STAFF SUMMARY FOR AUGUST 22-23, 2023

13. BULLFROGS AND NON-NATIVE TURTLES

Today's Item **Information ☒** **Action ☐**

Receive a background presentation in anticipation of a Wildlife Resources Committee (WRC) recommendation.

Summary of Previous/Future Actions

• Topic referred to WRC	December 12-13, 2018
• WRC received updates on stakeholder engagement process	2019-2022
• Final staff report received by WRC	May 16-17, 2023; WRC
• **Today receive background information**	**August 22-23, 2023**
• Potentially receive WRC recommendations	October 11-12, 2023

Background

Annually, approximately two million non-native American bullfrogs and 300,000 non-native turtles (mostly red-eared sliders and softshell turtles) are imported into California for food and the pet trade. While these species are not imported into California with the intent of being released, they have established wild populations that threaten native amphibians, fish, and wildlife by direct predation, competition for resources and habitat, and disease.

In December 2018, the Commission referred to the Wildlife Resources Committee (WRC) a stakeholder engagement plan to track progress addressing issues around non-native American bullfrog (commonly referred to simply as bullfrog) and turtles that are imported into California for food and the pet trade. The plan involved three independent groups developing situation analyses and strategies for addressing the threats, challenges, and opportunities posed by bullfrogs and non-native turtles and their impacts on native wildlife. The WRC received progress updates throughout the process.

For the situation analyses and strategies work, independent groups were formed, composed of representatives from three different spheres of California society that have a vested interest in bullfrog and non-native turtle concerns. The first group was composed of representatives from local, state, and federal government agencies, the second from environmental and animal welfare groups, and the third from various commercial sector and industry groups. The groups met separately and worked on the same task (in parallel) to analyze: (1) threats to California's environment posed by bullfrogs and non-native turtles, (2) benefits and cultural values of bullfrogs and turtles in California's communities and other intersections with human well-being values, (3) knowledge gaps in our understanding of the relevant systems and operative biological processes, and (4) opportunities for progress in addressing the issues posed by invasive bullfrogs and non-native turtles in California's environment.

The three groups used a flexible, comprehensive process called the *Open Standards for the Practice of Conservation* (see https://conservationstandards.org/about/ for more information) to guide their analyses. Each group independently developed a comprehensive analysis which included:

- a conceptual diagram which lays out conservation targets that experience some level of risk, the extant threats to those targets, and various strategies that may be implemented to address those threats;
- a ranking of proximate threats performed by the agencies group, with grids that outline how those assessments were developed;
- "results chains" for all strategies that enumerate the stepwise, logical process by which those strategies may be expected to work; and
- notes that expand, clarify and/or qualify certain elements of each assessment.

After completing their individual analyses, the three groups had several opportunities for cross-dialogue to clarify and discuss the approaches taken by the other groups. Additionally, a draft staff report synthesizing the analyses and recommendations from the three groups was provided to the participants, and they were able to provide feedback during these meetings. The final staff report is provided as Exhibit 1.

Today, the Department will provide an informational presentation on the biology of, and threats caused by, non-native frogs and turtles in California, and Commission staff will provide background on the stakeholder engagement process (Exhibit 2). Two additional materials are included to provide more background: A 2014 Department report on American bullfrog (Exhibit 3) and a report produced by the University of Tennessee One Health Initiative and the Pet Advocacy Network that assesses the U.S. amphibian pet trade (Exhibit 4).

WRC is anticipated to make recommendations for action during its September 2023 meeting, which will be received by the Commission for consideration at its October meeting. In October, staff will provide further information on the various options outlined in the staff report and the Committee's recommendation.

Significant Public Comments (N/A)

Recommendation (N/A)

Exhibits

1. *Staff Report on the American Bullfrog and Non-Native Turtle Stakeholder Engagement Process*, dated May 12, 2023
2. Department and Commission staff presentation
3. Department report: *Implications of Importing American Bullfrog (*Lithobates catesbeianus = Rana catesbeiana*) into California*, dated October 27, 2014
4. *Amphibian Consumer and Business Survey*, University of Tennessee One Health Initiative and Pet Advocacy Network, received May 17, 2023

Motion (N/A)

BULLFROGS AND NON-NATIVE TURTLES IN CALIFORNIA: OVERVIEW AND IMPACTS

2023

PRESENTED BY:

Ryan Peek, PhD

Wildlife Diversity Program

California Department of Fish and Wildlife

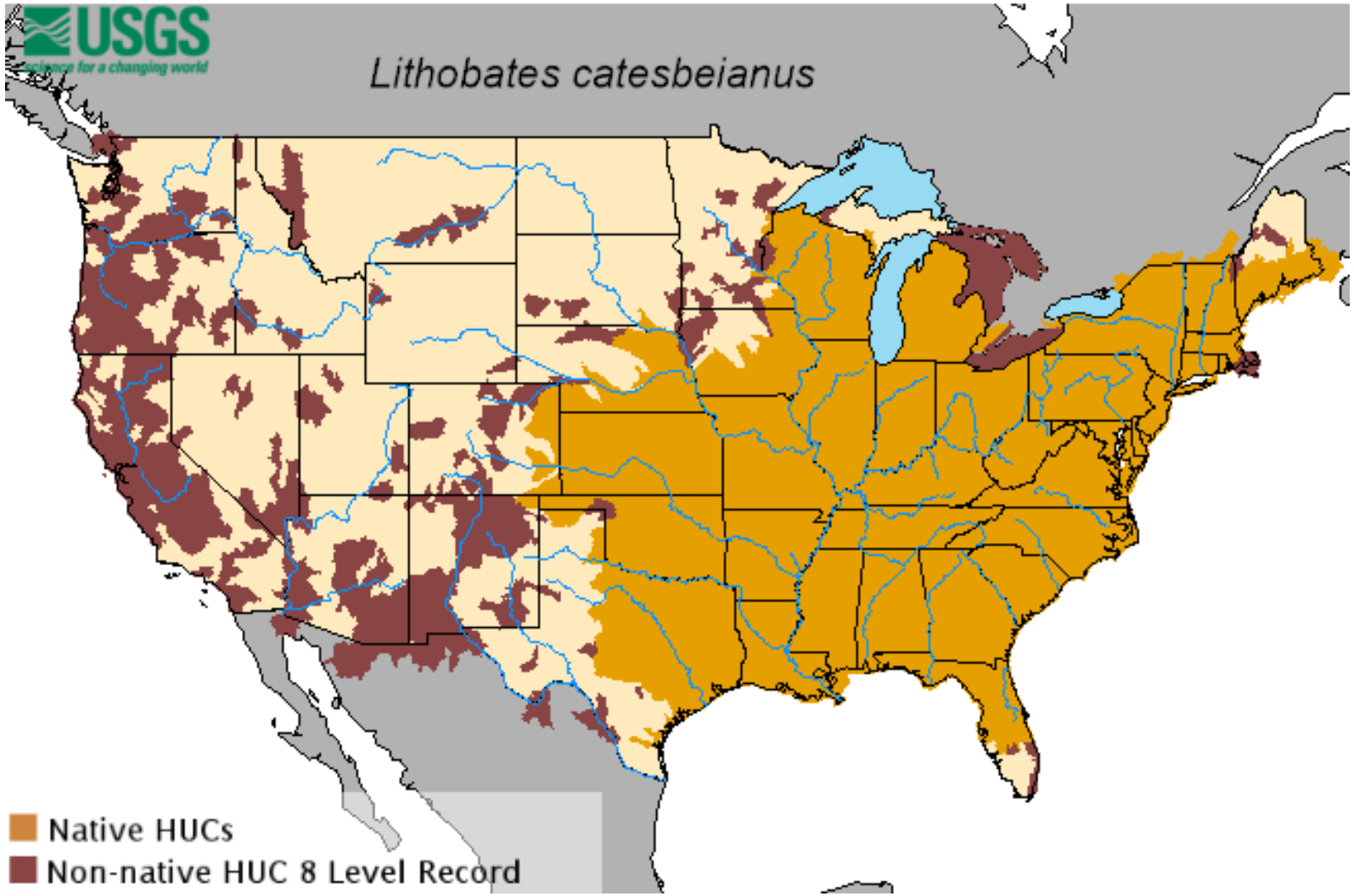
Invasive Species: **"A nonnative or alien species that invades an ecosystem and causes, or is likely to cause, economic, environmental, or public health damages."**

- Invasive species are a leading threat to native biodiversity
- Invasive species have been introduced across the globe
- Impacts include extinction of native species, loss of ecosystem processes, changes to food webs, and spread of disease

American Bullfrogs

Between 1986-2020, the global economic cost of invasive bullfrogs was $6 billion dollars *(Soto et al., 2022)*

American Bullfrogs: Distribution



- American bullfrog native range is primarily east of the Rocky Mountains
- They have proliferated throughout the west, introduced in CA as early as the late1800's as food source to replace CA red-legged frog
- Found in a variety of freshwater habitats, including ponds, marshes streams, man-made habitats like canals, reservoirs, and ditches

American Bullfrogs (*Lithobates catesbeiana*)

- Bullfrogs are the largest frog species in North America
- Voracious predators and disease vectors
- Bullfrogs compete with and prey on a wide range of native species
- Bullfrogs a major factor in declines of many native species in much of North America


Photo: D. Clark


Photo: D. Clark


Photo: R. Fisher

American Bullfrogs: Voracious Predators

Documented prey in California includes:

- Native frogs
- Turtles
- Fish (i.e., Coho salmon)
- Snakes
- Birds
- Mice/Voles
- Bumblebees/Invertebrates
- Salamanders
- Newts
- Toads
- Each other (cannibalistic)

American Bullfrogs: Successful Invaders

Traits that make them successful invaders:

- High tolerance to wide range of environments
- High reproduction (6,000-20,000 eggs per clutch per female per year)
- High dispersal (~10 miles in deserts)
- Easily outcompete native species
- Can be asymptomatic carrier of disease (e.g., chytrid which has caused amphibian declines globally and in California)

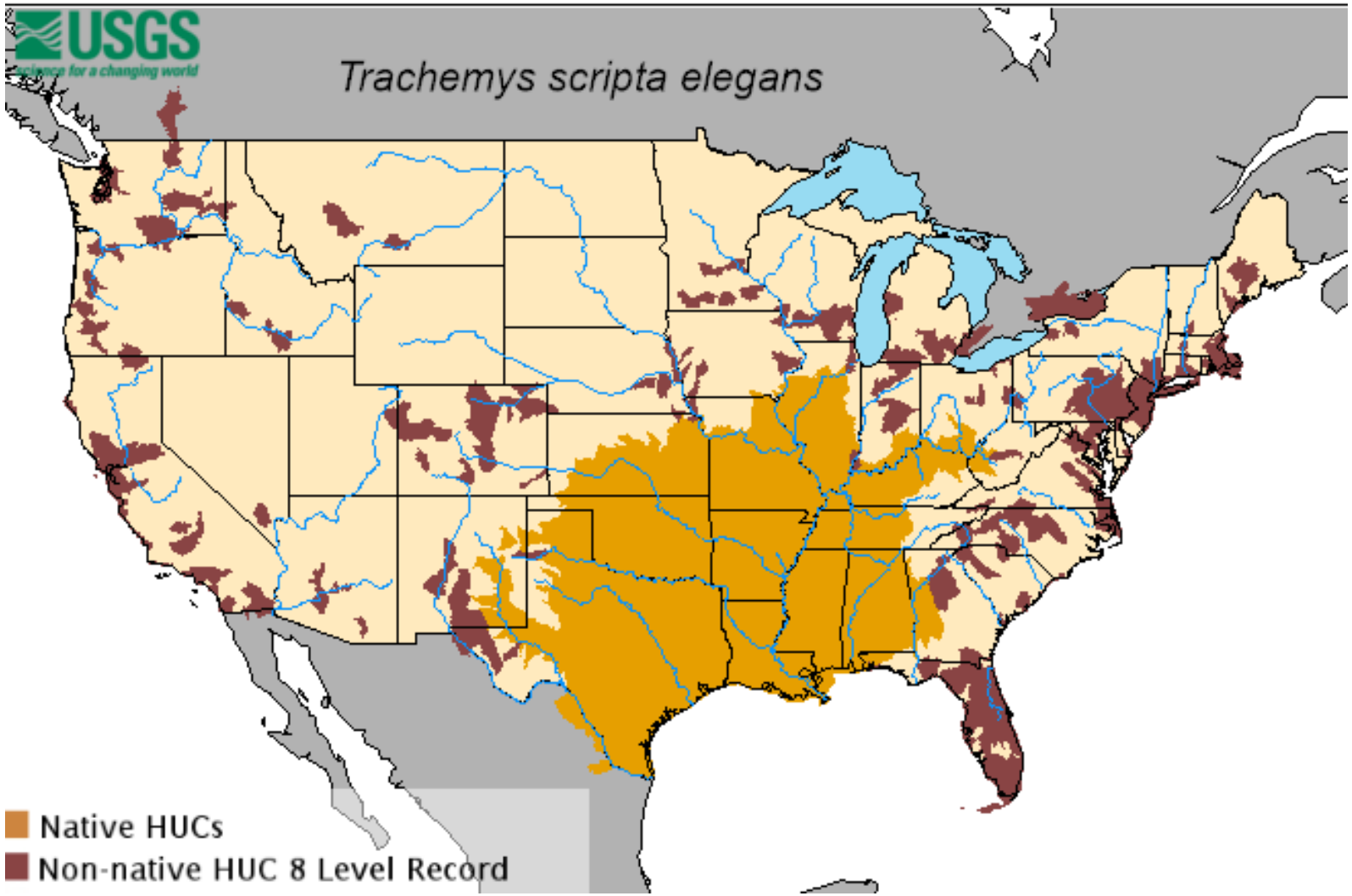
Invasive Turtles

Red-eared slider is listed as one of the world's worst 100 invasive species *(International Union for Conservation of Nature - IUCN)*

Red-eared sliders (*Trachemys scripta elegans*)

- Have been introduced into numerous counties in California
- Aquatic, omnivorous generalists, which rarely leave water except to bask
- Highly adaptable and can tolerate anything from brackish waters, to manmade canals, and city park ponds

Red-eared sliders: Distribution



- Has been introduced primarily through pet releases and escapes, and potential release via food and aquaculture
- Red-eared sliders have been introduced to many areas of the United States outside of their native range, as well as to other countries

Red-eared sliders: Habitat & Biology

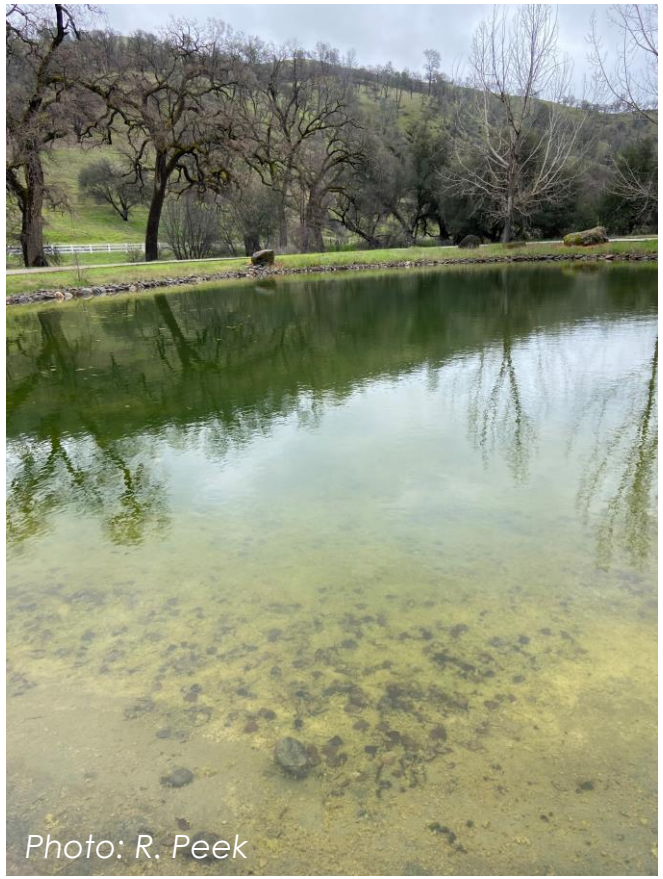
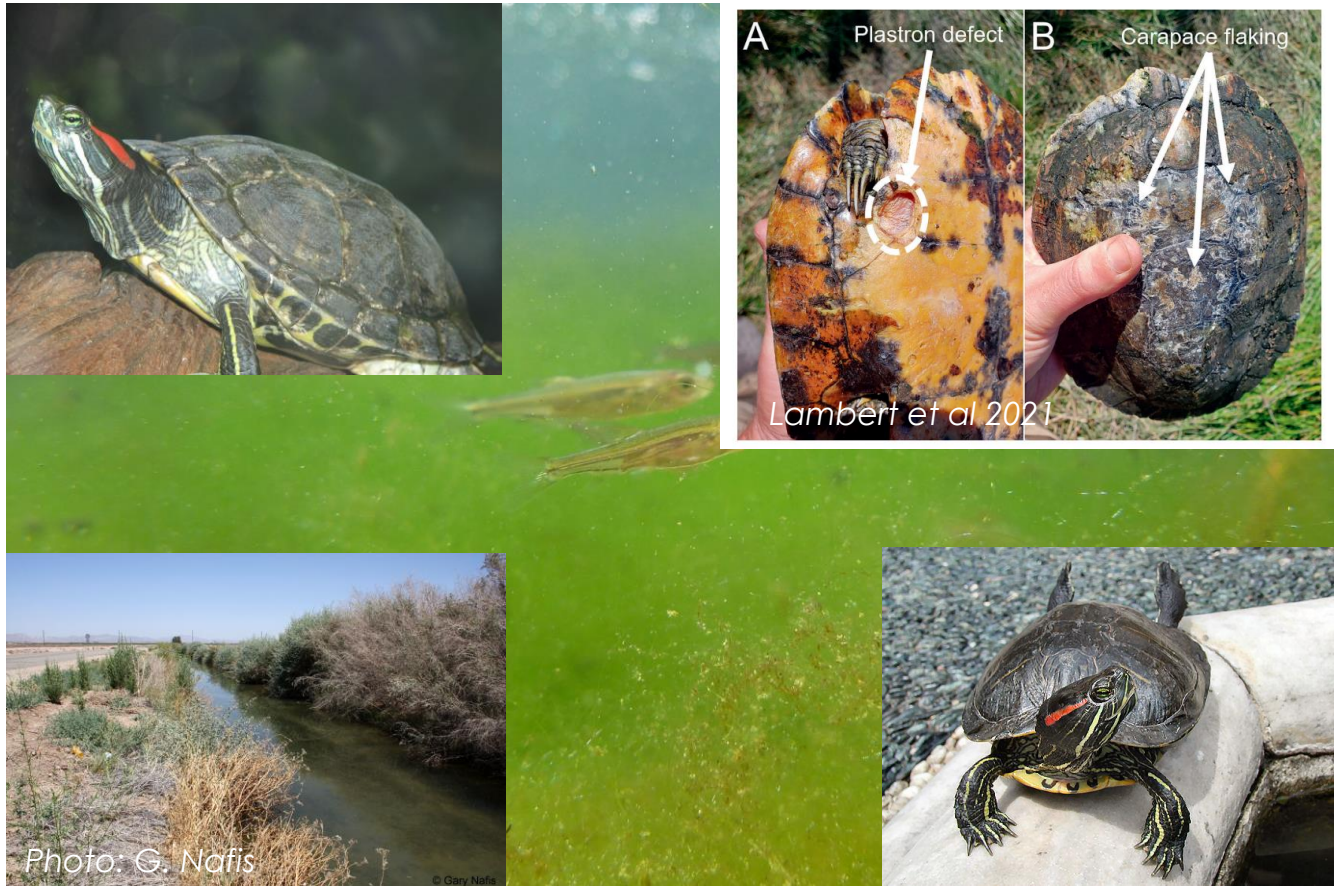


Photo: R. Peek

- **Generalist:** occupy wide a variety of natural freshwater habitats, as well as manmade areas such as canals or ponds
- **Spread rapidly**: Can rapidly colonize available habitat, aided by use of extensive system of manmade canals and irrigation ditches.
- **High reproduction:** Females lay 2-23 eggs per clutch

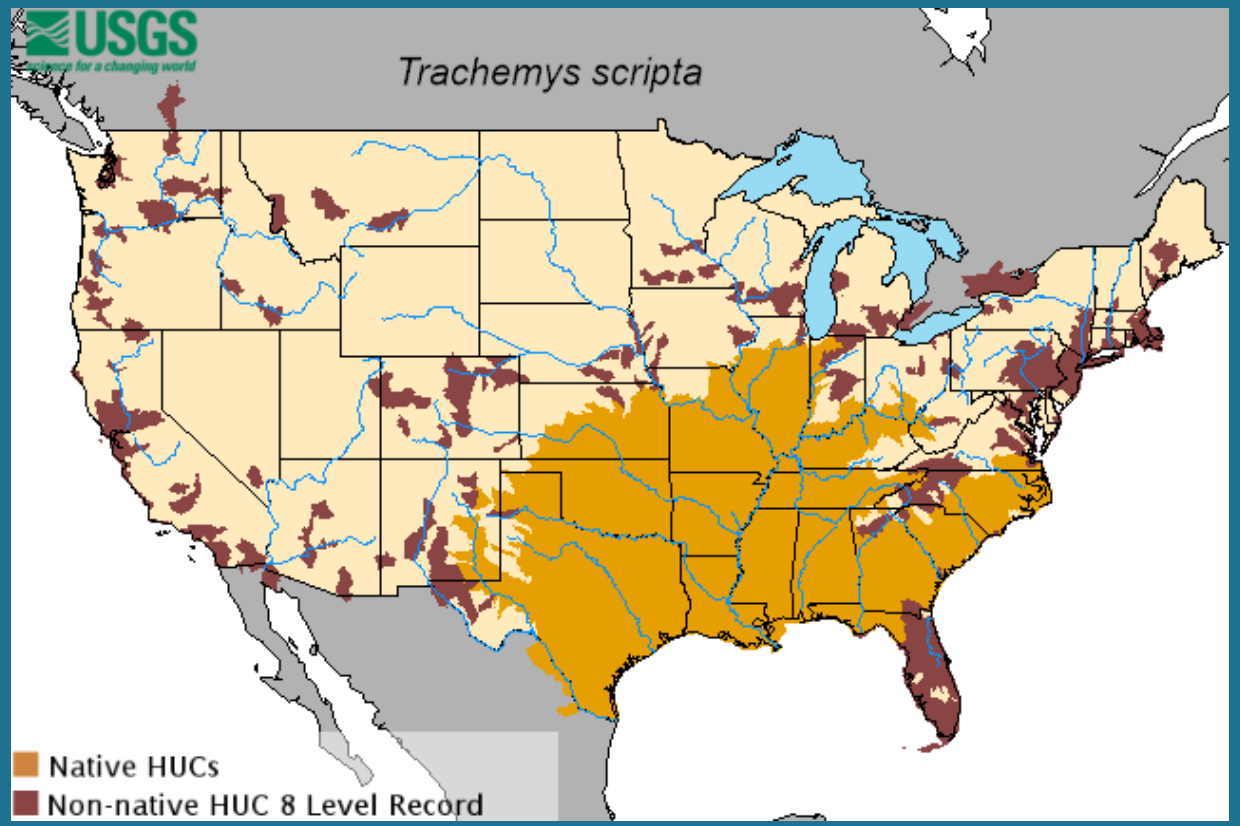
Red-eared sliders: Impacts



- **Competition:** Sliders outcompete native species like western pond turtles for food and habitat, egg-laying sites, and basking sites.
- **Disease Vectors:** Vectors of disease and can transmit parasites to native animal species and humans (*Salmonella*)
- **Rapid Expansion:** Introduced populations can expand rapidly, with female red-eared sliders able to lay up to 6 clutches per year

Additional Non-native/Invasive Species

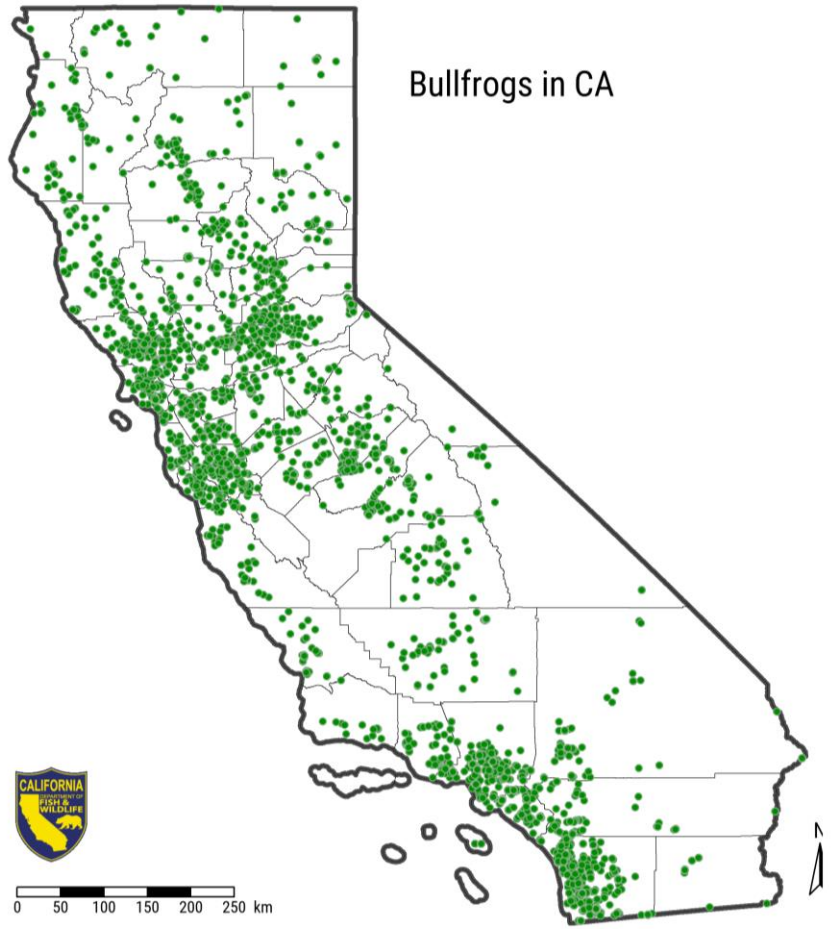
Over 20 additional species of non-native turtles. Majority of most widespread species are Trachemys (sliders) species.



African-clawed frog

Implications for Native Species in California

- Preventing establishment and/or removing non-native bullfrogs/turtles helps maintain native species and habitats
- Important to reduce or eliminate vectors of invasion or re-invasion
- Methods for management and removal have been implemented widely in other regions (OR, WA, AZ)
- Recovery of listed species requires active management, also provides an opportunity to keep at-risk species from needing protection under the federal and California Endangered Species Act



Effective control methods exist

- Effective and proven management techniques exist
- Are being implemented in other states and countries
- Requires current distribution and monitoring of invasive populations

Louette et al. 2014: Use of double fyke net to successfully remove American bullfrog larvae

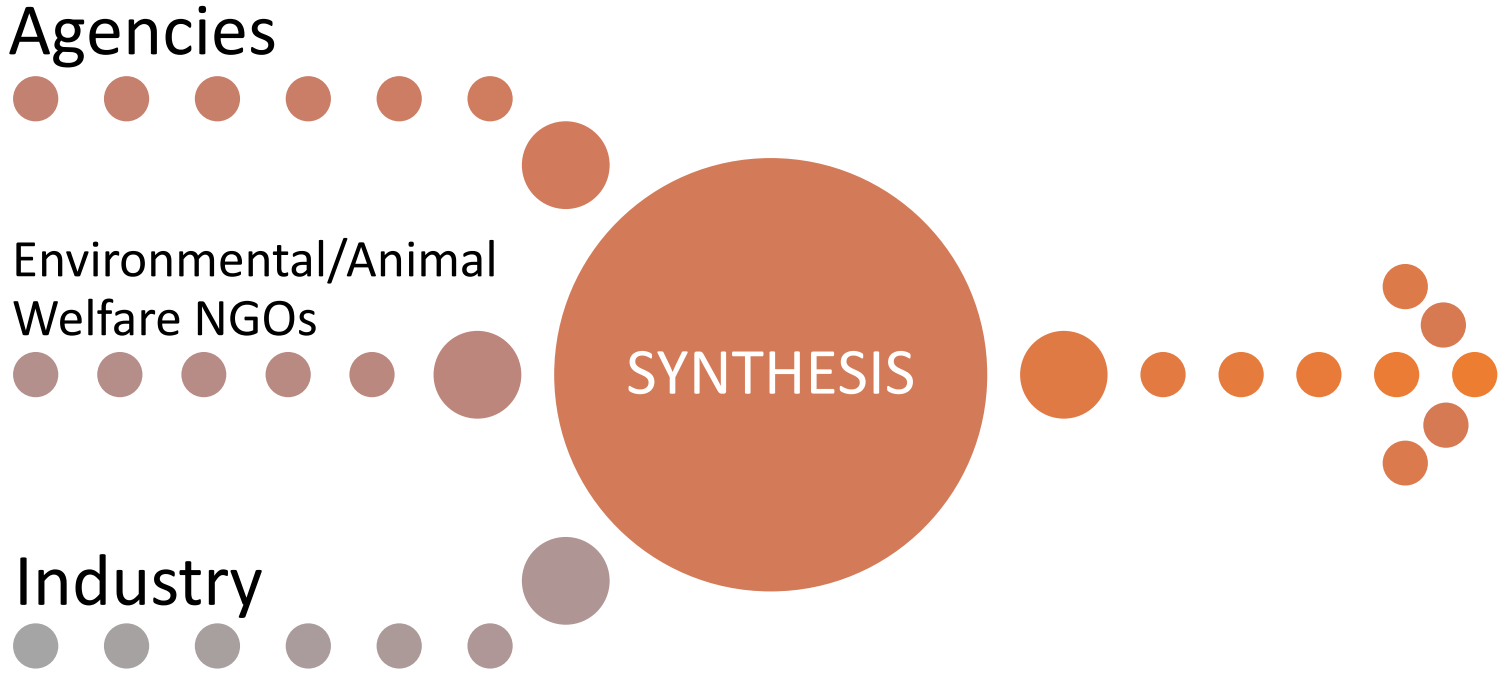
Key Needs for Control of Invasive Bullfrogs/Turtles

- Accurate location and detection information
- Roadmap/plan to develop, prioritize, and implement control programs
- Partnerships and collaboration across stakeholders/agencies

American Bullfrog and Non-Native Turtle Stakeholder Engagement Process

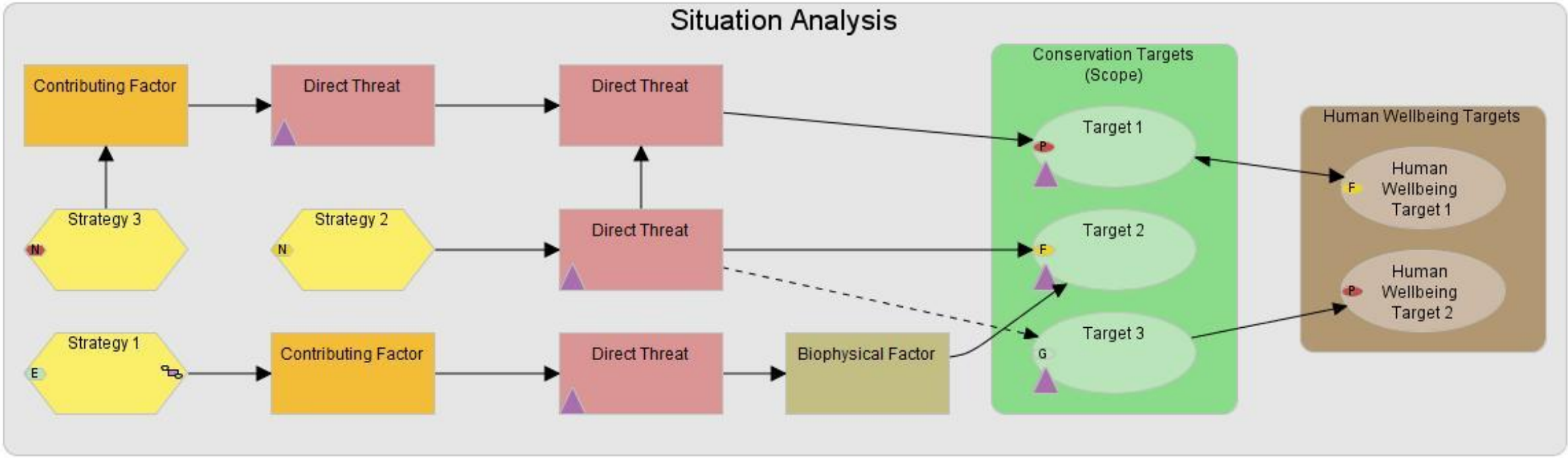
Presented by
Ari Cornman, Wildlife Advisor
California Fish and Game Commission

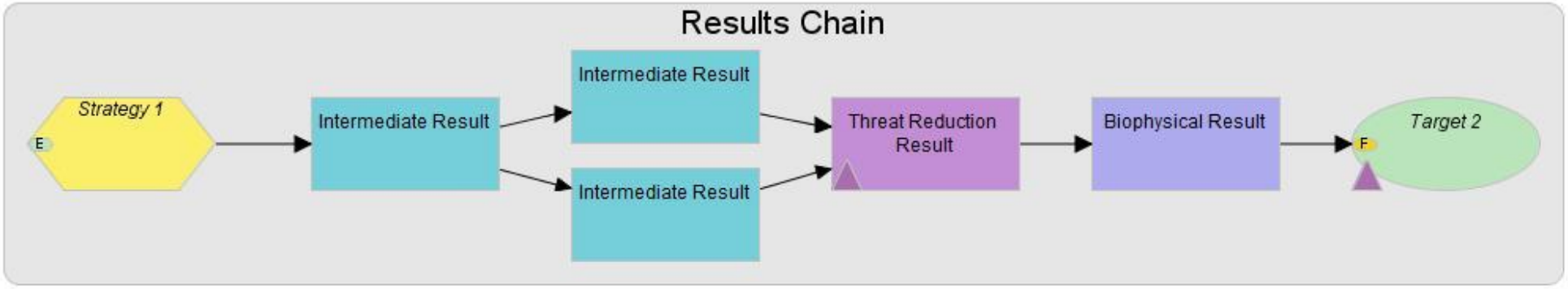
Stakeholder Engagement Process



- 2019 through 2022
- 7 government agencies
- 4 environmental/ animal welfare groups
- 9 industry groups and companies
- Engagement with California legislature

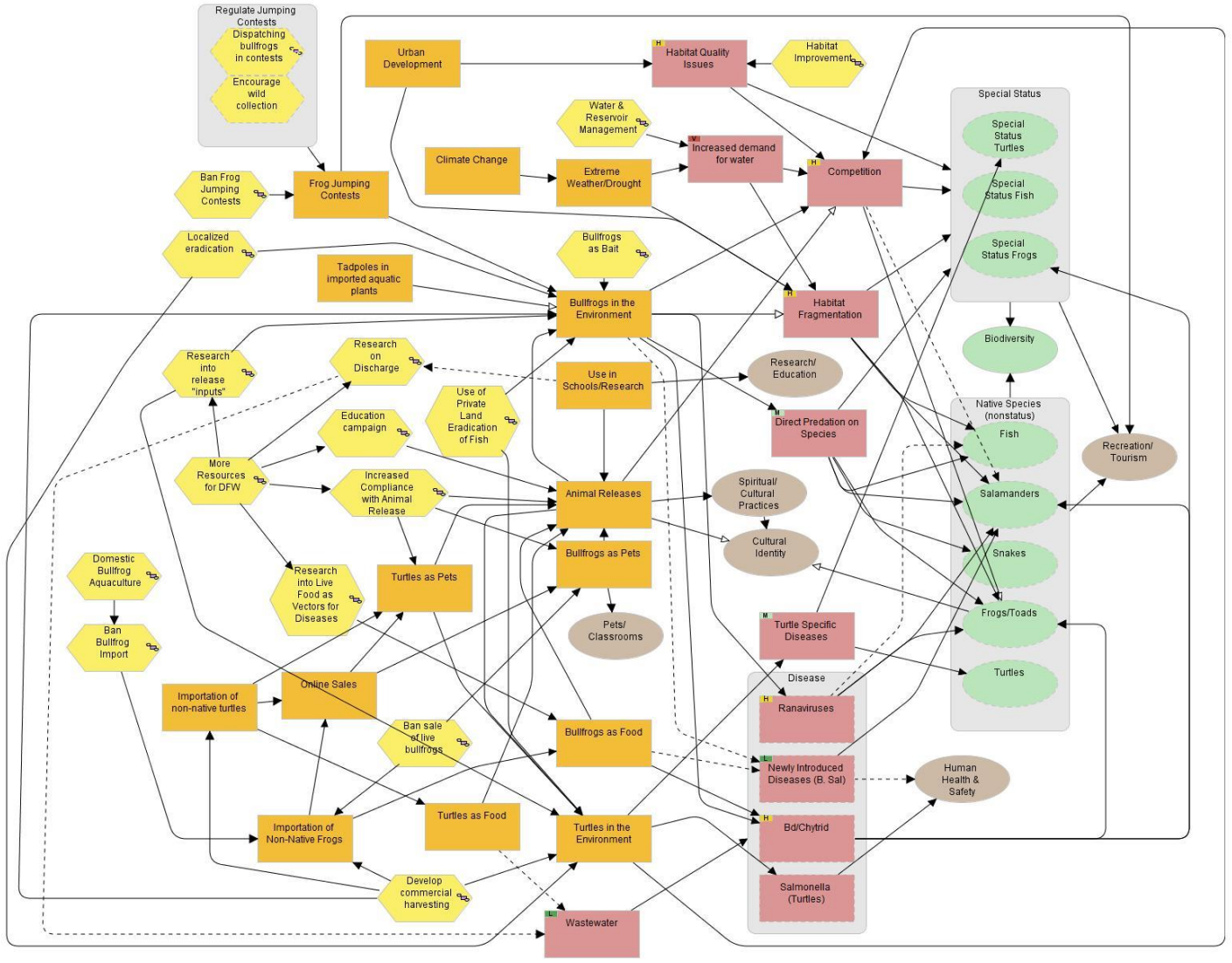
Open Standards for the Practice of Conservation

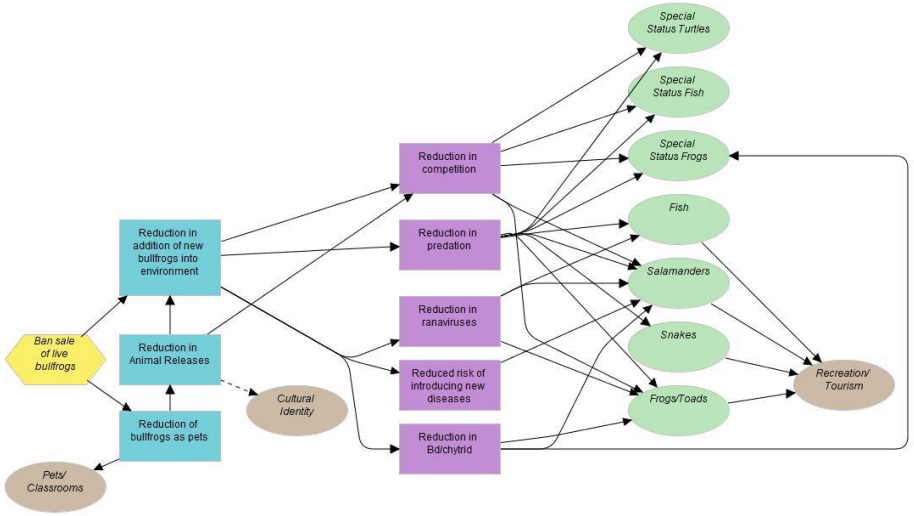




	Results Chain Defined
	Indicator Defined
	Causal Linkage
	Uncertain Link

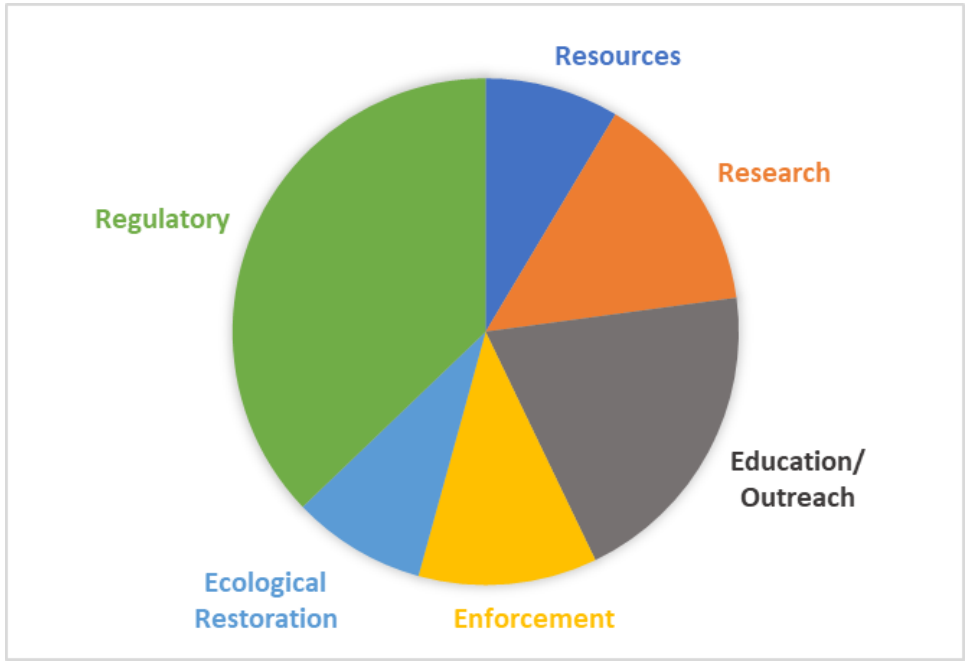
Agency Situation Analysis and Results Chain





Stakeholder Engagement Process Results

- Threat identification and ranking
- Identification of 34 different strategies across 6 categories



- Each strategy has a goal, effectiveness rating, and staff recommendation

Implications of Importing American Bullfrog (*Lithobates catesbeianus* = *Rana catesbeiana*) into California

Prepared by
Fisheries Branch
California Department of Fish and Wildlife
October 27, 2014

Acknowledgements

We would like to thank the CDFW Science Institute and the team of people who were instrumental in shaping and editing this report. Specifically, Susan Ellis, Dr. Garry Kelley, Dr. Kevin Kwak and Laura Patterson provided invaluable comments and guidance on early drafts. Dr. Robert Titus, Dr. Kevin Kwak and David Lentz leant expert advice while editing the late drafts. Finally, Dr. Robert Titus and Sarah Stinson provided crucial research assistance.

Executive Summary

The American bullfrog (*Lithobates catesbeianus)* is native to the eastern United States; however, the species has been spread beyond its native range and introduced throughout North America, Europe, South America, Asia, the Caribbean Islands, and Hawaii (Lever 2003). It was introduced to California in the 1910s for aquaculture production (Storer 1925) and has since become established throughout the state, where it is known to negatively impact several native California species (Fisher & Shaffer 1996; Kupferberg 1997; Kiesecker and Blaustein 1998; Kraus 2009; Fuller et al. 2011).

Approximately 2 million live bullfrogs are imported annually into California (California Department of Fish and Wildlife [Department], unpubl. data) and often sold in live food markets. Escapees from the trade of live bullfrogs have likely contributed to the spread of bullfrogs within California and may have contributed to the introduction of at least one strain of a devastating amphibian disease, *Batrachochytrium dendrobatidis* (Bd), to California (Schloegel et al. 2010; Schloegel et al. 2012).

Notably, the live amphibian trade may be the most significant introduction pathway for novel and emerging amphibian diseases, such as new strains of Bd and/or ranaviruses, the two infectious diseases with the largest contribution to global amphibian declines (Latney and Klaphake 2013). Bullfrogs have tested positive for the presence of Bd and ranaviruses at aquaculture facilities in countries of origin and in endpoint retail markets in the United States, including California (Mazzoni et al. 2003; Fisher and Garner 2007; Mazzoni et al. 2009; Schloegel et al. 2009).

In 2010 the Department amended its policies regarding the issuance of amphibian importation permits, requiring, amongst other things, that all animals sold be euthanized before leaving the retail premises. However, Department law enforcement officers have accumulated evidence of violations of this and other requirements of amphibian importation permittees. These violations suggest the current policy may not be effective without active enforcement.

Using concepts of invasive species biology, this paper argues that limiting or eliminating the issuance of amphibian importation permits is a reasonable alternative to the current policy. Reducing or eliminating live bullfrog importation will reduce the risk of introducing novel emerging amphibian diseases to California and reduce the risk of additional American bullfrog populations becoming established across the State, if not completely mitigate the risk. Broader policy which addresses additional imported species and introduction pathways will be more effective and should be considered.

Definition of Terms

- **Alien species**: a species that is not native to a given ecosystem or landscape.
- **Emerging disease**: a disease that has appeared in a population for the first time or is rapidly increasing in incident or geographic range.
- **Introduced species**: a species that has entered an ecosystem or landscape to which it is not native.
- **Introduction pathway**: the mode or vector by which a nonnative species is introduced into a new ecosystem or landscape.
- **Invasion pathway**: the mode or vector by which an invasive species enters a new ecosystem or landscape.
- **Invasiveness**: the ability of an introduced species to establish itself, reproduce, and spread in an ecosystem or landscape to which it is not native.
- **Invasive species**: a nonnative or alien species that invades an ecosystem and causes or is likely to cause economic, environmental, or public health damages.
- **Naturalized population**: a viable population of an introduced species in an ecosystem or landscape to which it is not native.
- **Nonnative species**: a species that is not native to a given ecosystem or landscape.
- **Propagule pressure**: the number, frequency, and volume of introduction events of a species into a landscape or ecosystem to which it is not native.

Implications of Importing American Bullfrog (*Lithobates catesbeianus* = *Rana catesbeiana*) into California

The American Bullfrog as an Invasive Species

The American bullfrog is native to the eastern United States; however, the species has been spread beyond its native range and introduced throughout North America, Europe, South America, Asia, the Caribbean Islands, and Hawaii (Lever 2003). The Global Invasive Species Database (2009) has given special attention to the American bullfrog's success by including the species on their list, "One Hundred of the World's Worst Invasive Alien Species." Part of the bullfrog's invasion success is attributable to its adaptable and hardy biological character as well as the global demand for frog legs driving international trade (Lever 2003).

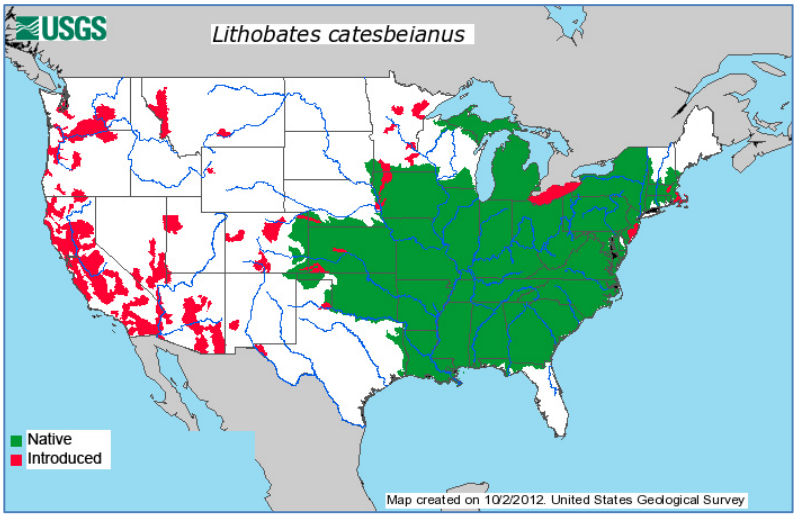


Figure 1. U.S. Geological Survey map of American bullfrog range in the United States. Native range is displayed in green while introduced range is shown in red (USGS, Accessed 7/18/2014).

Biology and Ecology

The American bullfrog is one of the largest frogs in the United States, reaching upwards of 8 inches in length. The frog is native to eastern North America, from Nova Scotia to central Florida and the Gulf of Mexico, westward to approximately the 100th meridian east of the Rocky Mountains (Figure 1) (Lever 2003; Stebbins 2003). It is highly aquatic and is commonly found in still water with thick aquatic vegetation but is known to occur in a variety of habitats with permanent water, including rivers and canals. Altered, degraded, or artificial habitats seem to be particularly suitable, including mill ponds, cattle ponds, and reservoirs (Stebbins 2003).

American bullfrogs have a broad temperature tolerance, preferring 15 – 32 degrees Celsius (Govindarajulu et al. 2006). They are capable of burrowing and hibernation when necessary, and will emerge in April or May and begin to form breeding choruses when air temperatures exceed 20 degrees Celsius (Govindarajulu et al. 2006).

The American bullfrog breeds in permanent aquatic habitats by external fertilization. A single female can lay up to 20,000 eggs in a clutch, and older females can lay multiple clutches per year (Schwalbe and Rosen 1999). Tadpoles typically metamorphose within two years (Govindarajulu et al. 2006). After breeding, bullfrogs tend to disperse locally from the host habitat and occupy new locations. Dispersals up to 3.2 kilometers have been observed, and longer distance dispersals are suspected (Schwalbe and Rosen 1999; Stebbins 2003).

Figure 2. An American bullfrog tests its own gape limit as it attempts to eat a Koi carp from a private pond.

As a gape-limited predator, the American bullfrog will eat anything it can swallow (Figure 2). Their diet primarily consists of invertebrates and small vertebrates. The frog will sit quietly, wait in ambush, and then lunge after a prey item (Schwalbe and Rosen 1988). Tadpoles are primarily herbivorous, consuming a variety of algae, aquatic plants, and occasionally invertebrates and egg masses of fish and amphibians. They intake large amounts of food and can grow to over six inches in length, especially in regions where bullfrog tadpoles require multiple seasons to metamorphose (Stebbins 2003).

American bullfrogs exhibit strong biological and behavioral defenses against predation. Adults and tadpoles produce a skin secretion that seems to be unpalatable to many predators, including many fish species (Walters 1975; Kruse and Francis 1977; Kats et al. 1988). Secondly, the ambush predation strategy of adult bullfrogs reduces the amount of unnecessary movement that might otherwise gain the attention of terrestrial or avian predators.

Global Spread

American bullfrogs have been introduced across the world largely due to the demand for frog legs (Lever 2003). In other cases, American bullfrogs have been deliberately introduced as a biological control for pest species; for use in jumping competitions; as pets; and through releases or unintended escapes of animals via the pet and aquarium trade (Lever 2003).

Due to the bullfrog's climatic tolerance, generalist diet, defense against predators, and large numbers of offspring, they have successfully established naturalized populations in Europe, Asia, Africa, the Middle East, North and South America, the Hawaiian Islands and the West Indies. All told, naturalized populations occur in 40 countries across four continents (Lever 2003). See Appendix 1 for a comprehensive list of documented American bullfrog introductions.

California Introductions and Spread

In the case of California, multiple bullfrog introductions to the San Joaquin Valley occurred between 1914 and 1920 (Storer 1922), probably by aquaculturists for food production (Storer 1925). Bullfrogs were deliberately moved from the Kings River into the San Joaquin River in 1929 and into Madera County in 1934 (Moyle 1973). Subsequently, bullfrogs spread into low elevation aquatic habitat throughout California (Storer 1925; Moyle 1973) and eventually became established in mid-elevation habitats in the Sierra Nevada foothills, Yosemite Valley, Shaver Lake, and Hume Lake (Moyle 1973). Currently, American Bullfrogs occur throughout California except in high mountain and desert regions (Figure 3).

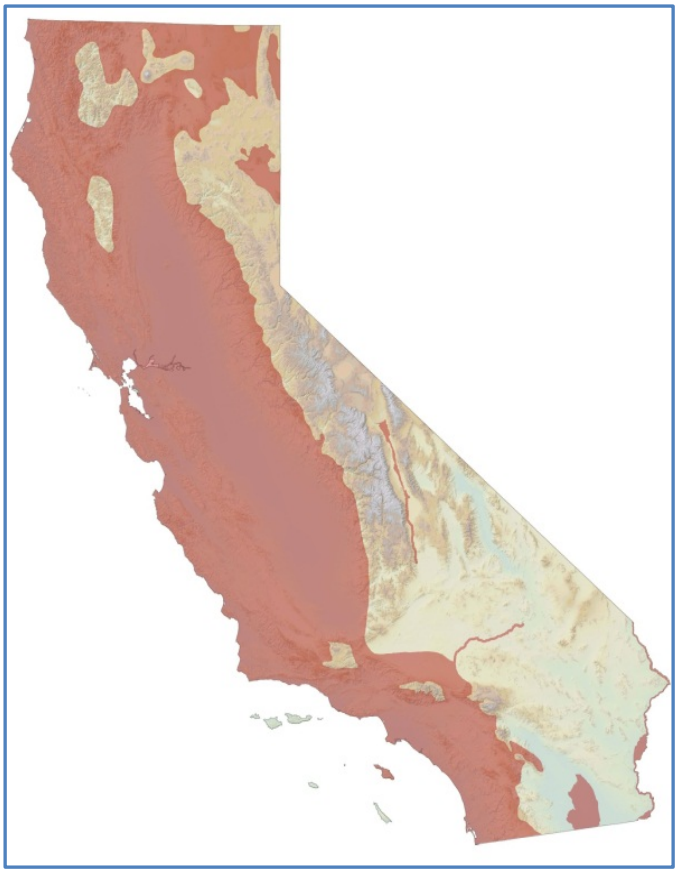


Figure 3. Current distribution of the American bullfrog in California displayed in red (California Wildlife Habitat Relationships Database, Accessed 6/15/2014).

Impacts of American Bullfrog Invasions in California

In California, the bullfrog has been implicated as a significant negative impact to many native aquatic species (Fisher and Shaffer 1996; Kupferberg 1997; Kiesecker and Blaustein 1998; Kraus 2009; Fuller et al. 2011) and identified as one of the principal threats to the continued survival of several special-status species. These include, but are not limited to, state and/or federally listed threatened or endangered species like the California red-legged frog (*Rana draytonii)* (Moyle 1973; U.S. Fish and Wildlife Service 2002), California tiger salamander (*Ambystoma californiense*) (U.S. Fish and Wildlife Service 2009), arroyo toad (*Anaxyrus californicus*) (U.S. Fish and Wildlife Service 1999a), giant garter snake (*Thamnophis gigas*) (U.S. Fish and Wildlife Service 1999b), and Species of Special Concern such as the foothill yellow-legged frog (*Rana boylii*) (Kupferberg 1997).

The predation habits of the American bullfrog are well documented. Any animal is potential prey that does not exceed the bullfrog's gape limit and wanders close enough for the frog to ensnare it with its muscular tongue (Schwalbe and Rosen 1988; Stebbins 2003). In addition to the species listed above, anecdotal reports claim the American bullfrog has been observed preying upon juvenile waterfowl, juvenile salmon (*Oncorhynchus* spp.), reptiles, Pacific chorus frogs (*Pseudacris regilla*), and small mammals.

California native amphibians are particularly susceptible to bullfrog predation since they often occupy the same habitat, thereby increasing interactions and encounters between species. For instance, the California red-legged frog prefers similar habitat to the bullfrog but does not grow as large. As a result, where bullfrogs and California red-legged frogs co-exist, all life stages of California red-legged frogs are preyed upon by bullfrogs (Moyle 1973; Fisher and Shaffer 1996). Although bullfrogs are not the only stressors contributing to the decline of the California red-legged frog, it is noteworthy that the red-legged frog has been excluded from nearly all habitats currently occupied by bullfrogs (Fisher and Shaffer 1996).

In addition to direct predation, bullfrogs negatively impact native species by out-competing for food and space (Kiesecker et al. 2001). The same reasons bullfrogs are effective predators of native frog species also applies to the prey shared by native frog species and bullfrogs. Native

amphibians suffer the largest impact compared to other taxa since bullfrogs are able to prey upon the same available diet. Furthermore, American bullfrogs grow larger than any native California amphibian and can consume high volumes of food relative to other native amphibians. Similarly, bullfrog tadpoles out-compete native amphibian larvae for the same available diet. Although tadpoles are not territorial, they still compete with native amphibian larvae for the best foraging and basking habitat (Kupferberg 1997).

Furthermore, American bullfrogs exhibit fierce territoriality as a display of sexual selection. They will attempt to, and often successfully, exclude other animals of their chosen territory. If another frog enters the territory of an American bullfrog, the bullfrog will attempt to shove, wrestle, and bite the trespasser until it leaves. This behavior results in the largest bullfrogs excluding other smaller frogs from the best foraging and breeding habitat (Howard 1978).

Lastly, California red-legged frogs have been observed attempting to breed with American bullfrogs. This may represent breeding interference by preventing frogs of the same species from successfully breeding where populations of native frogs co-exist or overlap with bullfrogs (Pearl et al. 2005; D'Amore et al. 2009).

What is an Invasive Species?

To understand the threat to California wildlife posed by the importation of American bullfrogs, we must identify what an invasive species is and how they become established. This, in turn, will improve strategic measures to minimize risks associated with the importation of American bullfrogs to native California wildlife.

The National Invasive Species Council (2001) defines an invasive species as a nonnative or alien species that invades an ecosystem and causes, or is likely to cause, economic, environmental, or public health damages. This definition implies the species is able to 1) enter an ecosystem, 2) establish a population, and 3) spread. These three points also serve to outline the process by which species invade (Kraus 2009).

Many species have been, and continue to be, introduced to California, most of which do not establish a population or spread (Davis 2011). These species are not considered invasive because they have accomplished only the first of the three-step invasion process. While most species introductions in California fall into this category (Kraus 2009), they largely cause no harm and therefore go unnoticed and undocumented, making it difficult to provide examples or estimates.

Of those many species that are introduced to California, a small portion is able to gain a foothold and establish naturalized populations. However, most do not effectively spread from the point of introduction without human assistance (Davis 2011) and, therefore, are not invasive. California agricultural crops, domesticated dogs, ornamental flowers, livestock, and the wild parrots of San Francisco's Telegraph Hill, are just a few examples of introduced species that are not invasive in California. Incidentally, once a population is established it becomes much easier

to notice the introduction and as a result this category accounts for most documented introductions worldwide (Kraus 2009).

A minority of species that establish naturalized populations spread from the introduction site and invade neighboring habitats and ecosystems. The ability to spread, occupy new habitats, and establish additional naturalized populations is what separates an invasive species from other introduced species (Kraus 2009). The New Zealand mudsnail (*Potamopyrgus antipodarum*), ice plant (*Carpobrotus edulis*), sudden oak death (*Phytophthora ramorum*), Argentine ants (*Linepithema humile*), salt cedar (*Tamarix spp.*), and the American bullfrog are just a few examples of invasive species in California.

With a basic understanding of invasive species, it is worth looking at the invasion process in more detail, connect the theoretical underpinnings of the invasion process to the American bullfrog invasion of California, and identify the role that importation of live bullfrogs has played.

1) *"…Enter an ecosystem…"*
   The first step in an invasion process requires a species to enter an ecosystem to which it is not native. This is also called *introduction*. The vector or pathway by which the species was introduced is dubbed the *introduction pathway* or *invasion pathway*. There are at least 10 invasion pathways that account for the majority of all documented herpetofauna invasions globally: aquaculture; bait use; biocontrol; cargo; food; "intentional"; nursery trade; pet trade; research; and zoo trade (Kraus 2009). What is most noteworthy is that the majority of pathways are associated with trade (underlined items).

   In fact, trade related pathways are the most significant for the majority of all documented invasions worldwide (Levine and D'Antonio 2003; Kraus 2009) regardless of taxa. As international markets have increased in number and volume, so have the frequency and number of species invasions (Levine and D'Antonio 2003; Davis 2011; Perrings 2011). Every shipment of goods or human travel from one locale to another may serve as a carrier of a nonnative or alien species. A prime example is the well documented association of international trade and human travel to the spread of human disease such as HIV-AIDS, SARS, avian flu, swine flu, and West Nile Virus (Perrings 2011).

   In the case of American bullfrogs, the production and trade of frog legs were largely responsible for introductions across the world (Lever 2003). The bullfrog's large, meaty hind legs, high reproductive capacity, and broad environmental tolerances make it an ideal candidate for aquaculture production (Moyle 1973). California is no exception; multiple introductions to the San Joaquin Valley occurred between 1914 and 1920 (Storer 1922), probably by aquaculturists for food production (Storer 1925).

2) *"…Establish a population…"*
   For a species to be invasive it must establish a naturalized population in an ecosystem to which it is not native. This means that the species must not only occupy and utilize a naïve

ecosystem but it must be able to successfully reproduce and sustain a population across generations. This step is pivotal in determining whether a species introduction goes unnoticed as harmless, as most do, or results in an invasion with economic and ecological consequences (Kraus 2009). For this reason, the topic is worth exploring in more detail.

The likelihood that a species introduction will result in an established naturalized population is a function of two variables (Davis 2011):

a) the degree to which a species is able to reproduce and spread from its introduction site, which is described as the *invasiveness* of the species (Rejmánek 2011); and
b) the number, frequency and volume of introduction events to a foreign ecosystem, the measure of which is called *propagule pressure* (Duncan 2011).

Invasiveness of the American Bullfrog

The American bullfrog exhibits many biological characteristics which contribute to its *invasiveness*. American bullfrogs have a broad temperature tolerance, preferring 15 – 32 degrees Celsius (Govindarajulu et al. 2006). If conditions are unsuitable, they are capable of burrowing and hibernation (Govindarajulu et al. 2006). These traits account for the bullfrog's broad environmental tolerance and have facilitated bullfrogs becoming established at northerly and southerly latitudes, as well as elevations up to 1,600 meters (5,250 feet).

The bullfrog's diet primarily consists of invertebrates and small vertebrates, but as a gape-limited predator it can eat anything it can swallow (Stebbins 2003). This generalist feeding behavior allows the frog to utilize prey items available in foreign habitats, rather than relying on specific food from its native environs. Moreover, bullfrogs have an effective predator defense; adults and tadpoles produce a skin secretion that seems to be unpalatable to many predators, including many fish species (Walters 1975; Kruse and Francis 1977; Kats et al. 1988).

The bullfrog, like many amphibians, is particularly fecund. A single female can lay up to 20,000 eggs in a clutch, and older females can lay multiple clutches per year (Schwalbe and Rosen 1999). After breeding, bullfrogs tend to disperse locally from the host habitat and occupy new locations. Dispersals up to 3.2 kilometers have been observed, and longer distance dispersals are suspected (Schwalbe and Rosen 1999; Stebbins 2003).

Propagule Pressure of the American Bullfrog Introduction to California

As mentioned earlier, bullfrogs were introduced to California by aquaculturists to meet the state's demand for frog legs (Storer 1925). Multiple introductions to the San Joaquin Valley occurred between 1914 and 1920 (Storer 1922), presumably into artificial habitats. We know that bullfrogs often disperse locally and occupy new habitats; therefore it is likely that bullfrogs "escaped" from aquaculture facilities into neighboring natural aquatic habitats. The propagule pressure was the number of escapees moving from an aquaculture facility into neighboring natural habitat. Of course, we cannot measure the propagule pressure of an introduction event that took place nearly 100 years ago, but the results are clear: bullfrogs established naturalized populations throughout the San Joaquin Valley (Figure 3).

The concept of propagule pressure can be similarly applied to the importation of live bullfrogs. The number of live imported bullfrogs that escape into California habitats represents the propagule pressure contributed by bullfrog importation. This pressure is expressed upon aquatic habitats neighboring ports of entry and/or aquatic habitats neighboring communities with high demand for live bullfrogs. Figure 3 illustrates the current distribution of bullfrogs in California and shows they are established in all areas adjacent to California's three largest ports: San Diego, Los Angeles, and San Francisco.

3) *"…Spread…"*

The ability to spread and occupy new habitats and establish additional naturalized populations is what separates an invasive species from other introduced species (Kraus 2009). The spread of an invasive species from its introduction site into a new habitat can be considered as a separate introduction event (Duncan 2011). These events follow the same general three-step invasion process and are driven by the same variables described above: the available invasion pathways, the propagule pressure expressed upon a new habitat, and the invasiveness of the species. However, spread events can have their own unique set of pathways and sources of progagule pressure, which may not be the same as the original introduction.

Kraus (2009) observed that over the course of years or decades, introduction pathways and sources of propagule pressure change. Specifically, trade related pathways account for the majority of introduction events and propagule pressure in the early stages of a herpetofauna species invasion. However, once an invasive herpetofauna species is well-established, trade related events diminish compared to aesthetically motivated releases, intentional releases for personal, ethical or religious purposes not otherwise related to pet or food trade. This pattern is evident with American bullfrogs in California; by the mid- to late-20th century, spread events from trade related pathways, such as aquaculture, decreased relative to spread events related to the pet trade, schools, and religious practices (Lever 2003).

Perhaps the most significant difference between the processes of invasive species introduction versus spread is the influence of existing naturalized populations. Not surprisingly, once an invasive species establishes a naturalized population, it is much easier for the species to spread into and occupy new habitat neighboring the population. This is due, in part, to the propagule pressure expressed by the naturalized population upon neighboring habitats. As the number of naturalized populations increases and/or a population(s) increases in size, so too does the propagule pressure upon neighboring unoccupied habitat (Duncan 2011).

In California, natural spread of bullfrogs from established populations is likely responsible for a significant portion of the observed distribution. Bullfrogs are particularly adept at spreading due to their fecundity and dispersal behavior. Only a small portion of the current distribution of bullfrogs (Figure 3) can be accounted for by the documented introduction and spread events (Appendix I). The majority of the spread of bullfrogs around California must

have been from undocumented events and/or the natural spread of bullfrogs from established naturalized populations.

Future Threats from the Importation of Live Bullfrogs

Continued Spread of American Bullfrogs within California

With an understanding of species invasion dynamics and American bullfrog biology, it is clear that American bullfrogs will continue to spread within California, establish additional populations, and broaden their current distribution. This will likely occur via three primary pathways:

1) dispersal and spread of existing naturalized bullfrog populations;
2) new introduction events from ethically motivated releases of captive frogs; and
3) new introduction events associated with live bullfrog importation and trade.

Each pathway's influence on the future spread of bullfrogs is a function of the pathway's propagule pressure expressed onto California aquatic habitats. Unfortunately, there have been few attempts to quantify these variables, making it difficult to predict areas most at-risk of being invaded by bullfrogs. However, by applying the theories of invasive species dynamics, it is possible to describe the areas at-risk, even if we cannot pinpoint the locations.

By its definition, spread can only occur into habitat not currently occupied by a naturalized bullfrog population; therefore, unoccupied habitat is at greater risk of invasion than occupied habitat. Propagule pressure can vary by distance from the introduction pathway (biological invasion) such that aquatic habitats neighboring one or more introduction pathways experience higher propagule pressure than habitats farther away. Similarly, habitats near multiple introduction pathways and/or near large, high volume introduction pathways experience more propagule pressure compared to habitats near small, isolated introduction pathways (Duncan 2011).

Therefore, one can anticipate that propagule pressure expressed by dispersal of bullfrogs from established populations will be highest in unoccupied habitat near the largest existing populations or near the largest clusters of populations. Similarly, the propagule pressure of aesthetically motivated releases of bullfrogs will be higher in and around cities, towns, and schools, etc. Pressure will be highest near communities that actively use live bullfrogs, such as near schools that use bullfrogs in science instruction; around communities served by a pet shop that stocks bullfrogs; or near places of worship for practitioners that use bullfrogs in ceremony. Lastly, propagule pressure from live bullfrog importation will be highest near ports of entry, live animal markets, and communities that have high demand for live bullfrogs.

These points imply that the habitats at highest risk of bullfrog invasion are unoccupied aquatic habitats located near existing bullfrog populations, near large cities or other population centers, and near a port of entry and/or live animal market. Therefore, we cannot only expect that bullfrogs will continue to spread within California, but they are likely to spread most rapidly in unoccupied habitat neighboring coastal California cities.

Introduction of Wildlife Diseases

While the proposition that bullfrogs will continue to spread throughout California and establish new populations is cause for concern, perhaps an equal threat to California wildlife posed by the importation of live bullfrogs is the introduction and spread of emerging and novel wildlife diseases. The ongoing movement of animals and wildlife by humans into California serves as potential pathways for the unintentional movement of wildlife diseases. In the case of American bullfrogs in California, not only is the continuous importation of bullfrogs a potential pathway for the introduction of emerging and novel diseases, it has been recently implicated as a vector (Schloegel et al. 2010; Schloegel et al. 2012) and/or a carrier for an amphibian disease, *Batrachochytrium dendrobatidis* (Bd), that has already been introduced to California and decimated at least two California native amphibians.

Bd is an aquatic fungus that is the causative agent for the amphibian disease chytridiomycosis. Multiple strains of Bd have been isolated, including endemic Bd strains and emerging virulent strains (Schloegel et al. 2012). Bd has spread around the world and is implicated in the extinction of over 90 frog species globally (Skerratt et al. 2007). In California, it is thought to have been introduced in the 1960s by release of live imported nonnative amphibian species (Padgett-Flohr and Hopkins 2009) such as the American bullfrog (Schloegel et al. 2010; Schloegel et al. 2012) and the African clawed frog (*Xenopus spp.*) (Vredenburg et al. 2013). Bd has since spread across California and into the water bodies of the Sierra Nevada and the Transverse and Peninsular ranges of southern California (Figure 4), where it has contributed to the precipitous decline of two species of mountain yellow-legged frog endemic to California (Figure 5): the Sierra Nevada yellow-legged frog (*Rana sierrae)* and the southern mountain yellow-legged frog (*Rana muscosa*) (Rachowicz et al. 2006; Vredenburg et al. 2010; Briggs et al. 2010;

Figure 4. Current distribution of Bd in California. Bd-positive localities are colored red while Bd-negative localities are displayed in white and blue (www.bd-maps.nets, Accessed 8/5/2014).

Figure 5. Southern mountain yellow-legged frog (*R.* muscosa) Bd mortality event at Sixty Lakes Basin, California (photo: Vance Vredenburg, 2008).

Bonham 2011). Over 90% of the remaining mountain yellow-legged frog populations have tested positive for the presence of Bd, and many of those populations remain at risk of extirpation (Bonham 2011).

American bullfrogs can carry Bd and spread zoospores but rarely develop chytridiomycosis themselves, thereby serving as an ideal disease reservoir (Hanselmann et al. 2004; Pearl et al. 2007; Latney and Klaphake 2013). Due to the bullfrog's dispersal behavior, they may serve as a vector for the spread of Bd from one water body to another. In California, naturalized bullfrog populations have tested positive for Bd and, in at least one case, have developed chytridiomycosis (Clifford et al. 2012).

The case of Bd in California illustrates a key point that emerging diseases are invasive species. By documenting the spread of Bd, it is clear that Bd has met the definition of an invasive species and followed the pattern of invasion as described by Kraus (2009). Therefore, the invasion of Bd, or any wildlife disease newly introduced to California, is driven by the same variables described above: the available invasion pathways, the propagule pressure expressed upon a new habitat, and the invasiveness of the species. This has important implications for policy makers or managers attempting to reduce or mitigate risks associated with live bullfrog importation.

Live Bullfrog Importation as an Introduction Pathway for Emerging Diseases

Ranavirus and Bd are considered the most significant infectious diseases contributing to global population declines in amphibians (Latney and Klaphake 2013). Although Bd has already been introduced to California, different virulent strains have been identified globally (Schloegel et al. 2012), which may still pose a threat to native amphibians if introduced to California. Currently, California imports approximately two million American bullfrogs annually, most of which originate from farms in Asia and South America (Schloegel et al. 2009). Notably, there is mounting evidence that the food trade is the most significant introduction pathway for Bd and ranaviruses into California.

Bd has been detected in South America at bullfrog farms (Mazzoni et al. 2003) and in other frog species traded for food (Fisher and Garner 2007). Ranaviruses were detected at bullfrog aquaculture facilities in China (Schloegel et al. 2009) and in Brazil (Mazzoni et al. 2009). Schloegel et al. (2009) found evidence of both pathogens from live food markets in Los Angeles, San Francisco, and New York and found 64% of 1,148 samples tested positive for Bd and 7.9% tested positive for ranavirus infection. The results for American bullfrogs, specifically, show 29.7% of American bullfrog samples tested positive for Bd. These findings suggest Bd

and ranaviruses are present at aquaculture facilities in countries of origin and in endpoint retail markets in the United States.

Ranaviruses are a group of emerging amphibian diseases that have been identified as the responsible agent for amphibian mass death events worldwide (Daszak et al. 1999), and result in up to 90% mortality rates within frog populations (Gray et al. 2009). Members of the group have been detected in amphibian populations in the United States and California. For example, Green et al. (2002) studied 44 amphibian mortality events across the United States and found ranavirus infections were the sole cause of 48% (21) of those mortality events. Members of the *Ranavirus* genus are common pathogens for other taxa including reptiles and fish (Daszak et al. 1999) and several ranaviruses infect multiple taxa and are known to host-switch (Duffus et al. 2008; Picco et al. 2010; Abrams et al. 2013; Brenes et al. 2014). Lastly, and perhaps most concerning, emerging and pathogenic ranaviruses continue to be discovered, such as *Rana catesbeiana* virus Z (Majji et al. 2006).

The ability of some ranaviruses to host-switch and the evidence of recent selective pressure resulting in host-switching adaptions (Abrams et al. 2013) demonstrate that ranaviruses threaten California wildlife in multiple ways. Ranaviruses can not only infect a single amphibian species but potentially jump to another host that it did not initially affect. In describing the potential threat, it is worth noting that the Centers for Disease Control and Prevention estimate that zoonotic diseases, those that jump from animals to humans, such as HIV, account for 75% of all emerging infectious threats to humans.

Policy Recommendations

California imports approximately 2 million American bullfrogs annually (California Department of Fish and Wildlife [Department], unpubl. data), which pose threats to native wildlife by contributing to the establishment of additional bullfrog populations throughout the state and by providing an introduction pathway for novel and emerging amphibian diseases. The importation of live bullfrogs may have contributed to the introduction of at least one strain of Bd into California and may be the most significant introduction pathway for new strains of Bd and ranaviruses. Researchers have observed Bd and ranaviruses at aquaculture facilities in countries of origin and in endpoint retail markets in the United States. Incidentally, these two diseases are considered the most significant infectious diseases contributing to global amphibian declines. Lastly, naturalized American bullfrog populations are well established throughout the State and are known to negatively impact populations of native wildlife. This paper has argued, using the concept of propagule pressure, that the severity of these risks is positively correlated to the amount of live American bullfrogs imported into California.

In 2010, the Department amended its policies regarding the issuance of amphibian importation permits, requiring, amongst other things, that all animals sold be euthanized before leaving the retail premises. This provision was included to avoid the spread of diseases and invasive species. However, the Department has received anecdotal reports of violations and

Department law enforcement officers have accumulated evidence of violations of this and other requirements of amphibian importation permittees.

Restricting the issuance of importation permits may be more effective and require less enforcement effort. Reducing or eliminating importation of live bullfrogs will proportionally reduce propagule pressure of American bullfrogs and novel emerging amphibian pathogens into California, thereby reducing threats to California wildlife. It is reasonable to expect the larger and more comprehensive the ban or reduction, the greater the benefits realized to California wildlife.

It is important to note that importation of live American bullfrogs is just one of many pathways for the introduction of amphibian diseases into California. For example, ranaviruses have been detected in non-native tiger salamanders sold as fishing bait in California (Picco et al. 2007). Similarly, importation of live bullfrogs is one of several sources of propagule pressure contributing to the continued spread of bullfrogs across California. Reducing or eliminating live importation of bullfrogs will not remove these threats; it will, however, reduce the risk that these threats will result in catastrophic, negative impacts to California wildlife.

Unfortunately, it is not possible to estimate or quantify the reduction in risk that may be gained by reducing or banning importation. There are few efforts to measure the scale of introduction pathways and, therefore, it is difficult to compare, for instance, the degree to which live bullfrog importation contributes to the risk of introducing a novel disease to California against other amphibian disease introduction pathways. In any case, adopting a live animal importation policy that addresses not just bullfrogs, but multiple species and introduction pathways, would be a more comprehensive approach to minimizing threats posed to California wildlife.

In summary, there is growing evidence that the live amphibian trade is the primary invasion pathway for the introduction of novel amphibian diseases into California. Moreover, the live amphibian trade has been implicated in the introduction of Bd into California. Due to the serious threat emergent diseases pose to California's wildlife, the Department holds that importation of live American bullfrogs poses a significant threat to the wildlife of California. Current importation policy may not effectively limit or avoid the spread of diseases and invasive species, as evidenced by significant incidents of violations. As a result, the Department believes that a significant reduction or elimination of importation permits for live American bullfrogs would reduce the risks to California wildlife.

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Appendix I - Comprehensive List of Documented American Bullfrog Introductions (Kraus 2009)

Locality Introduced	Success?	Number of Events	Pathway	Dates
Argentina	Y	4	Food (1)	Unknown
Austria	N	1	Unknown	1927
Belgium	Y	12	Pet trade (6)	1980s (2), 1990s (2)
Brazil	Y	2	Food (2)	1935, mid-1980s
Canada: British Columbia	Y	2	Food (2)	1930s (2)
Canary Islands	Unknown	1	Unknown	Unknown
Chile	Y	1	Food	Unknown
China	Y	2	Food (2)	1960s
Columbia	Y	1	Food	1986
Cuba	Y	1	Food	1915
Denmark	N	2	Pet Trade (2)	1990s (2)
Dominican Republic	Y	1	Food	1955
Ecuador	Y	1	Food	Late 1990s
France	Y	6	Food (2), pet trade (3)	Late 1800s (2), 1968, 1981, 1990, 2002
Germany	Y	17	Biocontrol (1), food (3), pet trade (13)	1911, 1927, 1934, 1978 (2), 1980 (3), 1985-1990 (2), 1987, 1988, 1990 (3), 1992, early 1990s
Great Britain	N	3	Intentional, pet trade (2)	1905, 1996

Appendix I - Comprehensive List of Documented American Bullfrog Introductions (Kraus 2009) (cont.)

Locality Introduced	Success?	Number of Events	Pathway	Dates
Greece: Crete	Y	1	Food	1997
Guyana	Y	1	Unknown	Unknown
Haiti	Y	1	Food	Unknown
Indonesia	Y	1	Food	1970
Israel	Y	1	Unknown	Unknown
Italy	Y	5	Food (2)	1935, mid-1930s, 1966, late 1960s, 1970s (2)
Jamaica	Y	3	Food (2)	1967
Japan: Izu Islands	Y	1	Food	1952
Japan: mainland	Y	2	Food (2)	1920s (2)
Japan: Ogasawara Islands	Y	1	Unknown	Unknown
Japan: Ryukyu Islands	Y	8	Food (8)	1953 (5), 1954 (2), late 1950s
Malaysia	Unknown	1	Unknown	Unknown
Mexico	Y	2	Food (2)	1945, 1970
Namibia	Y	1	Unknown	Unknown
Netherlands	N	47	Aquaculture contaminant, pet trade	1986
Peru	Y	1	Unknown	Unknown
Puerto Rico	Y	1	Food	1935

Appendix I - Comprehensive List of Documented American Bullfrog Introductions (Kraus 2009) (cont.)

Locality Introduced	Success?	Number of Events	Pathway	Dates
Russia	Y	1	Unknown	Unknown
Singapore	Unknown	1	Food	1980s
South Korea	Y	1	Unknown	Unknown
Spain	N	3	Food (2)	1980s, 2000
Sri Lanka	Y	1	Unknown	Unknown
Tadjikistan	Y	1	Unknown	Unknown
Taiwan	Y	2	Food (2)	1924, 1951
US: Arizona	Y	1	Unknown	Unknown
US: California	Y	6	Food (5), lab release	1896, 1910s, 1912 (2), 1914, 1915
US: Colorado	Y	3	Food (2)	1913, 1914
US: Hawaii	Y	2	Biocontrol, food	1897-1899, 1902
US: Idaho	Y	1	Unknown	1890
US: Iowa	Y	1	Food (2)	1930s, 1960s
US: Kansas	Y	1	Unknown	Unknown
US: Massachusetts	N	2	Unknown	Unknown
US: Minnesota	Y	1	Unknown	Unknown
US: Montana	Y	1	Unknown	1920
US: Nebraska	Y	1	Food	Unknown
US: Nevada	Y	5	Unknown	1920, 1934, 1935, 1936, 1938
US: New Mexico	Y	1	Unknown	1885
US: North Dakota	N	1	Unknown	Unknown

Appendix I - Comprehensive List of Documented American Bullfrog Introductions (Kraus 2009) (cont.)

Locality Introduced	Success?	Number of Events	Pathway	Dates
US: Oklahoma	Y	1	Unknown	Unknown
US: Oregon	Y	1	Unknown	1931
US: South Dakota	Y	1	Unknown	Unknown
US: Texas	Y	3	Food	1927
US: Utah	Y	1	Unknown	Unknown
US: Washington	Y	3	Food	1910
US: Wyoming	Y	2	Unknown	Unknown
Venezuela	Y	1	Unknown	1990s

Amphibian Consumer and Business Survey

Contributors: Kevin Cavasos, Neelam C. Poudyal, Matthew Gray, Alexa Warwick, Jesse Brunner, Jonah Piovia-Scott, Nina Fefferman, Molly Bletz, Julie Lockwood, Joshua Jones

Acknowledgements: This study was funded by the University of Tennessee One Health Initiative. Investigators thank industry partners Pet Industry Joint Advisory Council (PIJAC), Josh's Frogs, Reptiles by Mack, and their representatives.

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EXECUTIVE SUMMARY

Overview: An online survey of amphibian pet owners and businesses engaged in amphibian trade and ownership was conducted from July 2021 to September 2021 to understand the size and structure of U.S. amphibian pet trade, the husbandry practices of amphibian pet dealers and owners, and the value they place on maintaining healthy amphibian populations in the wild. In partnership with PIJAC, Josh's Frogs and Reptiles by Mack, amphibian pet owners and businesses engaged in the amphibian pet trade were invited to complete the survey. Of the 478 respondents who initiated the survey, 392 finished the survey. Of the 469 respondents that responded to the question, 401 (85%) identified themselves as amphibian pet owners/consumers, 85 (18%) as amphibian breeders, 81 (17%) as retailers, 20 (4%) as wholesalers, and 7 (1%) as amphibian importers.

Ownership history: Ninety-five percent of consumers indicated they currently own or have previously owned a frog or toad, while 38% reported owning, or having owned a newt/salamander. Eighty-one percent of consumers reported also either currently or previously owning a reptile(s). Thirty-five percent indicated they had owned amphibians for over 10 years.

Acquisition: Ninety-two percent of all consumers indicated they had purchased their pet amphibians while 24% indicated they had rescued or found their pet amphibian and 19% reported they had collected their pet amphibian from the wild. The majority (59%) of consumers reported having purchased amphibian(s) from an "In-store retailer/pet store", while 49% reported having purchased from an online retailer. Almost half (49%) of consumers indicated they spent between $26-$75 for their most recently acquired amphibian. One-half (50%) of consumers reported paying $1-$25 per month to care for their pet.

Ownership importance: Consumers were presented with 7 factors potentially influencing their decision to own their most recently acquired amphibian. Religious significance, cultural significance, and family favorites were least important, while scientific or educational value, sense of companionship, and aesthetic and environmental values were relatively more important. Most consumers were at least

moderately familiar with general knowledge of amphibians, the role of amphibians in the environment, status/trends of amphibian populations, and benefits to humans from amphibians.

Care and disposal of amphibians: Consumers mainly acquired information about caring for their pet amphibian(s) from websites (92%), personal experience (87%), and scientific journals (61%). Ninety-one percent of consumers indicated they had never become unable to keep or been forced to get rid of a pet amphibian. Of those that had been forced to get rid of an amphibian, the most common reason (41%) was family relocation, followed by "unable to care for it" (22%). No consumers indicated they had released the animal into nature.

Sixty-three percent of consumers indicated using diagnostic tests as needed. Seventy-nine percent of consumers reported having had a pet amphibian die. The majority (61%) of those who had had an amphibian die buried the dead. Ninety-nine percent of consumers indicated a willingness to seek veterinary care or administer treatment at home if their pet amphibian showed signs of illness.

Awareness of and concerns regarding pathogens: Most consumers (63%) indicated that before reading the survey they were unaware of *Bacillus mycoides* or other beneficial microbes and their ability to kill harmful microbes and increase disease resistance in amphibians. Seventy percent of consumers indicated, prior to reading the survey, they were aware that the *Bd*, *Bsal*, and *Rv* pathogens can be transmitted through the pet trade. Most consumers (64%) indicated they were "Not at all concerned" when acquiring their most recent amphibian that the animal may have been previously infected with *Bd*, *Bsal*, or *Rv*, while 23% reported being "Very concerned". Ninety-six percent of consumers reported having never detected harmful pathogens in their amphibians

Perception of threats: Most of the consumers indicated they believe the threat of transmission of harmful pathogens from pets to natural areas is serious, protecting natural populations is important, and that they have a role to play in protecting natural populations. However, time, knowledge, and financial constraints may be barriers preventing amphibian owners from further implementing biosecurity practices. Most consumers indicated they were extremely likely to take actions to mitigate the transmission of harmful pathogens.

Value of pathogen-free amphibians: Seventy-nine percent of consumers indicated it would be extremely or very important that the animal they acquire be free of the *Bd*, *Bsal*, and *Rv*. Seventy-six percent indicated they would be willing to pay more for an animal that is certified free of the *Bd*, *Bsal*, and *Rv* pathogens.

Business Survey

Business characteristics: Of the 143 businesses that responded to the question, 85 (59%) identified themselves as amphibian breeders, 81 (57%) as amphibian retailers, 20 (14%) as wholesalers, and 7 (5%) as amphibian importers. Seventy-five percent of businesses indicated they deal with both reptiles and amphibians, while 16% deal with amphibians only. Eighty-one percent of amphibian businesses reported obtaining their amphibians from breeders, followed by hobbyists (66%), wholesalers (60%), retailers (26%), importers (24%) and wild caught (14%). Eighty-four percent of business respondents indicated they sell to hobbyists, 67% to households, 29% to breeders, 26% to retailers and 11% to wholesalers. Almost a third (29%) of business respondents indicated they had been in the amphibian business for

over 20 years while 19% reported having been in business for 11-20 years. Another 19% indicated they had been in business for 6-10 years.

In terms of annual sales, 30% reported less than $5,000, 20% reported annual over $1,000,000. Of the remaining, 17% indicated $5,000-$50,000, 13% reported $500,000-$1,000,000, 11% reported $200,000-$500,000, and 8% reported $50,000-$200,000. The Midwest region of the country accounted for the most business respondents to the survey (33%), followed by the Southeast (20%), with respondents being relatively evenly distributed across the other regions of the country. Eighty-two percent of respondents indicated they only conducted business with buyers and sellers of amphibians in the United States.

Share of amphibian business: Approximately half (51%) of 106 amphibian businesses indicated that amphibian sales accounted for less than 10% of their total sales. Similarly, 23% reported 10%-25%, 12% reported 76%-100%, 8% reported 26%-50% and the remaining 6% reported amphibian sales accounted for 51%-75% of their total sales.

Factors important in business decisions: When asked to rate the importance of the factors in making business decisions, high level of importance was placed on issues of ethics, social concerns, and legal compliance.

Awareness of and concerns regarding pathogens: Most businesses (53%) indicated that before reading the survey they were unaware of beneficial microbes, such as *Bacillus mycoides*, that can kill harmful microbes and increase disease resistance in amphibians. Almost half (47%) of businesses indicated that they would definitely consider administering treatment to their pet amphibian(s) using "probiotics" such as *Bacillus mycoides,* while 53% indicated needing more information. Eighty-one percent of businesses indicated, prior to reading the survey, they were aware that the pathogens *Bd*, *Bsal*, *Rv* can be transmitted through the pet trade.

Perception of threats: More than half (55%) of businesses indicated they were very concerned that transmission of pathogens through the trade network of pets or pet products may impact the amphibian(s) in their facility.

Biosecurity practices: While most businesses indicated they use disinfectants to clean surfaces and tanks (92%), use gloves when handling animals (60%) and quarantine new animals in a separate room (66%), fewer businesses test new acquisitions for pathogens (18%), conduct testing to monitor for disease (22%), or treat recirculating water (23%) or wastewater (24%) prior to disposal.

Value of pathogen-free amphibian: Eighty-six percent of businesses indicated it was extremely or very important that an animal be healthy and free of the *Bd*, *Bsal*, and *Rv* pathogens when introducing it to their facility. Ninety-seven percent indicated they would be interested in acquiring an animal that is certified as free of the *Bd*, *Bsal*, and *Rv* pathogens and 59% indicated they would be willing to pay more for an animal that is certified. Of those willing to pay more, 22% indicated they would be willing to pay 1%-5% more, 36% indicated 6%-10% more, and 28% indicated 11%-20% more. Business responses indicated the mean loss resulting from the illness or death of an animal was $939.80, with a minimum and maximum value of $0 and $20,000, respectively.

INTRODUCTION

With growing concerns over continuous decline of amphibian populations in recent decades, researchers and stakeholders in the wildlife trade network have become increasingly interested in developing a deeper understanding of the scope of the amphibian trade and the husbandry practices and potential for pathogen transfer and spillover at various stages of the supply chain. To fill this gap in knowledge, the need for conducting a comprehensive survey of all business types in the amphibian trade, including importers, breeders, retailers, wholesalers, and consumers (i.e., pet owners) was realized.

The University of Tennessee Institute of Agriculture (UTIA) collaborated with the Pet Industry Joint Advisory Council (PIJAC) and other partners to identify science-based solutions that promote and foster animal wellbeing and environmental stewardship, minimize revenue losses due to harmful pathogens, and decrease opportunities for microbial spillover from captive to wild populations. UTIA and PIJAC established a Memorandum-of-Understanding (MOU) and Memorandum-of-Agreement (MOA) in 2021 to guide this project, with financial support provided by the UT One Health Initiative. Other amphibian care community partners and collaborators include Josh's Frogs, Reptiles by Mack, Washington State University, Michigan State University, University of Massachusetts-Boston, and Rutgers University.

Information collected in the surveys will be used to identify potential opportunities and barriers to developing an industry-led healthy trade program that ensures animal well-being, reduces disease-related financial losses for businesses and increases customer satisfaction. Additionally, information will enable researchers to provide recommendations on best strategies to minimize the likelihood of spillover of harmful microbes from the pet trade to the wild.

The specific objectives of the study were to: 1) characterize the size and composition of the U.S. pet businesses that are engaged in the pet amphibian trade; 2) understand the awareness and attitudes that amphibian pet businesses and owners have with respect to harmful and beneficial microbes; 3) estimate the value businesses and owners place on amphibians free of pathogens such as *Bd*, *Bsal*, and *Rv; and 4)* characterize the current husbandry practices of amphibian pet dealers and owners and their willingness to engage in proactive strategies that promote beneficial microbes and reduce harmful microbes in their facilities and the broader amphibian pet trade.

METHODOLOGY

Data needed to meet the objectives of this project were collected by designing and administering an online survey of businesses and owners in the amphibian pet trade industry. In collaboration with the industry partners (PIJAC, Josh's Frogs, Reptiles by Mack), the investigators developed a semi-structured questionnaire survey that included questions addressing aspects of the amphibian trade ranging from awareness and knowledge of pathogens (*Bd*, *Bsal*, and *Rv*), current husbandry and disposal practices, agreement with statements regarding biosecurity practices, and attitudes and values (willingness-s-to purchase, willingness-to-pay etc.) regarding acquiring pathogen-free amphibians.

The anonymous and voluntary survey instrument and protocols were reviewed and approved by the UTK Institutional Review Board for human subjects' research (Approval#: UTK IRB-21-06494-XM). The survey questionnaire was then formatted and administered using the Qualtrics online survey platform.

The survey was initially launched in mid-July 2021 with an email message sent from our industry partners to businesses and consumers in their membership list and contacts within their business network. A link to complete the survey was also placed on the project website (https://onehealth.tennessee.edu/pijac/) located in the public domain of University of Tennessee. The first question on the survey was a screening question for respondents to identify their role or relationship with the amphibian trade network. Those who identified themselves as consumers or pet owners only were directed to a module specific to consumers only, whereas those identifying themselves as business only were sent to a separate module specific to amphibian businesses. Those who identified themselves as both consumer and business were given an opportunity to complete both modules.

This report presents the results from all the responses completed by September 10, 2021. Of the 478 respondents who initiated the survey, 392 finished the survey. Of the 469 respondents that responded to the question, 401 identified themselves as amphibian pet owners/consumers, 85 as amphibian breeders, 81 as retailers, 20 as wholesalers, and 7 as amphibian importers.

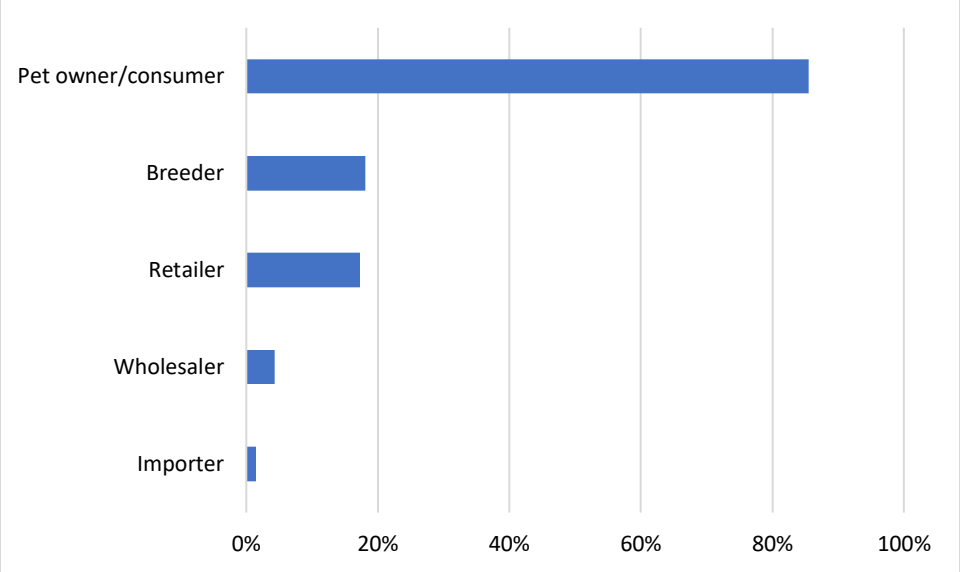
RESULTS AND DISCUSSION

Respondent characteristics

Of the 469 respondents that responded to the initial screening question "Which of the following best describes your role in the industry?", 86% identified themselves as amphibian pet owners/consumers, 18% identified themselves as amphibian breeders, 17% as retailers, 4% as wholesalers, and 1% as amphibian importers (Fig. 1).

In terms of demographics, of 357 respondents, 48% reported being under the age of 35, 34% indicated they were 35-54 and 17% were over the age of 55. One-half (50%) of respondents were female, 40% male, 6% non-binary / third gender, and 3% preferred not to say. Eighty-eight percent of respondents identified themselves as White, 1% Asian, 1% Black or African American and 8% identified as "Other".

In terms of education attained, of 356 respondents, 38% reported attending "Some college", 31% reported having completed a bachelor's degree, 18% completed a graduate degree, and 12% completed high school.



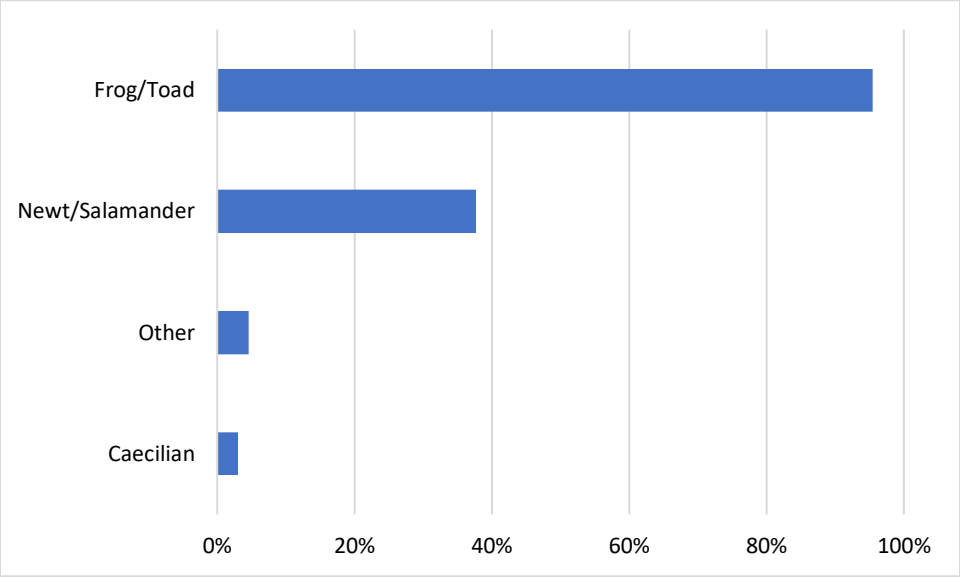
**Sum of percentages exceeds 100% as some respondents belong to multiple categories*
Figure 1. Respondent roles in the amphibian industry (n=469)

Consumers/amphibian pet owners survey

Amphibian Acquisition and Ownership

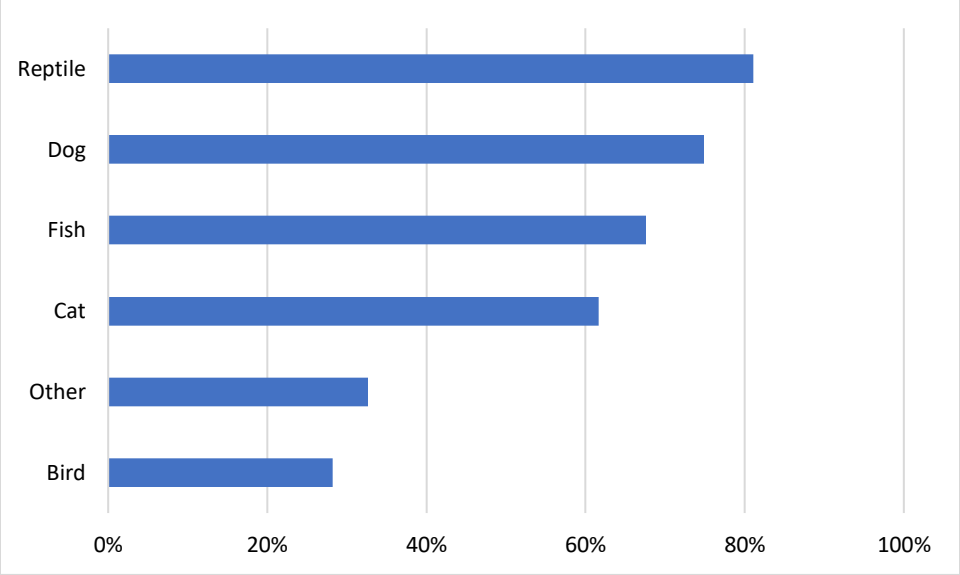
When asked about current or previous amphibian ownership, 95% of 393 respondents indicated they currently own or have previously owned a frog or toad, 38% own, or have owned, a newt/salamander,

3% own or have owned a caecilian, and 5% respondents indicated owning other types of amphibians (Fig. 2).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 2. Type(s) of amphibians owned by respondents (n=393)

When asked about ownership of pets other than amphibians, 81% indicated they currently or had previously owned reptiles, 75% reported owning dogs, 62% reported owning cat (Fig. 3). Similarly, 68% reported fish, 28% birds, and 33% respondents indicated ownership of other types of pets.



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 3. Other types of pets currently or previously owned by survey respondents (n=386)

In terms of the duration of ownership, 42% of the respondents indicated they had owned amphibians for 1-4 years, 15% reported 5-7 years, 7% reported 8-10 years and the remaining 35% reported having owned amphibians for over 10 years (Fig. 4).

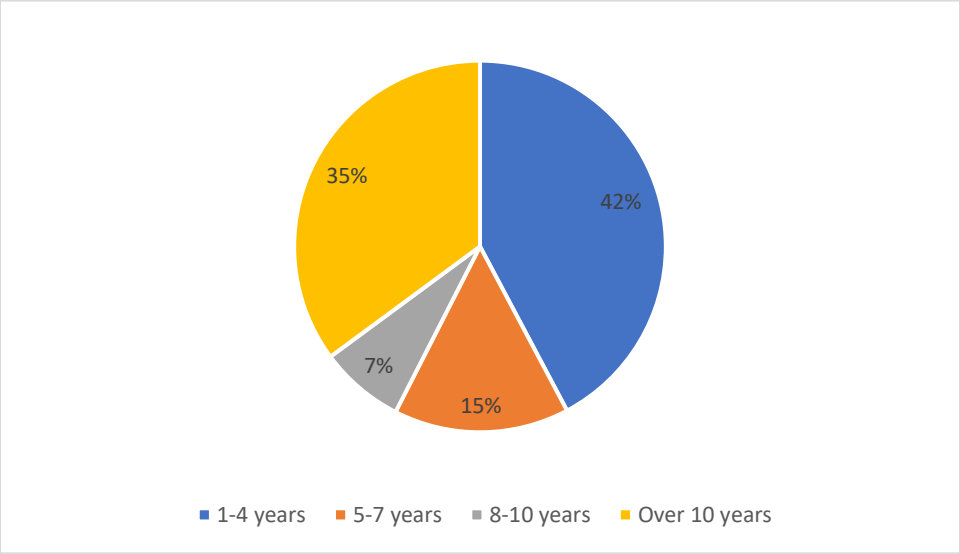


Figure 4. Length of amphibian ownership (n=393)

Regarding the total number of amphibians owned over the course of this duration, 37% reported having owned more than 10 amphibians, 27% indicated they had owned 2-4 amphibians, 18% have owned 5-7 amphibians, 10% have owned 8-10 amphibians and the reamining 9% of the respondents reported having owned only one amphibian (Fig. 5).

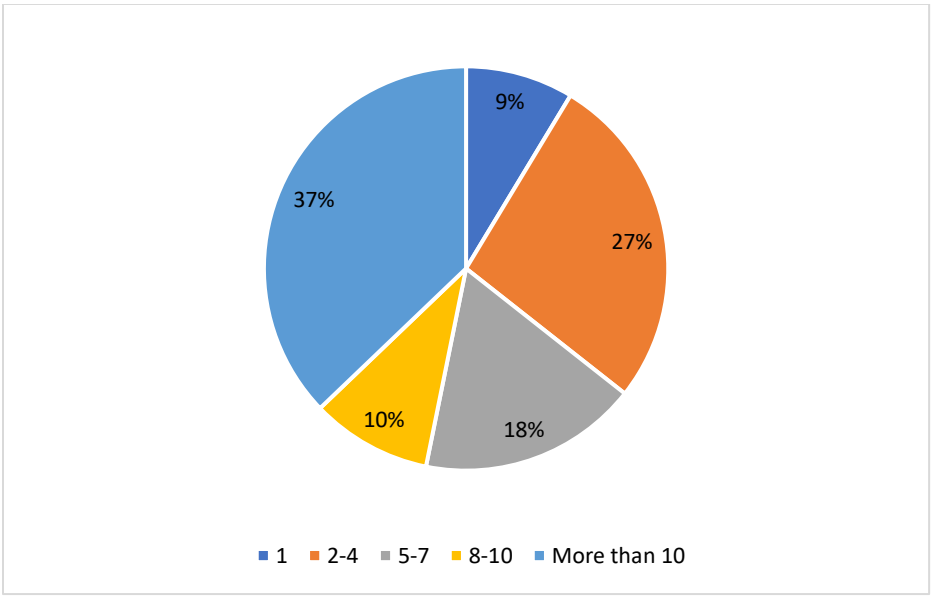
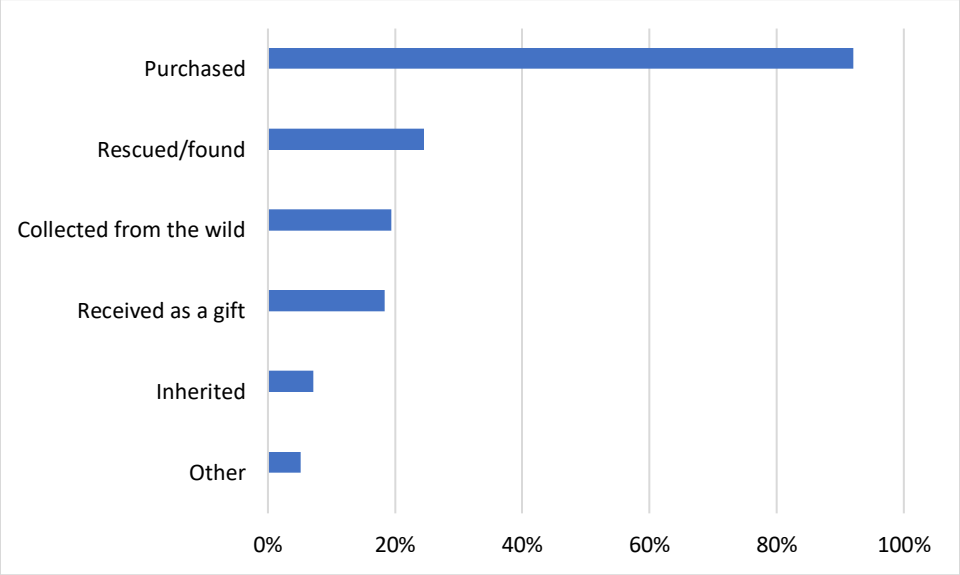


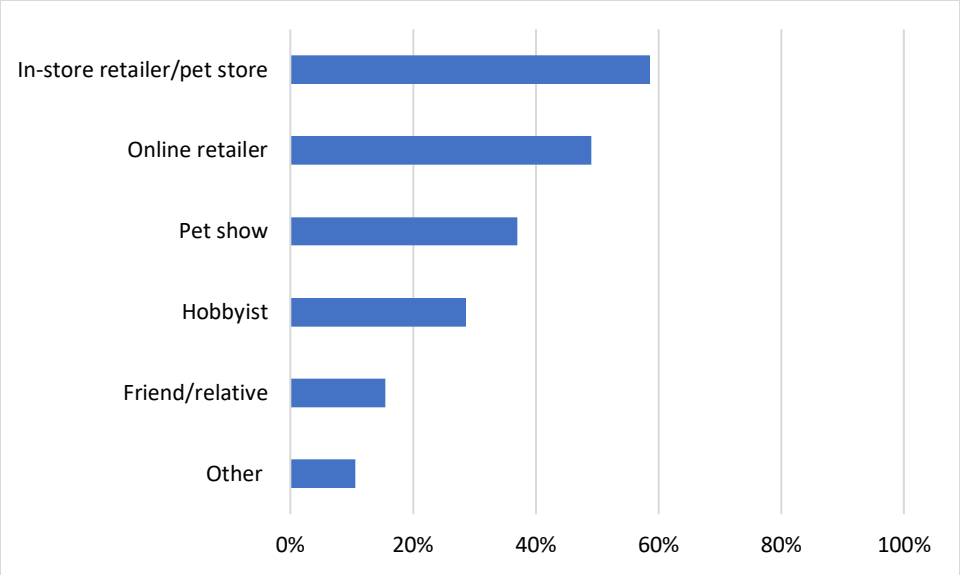
Figure 5. Number of amphibians owned. (n=393)

When asked about the sources from where they acquired their pet amphibians, 92% indicated they had purchased their pet amphibian, 24% indicated they rescued/found their amphibian(s), 19% collected them from the wild, 18% received them as a gift, 7% inherited their amphibians, and 5% respondents reported acquiring their amphibian(s) by other means (Fig. 6).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 6. Mode of acquisition of pet amphibian(s) (n=392)

When asked where they acquired their amphibians(s), of 387 respondents, 59% indicated they had purchased their pet amphibian from an in-store retailer/pet store, 49% indicated they had purchased their pet amphibian from an on-line retailer, 37% from a pet show, 29% from a hobbyist, 16% from a friend/relative, and 11% from other sources, which included breeding at home, expos and trade shows.



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 7. Sources of amphibian acquisition (n=387)

When asked about the cost of their most recently acquired amphibian, 49% indicated they had paid $26-$75 for their most recently acquired pet amphibian, 21% reported paying $1-$25, 12% paid $76-$125, 10% paid nothing and the remaining 8% reported paying over $125 (Fig. 8).

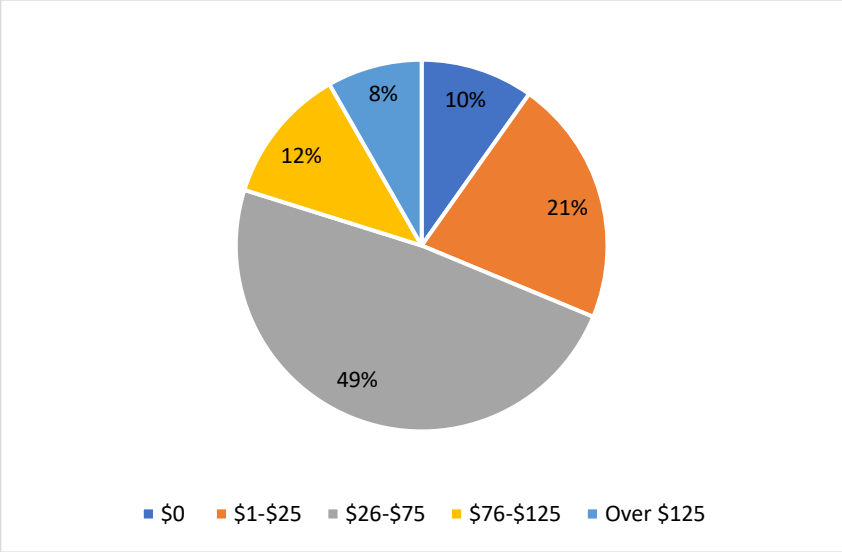


Figure 8. Cost of most recently acquired pet amphibian (n=387)

When asked to report the average monthly expense of care (feed, medical care, insurance, etc.) for their pet amphibian, 50% reported paying $1-$25 per month to care for their pet amphibian, 39% reported

paying $26-$75, 8% indicated they paid $76-$125 and 4% paid over $125 per month to care for their pet amphibian (Fig. 9).

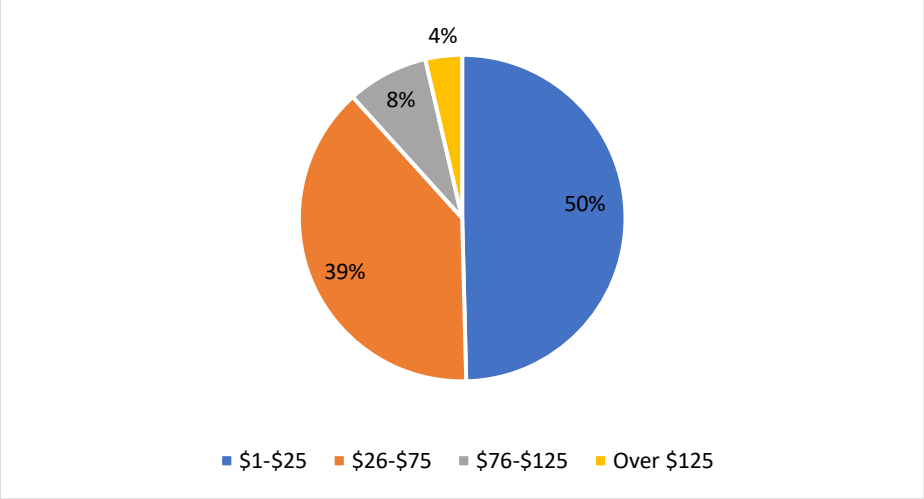


Figure 9. Average monthly cost of pet amphibian care (n=385)

Respondents were asked to indicate how important each of the following factors were in their decision to own their most recent pet amphibian. Religious significance, cultural significance and family favorite were clearly not very influential, while the influence of other factors on respondents' decisions to own their most recent pet amphibian was relatively evenly distributed (Fig. 10). Relatively more important

factors were scientific or educational value, sense of companionship, and aesthetic and environmental values.

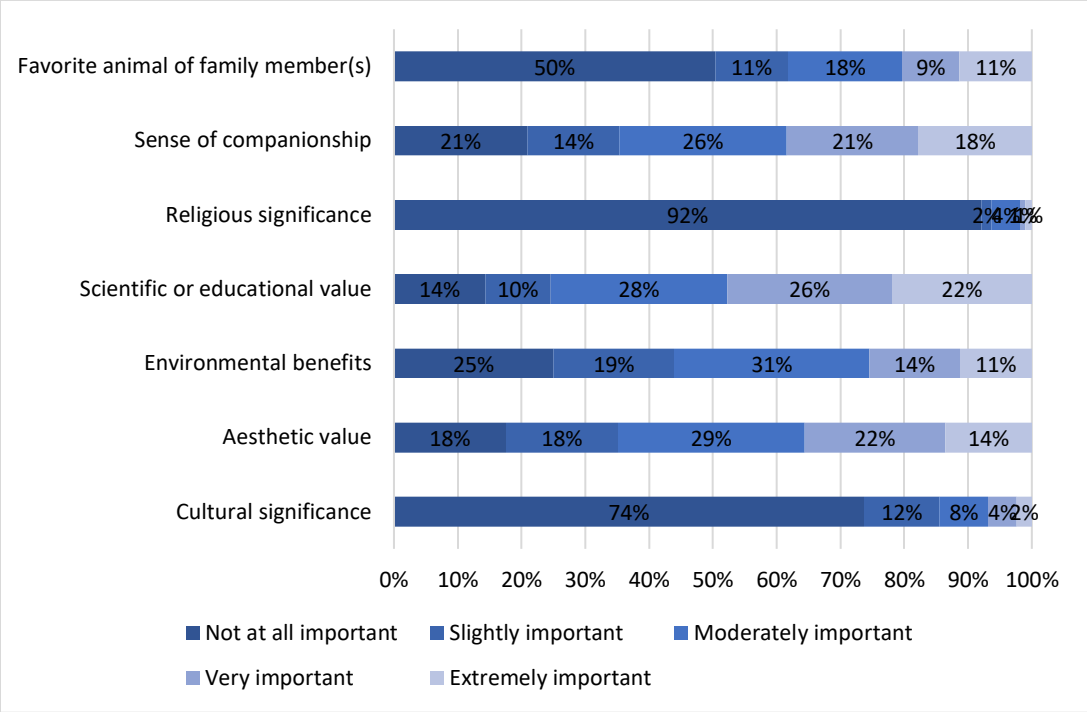


Figure 10. Importance of various factors in amphibian pet ownership (From top: n1=380, n2=382, n3=380, n4=383, n5=379, n6=383, n7=379)

Respondents were asked to indicate the extent to which they were familiar with various aspects of amphibians prior to reading the survey. In general, most respondents are at least moderately familiar with the aspects presented. For example, 84% indicated being at least moderately familiar with the status and trends of amphibian populations (Fig. 11).

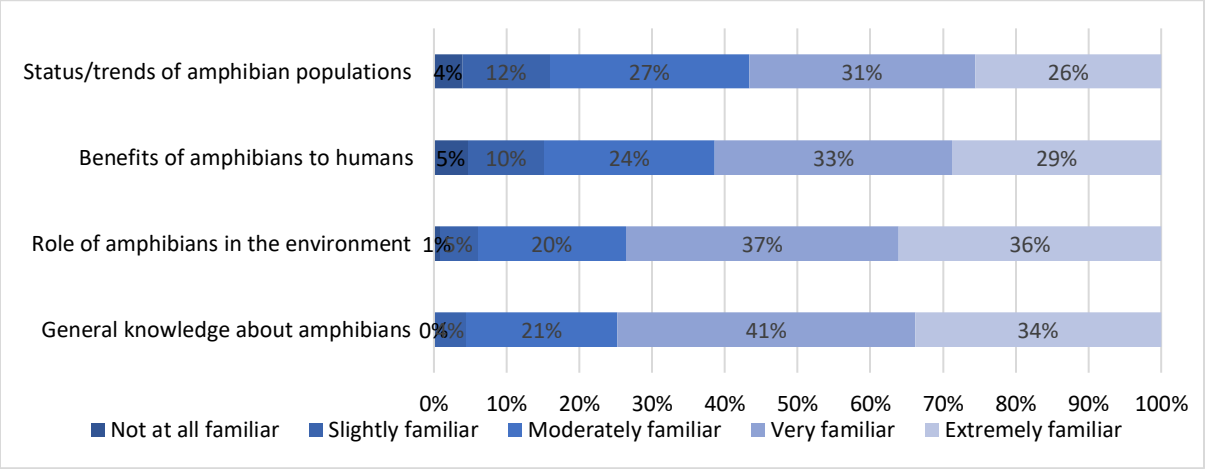


Figure 11. Familiarity with various aspects of amphibians prior to reading survey. (From top: n1=384, n2=382, n3=383, n4=383)

Experience with amphibian health

Respondents were asked if they had ever become unable to keep a pet amphibian or been forced to get rid of a pet amphibian for any reason. About 91% indicated they had never become unable to keep a pet amphibian or been forced to get rid of a pet amphibian for any reason, while the remaining 9% reported having been forced to get rid of a pet amphibian (Fig. 12).

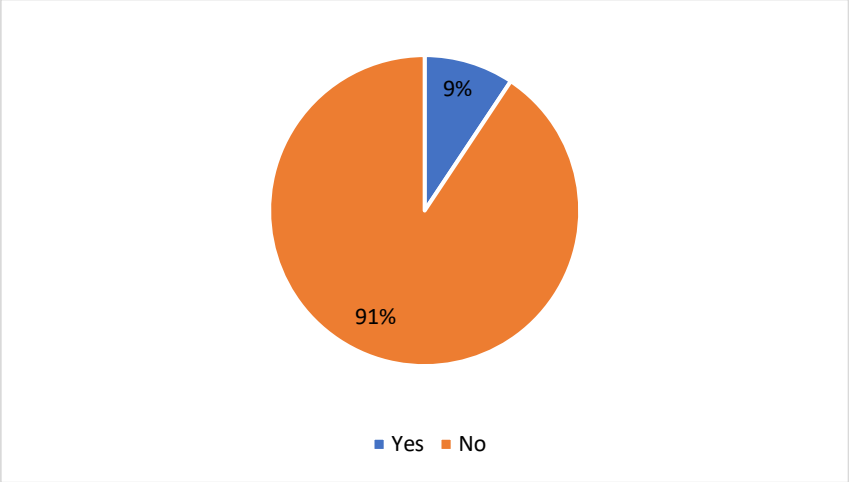
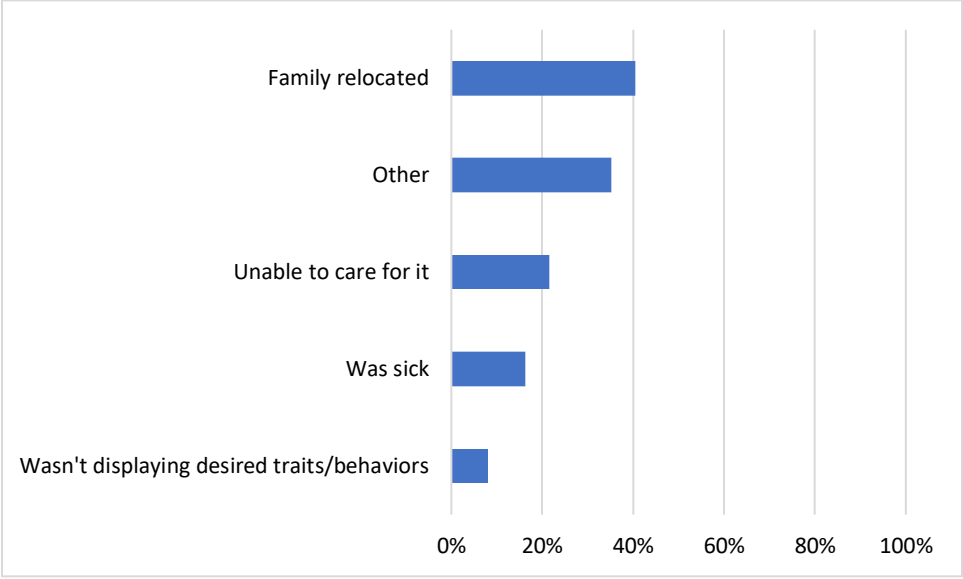


Figure 12. Percentage of owners forced to ever get rid of pet amphibian (n=385)

Respondents that reported having been forced to get rid of a pet amphibian were asked to indicate the reason. Forty-one percent indicated the reason they had been unable to keep a pet amphibian was family relocation, 22% indicated they were unable to care for the animal, 16% reported that the animal was sick, 8% indicated the animal was not displaying desired traits or behaviors and 35% respondents indicated having been forced to get rid of their pet amphibian for "other" reasons including family problems and conflicts with other animals (Fig. 13).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 13. Reason(s) owners forced to get rid of pet amphibian(s). (n=37)

Most (59%) of the respondents reported having been forced to get rid of a pet amphibian indicated they had given away or sold their animal (Fig. 14). Eight percent each indicated they had taken their animal to a rescue facility/pet amnesty event and returned to where it was acquired from. Similarly, 5% indicated to have euthanized the animal. No respondents reported having released their animal into nature.

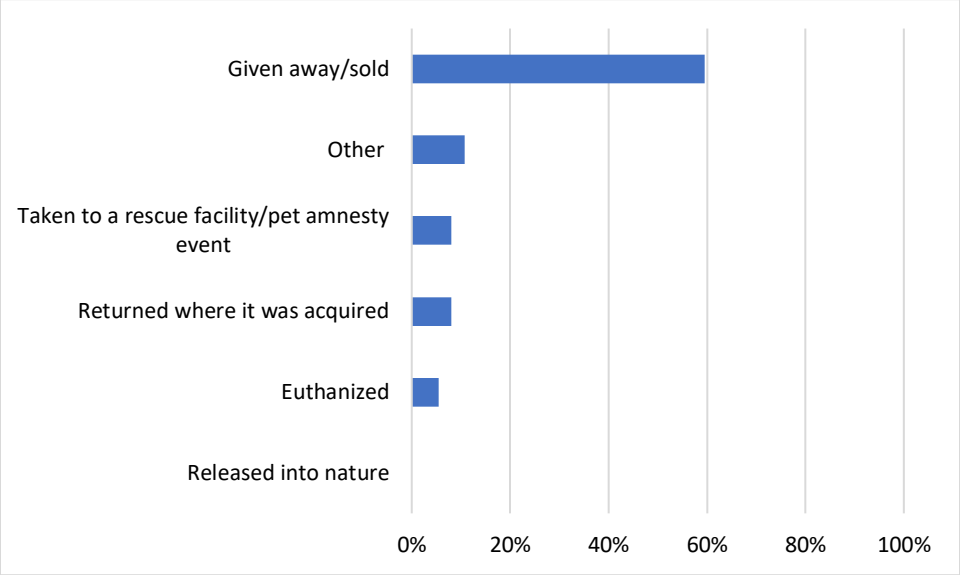
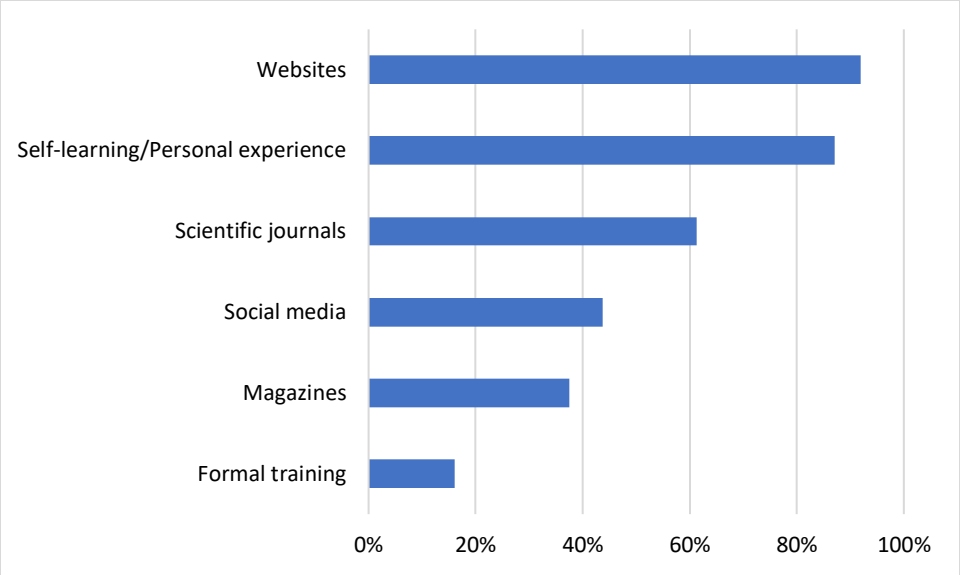


Figure 14. Method(s) used to dispose of animal(s) (n=37)

Respondents were asked to indicate from which of the following sources they typically acquire information about caring for their pet amphibian. Most (92%) of the respondents indicated they typically acquire information about caring for their pet amphibian(s) from websites, 87%) cited self-learning/personal experience, 61% reported getting their information from scientific journals (Fig. 15). The other sources frequently mentioned were social media (44%), magazines (37%), and formal training (16%).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 15. Sources of information for amphibian care (n=387)

When asked how frequently their pet amphibian(s) receive veterinary care, of 358 respondents, 63% indicated their amphibian(s) receive veterinary care or diagnostic tests as needed (Fig. 16). Similarly, 5% indicated regularly receiving care and test, 3% indicated occasionally, and 30% indicated reported never receiving care or tests. When asked if they had a death of amphibians in possession, nearly 80% of the respondents reported having had a pet amphibian die.

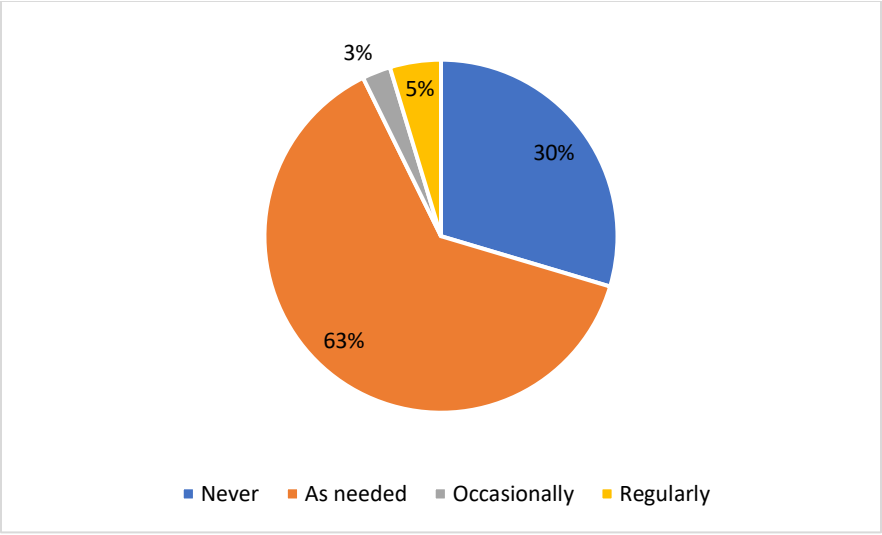


Figure 16. Frequency of veterinary care and diagnostic tests. (n=385)

Of 387 consumers responding, seventy-nine percent indicated they had had a pet amphibian in their care die.

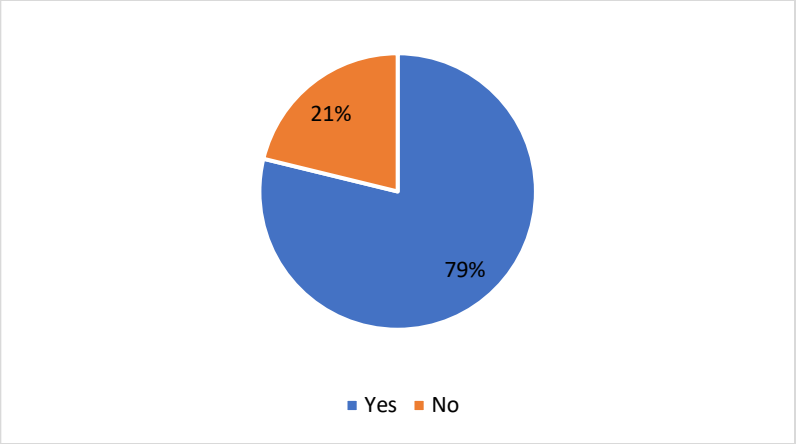
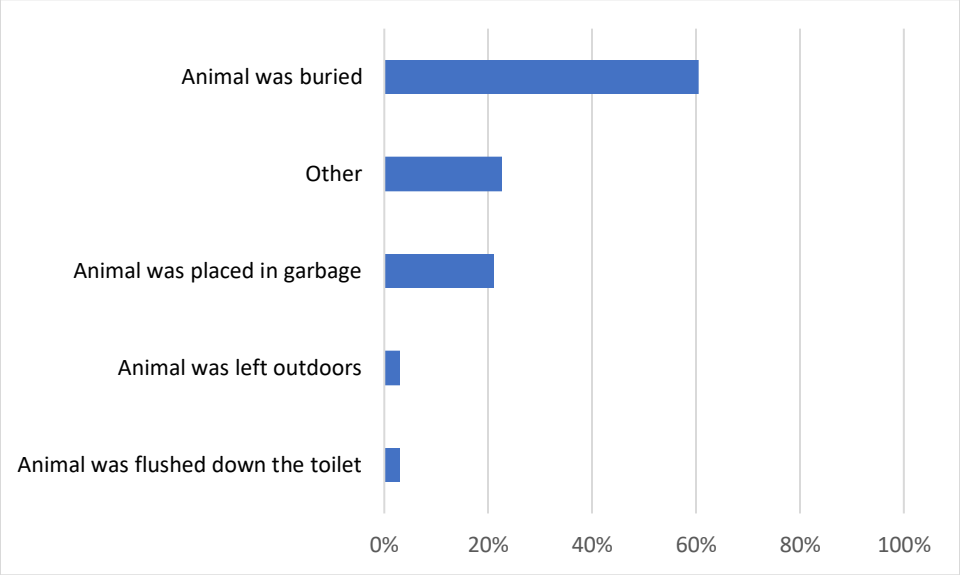


Figure 17. Percentage of amphibian pet owners who have had a pet amphibian in their care die (n=387)

Of 304 respondents reporting having had an amphibian die, 61% indicated the deceased animal was buried, 21% indicated the animal was placed in the garbage, 3% reporting flushing the animal down the toilet, 3% left the animal outdoors, and 23% respondents indicated the animal was disposed of through other means including cremation.



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 18. Methods for disposing of deceased animal(s) (n=304)

When asked about their intention to seek veterinary care or administer treatment at home for animal showing signs of illness, 99% indicated, assuming costs were not a concern, they would be willing to seek veterinary care or administer treatment at home if their pet amphibian showed signs of illness (Fig. 19).

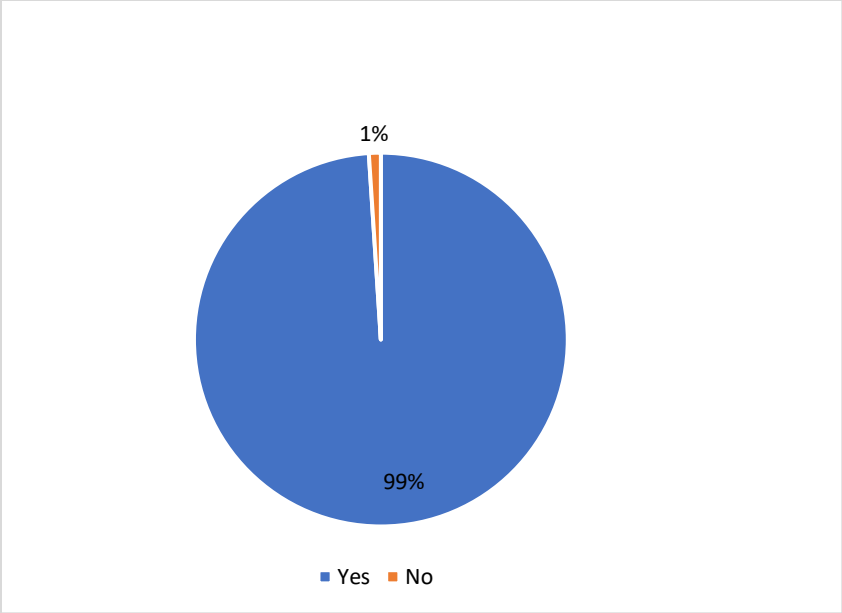


Figure 19. Willingness to seek veterinary care or administer treatment at home if pet amphibian(s) show signs of illness. (n=387)

Familiarity and Experience with Beneficial Microbes and Harmful Pathogens

Respondents were asked if, before reading the survey, they were aware of beneficial microbes, such as Bacillus mycoides, that can kill harmful microbes and increase disease resistance in amphibians. A majority (63%) reported that they were unaware of *Bacillus mycoides* or other beneficial microbes and their ability to kill harmful microbes and increase disease resistance in amphibians (Fig. 20).

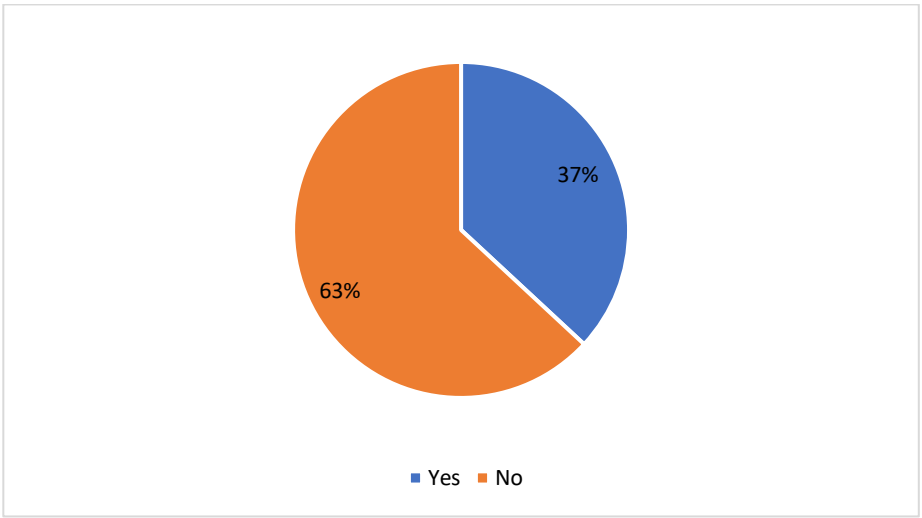


Figure 20. Percentage of respondents aware of beneficial microbes, such as Bacillus mycoides, that can kill harmful microbes and increase disease resistance in amphibians (n=382)

When asked whether they would consider administering treatment to their pet amphibian using "probiotics" such as *Bacillus mycoides,* 40% indicated "Definitely Yes", and the other 60% indicated "Maybe, but I need more information" (Fig. 21). Only 1 respondent indicated "Definitely not".

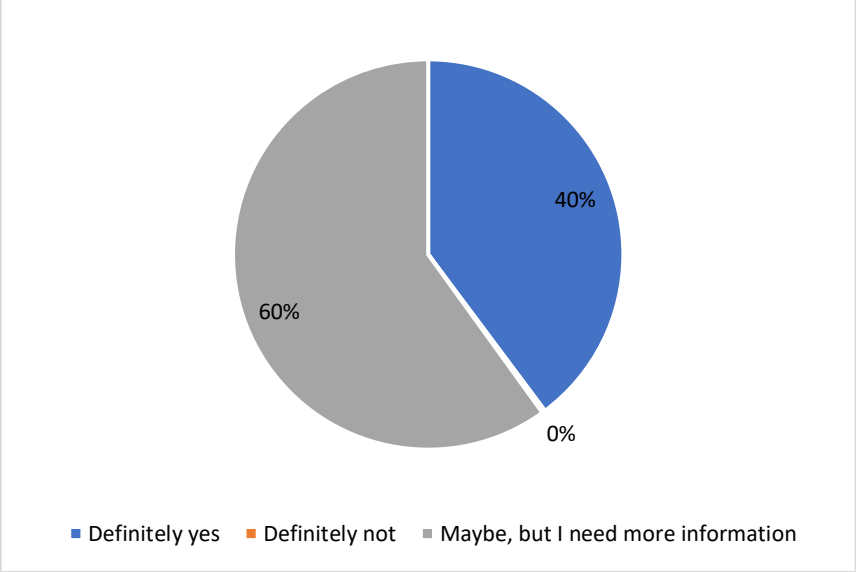


Figure 21. Percentage of respondents who would consider administering treatment to your pet amphibian using "probiotics" such as Bacillus mycoides (n=382)

When asked whether, before reading the survey, they were aware that the *Bd*, *Bsal* and *Rv* pathogens can be transmitted through pet trade, 70% indicated they were (Fig. 22). The remaining 30% were not aware that the *Bd*, *Bsal* and *Rv* pathogens can be transmitted through pet trade

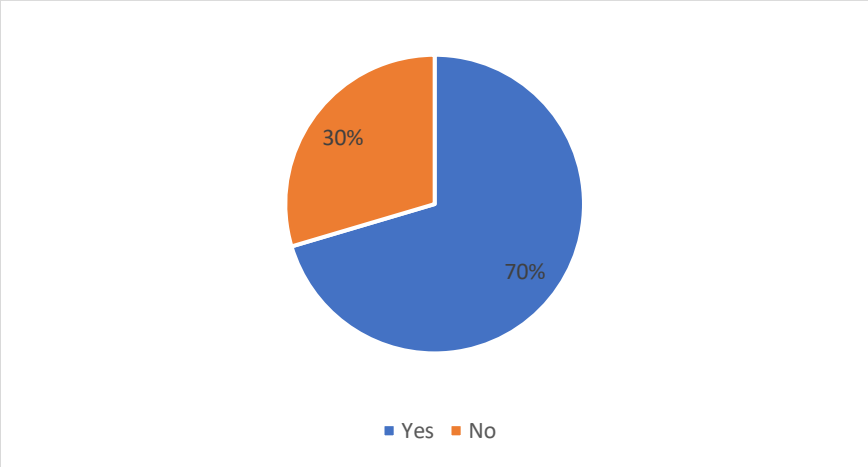


Figure 22. Percentage of respondents aware that the Bd, Bsal and Rv pathogens can be transmitted through pet trade (n=382)

When asked their level of concern when acquiring their most recent amphibian that the animal may have been infected with *Bd*, *Bsal* or *Rv*. Approximately two-third (64%) reported they were not at all

concerned whereas one-third (30%) were slightly concerned and the remaining 6% were very concerned (Fig. 23).

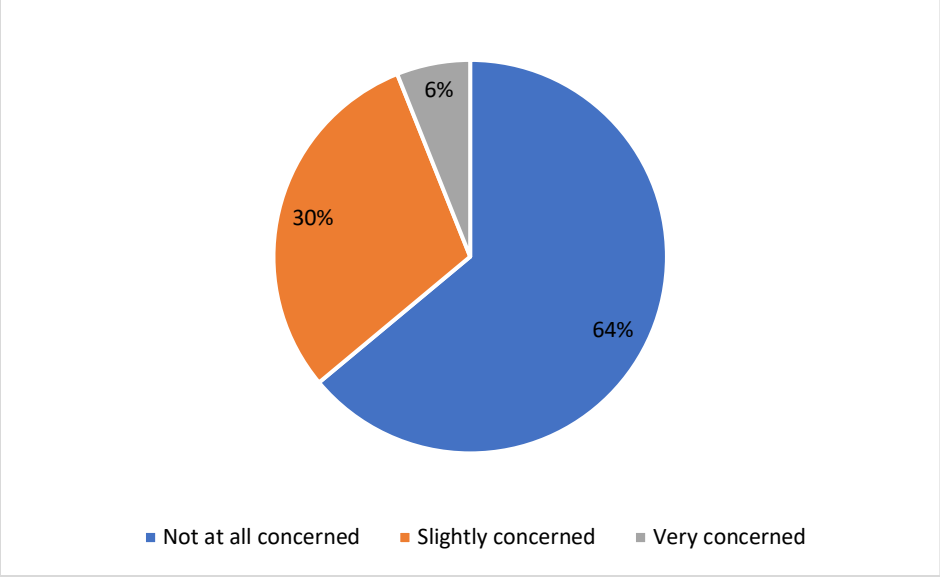
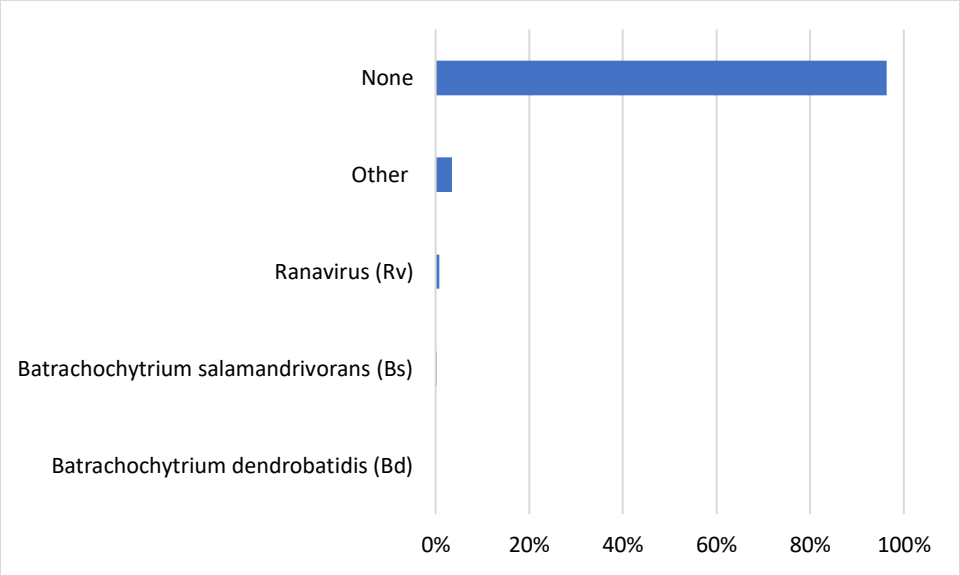


Figure 23. Level of concern that most recent amphibian purchase may have been infected with Bd, Bsal, or Rv prior to acquisition (n=380)

Nearly all (96%) respondents reported having never detected a pathogen in their pet amphibian(s) (Fig. 24). Less than 1% of consumers reported having detected either *Rv* or *Bsal.* Although about 3% respondents responded "other" pathogens had been detected in their pet amphibians, only three respondents specified actual illnesses or disease, which included "skin infection", "Red leg disease in Pac-Man frogs", and "reptiles with salmonella".

While one consumer indicated the *Bsal* pathogen was detected in their amphibian(s), to date, *Bsal* is not known to have been found in North America in the wild or archived museum and DNA samples. However, requisite levels of surveys and monitoring have not yet been conducted in order to state conclusively that *Bsal* is not yet here, undetected (salamanderfungus.org).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 24. Pathogens detected in respondent pet amphibians (n=378)

When asked if they were to acquire another pet amphibian in the future, how important it would be that the animal is free of the *Bd*, *Bsal*, *Rv* pathogens mentioned in the previous question, over half (52%) indicated it extremely important and another 27% indicated very important (Fig. 25). Only 2% indicated it to be not at all important.

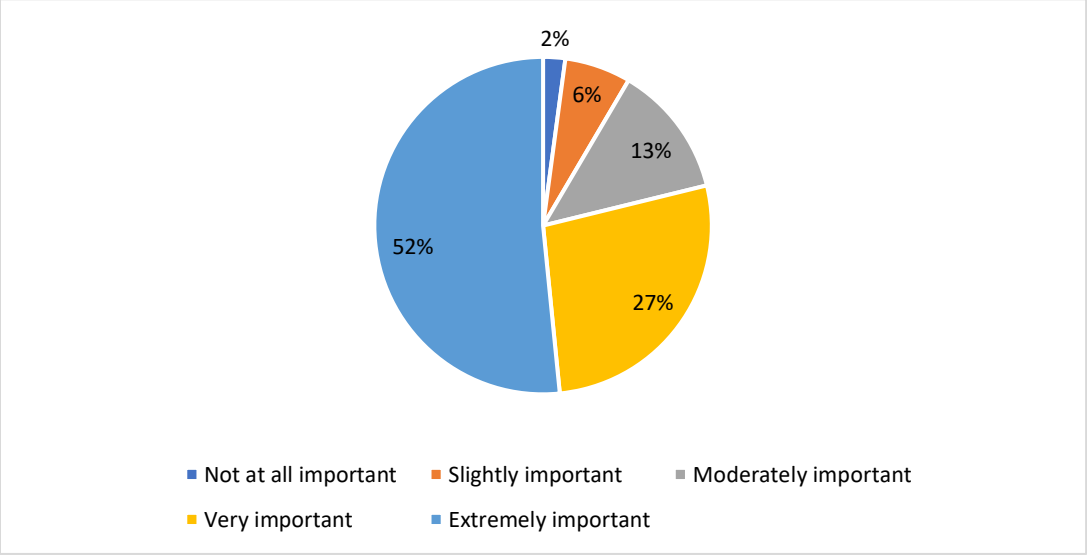


Figure 25. Level of importance that an amphibian acquired in the future is free of the Bd, Bsal, and Rv pathogens (n=378)

Willingness to pay for pathogen free amphibian

Three-quarters (76%) of the respondents indicated that, when acquiring an amphibian, they would be willing to pay more for an animal that is certified free of the *Bd*, *Bsal* and *Rv* pathogens (Fig. 26). About 20% indicated they were not sure about paying more whereas the remaining 4% were not willing to pay more.

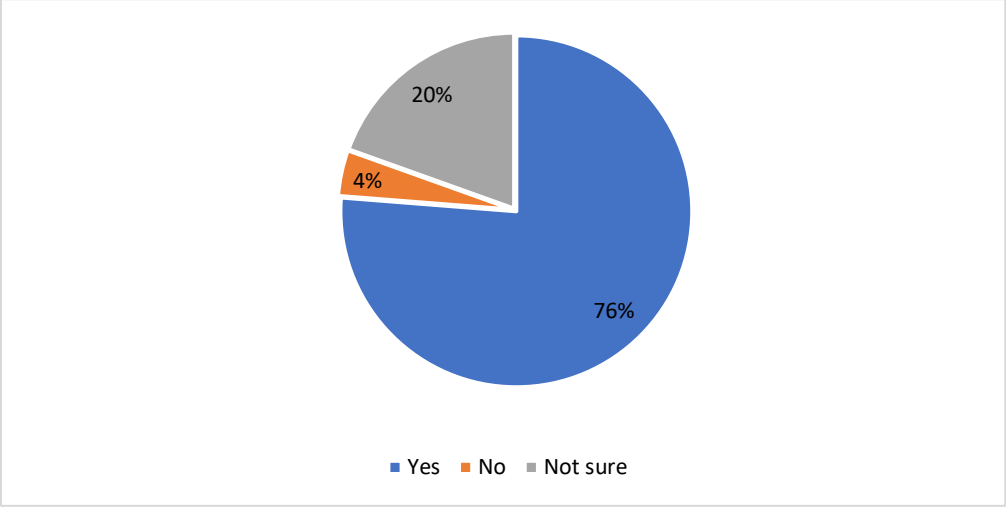


Figure 26. Willingness to pay more for an animal that is certified free of the Bd, Bsal, and Rv pathogens (n=379)

Respondents were presented with a randomly selected dollar amount ($1, $2, $3, $5, $7, $10, $15, $20, $30, $50) and asked whether they'd be willing to pay the presented amount extra to acquire an amphibian that is certified free of the *Bd*, *Bsal*, and *Rv* pathogens compared to the price for not certified or not confirmed to be free of these pathogens. Overall, ninety percent of respondents indicated they would be willing to pay the amount presented (Fig. 27).

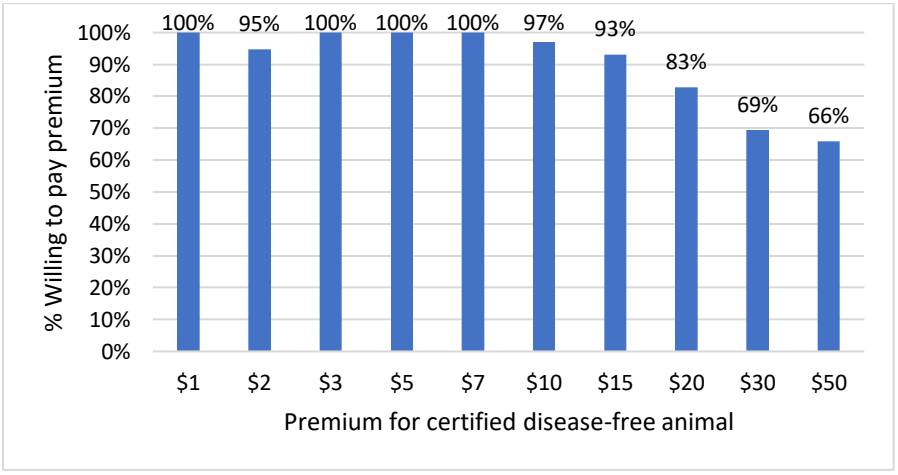


Figure 27. Percent of respondents willing to pay extra for certified disease-free animal (n=364)

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Among the respondents who were not willing to consider paying extra for a certified animal compared to a non-certified animal, 38% indicated that they cannot afford to pay the extra amount being

proposed, 40% indicated they do not think they should be responsible for this expense and the remaining 22% indicated it is not worth paying (Fig. 28).

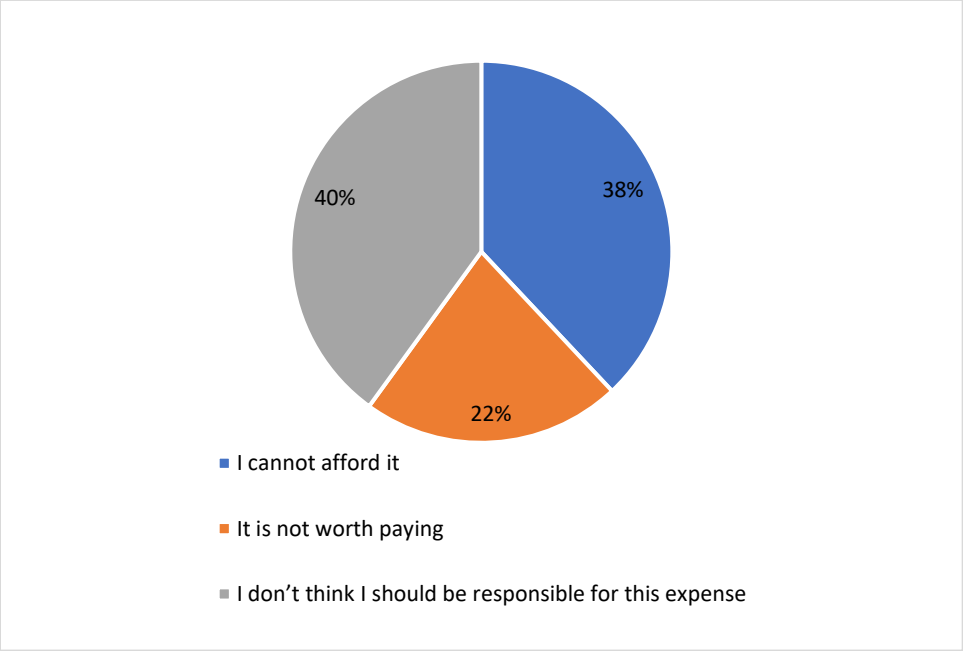


Figure 28. Reasons for unwillingness to pay extra for an animal certified free of Bd, Bsal and Rv compared to a non-certified animal (n=50)

Attitudes Toward Pathogen Transmission and Likelihood of Adopting Mitigating Actions

Respondents were asked to report their level of agreement with a series of statements pertaining to pathogen transmission in the pet trade (Fig. 29). Most of the respondents indicated they believe the threat of transmission of harmful pathogens from pets to natural areas is serious, protecting natural populations is important, and that they have a role to play in protecting natural populations. However, time, knowledge, and financial constraints may be barriers preventing amphibian owners from further implementing biosecurity practices.

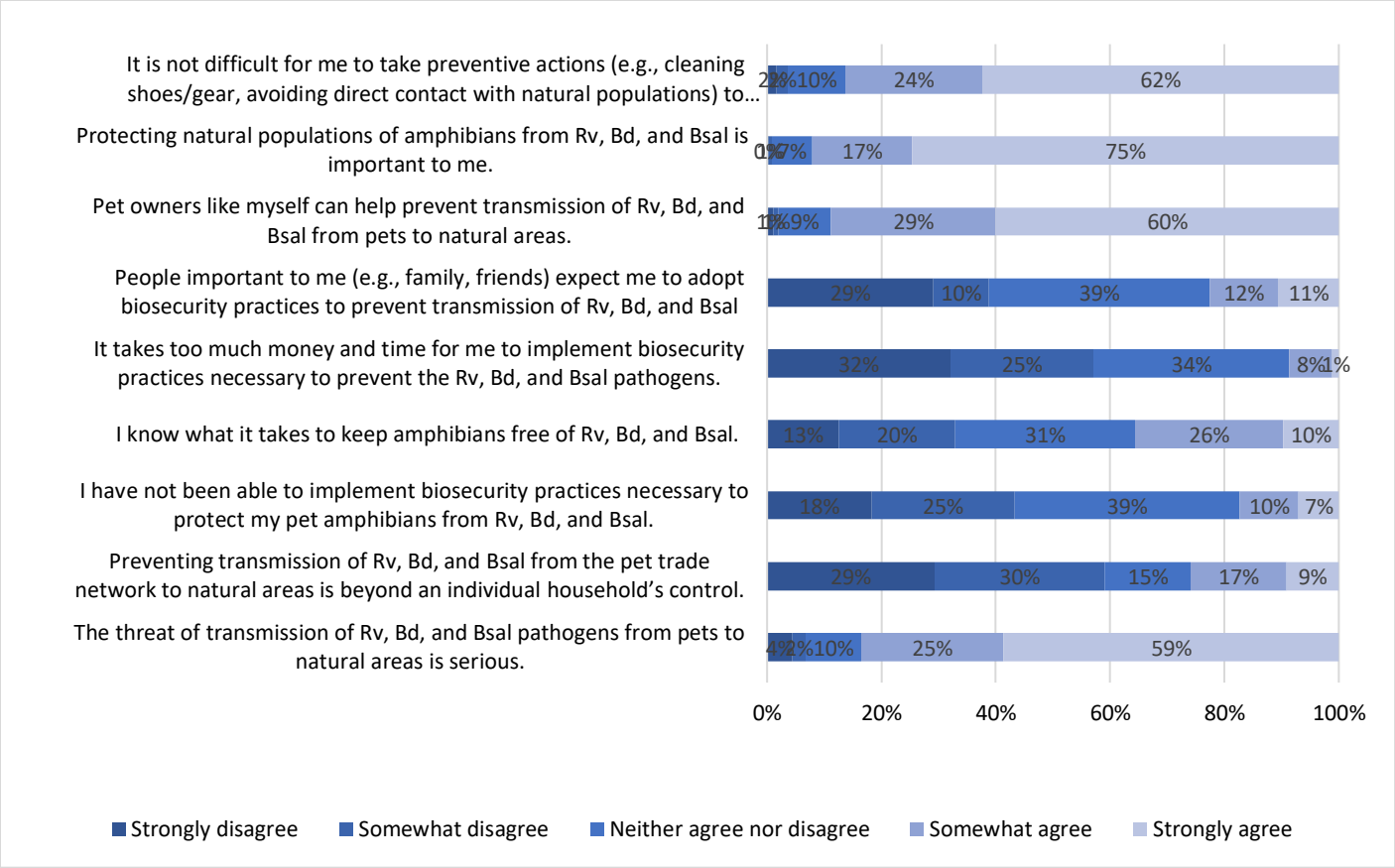


Figure 29. Level of agreement and disagreement with statements related to Bd, Bsal and Rv transmission (From top: n1=358, n2=359, n3=356, n4=359, n5=359, n6=358, n7=358, n8=355, n9=359),

When asked about the likelihood of taking various steps to limit the spread of harmful pathogens, most respondents indicated they were extremely likely to take the steps listed (Fig. 30).

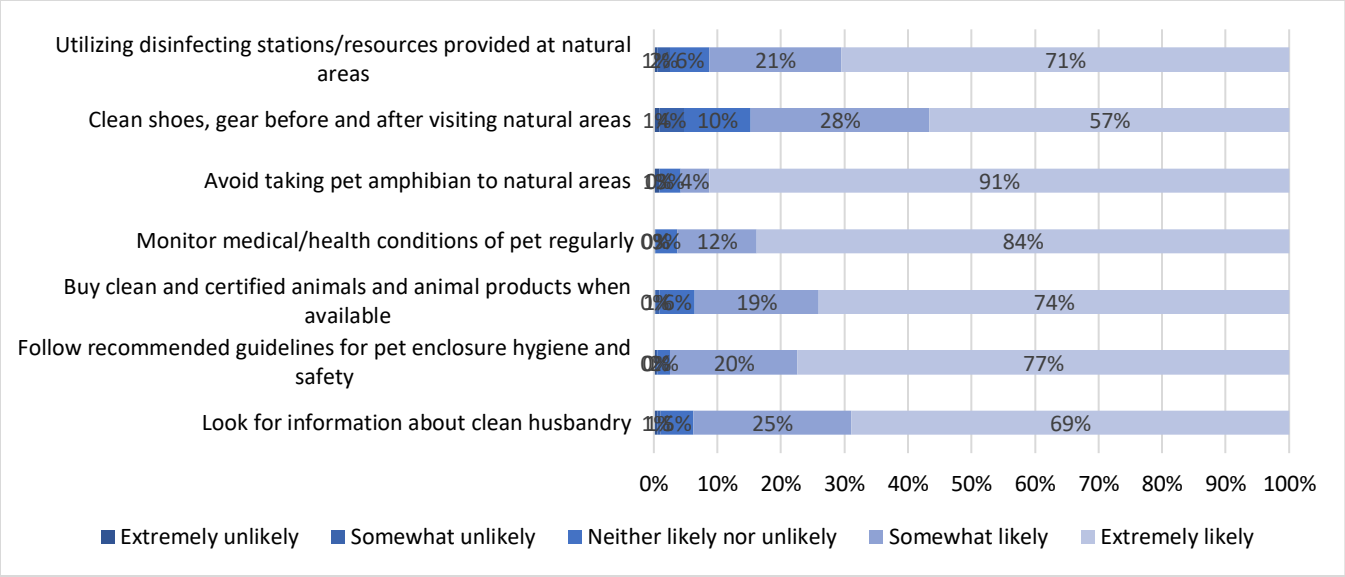


Figure 30. Likelihood of taking various steps to limit the spread of harmful pathogens (From top: n1=354, n2=350, n3=355, n4=353, n5=357, n6=356)

Correlations between responses

Results suggest a positive relationship between length of amphibian ownership and number of amphibians owned with 68% of respondents that have owned amphibians for over 10 years indicating they have owned more than 10 amphibians, while only 17% of respondents who have owned amphibians for 1-4 years reported owning more than 10 amphibians (Fig. 31).

	Number of amphibians owned				
Years owned amphibians	1	2-4	5-7	8-10	More than 10
1-4 years	16%	40%	19%	8%	17%
5-7 years	2%	30%	22%	15%	32%
8-10 years	0%	14%	28%	10%	48%
Over 10 Years	1%	7%	14%	10%	68%

Figure 31. Number of amphibians owned by years of ownership

Overall, seventy-nine percent of all consumer respondents (305) indicated they had had an amphibian in their care die. Ninety-five percent of consumers that had owned amphibians for more than 10 years had had a pet amphibian die, 89% of those owning amphibians for 8-10 years had had an amphibian die, 86% of those owning 5-7 years and 61% of those owning amphibians owning 1-4 years (Fig. 32).

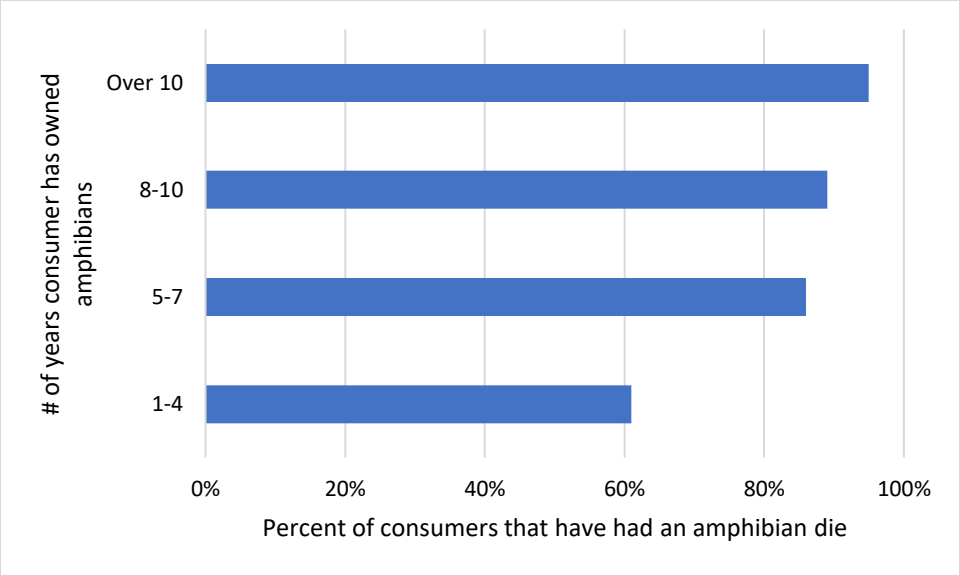
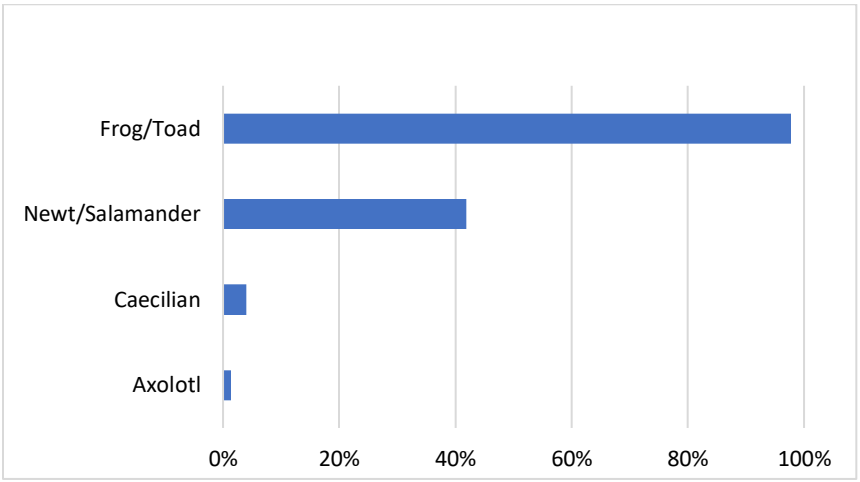


Figure 32. Percent of consumers indicating they've had a pet amphibian die by years of amphibian ownership. (From top, n1=163, n2=59, n3=28, n4=137)

Of the 19% (76) consumers indicating they had collected an amphibian from the wild, 98% indicated they had collected a frog or toad, 42% a newt or salamander, 4% a Caecilian, and 1% indicated they had collected an axolotl (Fig. 33).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*

Figure 33. Amphibian ownership by respondents indicating they've acquired amphibians by collecting from the wild. (n=76)

Tables 34-38 report the additional amounts of money consumer respondents indicated they would be willing to pay for an amphibian certified to be free of *Bsal*, *Bd* and *Rv* by the amount the consumer paid for their most recently acquired amphibian.

Respondents that paid $0 for their amphibian	
Premium for certified animal ($)	Consumers WTP premium (%)
1	19%
2	3%
3	13%
5	6%
7	19%
10	9%
15	6%
20	16%
30	3%
50	6%

Figure 34. WTP for certified disease-free animal for respondents paying $0 for most recently acquired amphibian (n=32)

Respondents that paid $1-$25 for their amphibian	
Premium for certified animal ($)	Consumers WTP premium (%)
1	13%
2	12%
3	13%
5	10%
7	7%
10	10%
15	12%
20	6%
30	7%
50	9%

Figure 35. WTP for certified disease-free animal for respondents paying $1-$25 for most recently acquired amphibian (n=68)

Respondents that paid $26-$75 for their amphibian	
Premium for certified animal ($)	Consumers WTP premium (%)
1	12%
2	12%
3	9%
5	11%
7	12%
10	11%

15	9%
20	7%
30	9%
50	7%

Figure 36. WTP for certified disease-free animal for respondents paying $26-$75 for most recently acquired amphibian (n=161)

Respondents that paid $76-$125 for their amphibian	
Premium for certified animal ($)	Consumers WTP premium (%)
1	7%
2	12%
3	17%
5	17%
7	15%
10	5%
15	5%
20	10%
30	5%
50	7%

Figure 37. WTP for certified disease-free animal for respondents paying $76-$125 for most recently acquired amphibian (n=41)

Respondents that paid over $125 for their amphibian	
WTP Amount ($)	% Of respondents WTP
1	7%
2	11%
3	7%
5	15%
7	11%
10	11%
15	4%
20	15%
30	11%
50	7%

Figure 38. WTP for certified disease-free animal for respondents paying over $125 for most recently acquired amphibian (n=27)

Amphibian Business Survey

Respondent Business Characteristics

Of 122 amphibian businesses responding to the survey, 75% reported dealing with amphibians and reptiles, 16% indicated they deal with amphibians only, and the remaining 8% deal with reptiles only (Fig. 39).

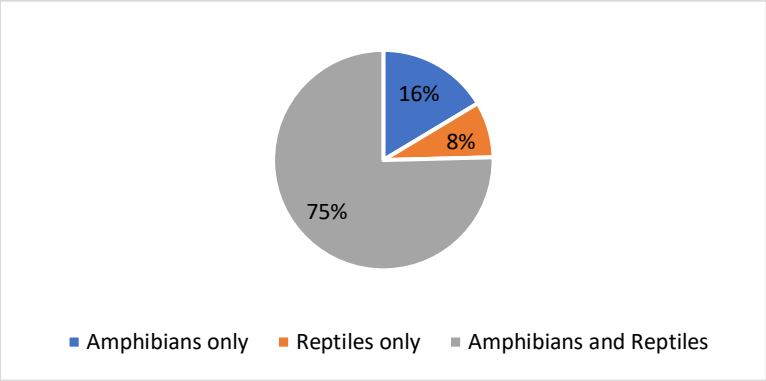
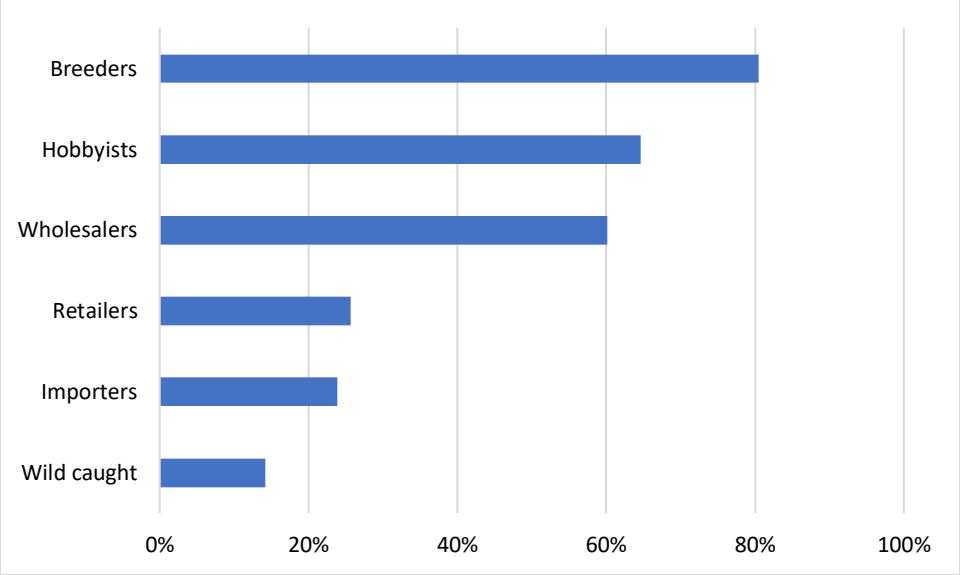


Figure 39. Types of animals business deals with (n=122)

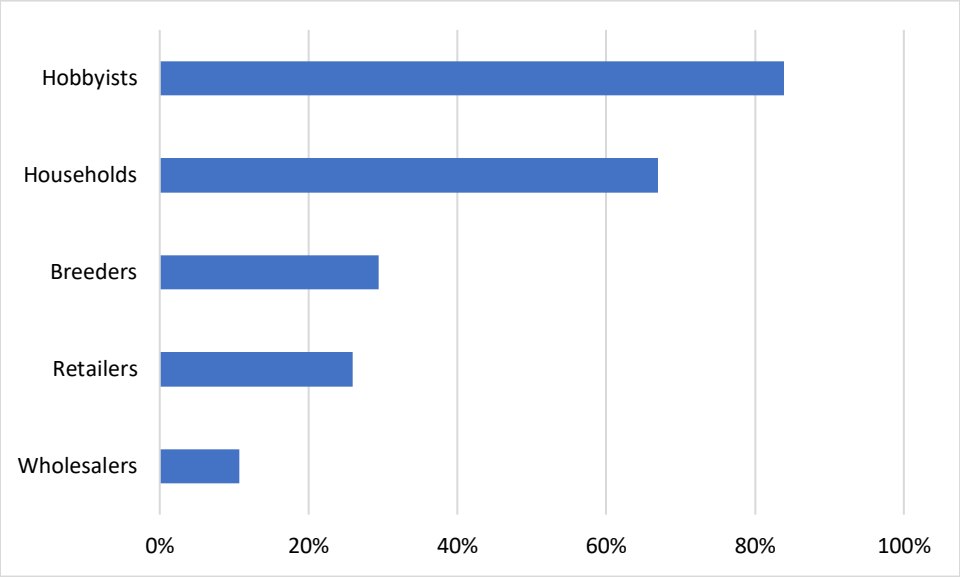
Of 113 amphibian businesses, 81% indicated the most used source for obtaining their amphibians is breeders, followed by hobbyists (76%), wholesalers (60%), retailers (26%), importers (24%), and wild caught (14%) (Fig 40).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*

Figure 40. Where respondent businesses acquire amphibians (n=113)

Amphibian businesses reported the most common purchasers of their amphibians are hobbyists, with 84% of businesses selling to hobbyists, followed by households (67%), breeders (29%), Retailers (26%), and wholesalers (11%) (Fig. 41).



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 41. Parties business sells amphibians to (n=112)

About 29% of the businesses responding indicated to have been in the amphibian business over 20 years, whereas the other 26% indicated being in the business for 1 to 5 years only (Fig. 42).

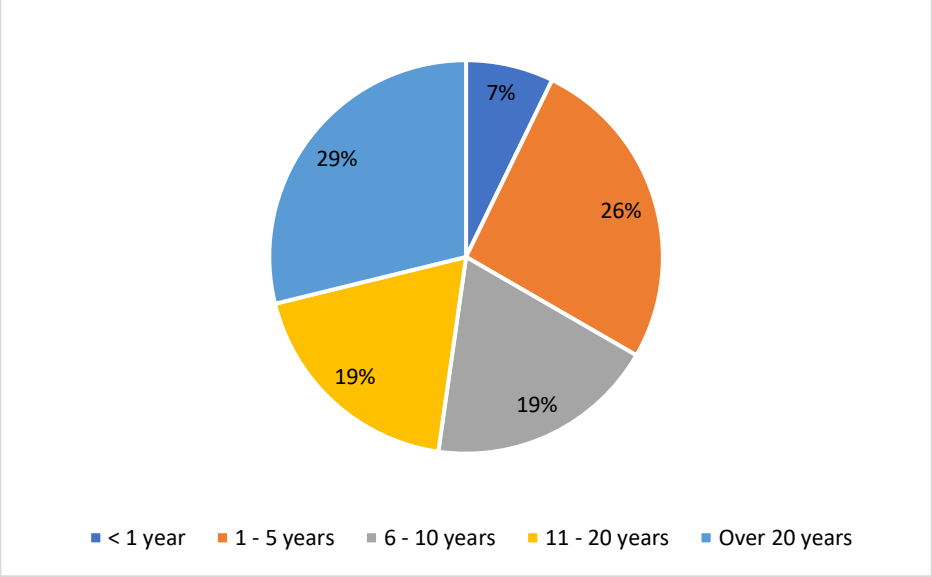


Figure 42. Number of years in the amphibian business (n=111)

In terms of the annual sales volume, approximately one-third reported less than $5,000 and another one-third (36%) indicated somewhere between $5,000 and $500,000). The remaining one-third (33%) indicated over $500,000 of annual sales (Fig. 43).

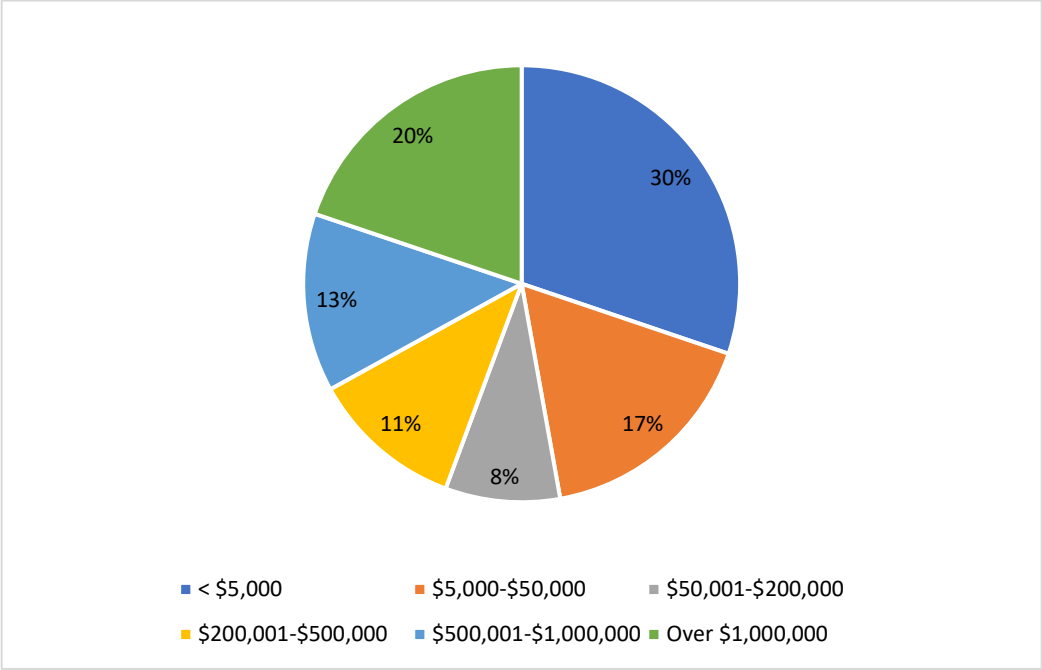


Figure 43. Annual sales of business (n=106)

Approximately half (51%) of amphibian businesses responding indicated that amphibian sales accounted for less than 10% of their total sales. Twenty-three percent reported amphibian sales accounted for 10%-25% of total sales, 14% businesses reported 26%-75%. The remaining 12% reported amphibian sales accounted for 76%-100% of their total sales (Fig. 44).

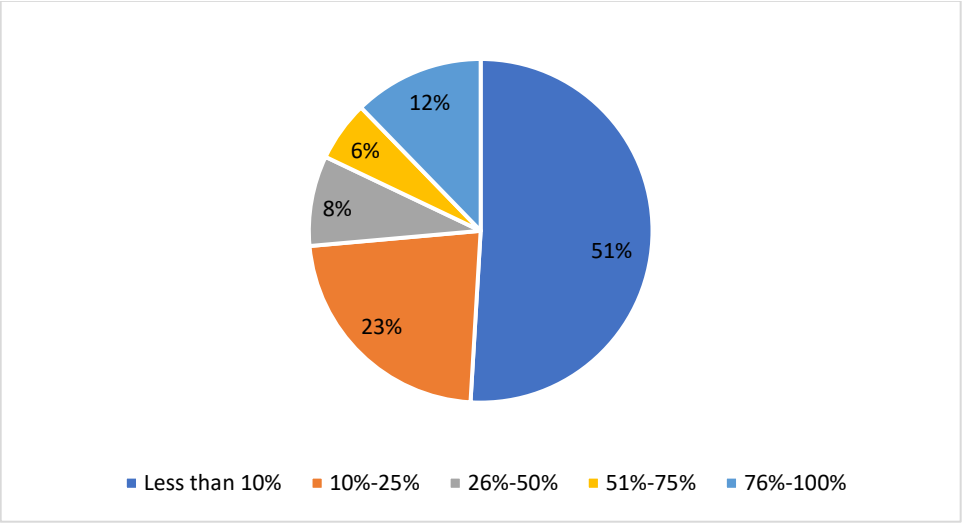


Figure 44. Percentage of total sales attributed to amphibians (n=106)

In terms of the employee size of the businesses responding to the survey, 45% reported having 1-2 employees, 17% had 3-5 employees, 25% had 6-20 employees, and the remaining 12% reported having more than 20 employees (Fig. 45). Nearly half of the responding businesses reported being operated as sole proprietorship (Fig. 46).

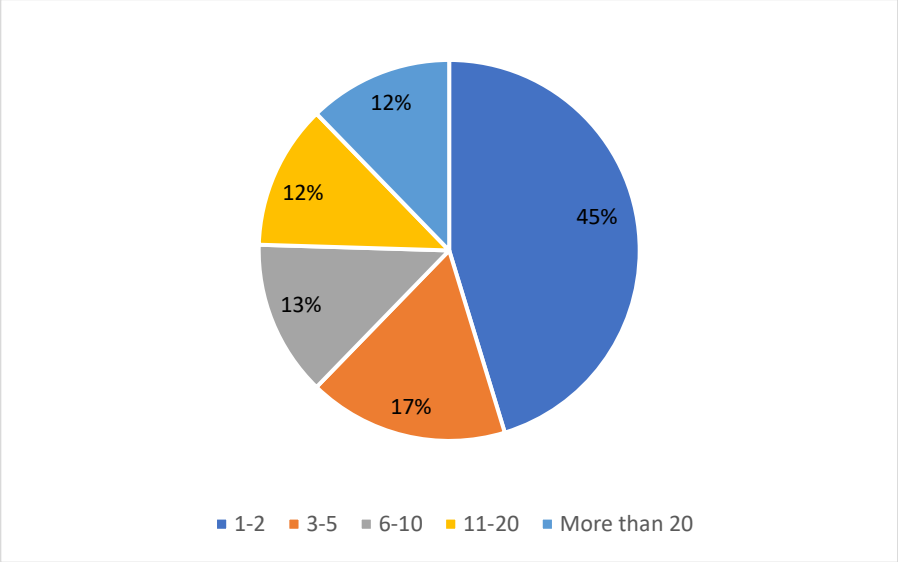


Figure 45. Number of employees at the responding businesses (n=106)

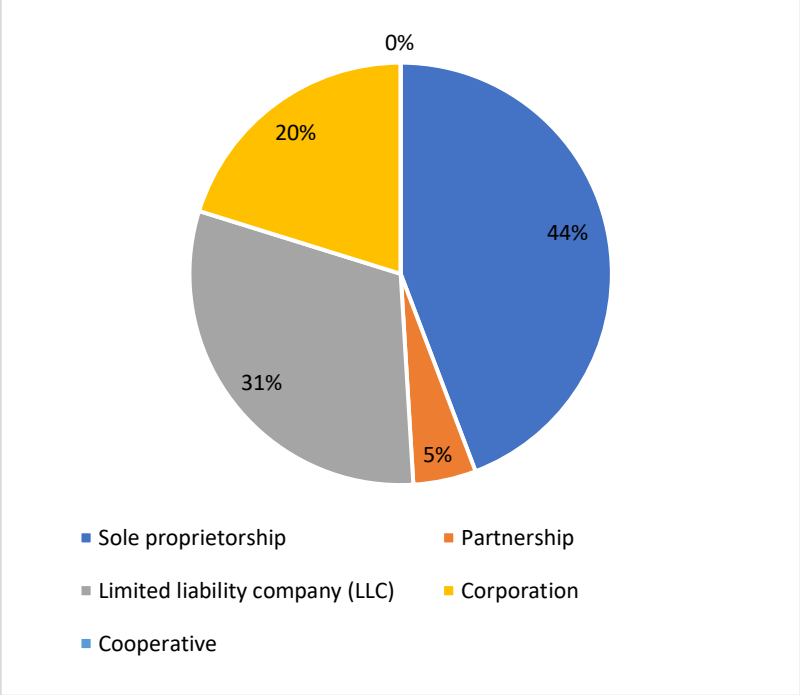


Figure 46. Type of ownership of business (n=104)

One-third of the responding businesses indicated they are in the Midwest, 20% indicated they are in the Southeast, 13% indicated they were in each of the Northeast, Southwest, and Pacific Northwest regions (Fig. 47). The final 8 % reported being in the Rocky Mountain region.

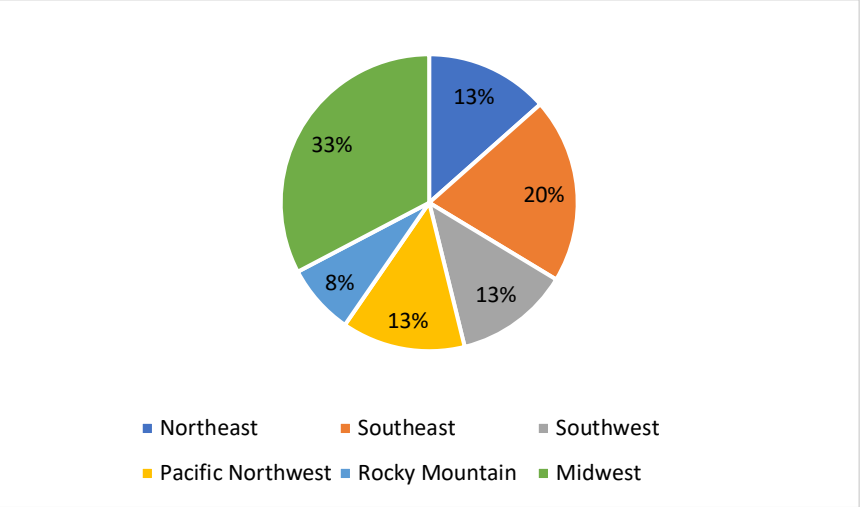


Figure 47. Geographic region of business location (n=104)

When asked whether their organization conducts business with buyers or sellers of amphibians outside the country, 82% responding businesses indicated “No” and the other 10% responded they do business with buyers and sellers outside the country. The remaining 8% indicated did not know (Fig. 48).

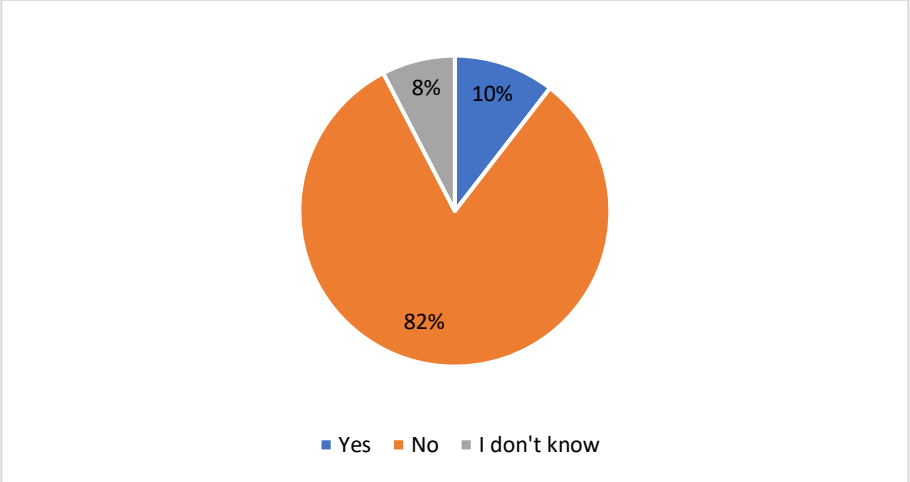


Figure 48. Business with buyers and sellers outside of the United States (n=105)

Fifty-eight percent of the responding businesses indicated that there were, to their knowledge, other businesses, or organizations similar to theirs currently operating within the same state Fig. 49). Similarly,

38% indicated they were not sure, while the remaining 4% indicated they were not aware of other businesses in the state.

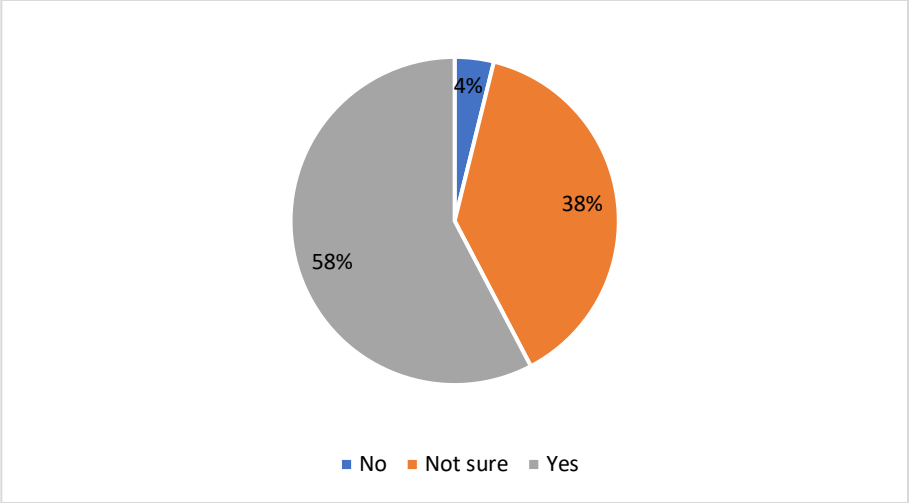
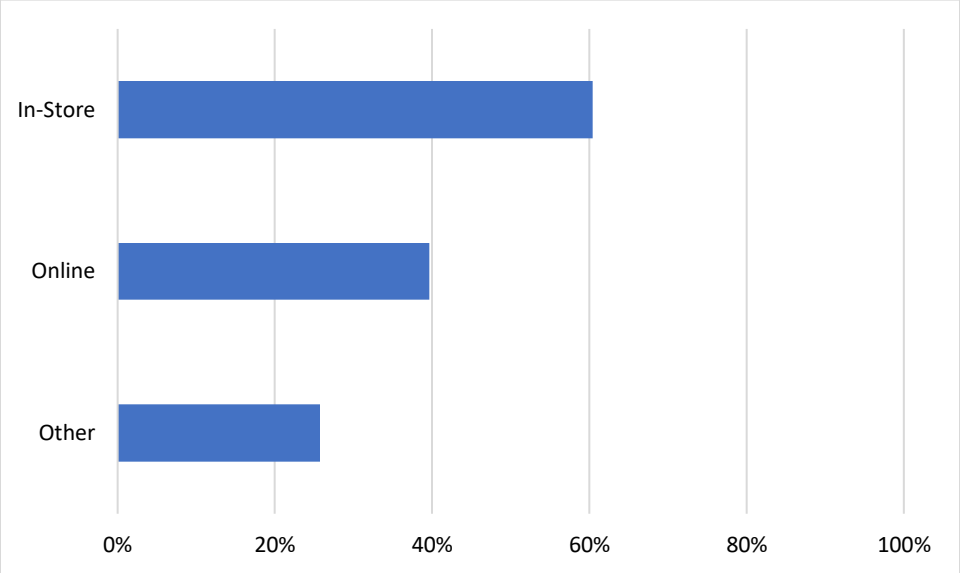


Figure 49. Knowledge of businesses like respondents operating within same state as respondent

Regarding the mode of sales operation of their business, in-store operation was reported by the majority (60%) of respondents and online operation was indicated by less than half (40%). About 26% reported selling/supplying amphibians by other means including expos and trade shows (Fig. 50).



Sum of percentages exceeds 100% as multiple responses may be selected by each respondent
Figure 50. Mode of selling amphibians (n=101)

Businesses were asked to rate the importance of the following factors in making business decisions. Relatively high level of importance was placed on following ethical business practice, legal compliance, improving public image and profit, and responding to issues of social concerns (Fig. 51).

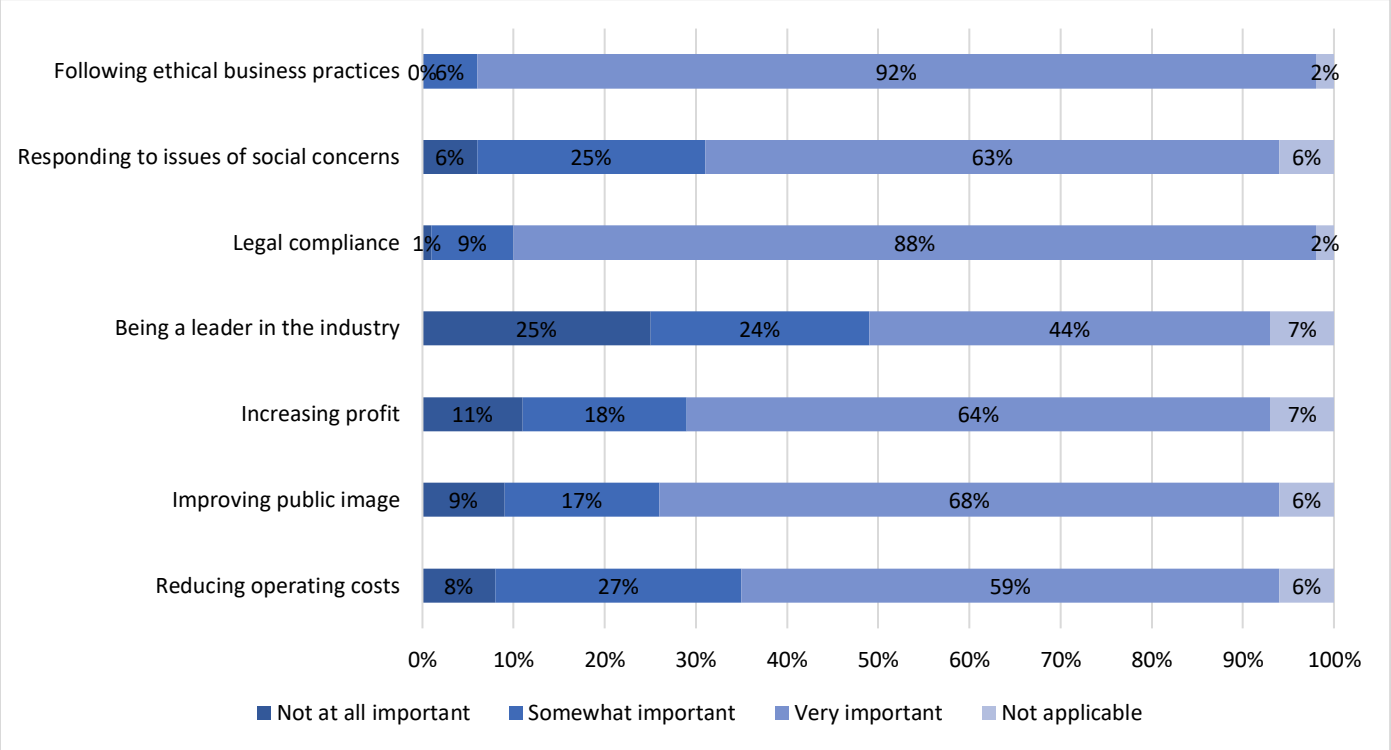


Figure 51. Importance of factors in making business decisions (From top: n1=100, n2=100, n3=100, n4=100, n5=100, n6=100, n7=100)

Awareness of Pathogens and Adoption of Biosecurity Practices

Slightly less than half (47%) indicated that before reading the survey they were unaware of beneficial microbes, such as *Bacillus mycoides*, that can kill harmful microbes and increase disease resistance in amphibians (Fig. 52). When asked if they would consider administering treatment to their pet amphibian using "probiotics" such as *Bacillus mycoides*, 47% indicated they will definitely do so, whereas the remaining 53% indicated they may be interested but need more information (Fig. 53).

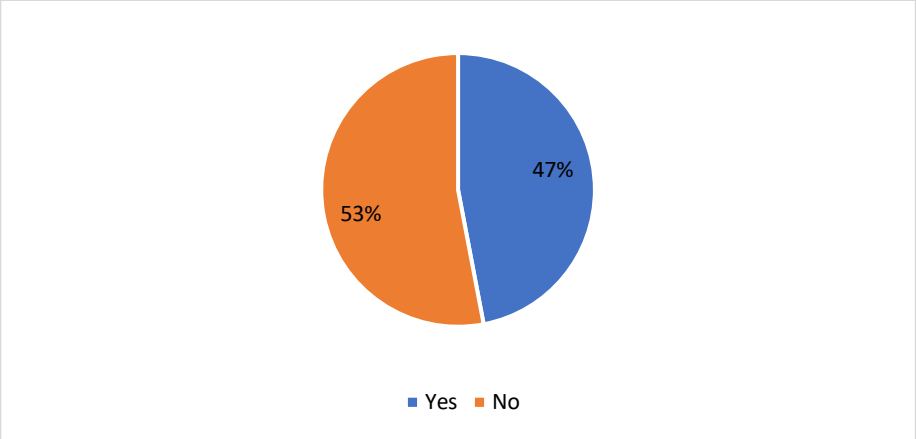


Figure 52. Percentage of respondents aware of beneficial microbes like Bacillus mycoides prior to reading this survey

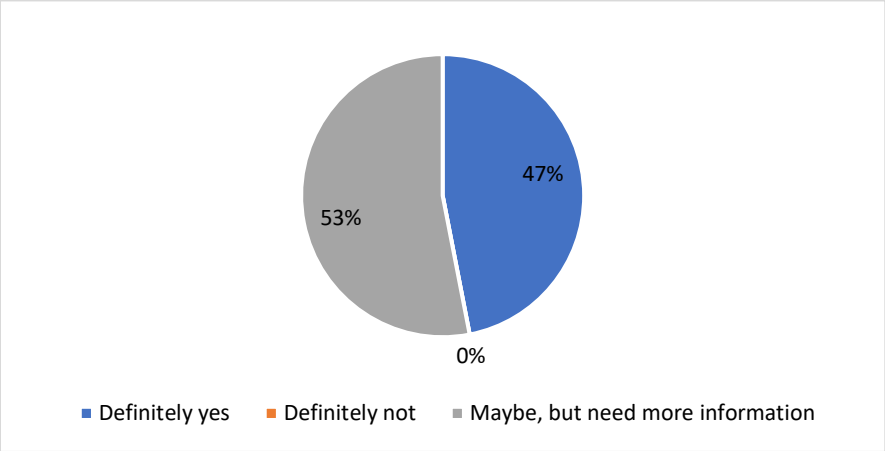


Figure 53. Percentage of respondents that would consider administering treatment to their pet amphibian using "probiotics" such as Bacillus mycoides (n=98)

Prior to reading the survey, 81% were aware that the pathogens *Bd*, *Bsal*, *Rv* can be transmitted through the pet trade, while the remaining 19% indicated they were not (Fig. 54).

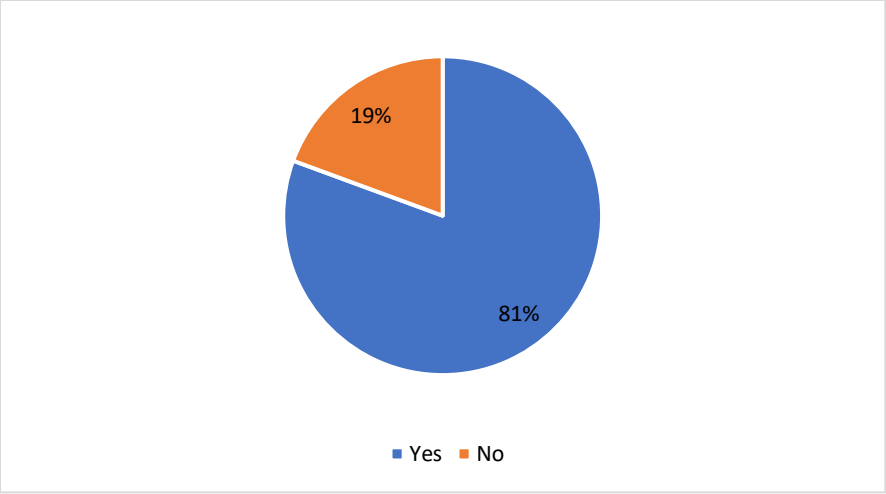
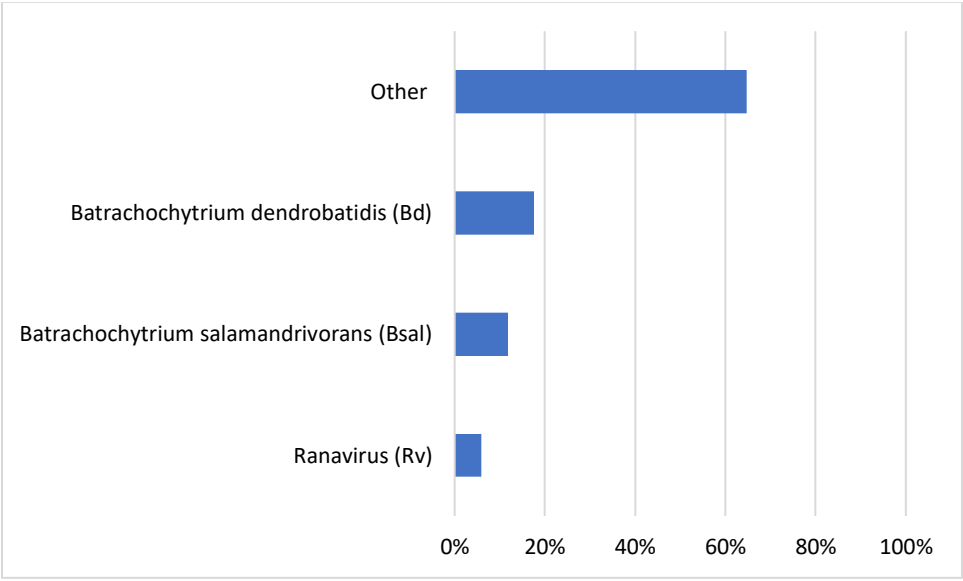


Figure 54. Percentage of respondents aware prior to reading survey that Bd, Bsal, and Rv can be transmitted through the pet trade (n=98)

Seventeen businesses indicated a pathogen had been detected in amphibians at their facility. Of those, 18% percent indicated *Bd* had been detected, 12% (2 businesses) indicated *Bsal* had been detected, 6% indicated *Ranavirus* had been detected and 65% reported the detection of another type of pathogen (Fig. 55).

To date, *Bsal* is not known to have been detected in North America in the wild or archived museum and DNA samples. However, requisite levels of surveys and monitoring have not yet been conducted in order to state conclusively that *Bsal* is not yet here, undetected (salamanderfungus.org).



Sum of percentages exceeds 100% as multiple responses may be selected by each respondent
Figure 55. Percentage of respondents that have detected pathogens at their facility (n=17)

Sixty-three percent of businesses indicated they had had an amphibian die from illness or disease in their business facilities, while 37% indicated they had not (Fig. 56). Businesses indicated the average value of total loss resulting from the illness or death of an animal at their business facility was $145, with minimum and maximum values of $5 and $700, respectively.

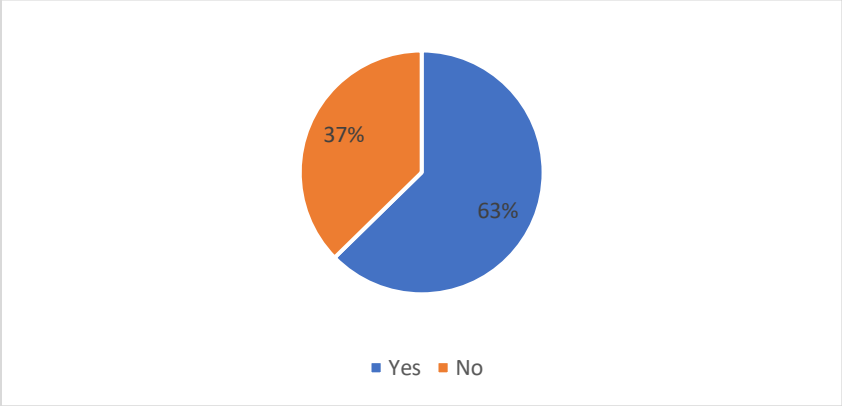


Figure 56. Percentage of businesses that have had an amphibian die from illness or disease (n=75)

Fifty-five percent of the responding businesses indicated they were very concerned that transmission of pathogens through the trade network of pets or pet products may impact the amphibian(s) in their facility. Thirty-one indicated being slightly concerned and the remaining 13% were not concerned (Fig. 57)

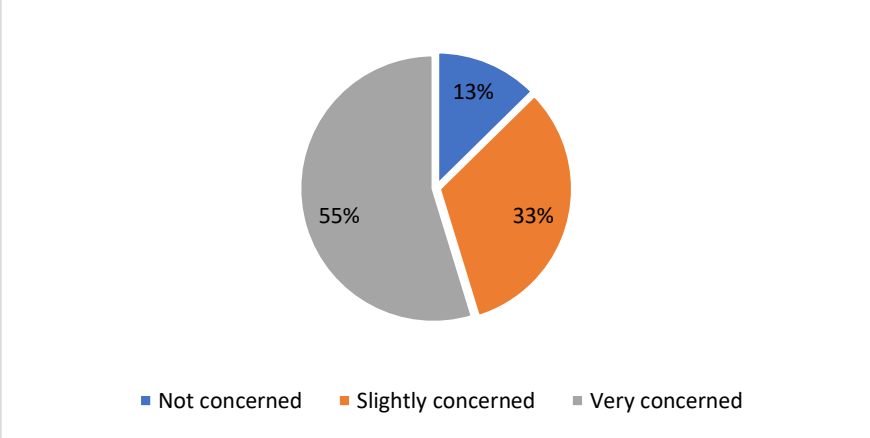
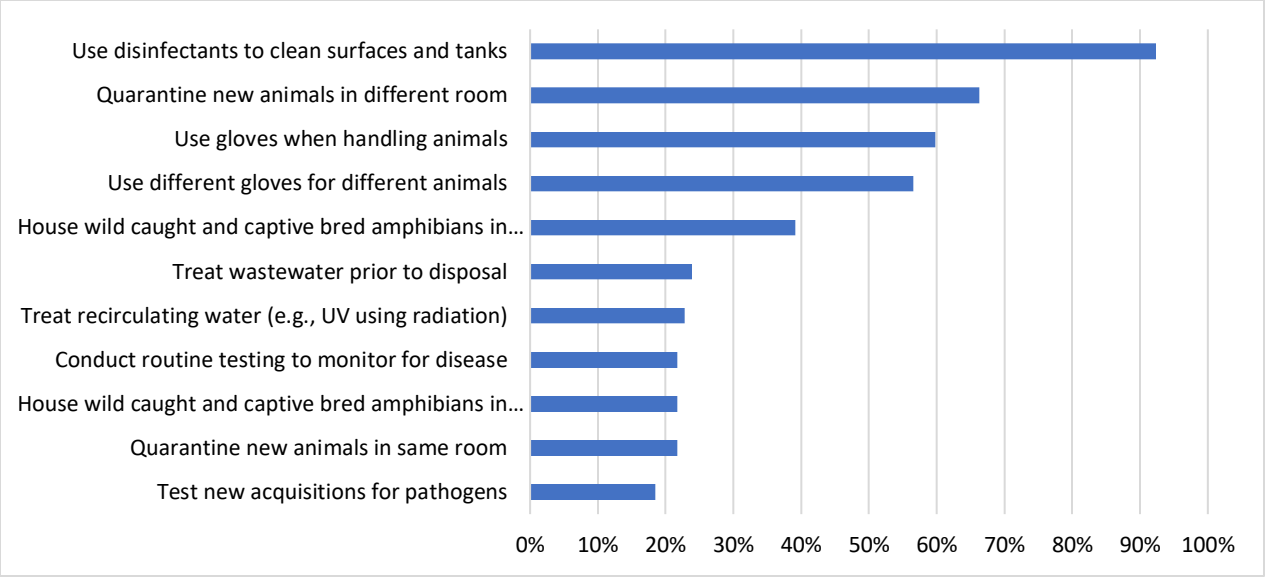


Figure 57. Level of concern transmission of pathogens through the trade network of pets or pet products may impact amphibians in respondent's facility (n=95)

Businesses were asked whether they took the following biosecurity measures at their facility. While most businesses indicated they use disinfectants to clean surfaces and tanks, use gloves for different animals and quarantine new animals in a separate room, few businesses test new acquisitions for pathogens, conduct testing to monitor for disease, or treat recirculating water or wastewater prior to disposal (Fig. 58).



Sum of percentages exceeds 100% as multiple responses may be selected by each respondent
Figure 58. Percentage of businesses taking various biosecurity precautions (n=92)

When asked about the importance of amphibian health, 54% indicated it was extremely important to them that an animal be healthy and free of the *Bd*, *Bsal*, and *Rv* pathogens when introducing it to their facility, 32% indicated it is very important (Fig. 59).

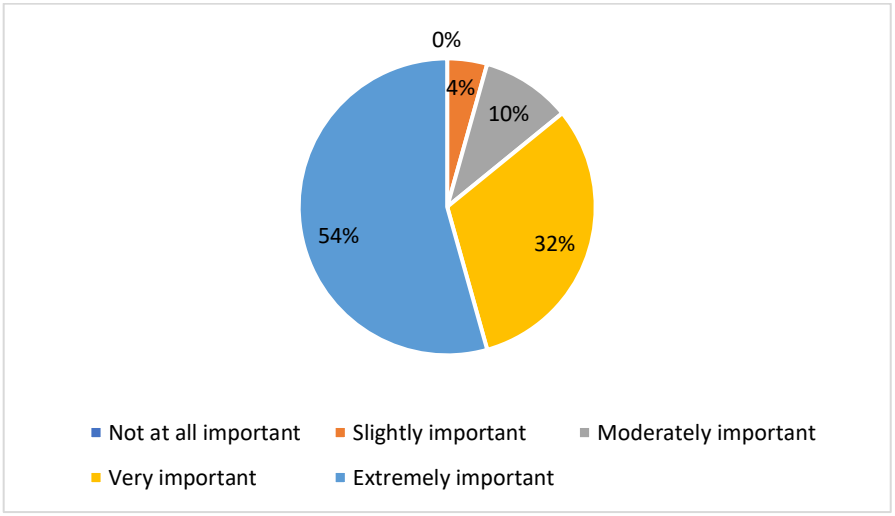


Figure 59. Level of importance placed on amphibians that are healthy and free of Bd, Bsal and Rv (n=92)

Business willingness to pay for pathogen-free amphibian

Nearly all (97%) of the businesses responding this survey indicated that, when introducing a new pet amphibian to their business, they would be interested in acquiring an animal that is certified as free of the *Bd*, *Bsal*, and *Rv* pathogens (Fig. 60).

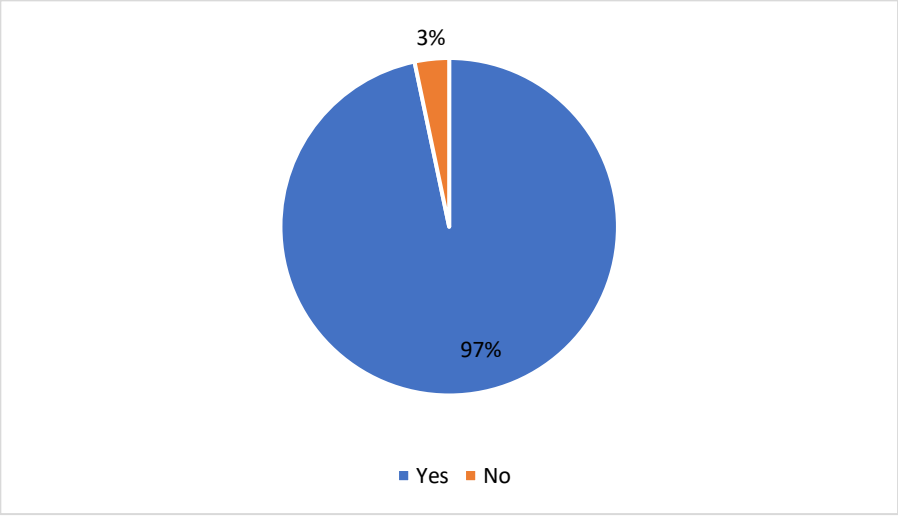


Figure 60. When introducing a new animal to their business, the percentage of respondents interested in acquiring an animal that is certified as free of the Bd, Bsal and Rv pathogens (n=91)

When asked if they would be willing to pay more for healthy animal, 59% indicated they would be willing to pay more for an animal that is certified as free of the *Bd*, *Bsal*, and *Rv* pathogens, while 35% indicated they were not sure (Fig. 61). Only 5% declined to pay more for an animal that is certified as pathogen free.

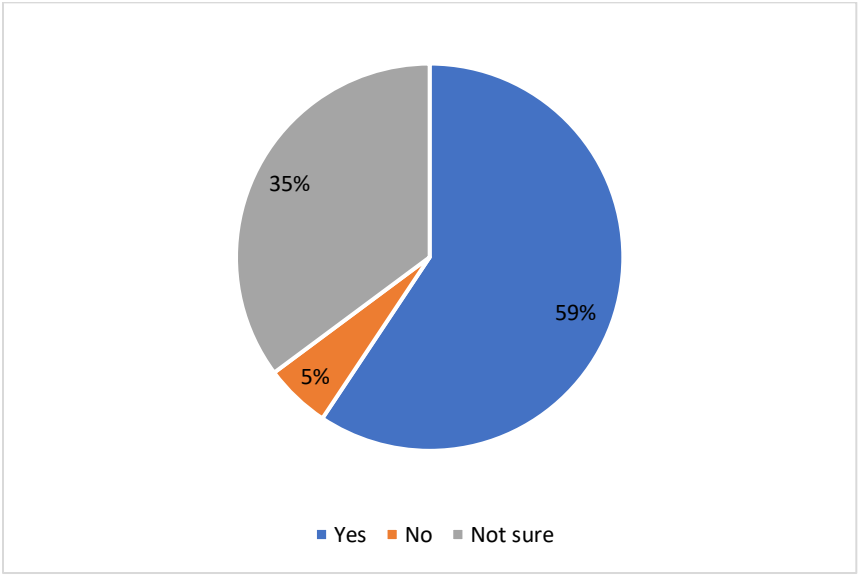


Figure 61. Percentage of respondents according to their willing to pay more for an animal that is certified as free of the Bd, Bsal and Rv pathogens (n=91)

The respondents who were willing to pay more for a certified animal were asked the approximate amount they would be willing to pay in addition to the price for non-certified animal. About 22% indicated they would be willing to pay 1-5% more, 36% indicated 6-10% more, and 28% indicated they would be willing to pay 11-20% more (Fig. 62). Similarly, 9% indicated a willingness to pay somewhere between 21-100% more and the remaining 5% indicated a willingness to pay over 100% more than the price of an animal that is not certified free of the *Bd*, *Bsal*, and *Rv* pathogens.

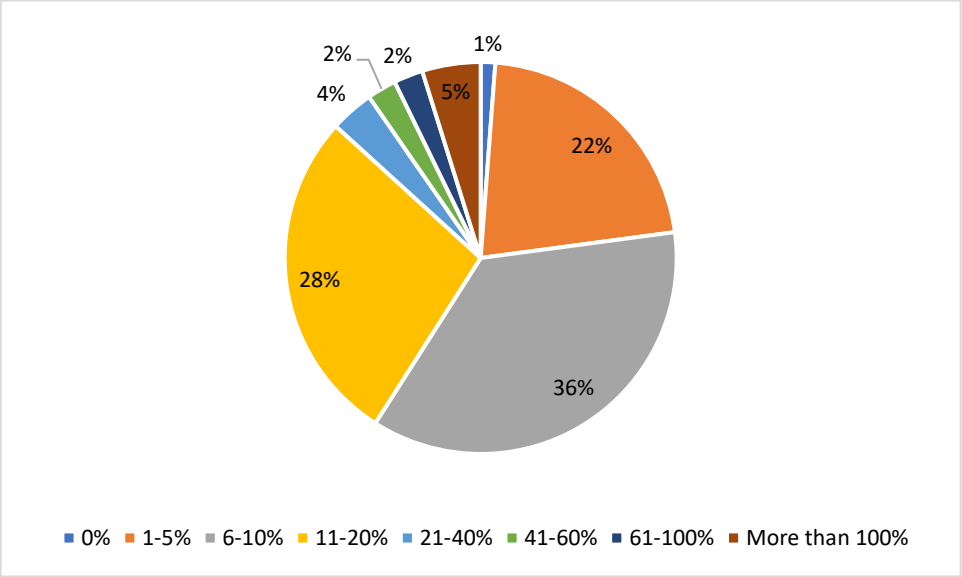


Figure 62. Percentage of respondents willing to pay various increases in price to acquire amphibian that is certified free of the Bd, Bsal, and Rv pathogens (n=83)

Respondents were asked about their perceived ability to improve the biosecurity at their facility without increasing the sales price. Slightly less than half (46%) indicated they were not sure but 25% indicated they can do so without increasing the selling price to their consumers. The remaining 29% indicated they cannot do so (Fig. 63).

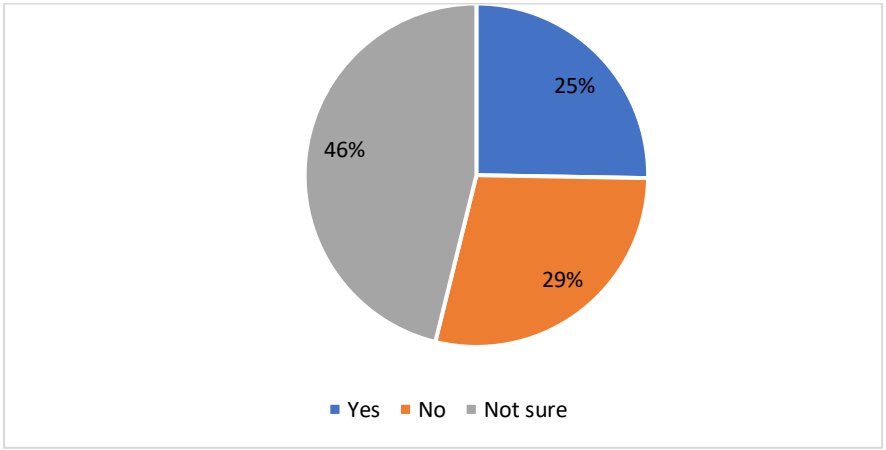


Figure 63. Percentage of respondents who believe they can improve biosecurity practices without increasing costs (n=91)

When businesses were asked how much they would expect the average amphibian sales price to increase if they were to ensure the animal was free of *Bd*, *Bsal*, and *Rv,* 27% indicated "Not sure", 27% indicated 11-20%, and 24% indicated 6-10% (Fig. 64).

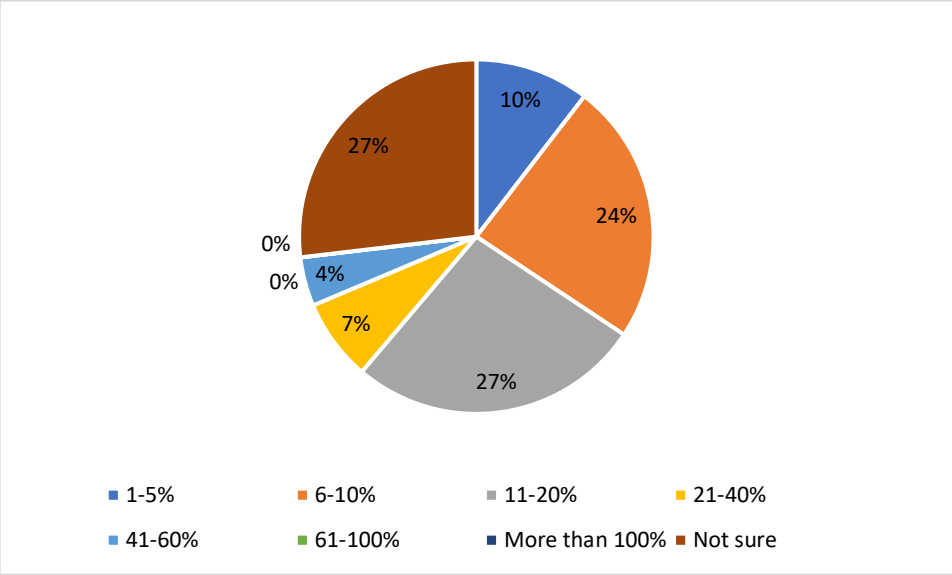


Figure 64. Respondents' perception of needed increase in price to ensure animal is free of Bd, Bsal, and Rv (n=67)

When asked if increasing sales price is not an option, what the maximum increase in operating cost their organization may be willing to accept and still adopt improved biosecurity practices to keep the amphibians in their facility free of the *Bd*, *Bsal*, and *Rv* pathogens, 30% indicated "Not sure", 24% indicated 6-10%, and 21% indicated 1-5% (Fig. 65).

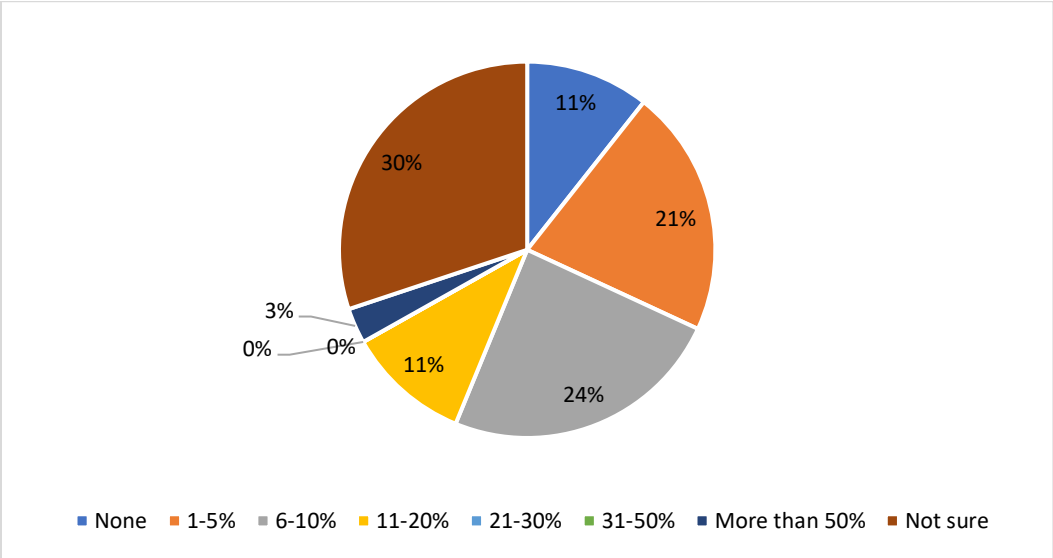


Figure 65. Maximum acceptable increase in operating costs to improve biosecurity practices (n=66)

Attitudes and Intentions Regarding Adoption of Mitigating Actions

Overall, the majority (85%) of responding businesses indicated they believe the threat of the spread of *Bd*, *Bsal*, and *Rv* is serious (Fig. 58). Also, respondents have a responsibility to mitigate their spread to protect natural amphibian populations, and 90% believe that businesses should take part in preventing the transmission of those pathogens in the trade network (Fig. 66).

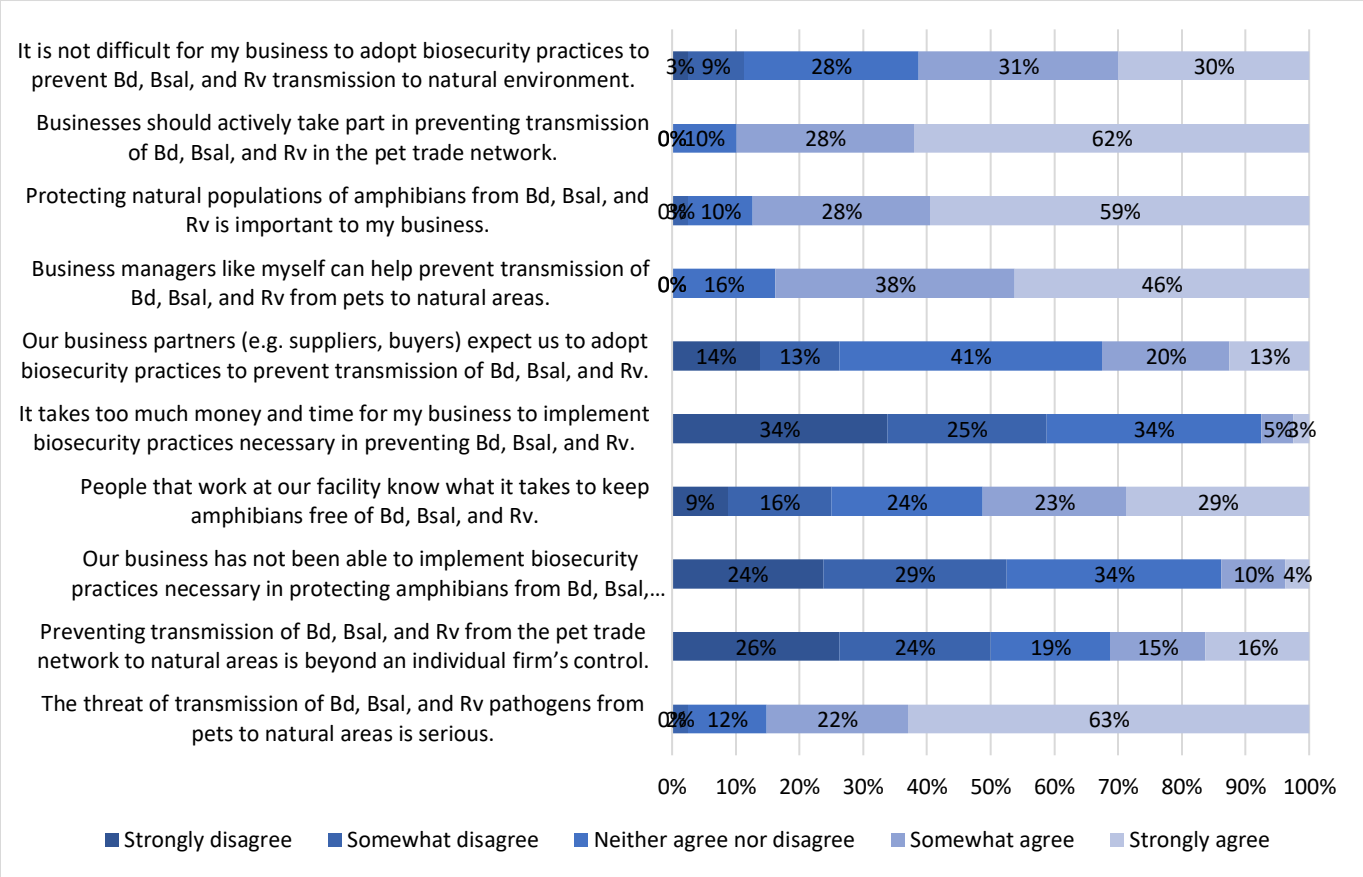


Figure 66. Business attitudes toward biosecurity measures and pathogen transmission (From top: n1=81, n2=80, n3=80, n4=80, n5=80, n6=80, n7=80, n8=79, n9=79, n10=80)

Overall, businesses indicated they believe they will benefit from adopting biosecurity practices to mitigate the spread of harmful pathogens, with the majority (68%) indicating they intend to implement practices at their facility to contain *Bd*, *Bsal*, and *Rv* (Fig. 67). While more than three-quarters (82%) agreed that keeping their facility free of pathogens will enhance the public image, just over half (52%) agreed it will impact their profit.

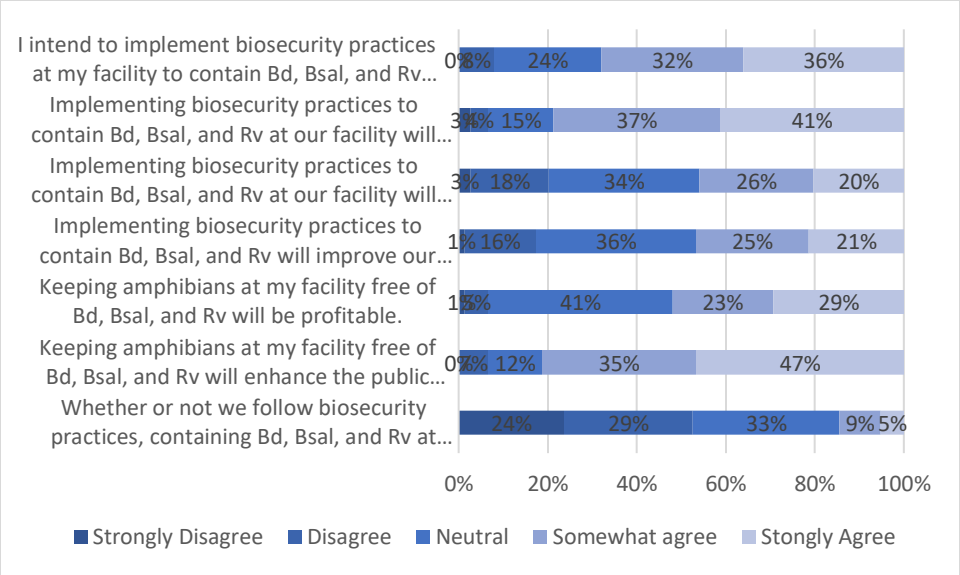
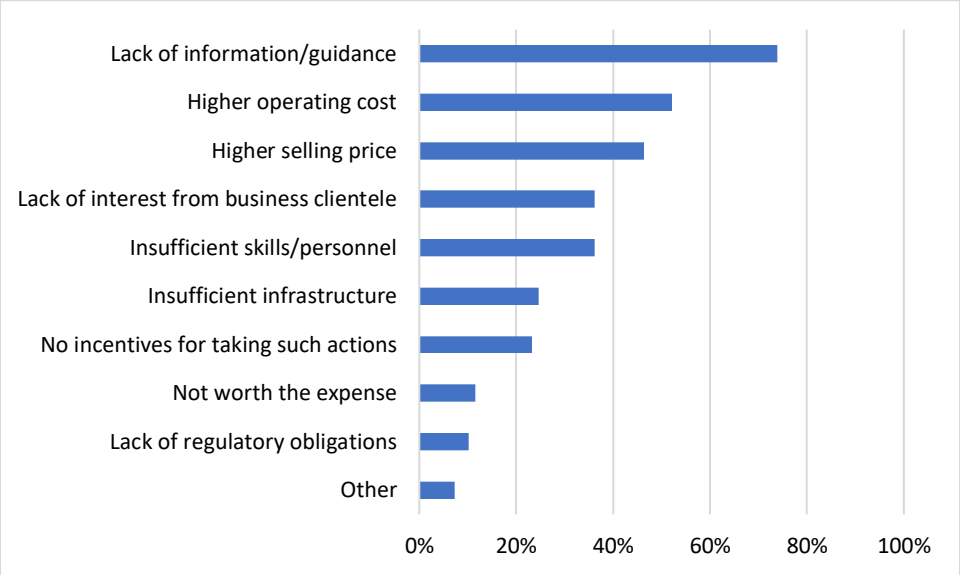


Figure 67. Business attitudes toward adopting practices to mitigate pathogen transmission (From top: n1=75, n2=75, n3=74, n4=75, n5=75, n6= 75, n7=76)

When asked which of the following, if any, would be considered barriers to adopting biosecurity practices to prevent or contain *Bd*, *Bsal*, and *Rv* in their facility, the most frequently cited barriers were lack of information/guidance (74%), higher operating cost (52%), and higher selling price (46%) (Fig. 68). About one-third (36%) also cited lack of interest from their business clientele and insufficient skills/personnel, whereas about one-quarter mentioned lack of infrastructure and lack of incentives for taking such actions.



**Sum of percentages exceeds 100% as multiple responses may be selected by each respondent*
Figure 68. Barriers to adopting biosecurity practices (n=69)

APPENDIX

Text responses provided to survey questions with "Other" as an answer choice.

What other type of amphibian(s) do you currently own or have you previously owned?

Tortoise
Axolotl
gecko
Turtle
Gecko
Axolotl
Mourning geckos
Snake, lizards
Axolotl
Veil chameleon
Snakes
axolotl
Siren
Bearded dragon
Axolotl
I mostly do lizards (reptiles) I just have a slight scattering of amphibians, mostly from my local region.
Axolotl

What other type(s) of pet(s) currently resides or previously resided in your household?

tarantulas, vinegarroon, and copepods
Opossums
Invertebrates
several invertebrates
Invertebrates
Hedgehog
Shrimp
invertebrate
Rabbits, hamsters, gerbils, guinea pigs
Rabbit, tarantula (5)
Tarantulas
Equine
Spiders
Arachnids
squirrels
Alpacas, chinchilla, hedgehogs, lizards snakes
Sugar Gliders
Snail, spider, crayfish
Rodents, hedgehogs, insects, and tarantulas
sugar glider

coral
Ferret
Rats, hamsters, mouse and gerbil
Hermit crabs, wolf spider, isopods
Gerbils
Spiders/Mantis/Millipedes
pill bugs and ant colonies
Bugs
rabbit, chinchilla
arachnid
chickens
Skink
Guinea pig
Horses, gerbils, guinea pigs
Hamster
Guinea pig
Rats
scorpion
tarantulas
Arthropod
Coral
Hedgehog, hamster
Pigs, rabbits, rats, mice, chickens, axolotls and tortoises
Rabbit, Guinea pig, invertebrates
Snails and tarantula
Chinchilla, Rabbit, Hamster
Rabbit, Chinchilla, Hamster
Axolotl
Rabbit
Rats, Guinea Pigs, Mice, Hamsters, Hedgehogs, Rabbits
Rabbit
Chickens
Horse, goats, chickens, ducks
Axolotls
Invertebrates
Tarantulas
Horse, cattle
Large farm animals, chinchillas, rabbits, rodents
Gecko
Arachnids, shrimp and isopods
Tarantulas
Rats
Isopods spiders scorpions and mantids
Rat
Chickens, pheasants, quail, rabbit
Isopods, tarantulas, scorpion
Invertebrates
small animals

invertebrate
small mammal
Skunk, sugar gliders
Various small animals/rodents
scorpion, tarantula
Horse
Rodents
Arthropods (tarantula, insects, etc.)
Hedgehog
Rabbit
Guinea pigs, rabbits
Skunk
Rabbit
inverts and small mammals
Ferret, Crabs
Praying mantis and hermit crab
Arachnid
Rabbit, hamster
tarantula, snails
hamster
Hermit crabs, bugs
Rabbit
Invertebrates
Small mammals
Rabbits
Ferret
Centipede
gerbils, hamsters, rats, mice, guinea pigs, rabbits
Various invertebrates
Insect
Rat, Invertebrate
Isopods - and the birds are livestock and outside 99% of the time.
Rodents (Guinea pigs)
Gerbils
Small mammals
Arachnids
Pig
inverts
Hamster,
Tarantula
Tarantulas, scorpions
Chinchilla, Rabbit, Guinea Pig, Rat
Rabbit
Tarantula
Pig, rabbits, hamsters
Tarantula
dormice, pygmy mice, many inverts
Hamster, guinea pigs, chinchilla

Snails, slugs, spiders of all kinds
small mammal
Arachnid
Invertebrates
Isopods and spiders
Rodent & marsupial
scorpion
Inverts
Sulcate tortoise

How did you acquire your amphibian(s)?

reproduced on site
Accidentally bred
acquired tadpoles from a fish hatchery
Bred my own
From State Facility
Reptile show
Traded with friends who keep and breed
reptile fair from breeder
Reptile Shows
From a f rog specific stores like Josh's frogs
Reptile expo
zoo employee
they just come to my pond
Took in when prior owners were unable to care for.
Purchased through Craigslist ad
traded
Most of my amphibians were caught my small children in the area - I have no clue what all they have been exposed to, so I don't release them back usually.
Breeders
Expo

Which of the following best describes from where you acquired your pet amphibian?

Wholesaler
Daughter’s biology group project
Collected
In our yard
wild/ rescue
fish hatchery... rescue
Someone selling tadpoles online locally, salamander from bait shop
Bait shop
student
Irresponsible pet owner
Captive Breeder

reptile shows
At a public park
Backyard pool
School
Professional Breeder
Rehomed
Wild
outside invasive species
Wild
field collected, wholesalers
they just come to my pond
Wild
Wild
rescued a baby toad that was drowning in a swimming pool
Wild
Yard
Rescue
Breeder
under a pile of yard wastes; at the bottom of a stairwell that had to be cleaned out
Rescue
Young students - I use to teach middle school science. I have an unknown frog species which was donated to my classroom, and I bought a poison dart frog once for fun.
Yard
Bait shop
Pool
Set up small pond for breeding natives outside.
Craigslist

Which of the following best describes the reason you were unable to keep your pet amphibian?

Someone else wanted it
Laws changed
Needed money
Too many babies
Bred axolotls, placed offspring in new homes
left zoo job
grew too large to keep in the setup we had
1st one I was very young and my parents wouldn't let me keep it--unable to care for it; Second time I had them, I was wintering them over because when I found them, it was too far into winter for them to survive long enough to build a burrow to hibernate.
Kept at work, employer decided to have fish tanks instead of axolotl tank
my house caught fire
conflicts with other animals

Family problems
It began starving itself, and a friend thought he could get it to eat (larval tiger salamander)

Which best describes what was done with the animal?
Moved to specialized caretaker and quarantinable area
Adoption
First time I released it into a similar type of habitat. Second time, once spring sprung, I release them into the exact same area where I found them...but the woods, not the bottom of the stairwell.
a friend took care of them for me
Placed on display.....not for sale

Which of the following best describes what happened to the animal? Follow-up to: Have you ever had a pet amphibian die?
Disease
taxidermy preservation
Buried in indoor plants
animal was left in vivarium and biologically absorbed
stored in formalin sent to university
DE fleshed and skeleton kept
Drowned in water bowl
Wild caught and thought it was captive bred
We returned one to our yard
Bad husbandry
Old age
Buried in plant pot
Natural cause
Sent to vet for necropsy
Frozen for several days then placed in garbage inside sealed bag. I did not want to spread parasites.
I do not know, it happened when I was out of town.
Animal was burned/ cremated
Animal was frozen for a week then disposed of in the garbage in a sealed bag
Decomposed in enclosure
Disposed of by Veterinarian
Frozen
Put into a vivarium for natural decomposition
Frozen for 1 week prior to disposal in trash
Animal was buried in an bioactive terrarium that housed only plants and invertebrates.
had 6 tadpoles and 1 was a salamander and it ate all the frogs.
Contained in a separate freezer until a necropsy could be performed
Animals were taxidermized
Composted/feed to CUC
for study
frozen
Frozen
Put it in the freezer
Animal was fed to isopod colony
Animal was accessioned into teaching collection at UCF

dried
Cremation after Euthanasia
Euthanized and then buried
Cremated
Frozen
old age
preservation/taxidermy
necropsied for cause
Cremated
Cremated and ashes spread
Necropsy
drowned
placed in freezer after death
Dried out due to lack of humidity
Gave back to pet store
Let our invertebrate clean up crew
escape
After dying, was fed to captive bred isopod colony.
Shadow box memorial
preserved
Incinerated
Preserved as wet specimen in formalin
Frozen and incinerated to keep novel pathogens from entering the environment.
Not exactly sure what happened - was a poison dart frog - humidity was slightly off, parts of cage were still wet, but it was in a dry area and had dehydrated. I dispose of any of my amphibians and reptiles as biological waste carefully.
Was eaten by tank mate
tumors and full of unfertilized eggs
Frozen after death and then placed in garbage
Preserved as a wet specimen
Dried
Frozen, then disposed of in trash
Cremation

Have any of the following pathogens ever been detected in your pet amphibians?
not that I know of
based on symptoms I suspected Bd so I treated for it and then tested after treatment was done and it came back negative.
Never had them tested so I don't know
Unknown
Skin infection multiple pathogens as tested by a vet
Parasite; unsure which one
Lead from paint on aquarium plastic plants
Don't know
Never tested
They were wild rescues that were released as soon as possible.
Not for any of my amphibians, but there is no exotic vets within a days travel of where I live. I have had reptiles with salmonella

Other bacterial infection unable to identify
Red leg disease in Pac-Man frogs

To your knowledge, are there other businesses or organizations similar to yours that are currently operating within your state? Please specify the number.

20
100-1000
5
lots
5
20
Many
50
5
10
10+
100+
12
At least 10
15
50
50
50+
5
3
?
2
4
10
8
more than 20
50
12?
50
100
20
Many pet Stores
Several hobbyists
10000
5
1
Unsure
2
25
50
not sure 6ish ??
1

20
10
6
30

Which of the following describes the mode of your business operation in selling/supplying amphibians?

wholesale
Phone sales
Reptile Show
Shows
I am not in the business per say, but I would sell the long-toed salamanders that breed in my yard to reputable hobbyists or businesses.
Selling to friends
In person
Trade show
Reptile/amphibian shows
Reptile Expos
Peer to peer, social media
Expos
word of mouth
Expositions
wholesale
At expos
Contracts
Face to face
Expos
Advertise locally, sell to local pet stores.
Expos
wholesale
Reptile shows
Distribution to wholesalers and retailers
Local sales
Person to person

Have you ever detected any of the following pathogens in amphibians at your facility?

No
none
na
N/A
none
No
None
None
None
No

none

What is the estimated approximate value of total loss resulting from the illness or death of an animal at your business facility? (Including cost incurred in treatment, care and disposal, if any)?

100
10
100
500
65
120
500
80
500
65
20
60
50
200
0.25
50
20
10
50
5
400
40
100
500
200
50
0
25
20
1000
20000
50
10000
15
1
30
75
700

Mean	939.8
Min.	0
Max.	20000