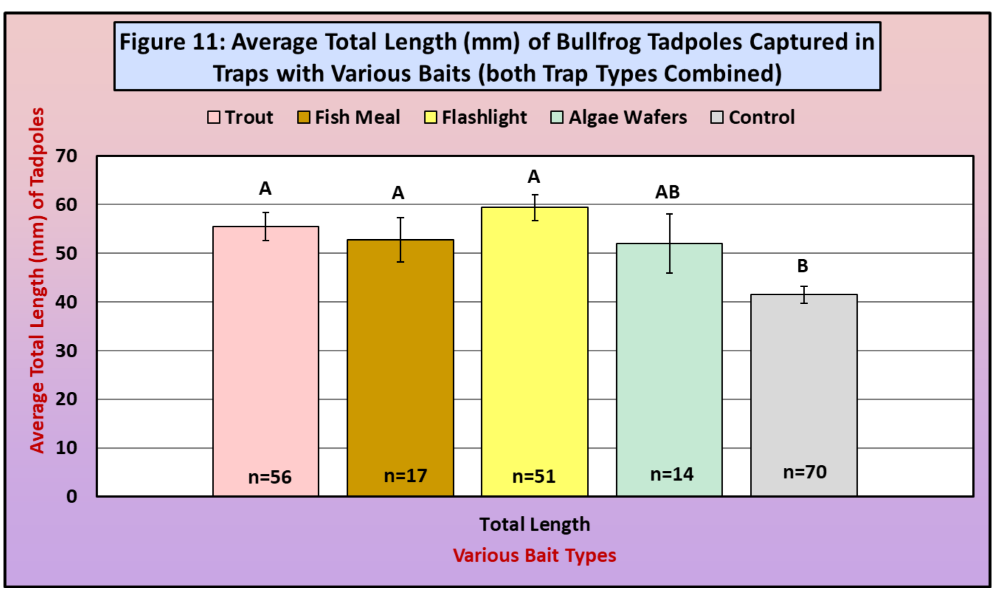


Figure 11 demonstrates the average body length of captured bullfrog tadpoles regardless of trap type for each attractant type. Overall, this data suggests that there is no significant difference between the tadpole sizes given different baits. However, the control caught significantly smaller tadpoles.

Table 8: T-Test p-Values for Bait vs Bait Total Length Comparisons in the Field (Trap Types Combined)		
Comparison (Bait vs Bait)	**T-Test p-Values**	**Significance for Bait vs Bait Body Length?**
Trout vs Fish Meal	0.609	Not Significant
Trout vs Flashlight	0.322	Not Significant
Trout vs Algae Wafers	0.603	Not Significant
Trout vs Control	**0.000**	**Trout Caught Longer Tadpoles**
Fish Meal vs Flashlight	0.216	Not Significant
Fish Meal vs Algae Wafers	0.920	Not Significant
Fish Meal vs Control	**0.030**	**Fish Meal Caught Longer Tadpoles**
Flashlight vs Algae Wafers	0.275	Not Significant
Flashlight vs Control	**0.000**	**Flashlight Caught Longer Tadpoles**
Algae Wafers vs Control	0.113	Not Significant

Table 8 corresponds with **Figure 11**, showing significance of tadpole total length between different bait types. When trout, fish meal, and the flashlight were compared to the control, each bait caught bigger bullfrog tadpoles. In addition, when baits were compared against other baits and not a control, there was no significance present.

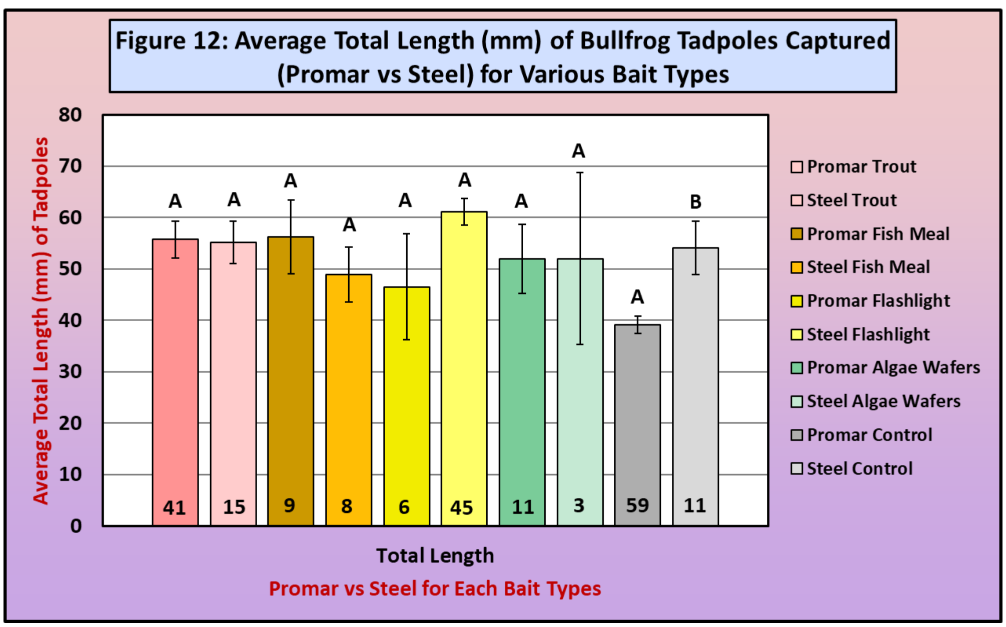


Figure 12 depicts the average total length of all captured tadpoles for each bait and trap type. The total length had little variation between each trap type with each bait. However, the steel control revealed that it caught larger animals than the Promar control.

Table 9: T-Test p-Values for Trap vs Trap Total Length with Various Bait Types in the Field		
Comparison (Trap vs Trap)	**T-Test p-Values**	**Significance for Trap vs Trap Body Length?**
Promar Trout vs Steel Trout	0.921	Not Significant
Promar Fish Meal vs Steel Fish Meal	0.428	Not Significant
Promar Flashlight vs Steel Flashlight	0.220	Not Significant
Promar Control vs Steel Control	**0.017**	**Promar Caught Smaller Tadpoles**

Table 9 corresponds with **Figure 12**, demonstrating the comparision and p-values between trap types given the same attractants. There was no significance found with the exception of the Promar and steel controls compared. The Promar control caught smaller tadpoles while the steel control caught larger tadpoles.

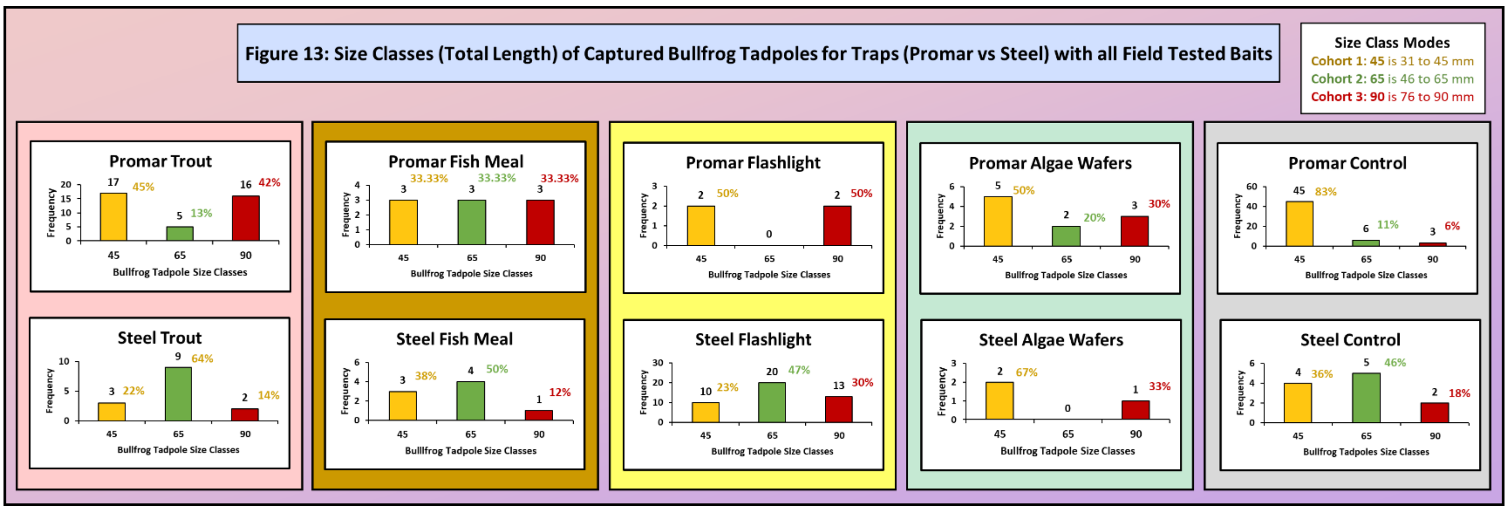


Figure 13: Size Classes (Total Length) of Captured Bullfrog Tadpoles for Traps (Promar vs Steel) with all Field Tested Baits

Figure 13 demonstrates a breakdown of each trap type with a specific bait and the frequency of each size class discovered. Overall, the Promar traps had a high capture rate of Cohort 1 (smallest) and Cohort 3 (biggest) while the steel traps mostly caught Cohort 2 (medium size) with the exception of steel combined with algae wafers.

Discussions & Conclusions

The American Bullfrog exhibits many biological characteristics that contribute to its invasiveness. This included having a broad temperature tolerance, the ability to adapt quickly, having a diet that consists of anything they can swallow, high fecundity, and the ability to aggressively disperse locally and occupy new habitat (Oregon State University, 2014). The American Bullfrog is one of many invasive species that has dispersed and caused significant negative ecological impacts, including contributing to the decline of native anuran species as well as many other vertebrate groups. In addition, bullfrogs carry and spread Chytrid Fungus and Ranavirosis, diseases that are known to impact anuran species internationally (Daughterty, 2023).

Because the American Bullfrog is a species that establishes quickly and effectively, there is little to no management plans that are completely efficient in removing bullfrogs from an infested area. Management plans typically include informing the public to hand net, remove, and kill American Bullfrogs in specific states (Oregon State University, 2023). Despite these general directives, haphazard removal of adult bullfrogs does not stop the hatching of eggs masses from mature adult females, which can be up to 47, 840 eggs. Additionally, some older female bullfrogs can even produce two clutches of eggs each year (Hammerson, 1999).

In this study, instead of focusing on the capture of adult bullfrogs, the aquatic form (tadpoles) was targeted due to several factors that occur during winter months. In the summertime, adult bullfrogs have the ability to disperse anywhere within a given area, either terrestrially or aquatically. Bullfrog tadpoles, however, are limited to water and furthermore, are one of the only frog species that overwinter as a tadpole, especially in the intermountain west. Other research studies have demonstrated that in winter, bullfrog tadpoles will congregate in specific areas in a given pond and understanding and taking advantage of this behavior could potentially "provide opportunities for effective control efforts (Sepulveda & Layhee, 2015)."

Because of these factors, the collection of bullfrog tadpoles occurred in early December in this study from an infested pond within Blanca Wetlands, CO (a BLM managed property, containing a series of wetlands infested with Bullfrogs). Abiotic and biotic attractants were selected based on a variety of background information, including protein based (shrimp pellets, freeze dried minnows, fish meal, freeze dried beef liver, fish meal, freeze dried beef liver, freeze dried daphnia, and trout), plant based (algae wafers, dried spinach, and plant protein), commercial based (cheese power bait, salmon eggs, super scent, and magic bait) and abiotic baits (oyster shell, diving flashlight, and heat). These attractant types were tested in a lab setting using an originally designed choice chamber.

At first, tadpole trials were conducted individually. However, there was minimal activity occurring and no choices were being made: tadpoles would simply rest in the center chamber. After considering that wild tadpoles almost always congregate in groups, trials were then conducted as groups of four individuals, and movement and subsequent choices became more frequent. After running an analysis on choice order, it was determined that the first tadpole's choice did not influence the second tadpole's choice. The distribution of tadpole choice chamber decisions were analyzed using Chi Square and only three out of sixteen baits appeared to be attractive: fish meal (p-value of 0.000), algae wafers (p-value of 0.000) and magic bait (p-value of

0.003). The control trials (with no attractants on either side, to control for unknown factors relating to the tank design) resulted with a p-value of 0.000. However in the control trials, 38 out of 44 tadpoles stayed in the middle. This suggests that when bullfrog tadpoles are exposed to different attractant types, they are making decisions based on the influence of the specific bait type, and not from unknown influences relating to the chamber set-up. This provides evidence that during attractant trials, tadpoles choosing the side with no bait could possibly be repelled by the offered bait, while tadpoles choosing the bait could be genuinely attracted to it.

When referring to **Figure 1** and combining the success rate for any particular bait category (plant, protein, commercial, or abiotic), a higher number of bullfrog tadpoles chose the plant-based baits than the other categories. Protein-based baits were a close second to the plant-based baits, and abiotic baits attracted the least amount of tadpoles in sum total. The attractants that were significant for lab testing were chosen for use in field trials. Although trout was not significant in the lab, it was tested in the field due to strong evidence that mature bullfrog tadpoles will feed on dead fish when given the opportunity (photo of tadpoles feeding on a deceased carp, captured by Rombough, 2023).

Figure 1 data was further examined, and choice time analysis was conducted for each bait category. For protein-based attractant types, bullfrogs made the fastest decision during the shrimp trials (95 s for bait and 80 s for no bait). However, tadpoles were slowest to decide during the trout trials (420 s for bait and 155 s for no bait). Overall, there was no significant difference between decision time for the protein-based bait types, with the exception of dried daphnia (p-value of 0.048) suggesting that bullfrogs were repelled by this bait type. In addition, no statistics were run for trout due to insufficient sample size for tadpoles choosing the bait side.

For plant-based attractants, bullfrog tadpoles made the quickest decision when exposed to algae wafers (110 s for bait and 80 s for no bait) and chose most slowly when exposed to plant protein (190 s for bait and 360 s for no bait). For the commercial baits, the decision time was distributed evenly across all types. This indicates that bullfrog tadpoles are not especially attracted to this kind of bait in a lab setting. Furthermore, the abiotic time analysis demonstrated that bullfrogs made the quickest decision when exposed to the diving flashlight (98 s for bait and 90 s for no bait). Overall, running the time analysis for all categories of bait tested indicated that bullfrog tadpoles do not necessarily choose faster or slower when introduced to baits they like or dislike.

Attractive bait types were deployed in two different trap types (Promar and Gee Minnow steel) in the field at a geothermal artesian pond in the San Luis Valley. As mentioned in the **Methods** section, pilot trials were conducted on the main pond with all four bait types; however, nothing was captured after several days. It was assumed that the main area was devoid of bullfrog tadpoles or were located at an inaccessible part of the pond. Because of this, field trapping was moved to a slough historically known to contain high numbers of bullfrogs east of the main pond. After the remaining trapping sessions, a total of 208 bullfrog tadpoles were captured over three 40-hour deployment periods. When data was analyzed regarding the average number of bullfrog tadpoles captured, Promar traps combined with trout had the highest frequency of capture at 20.5 (and was statistically significant with a p-value of 0.014). On the other hand, steel traps captured the highest average of bullfrog tadpoles when combined with the diving flashlight (22.5, statistically significant with a p-value of 0.000). It is possible that this combination was effective due to the steel becoming shiny once illuminated with light. There has been research conducted on bullfrog tadpole attractiveness to glow sticks. However, the behavioral mechanism that attracts bullfrog tadpoles to this type of diffuse light is unclear. The data was analyzed further, and the average number of tadpoles captured between a baited trap and the same trap type's control was compared. On average, Promar with algae wafers and the Promar control performed the worst in terms of capture rate. Promar with algae caught 5.5 tadpoles while the promar control caught 20 tadpoles. Overall, the Promar control traps caught more tadpoles than the steel control traps, indicating that the Promar trap itself is effectively attractive.

In the field, it was observed that the Promar traps were capturing smaller tadpoles while the steel traps were catching larger tadpoles. Because of this observation, tadpoles captured from the field were measured with an originally constructed device (similar to a fish measuring board) in the lab. Body length measurements were recorded (from nose to vent) as well as total length measurements (from nose to tail tip). This data was analyzed with a correlation scatterplot, and this revealed that three different size classes of bullfrog tadpoles were captured from the field in this study. Data indicated that three cohorts (likely different hatch years) were represented. The first cohort demonstrated a total length size class mode between 31-45 mm, the second cohort being 46-65 mm and the third cohort being 76-90 mm (pictures down below).


Cohort 1


Cohort 2


Cohort 3

The pictures below were taken of the smallest bullfrog tadpole captured (Cohort 1). When this size class was obtained from the field, it could not be identified. However, the bullfrog tadpole characteristics were present (having black sharp-edged dots, black dorsum, & gold crossbands (Hammerson, 1999)). Despite this, no research was found on a bullfrog tadpole this small with this underside coloration.



Tadpole Temporarily Held for Photos

Although the Promar trap captured more tadpoles overall, the size class data suggested that both trap types are important for capturing all size classes of bullfrog tadpoles. This finding is important to gauge what trap types need to be deployed to successfully capture the greatest number of tadpoles possible. The average body and total length of all captured bullfrog tadpoles were separated between Promar and steel, regardless of bait types. Overall, the Promar trap caught smaller tadpoles (with a total length of 47 mm) while the steel caught larger tadpoles (with a total length of 78). When statistically analyzed, the body length and total length for Promar compared to steel had a p-value of 0.000. Furthermore, this data was analyzed between bait types regardless of trap type. These results demonstrate that there was no significant difference between the total length of captured tadpoles between different bait types. These results suggest that bait type doesn't influence the size of tadpoles that go into a trap. However, the traps chosen for deployment may play a big role in influencing different tadpole sizes that are captured. Furthermore, the average total length of captured tadpoles was analyzed between trap types with various baits. However, there was no significant difference between tadpole size classes and a combination of trap and bait. To conclude, all size classes of bullfrog tadpoles for each trap and bait type were displayed with histograms (**Figure 13**). This data revealed that the Promar traps mostly caught the smallest and largest size classes (31-45 mm and 76-90mm) of bullfrog tadpoles and the steel traps mainly captured the middle class (46 to 65 mm) of bullfrog tadpoles. Again, this data indicates that both trap types used in this study should be deployed to effectively capture all bullfrog tadpoles present in a pond.

Application & Future Work

This research contains many components; however, there is a lot more to be discovered. In this study, I was able to successfully find different attractants that are effective at capturing bullfrog tadpoles and combine them with commercial traps for tadpole removal. One limitation in this research was that the lab trials could not completely simulate a highly variable field setting and therefore, findings may vary from what is learned in the field.

This study was geared to find a solution at the Blanca Wetlands Area, CO however, these findings can be applied anyplace where bullfrog infestation is occurring. This research is critical for understanding bullfrog tadpole behavior, particularly wintertime congregation, which can be used to significantly reduce their spread and remove them before they metamorphose into adults, while simultaneously avoiding the capture of natives. Although this study represented an initial lab analysis and field trapping effort, the implications for large-scale deployment utilizing multiple combinations of baits and trap types is very encouraging.

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