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

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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).

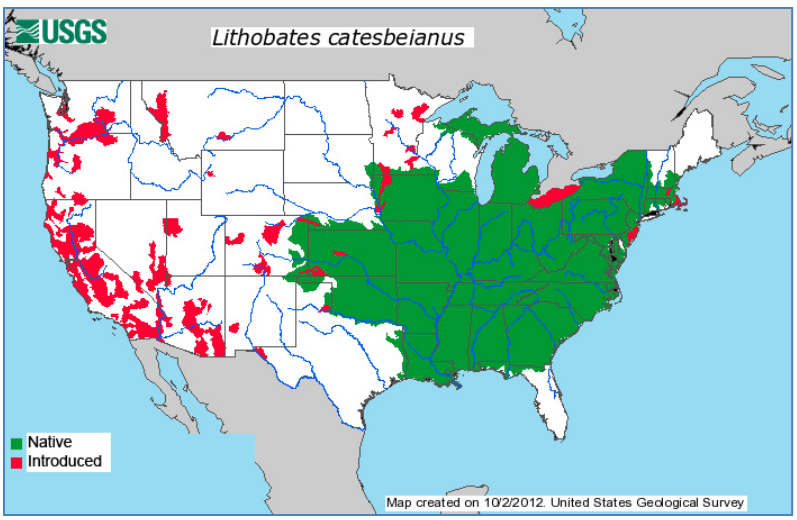

Figure 1. U.S. Geological Survey map of American bullfrog range in the United States. Native range is displayed in green while introduced range is shown in red (USGS, Accessed 7/18/2014).

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).

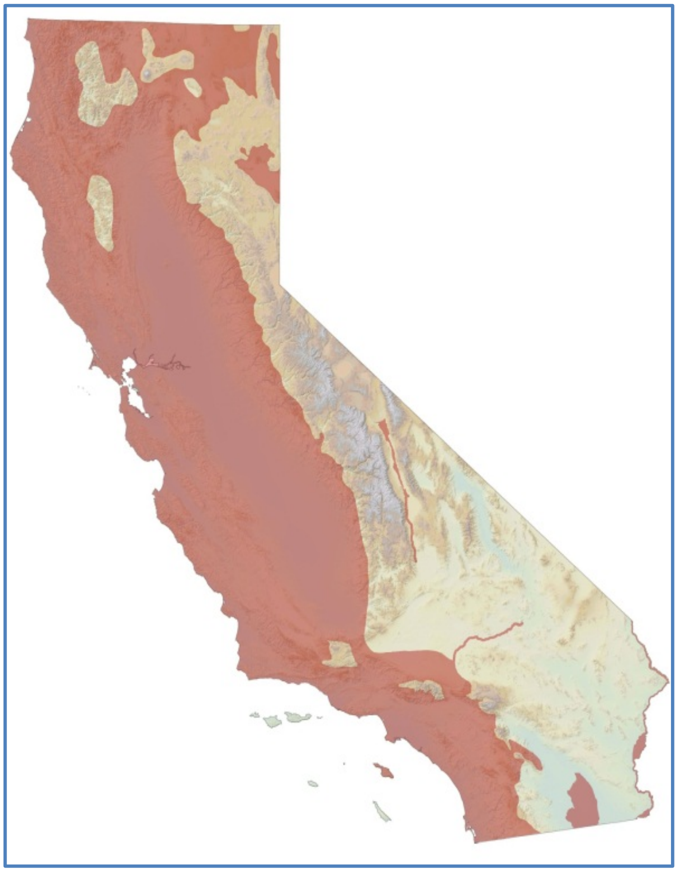

Figure 3. Current distribution of the American bullfrog in California displayed in red (California Wildlife Habitat Relationships Database, Accessed 6/15/2014).

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: <u>aquaculture</u>; <u>bait use</u>; biocontrol; <u>cargo</u>; <u>food</u>; "intentional"; <u>nursery trade</u>; <u>pet trade</u>; research; and <u>zoo trade</u> (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