

Assessing the Biogeography of Parasites of the American Bullfrog (*Lithobates catesbeianus*) in
the Native and Introduced Ranges

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Abstract

The American bullfrog, *Lithobates catesbeianus*, is a well-established invasive species found throughout the world. While means and implications of introduction are well studied, reasons behind successful establishment of invasive bullfrogs are not well known. In order to address whether a release from natural enemies may be behind successful bullfrog invasion, we examined parasite species abundance and diversity of bullfrog populations. A dataset of bullfrog parasites from 22 locations was compiled through (i) firsthand collection from bullfrog dissections and (ii) a literature review. We examined the effects of latitude, host range (native versus invasive), and distance from the native range as site level predictors for the variance in parasite richness (number of different species represented) per site and sum prevalence (total percentage of the individual parasite species observed), respectively. While parasite richness was not significantly different between the native and invasive ranges, GLM analysis demonstrated that the biogeographic factors latitude and distance from the native range together predicted variability in parasite species richness, but not sum prevalence. Parasite species richness increased with increased latitude and decrease with increased distance from the native range. Difference in abundance of direct and complex life cycle parasites (infecting a single species versus infecting more than one host species) between ranges was marginally significant. Since the success of invasive bullfrogs may be related to a release from natural parasite enemies, understanding patterns of species diversity and the influence of biogeographic factors on parasite richness could help predict the probability and intensity of bullfrog invasions.

Introduction

The invasion of the American bullfrog, *Lithobates catesbeianus*, is thought to be one of the worst species invasions throughout the world. Wild populations can be found in more than 40 countries outside the native range, and the amount of destruction these invasive species bring to the native ecosystem is profound. Our research aims to understand the reasons behind the establishment of the extremely successful invasive bullfrog. It is often believed that invasive species leave behind their natural enemies, such as parasites, when they invade. To see if this pattern is true of the invasive bullfrog, we examined parasite diversity and community composition (the assortment of parasite species based on life cycle parameters) of bullfrog populations. We examined the effects of latitude, host range, and distance from the native range on parasite species richness. We predicted to see a decrease in the number of parasite species in the invasive range, with certain biogeographic factors influencing patterns of parasite species richness across all site locations.

Background

Invasive Species

Invasive species can be found in almost all ecosystems throughout the world. These species, also termed exotics, include plants, animals, and microorganisms that have been transported to habitats they historically did not naturally occupy (Mack 2000). In the United States alone, the estimated number of invasive species is approximately 50,000 (Pimentel 2004). Invasion by exotics occurs mostly through human mediated modes of transportation. While species are known to extend their ranges naturally, rates and extent of anthropogenic introduction far exceed natural invasive colonization rates (Cassey *et al.* 2004). Intentional introduction of

non-native species occurs through use of organisms for attempted biological control (method of mitigating pests through the use of natural enemies) or via importation of seeds and animals for horticulture and as food sources (Pimentel *et al* 2004; Cassey *et al.* 2004). Biological control agents (natural enemies used to mitigate pests) can easily become invasive species if they target not only the intended host. The western mosquitofish was introduced to many waterways around the world as an attempt to control mosquito populations (Lowe 2000). Failing to lower mosquito population densities, the mosquitofish turned out to be no more effective than natural predators. The mosquitofish has instead become a well-established invasive species, preying on eggs of economically desirable fish and rare, indigenous fish and invertebrates (Lowe *et al.* 2000).

Introduction of invasive species can also be unintentional; careless plant and animal stocking procedures for agriculture and aquaculture can bring along unintended organisms, and transportation vehicles can unknowingly carry species as stowaways (Mack *et al.* 2000). As an example, the disruption caused to the Great Lakes ecosystems including damage to water pipes, boats, and numerous water utilities within these areas, is the result of the introduced zebra mussel (*Dreissena polymorpha*). Native to the Black, Caspian, and Azov seas, zebra mussels were transported to North America through ballast water on a transoceanic ship in the late 1980s, and have since proliferated throughout the Great Lakes (Ricciardi *et al.* 1998). Irrespective of the means of anthropogenic introduction, relocating species into habitats where they do not belong always has the potential to impact the native ecosystem.

Even though organisms are transported around fairly often, only about ten percent actually successfully establish and become invasive, and only 2%-3% are able to expand their ranges once established (di Castri 1989). According to the National Invasive Species Council (2009), to be considered invasive, the organism must be able to “form a population that is self-

sustaining and does not need re-introduction to maintain a population base such that it continues to survive and thrive in its new environment.” Successful invasion is thus relatively difficult, and certain factors facilitate invasion success. Usually, successful invasion occurs in habitats similar to the organism’s native habitat (Williams & Meffe 1998). The faster the organism can cope with the new surroundings and challenges, the sooner it can devote energy toward growth and reproduction necessary to sustain a population. Furthermore, physical and biological traits distinguish successful exotics from failures. Invasive species are often characterized by rapid growth, short life cycles, high reproduction rates, high dispersal rates, and tolerance of a wide range of environmental conditions (Mack *et al.* 2000). Presence of these traits, however, does not guarantee an invader’s success, and absence of the traits does not exclude an organism from possibly becoming invasive. Since invasion success is a random event, with some invasions being predictable and others being a surprise, it is essential to understand underlying mechanisms that may confer a competitive advantage to invasive species in their introduced habitats.

The Implications of Species Invasions

Although a successful invasion rate of 10% seems small, the possible impacts of these situations should not be overlooked. There are many examples of beneficial introductions of non-native species. The introductions of corn, wheat, rice, domestic chicken, and cattle into the United States have all helped our economy and food availability (Pimentel *et al.* 2000). Most other introductions, however, negatively affect the areas they invade. In fact, the specific definition of invasive species according to Executive Order 13112 of the National Invasive Species Council (1999) is “an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health.” Ecological interactions between

native and non-native species may directly change the population biology of the native ecosystem (Sakai *et al.* 2001). Even small alterations to a single system may create cascade effects impacting many organisms within the community. Unnatural changes to native ecosystems caused by invasive species often translate directly into economic costs. A study by Pimentel *et al.* (2004) revealed that in the United States alone, the near 50,000 known invasive species generate costs of about \$120 billion/year in damage and control. Economic costs of ecosystem management arise through prevention, control, and repair strategies. Preventing the introduction of invasive species does not usually gain sufficient recognition and economic support to successfully work. Failure to prevent introduction of invasive species, in turn, frequently results in significant loss of economic output, such as a decline in agricultural production due to invasive weeds, insects, and pathogens, or damage repair costs including quarantine, eradication, further prevention, and maintenance control of invasive species once established (Leung *et al.* 2002; Mack *et al.* 2000; Pimentel *et al.* 2004). Alterations to native habitat and community structure are often so destructive that monetary repercussions will occur eventually when money is not initially spent on management and control.

With the potential to cause so much damage and modification to native ecosystems, it is no wonder that the invasion of exotics is currently the second leading cause of species endangerment and extinction (Cain *et al.* 2011). One concept to explain the success of invasive species is the idea that invasive species experience a release from their natural enemies in the new environment (Keane & Crawley 2002; Torchin *et al.* 2003; Mitchell & Power 2003). The enemies included in this proposed hypothesis (the enemy release hypothesis (ERH)) can be pathogens, parasites, or predators. This pattern has been studied on numerous invasive plants (Colautii 2004; Mitchell & Power 2003; Vila *et al.* 2004), invertebrates (Aliabadi & Juliano

2002; Calvo-Ugarteburu & McQuaid 1998), and vertebrate species (Dove 2000; Tompkins *et al.* 2003; Torchin *et al.* 2001). The influence of enemies on host populations, whether they are invasive or not, is well known; the pressures of pathogens, parasites, and predators help control population growth of all species in all ecosystems (Torchin *et al.* 2001). While the importance of enemies on host population dynamics is well accepted, previous studies of the ERH have mixed support (Colautti *et al.* 2004). The most extensive data currently available to address whether loss of parasites in the invasive range is a widely observed phenomenon across animal invaders comes from Torchin *et al.* (2003). Parasites of 26 different introduced species of mollusks, crustaceans, fishes, birds, mammals, amphibians, and reptiles were compared in the native versus invasive range, and the abundance of parasite species in the native population was found to be twice that in the introduced range. Understanding major factors attributing to the success of all types of invasive species, and the validity of the ERH, will allow for better prediction, control, and management of invasive species. One well-known invasive host species for which the pattern of enemy release has not been well studied is the globally invasive American bullfrog.

*Successful invasion of the American Bullfrog (*Lithobates catesbeianus*)*

The worldwide invasion of the American bullfrog (*Lithobates catesbeianus*) is a prime example of a particularly successful introduced species. According to the U.S. Geological Survey, the natural range of the bullfrog is the majority of the Eastern United States, roughly east of Colorado and south of the Great Lakes (Figure 1) (McKercher & Gregorie 2013). Currently, however, the American bullfrog can be found in ten US states west of Nebraska and 40 other countries around the world (McKercher & Gregorie 2013). The spread of bullfrogs into non-native areas has occurred mainly through human-mediated forms of transport. Bullfrog farming

is popular in many regions of the world for use in the international food and pet trade (Schloegel *et al.* 2009). Through accidental escape from unsecure farms (Govindarajulu *et al.* 2006) and the intentional release of unwanted larvae, bullfrogs have been able to successfully establish wild populations in areas around the world they are not known to naturally inhabit.

Not only are bullfrogs currently one of the most successful invasive species known, they are also believed to be one of the most harmful invasive species that exist today. Qualities such as high population densities, large body size (Pearl *et al.* 2004), and low resource need (Rosen 1995) have enabled bullfrogs to earn their high rank among the world's most harmful invasive species. Countless accounts of bullfrog invasions demonstrate the negative impacts of bullfrogs on native species and ecosystems through competition, predation, and pathogen transmission (Ficetola 2006; Schloegel *et al.* 2009). In Oregon, the decline of the native northern red-legged frog (*Rana aurora aurora*) and the native Oregon spotted frog (*Rana pretiosa*) is a result of resource competition and predation by the invasive bullfrog (Pearl *et al.* 2004). In the San Joaquin Valley of California, disappearance of the northern red-legged frog and decline of the yellow-legged frog (*Rana boylei*) are also thought to be due to competition and predation, along with habitat alteration, caused by bullfrog presence. This same pattern, of emergence of invasive bullfrog populations and decline of native amphibian populations, has also been documented in Arizona (Goldberg *et al.* 1998), British Columbia (Govindarajulu *et al.* 2006), Uruguay (Laufer 2007), China (Wang *et al.* 2007), and Brazil (Schloegel *et al.* 2010).

In the context of the enemy release hypothesis, the success of the American bullfrog may be partially attributed to the release from, or absence of, natural parasites found in the bullfrog's native range. It is thought that successful invasive species typically do not bring their native parasites along to invaded areas (Keane & Crawley 2002; Mitchell & Power 2003; Torchin *et al.*

2003). This may be attributed to the transport of uninfected host life history stages (seeds or larvae), the absence of other hosts in the introduced range that are necessary for completion of the parasite's life cycle (Torchin & Mitchell 2004), harsh conditions during transport, or human selection for "healthy-appearing" hosts (Colautti *et al.* 2004). Recently, an inventory list of all known helminth species (parasitic worms) associated with the American bullfrog was compiled by Mata-Lopez (2010). The inventory describes 159 different parasite species all found within bullfrog hosts. Significant infection with these parasites may be reducing the full potential of bullfrog physiology and population densities in the native range. The effect of parasite release on host physiology and population density has been studied for the invasive European green crab (Torchin *et al.* 2001). Crabs in the introduced versus native range exhibited significantly lower parasite infection, and were also larger and encompassed a greater biomass. If bullfrogs leave behind a significant amount of parasites when they invade, causing a similar effect as in the green crab, this may contribute to their ability to exploit non-native locations so effectively.

Parasites using bullfrogs as hosts exhibit both direct and complex life cycles. A direct life cycle parasite infects only a single type of host (Roberts & Janovy 2008); bullfrog direct life cycle parasites only infect bullfrogs. Complex life cycle parasites, on the other hand, must infect two or more hosts to complete their life cycles (Roberts & Janovy 2008). Common intermediate hosts (hosts briefly harboring an intermediate developmental stage of the parasite) of bullfrog complex life cycle parasites are snails, mosquitoes, and birds (Roberts & Janovy 2008). Most known bullfrog parasites have complex life cycles, often with two intermediate hosts, and therefore have diverse life histories. While some of these complex life cycle parasites can use multiple different organisms as intermediate hosts, others rely on hosts of a specific genus or even species to complete their life cycle. If successful invasive bullfrogs do in fact leave behind

the majority of their native parasites when they invade, these complex life cycle parasites are especially unlikely to make it to the new range. The absence of specific necessary intermediate hosts such as snails, mosquitoes and birds in the invasive range would not allow complex life cycle parasites to complete their life cycles.

A final differentiation of bullfrog parasites is their ability to use bullfrogs as either the definitive or intermediate host. When bullfrogs serve as the definitive host, the parasite reaches maturity and reproduces sexually within the bullfrog. When bullfrogs serve as the intermediate host, on the other hand, parasites are found within the bullfrog in the encysted larval form, and transmission to the definitive host occurs through consumption of the bullfrog, a process known as trophic transmission. This diversity in life histories of bullfrog parasites provides a good system in which to study how parasites are lost or maintained through the invasion process.

The purpose of the present study is to address the following three questions involving the presence of macroparasites (parasites visible to the naked eye) of the American bullfrog in its native versus invasive range: 1) Do invasive bullfrogs exhibit a higher parasite species richness (the number of different species represented at a single location) in their native range compared to in their invasive range? 2) How do factors such as latitude and distance from the native range influence the distribution of bullfrog parasites in both ranges? 3) Is there a relationship between geographic location within the invasive range and parasite community composition (the assortment of parasite species based on life cycle parameters)? Using an approach that combined collection of novel parasite data through bullfrog dissections, a literature review and meta-analysis (combining results from published studies), we compiled a dataset to address these questions. We expected to find higher parasite species richness among native range bullfrogs, and a decrease in all parasite species, especially complex life cycle parasites, as distance from

the native range increased. Finally, we addressed how biogeographical factors, such as latitude, influence parasite richness and community composition throughout both the native and invasive range.

Materials and Methods

Dissections

A total of 80 wild-caught adult bullfrogs (*Lithobates catesbeianus*) were obtained between August 2012 to January 2013 for use in Institutional Animal Care and Use Committees (IACUC) approved necropsies. Frogs were either hand caught using dip nets or purchased from biological supply companies from the following locations: 20 frogs hand caught from Boulder, CO; 23 wild-caught frogs from Fulton, NY purchased from Carolina Biological Supply Company; and 37 frogs purchased from Niles Biological Supply Company, 20 of which were wild caught from Louisiana and 17 of which were obtained from Taiwan. In preparation for dissection, the frogs were euthanized with MS-222 solution (tricaine methanesulfonate, 3g/L). Snout-vent length in centimeters and weight in grams were measured for each specimen when possible. The body cavity of each specimen was opened and the lungs, intestines, rectum, urinary bladder, and kidneys were removed and separately examined under a dissecting microscope. Each individual was also checked for ectoparasites (parasites that live on the external surfaces of hosts), parasites of the mouth (particularly in the Eustachian tubes), and parasites encysted on the musculature of the hind legs. Any and all parasites found were documented according to where they were found within the body cavity and by number present per host. Parasites were identified at the genus level as well as to the species level when possible. Newly encountered parasites were preserved using a 10% buffered formalin solution, and depending on size, either

photographed using a camera mounted on a compound microscope or transferred to 70% ethanol for storage.

Literature Survey

A meta-analysis of relevant literature was performed to compile a more in-depth data set of bullfrog parasites in both the native and invasive range. The intent was to obtain a sample size large enough to be considered representative of the overall native and invasive ranges. We performed a search on the ISI Web of Knowledge using the following search string: “(bullfrog* OR *Rana catesbeiana* OR *Lithobates catesbeianus*) AND (parasit* OR pathology OR pathogen*)”. After sorting through 239 research articles, thirteen relevant articles were found to meet our chosen criteria. The three criteria met by the thirteen articles are as follows: a sample size of ten or more bullfrog individuals, full necropsy and scan for all possible parasite species, and specific site location information to ascertain whether the site was from the native or invasive range. Parasite species richness along with prevalence and mean intensity were gathered when available. Prevalence refers to the proportion of the sample infected with a specific parasite. Mean intensity is the mean number of helminths per infected host.

Meta-analysis

To address the research questions outlined above, we used several statistical approaches. First, to assess whether bullfrogs have more parasites in the native range, we performed an ANOVA t-test, weighted by sample size to correct for sampling bias, comparing the mean parasite species richness observed between native sites and invasive sites. The same analysis was performed for mean sum prevalence. Sum prevalence is the total percentage of the individual

parasite species observed from a site. This metric provides a measure of the parasite pressure on a given host population (Torchin *et al.* 2003). We chose to use sum prevalence measures and not parasite intensity measures because most studies included from the literature survey provided prevalence measures but not parasite intensity measures.

To incorporate other biogeographic aspects of the bullfrog's ecosystem, we performed a general linear model analysis (GLM) with an information theoretic approach (Burnham and Anderson 2002). Given that within North America some of the invasive sites are significantly closer to the native range than others, we wanted to explore the 'distance from the native range' (measured as distance to the closest point within the native range on the longitudinal grid) as a potential variable that may affect parasite loss in the invasive range. Additionally, we wanted to include latitude as a potential factor of interest, given that latitude is often correlated with species richness for many animals. (We did not examine longitude since it is highly related to distance from the native range.) We performed two GLM analyses in a step-wise manner, one with parasite species richness per site as the response variable and the other with sum prevalence as the response variable. For each analysis, the response variable was weighted by sample size in order to correct for sampling bias. Predictor variables in the model included site range (native or invasive), distance from the native range, and degrees latitude. To choose the best model, Akaike's Information Criterion (AIC) values were generated, and the one with the smallest AIC value was selected. The AIC value is an estimate of the goodness of fit of a statistical model (Burnham & Anderson 2002).

Lastly, to compare parasite community composition between the native and invasive range, we examined the number of parasites with direct life cycles versus the number of parasites with complex life cycles per site. We performed an ANOVA t-test between the mean number of

direct life cycle parasites in the native versus invasive range and the mean number of complex life cycle parasites again between both ranges. In both cases, we weighted the response variable by sample size to correct for sampling bias.

Results

Of the 80 American bullfrogs dissected, the 43 from native sites (Fulton, NY and Louisiana) had a mean snout-vent length (a standard measurement of body size from the tip of the nose to the anus) of 11.84 cm. The 37 bullfrogs caught from invasive sites (Boulder, CO and Taiwan) had a mean snout-vent length of 11.8 cm. Mean weights were not calculated due to limitations on the weight capacity of the scale used. Sex and age were also not classified. The number of bullfrogs parasitized (infected with at least one helminth species) from each site was as follows: 22 (95.7%) from Fulton, NY, 15 (75%) from Louisiana, 19 (95%) from Boulder, CO, and 2 (11.8%) from Taiwan (Table 1). A total of 13 different parasite species were present among all four sites combined. Species richness was highest at the Louisiana location (nine different parasite species) while sum prevalence was greatest for the Fulton, NY site (2.048) (Table 1).

In addition to parasite information from the four above locations, parasite species richness was also determined for 18 other sample sites obtained from the 13 articles from our literature search. Of the 18 sites, six were of bullfrog populations within the invasive range and 12 were native bullfrog populations (Fig. 1). When available, mean intensity and prevalence values were also collected from these sites. Detailed information, including sample size, geographic coordinates, and distance of location from the native border, was combined in Table 2 for all 22 sample locations.

Results of a one-way ANOVA t-test of parasite species richness in native versus invasive ranges showed no significant difference in richness solely due to range ($P = 0.1069$, $R^2 = 0.125$) (Fig. 2). However, in assessing AIC values and obtaining a model with factors that significantly influence species richness, it was found that latitude and distance from the native range together account for 44% of variation in bullfrog parasite species richness across all sites ($R^2 = 0.4378$, $P = 0.0075$) (Table 3). Latitude showed a positive relationship with parasite species richness (Fig. 3), while distance from native range showed a negative relationship with species richness (Fig. 4). Two other models produced ΔAIC values of less than two: the model that included exclusively latitude and the model that included all three variables combined (latitude, distance from native range, and site location either native or invasive) (Table 3). Model analysis was also run using summed prevalence as the response variable (Table 4); however, the strength of the best fit model was very weak ($R^2 = 0.0518$), and therefore only the effects on parasite species richness are further discussed.

Difference in parasite community composition between native and invasive ranges was marginally significant (Figs. 5 and 6). The mean number of direct life cycle parasites was slightly higher in the invasive range versus the native range ($P = 0.0601$, $R^2 = 0.827$). On the other hand, the mean number of complex life cycle parasites was slightly higher in the native range ($P = 0.0667$, $R^2 = 0.1747$).

Discussion

After compiling a dataset totaling nearly 1,000 American bullfrogs sampled across 22 different locations, we found that the pattern of parasitism within the bullfrog's native and invasive range is much more nuanced and complex than expected. While we do find evidence for

the predicted general pattern that exotics exhibit a loss in parasite diversity in the invasive range (Torchin *et al.* 2003), our results do not show a simple relationship between parasite richness and range. Rather, we have identified biogeographic factors, namely latitude and distance from the native range, that contribute to the variability in overall bullfrog parasite species richness (Table 3). Currently, only one other comparison of parasite species richness in bullfrogs between native and invasive ranges exists. Dare and Forbes' (2012) compared parasite diversity in native and invasive populations of bullfrogs in Canada and did find a significant decrease in parasite species within the invasive range. The survey of 26 bullfrogs from Ontario (native range) and 50 bullfrogs from British Columbia (invasive range) showed that, on average, bullfrogs from British Columbia were infected by about four times fewer parasite species than native Ontario bullfrogs. While the latter results revealed a significant decrease in the invasive range, the comparison was only between single locations in the native versus the invasive range. In contrast, our study encompasses multiple sites in North America as well as one site outside of the continent, for a more representative sample of both the native and invasive range. This larger data set allowed us to evaluate differences and patterns among bullfrog parasites within native and invasive ranges with greater statistical power.

When we employed a simple t-test to examine differences in mean parasite species richness from native versus invasive sites, there was no statistical difference (Fig. 2). However, our general linear model analysis determined that the two biogeographic variables latitude and distance from the native range together explain significant variability in parasite species richness across sites. A positive relationship of latitude with parasite species richness and a negative relationship of distance from native range with parasite richness were observed. Since the two models that have ΔAIC values less than two both contain latitude and/or distance from native

range, these two variables do in fact have a significant impact on parasite species richness across all sites (models with ΔAIC value of less than two indicate a substantial level of empirical support in explaining variability and serve as good approximating models for the data).

Latitudinal effect on parasite species richness

The pattern of increased species richness at higher latitudes as found here, is the opposite of almost all other findings of overall species richness along a latitudinal gradient. The great abundance and diversity of life in the tropics is a well-established occurrence (Rohde 1992), and has been reported for many different plants and animals (McCoy & Connor 1980; Cushman *et al.* 1993; Kaufman & Willig 1998; Stevens & Willig 2002). However, this pattern of increased species richness with decreased latitude does not seem to be as straightforward for disease-causing agents such as parasites (see below). While research on the effect of latitude on parasite diversity is limited, some empirical data do exist. The most comprehensive report of how latitude affects human parasites and infectious diseases support the typical pattern, where richness increases from the poles to the equator (Guernier *et al.* 2004). Such a latitudinal gradient also exists for primate protozoan parasites (unicellular eukaryotic parasitic organisms) (Nunn *et al.* 2005) and for ectoparasites of teleost fish (Rhode & Heap 1998). For primate viruses and helminths (Nunn *et al.* 2005), and gastrointestinal helminths of teleost fish (Rohde & Heap 1998), latitude was not a significant predictor of variation in parasite species diversity. Finally, parasite species richness increased with latitude for carnivore parasites (Lindenfors *et al.* 2007), which is similar to our results with bullfrog parasites.

These differences among studies make the latitudinal effect on parasite species richness difficult to generalize, and the reasons behind the discrepancies warrant further study. For the

case of bullfrog parasites, we offer a few possibilities to explain the positive relationship between species richness and latitude seen here. In general, latitude has a large influence on host population densities. Biotic factors such as precipitation and temperature vary along the latitudinal gradient, creating differences in habitat preferential at different latitudes. Since higher host densities allow for richer parasite communities (Lindenfors *et al.* 2007), differences in host population densities of all hosts involved in bullfrog parasite life cycles may be creating the observed pattern of species richness. One example is the differences in population densities of bullfrog intermediate hosts. Intermediate hosts of bullfrog parasites often consist of ondonates (insect class including dragon flies and damsel flies) or other invertebrates (Novak & Goater 2013). In fact, many common parasites found in bullfrogs all have necessary intermediate invertebrate hosts. *Haematolechus* spp. use aquatic arthropods and ondonate intermediate hosts, while *Gorgoderina* spp., *Glypthelmins* spp., and *Megalodiscus* spp. all use snail intermediate hosts (Roberts & Janovy 2008). Since the diversity of these intermediate hosts decreases at higher latitudes, there exists potential for higher abundances of those species present. With fewer niches occupied and fewer resources to provision, organisms can afford to have larger population sizes. High densities of intermediate hosts necessary for transmission of common bullfrog parasites may be increasing overall vector competence (the ability to transmit parasites).

Another possible reason for increased parasite species richness at higher latitudes may be a greater abundance of bullfrogs themselves in the northern part of their range. Host population density is often identified as a factor influencing the ability of parasites to establish within a host; animals living at higher densities tend to have higher parasite species richness (Lindenfors *et al.* 2007). Ficetola *et al.* (2007) used models to predict successful population establishment and invasion of the American bullfrog, and found that precipitation is the most important climatic

factor influencing their success. The presence and availability of water is necessary for both reproduction and tadpole metamorphosis, and is thus essential for the existence of bullfrog populations (Graves & Anderson 1987). Precipitation rates are highest at the equator and decrease significantly at about 30° latitude (Ritter 2006). However, at mid-latitudes, corresponding with the higher latitude range of the bullfrog, precipitation increases again. This may allow for increased bullfrog population densities at higher latitudes and therefore higher parasite species richness. Furthermore, since overall amphibian richness is lower at the higher latitudes (Rohde 1992), corresponding with the northern part of the bullfrog range, bullfrogs have fewer amphibian competitors at higher latitudes, again allowing their populations to be more abundant.

A final possible reason for the increase in parasite species richness as latitude increases is potential seasonal variation in the density of frog-eating birds at higher latitudes. Many highly prevalent parasites in the bullfrog's northern range are trophically transmitted, using the bullfrog as an intermediate host in their life cycle (Roberts & Janovy 2008). Of the five sample location sites 43° latitude or higher (New Brunswick, Bishops Mills, British Columbia, Michigan, and New York), four have high prevalence of *Echinostoma* infection, a trophically transmitted parasite. Bullfrogs serve as intermediate hosts in the *Echinostoma* lifecycle, while primarily predatory birds, and some mammals, serve as the definitive host (Roberts & Janovy 2008). Many northern latitude locations serve as habitats for migratory birds during the summer months, causing bullfrog habitats to become swarmed with these birds. Many of the birds, including herons, hawks, and egrets, are predators of bullfrogs (Hammerson 1999). High density populations of bullfrogs, coexisting with high densities of predatory birds that are the definitive

host to certain bullfrog parasites, could be a reason for high parasite diversity in northern locations.

Effect of distance from native range on parasite species richness

The decrease in parasite species richness with increasing distance from the native range is a more readily explainable observation. Introduced species may fail to bring their parasites with them into the invasive range for many different reasons; one reason being harsh conditions during transport. According to Colautti *et al.* (2004), the proportion of parasites experiencing mortality during the transport process is significant. The farther away from the native range bullfrogs were transported, the greater the likelihood of host mortality becomes due to harsh travel conditions, especially for bullfrogs already weakened by parasite infection. Death of the host or parasite may be one reason accounting for the decrease in parasite species richness as bullfrog populations move further from native ranges.

Another reason for a decrease in parasite diversity may be based on the life history characteristics of the parasites found in the native range. The majority of bullfrog parasites have complex life cycles dependent on specific animals, or even specific species, as intermediate hosts. While host switching in complex life cycle parasites comes with risks, such as the uncertainty of transmitting to a definitive host for reproduction and life cycle completion, certain factors contribute to assuring successful transmission. One key factor is spatial overlap between the niches of all hosts involved (Parker *et al.* 2003). The farther away from the native range bullfrog populations colonize, the less potential overlap exists of necessary intermediate host species. Although our results only showed a marginally significant decrease in complex life

cycle parasites within the invasive range, the absence of niche overlap could still explain the decrease of parasite transmission within bullfrog populations far from the native range.

A final reason for the influence of both distance and latitude is the discrepancy in time since introduction into invasive locations and ecological time necessary for parasite species to establish within the host. A study by Goldberg *et al.* (1998), comparing helminth community structure between introduced bullfrogs in Arizona and two native ranid frogs (*Rana chiricahuensis* and *Rana yavapaiensis*), found that *L. catesbeiana* harbored none of the helminth species found in the native Arizona frogs, even though the frogs coexisted in the same water bodies. However, the parasites found only in the native Arizona ranids were close relatives (congenerics) of the parasites commonly found in many native bullfrog populations. This finding implies that parasites may exhibit a certain degree of local adaption to native host species, and that even though opportunity for transmission to invasive bullfrogs exists, native parasites have yet to establish in the invasive bullfrog. While Torchin *et al.* (2003) observed that a fairly large portion of native parasites colonized invasive species in the new territories subsequent to their arrival, the species studied may have been invasive for substantially longer within their introduced range than bullfrogs. Most bullfrog invasions have occurred only over the past 100 years. Perhaps with more time and interaction with native ranid species, helminth community structure between native and invasive bullfrog populations will become uniform.

Ecological implications

Biogeographic factors influencing patterns of parasite richness across sites may explain the success of bullfrog invasions on the basis of differences in richness. Release from parasites as a cause of invasion success is best understood through the effect of parasitism on the bullfrog

host in general. While some bullfrog parasites may not cause significant pathology, the pathology of many bullfrog parasites is not known. A release from pathogenic parasites, in agreement with the enemy release hypothesis, may give invasive bullfrogs an advantage in their new habitats. *Rhabdias* species are one example of highly pathogenic parasites of bullfrogs. Found within the lungs of the definitive frog host, *Rhabdias* causes pulmonary damage and pneumonia in amphibian hosts (Densmore & Green 2007). Infection of *Rhabdias* in bullfrogs is known to cause mortality when intensity of infection is high (Schmidt & Roberts 1989). From our data, *Rhabdias* is found in native bullfrog populations in New York, Louisiana, and Nebraska, while the only invasive location in which the latter parasite was present in was central-east Texas. Other pathogenic parasites are those that use the frogs as an intermediate host. This is logical since the parasite's goal is transmission to the definitive host via predation. Host bullfrogs negatively impacted by pathogenic parasites are easier prey for predators, thus increasing the likelihood of parasite transmission. Examples of common complex life cycle parasites that rely on bullfrogs for trophic transmission are *Echinostoma* species. *Echinostoma* is found encysted in the frog's kidneys, awaiting predation by a bird final host. High intensity infection of *Echinostoma* can cause increased morbidity (incidence or prevalence of a disease) and mortality (Schotthoefer *et al.* 2003), making these frogs easy prey. Although there was no significant decrease in richness of all parasite species, a release from highly pathogenic parasites, such as *Echinostoma* or *Rhabdias*, may contribute to successful invasion. Since the pathology of most parasites is unknown, bullfrogs may be escaping from other pathogenic parasites in their introduced range that give them advantages for successful invasion.

A release from pathogenic parasites, may explain why bullfrogs are so harmful in the locations they invade. Torchin *et al.* (2003) states that, when exotics are released from parasites

in their introduced range, they are able to attain high population densities and large body sizes enabling them to cause harm to the native ecosystem. As previously mentioned, introduction of invasive species is the second leading cause of species extinction and endangerment. The interaction of bullfrogs with native amphibian populations is especially concerning. Amphibians are currently the group most at risk of extinction and endangerment (Wake & Vredenburg 2008), which is due at least partially to the presence of non-native species. Bullfrogs coexist with other amphibian species in their native range by occupying and preferring separate niches. Bullfrogs typically inhabit the periphery of a body of water, allowing other species to inhabit the deeper regions of the same water source (Snow & Witmer 2010). Introduction into locations where this niche has already been filled by native amphibian species creates competition between bullfrogs and the natives. The naturally large body size of the bullfrog, which may possibly be even larger in the invasive range, due to release from pathogenic parasites, allows the bullfrog to outcompete native amphibians for limited resources. Bullfrogs are also known to directly prey on smaller native amphibians occupying their desired territory within the new location (Moyle 1973; Wang *et al.* 2007; Pearl *et al.* 2004). Successful invasion events often occur in a short time on an evolutionary scale. This allows bullfrogs to exploit native amphibians without allowing time for the emergence of coexistence through niche partitioning.

Conclusion

Understanding the differences in bullfrog parasite diversity between the native and invasive range, and recognizing biogeographic patterns affecting distribution of bullfrog parasites can be used as a tool to predict the strength and possibility of bullfrog invasions. If successful colonization is correlated to a release from parasite enemies, then locations at low

latitudes and far away from the native range are most at risk of invasion. Bullfrog activity within these latter areas can be monitored, and management strategies can be implemented based on predicted strength of invasion. With species invasion being such a threat to species extinction, especially for amphibians, understanding patterns of invasion and the factors that permit invasion success will be key for aiding in conservation efforts of native species.

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Figures and Tables

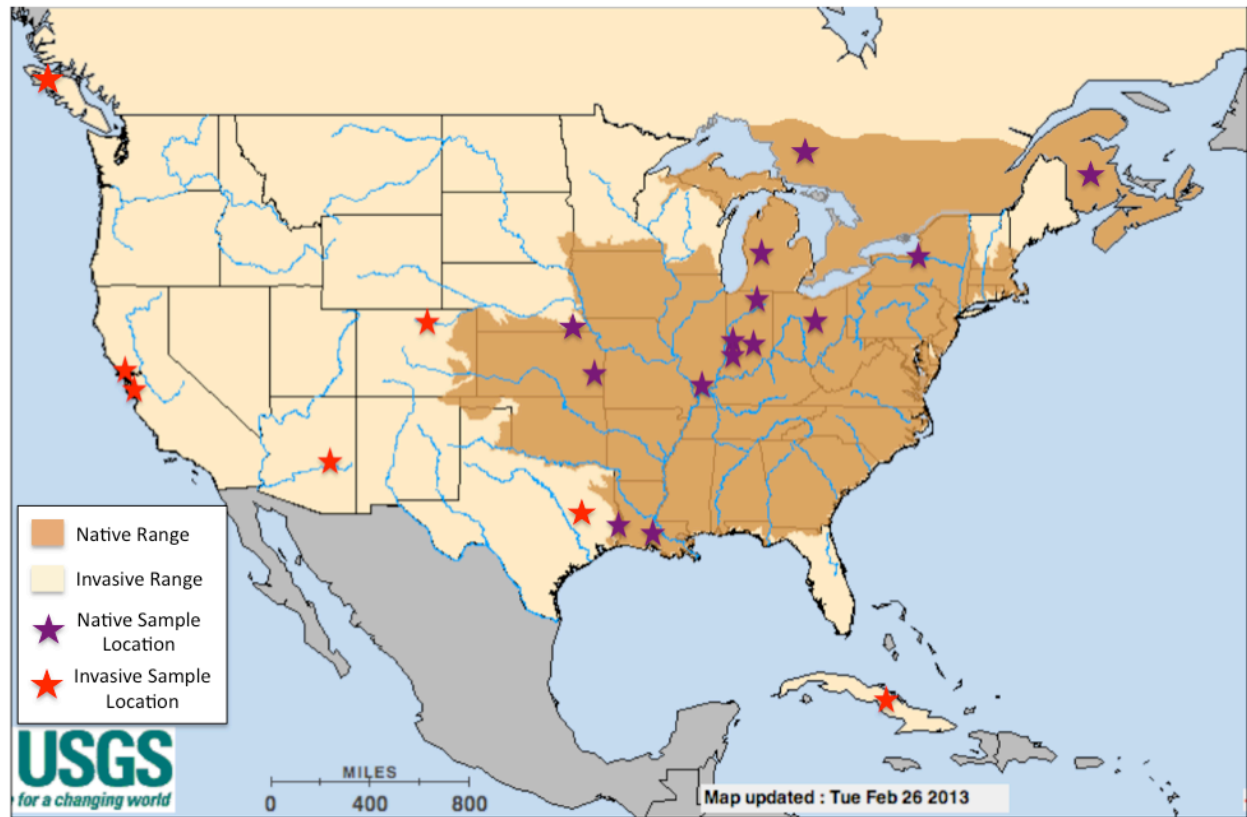


Figure 1. Map of the current native and invasive range of *Lithobates catesbeianus* in the United States and Canada (modified from USGS map, <http://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=71>). Note that bullfrog populations are not necessarily found in all areas throughout the ranges. Map includes 21 sample sites (excluding Taiwan) from which bullfrog parasite information was obtained.

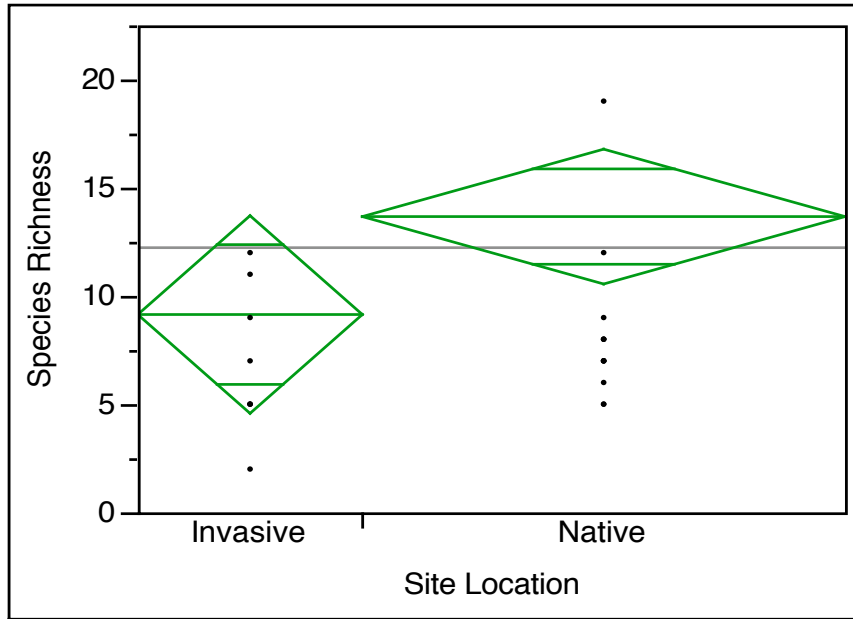


Figure 2. Results of a one-way ANOVA t-test of mean parasite species richness in the invasive range versus the native range ($P = 0.1069$, $R^2 = 0.125$). The top and bottom of the diamonds represent the 95% confidence interval for each group. The line across the middle of the diamonds represents the group mean.

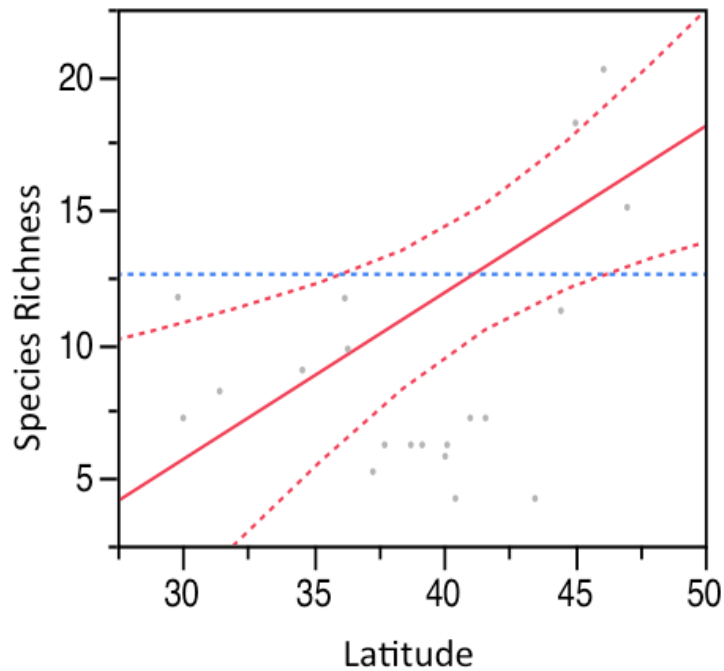


Figure 3. Graphical analysis of the positive relationship between the biogeographic factor latitude and parasite species richness at all location sites. As latitude increases, parasite species richness also increases. Of note is that location sites came from only within North America (Taiwan and Cuba were excluded for this analysis) and so this is only the assumed pattern between 25°N and 50°N.

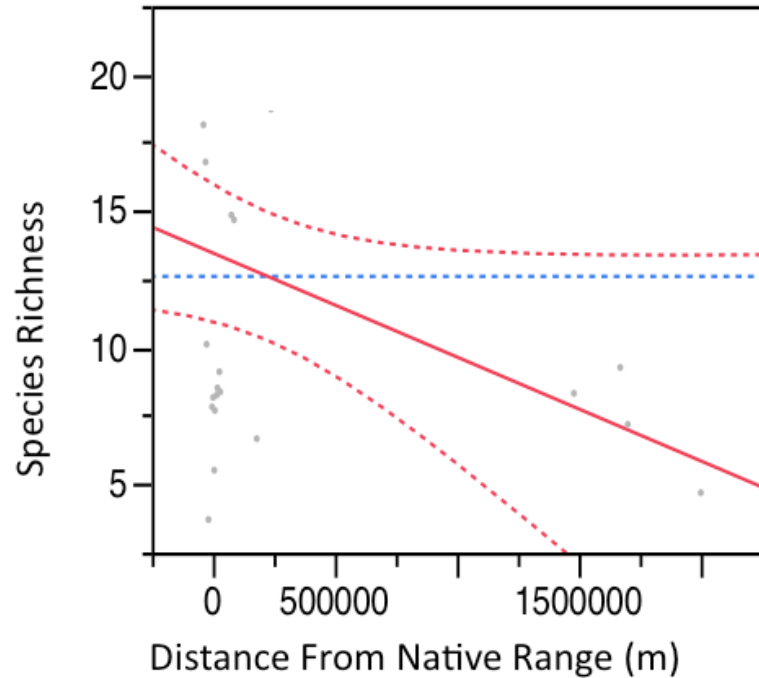


Figure 4. Graphical analysis of the negative relationship between the biogeographic factor 'distance from native range' and parasite species richness at all location sites. As distance increases, parasite species richness decreases.

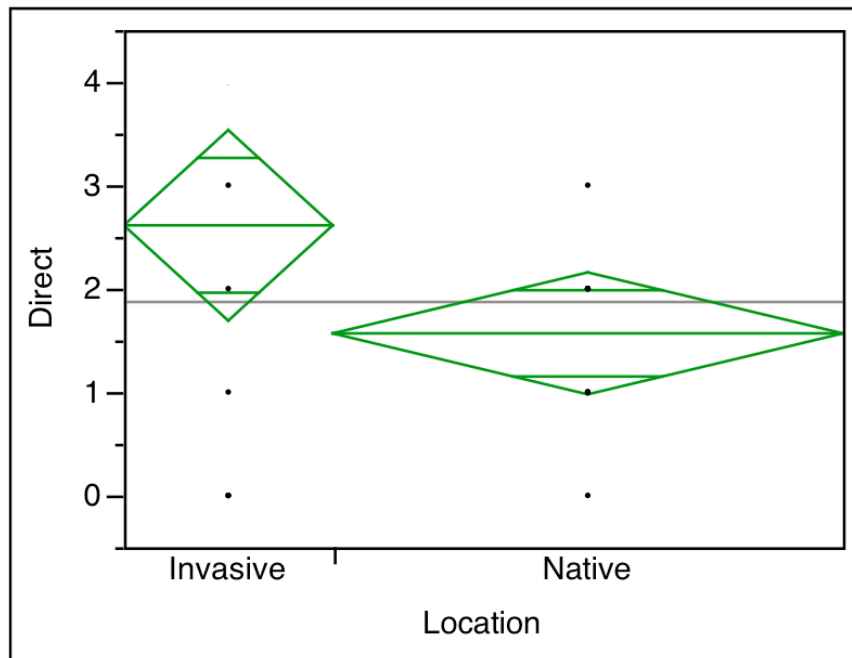


Figure 5. Results of a one-way ANOVA t-test of mean direct life cycle parasite abundance in the invasive range versus the native range ($P = 0.0601$, $R^2 = 0.827$). The top and bottom of the diamonds represent the 95% confidence interval for each group. The line across the middle of both diamonds represents the group mean. While difference in means is only marginally significant, it is important to note that the average number of direct life cycle parasites is higher in the invasive range than in the native range.

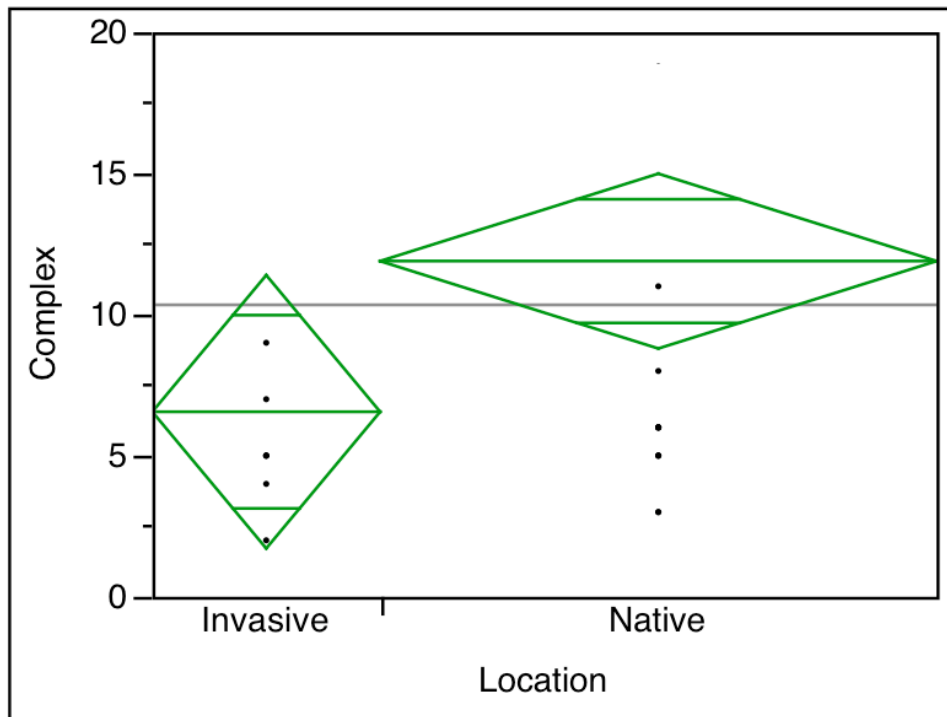


Figure 6. Results of a one-way ANOVA t-test of mean complex life cycle parasite abundance in the invasive range versus the native range ($P = 0.0667$, $R^2 = 0.1747$). The top and bottom of the diamonds represent the 95% confidence interval for each group. The line across the middle of both diamonds represents the group mean. While difference in means is only marginally significant, it is important to note that the average number of complex life cycle parasites is higher in the native range than in the invasive range.

Site Location								
	Native				Invasive			
	Fulton, NY		Louisiana		Boulder, CO		Taiwan	
Sample Size	23		20		20		17	
	Mean Intensity	Prevalence	Mean Intensity	Prevalence	Mean Intensity	Prevalence	Mean Intensity	Prevalence
<i>Cestoda</i>	-	-	1	0.125	-	-	-	-
<i>Echinostoma sp.</i>	-	-	-	-	105.26	0.95	175	0.059
<i>Fibricola sp.</i>	-	-	3.2	0.25	-	-	-	-
<i>Foleyella sp.</i>	-	-	1.2	0.25	-	-	-	-
<i>Glypthelmins quieta</i>	4.67	0.13	-	-	29.6	0.5	3	0.059
<i>Gorgoderia sp.</i>	-	-	-	-	4.5	0.1	-	-
<i>Haematoloechus sp.</i>	7.4	0.652	2	0.1	5.2	0.25	-	-
<i>Halipegus sp.</i>	13	0.05	-	-	13	0.05	-	-
<i>Megalodiscus temperatus</i>	5.47	0.739	1	0.05	-	-	-	-
<i>Oswaldocruzia sp.</i>			9.5	0.15	1	0.05	-	-
<i>Oxyurida sp.</i>	15.5	0.434	1	0.05	-	-	-	-
<i>Rhabdias ranae</i>	1	0.043	2	0.1	-	-	-	-
<i>Spirurida sp.</i>	-	-	2.89	0.45			-	-

Table 1. Mean intensity and prevalence values of all helminth species found from firsthand collection of bullfrog dissections. Of note, the Louisiana site had the highest parasites species richness (9 difference parasite species found).

Location	Sample Size	Species Richness	Mean Intensity	Summed Prevalence	Latitude	Longitude	Distance from Native Range (m)	Reference
Invasive Sites:								
Victoria, British Columbia	50	9	-	1.406	48.428	-123.366	2058154	Dare & Forbes 2012
Boulder, CO	20	5	17.4	0.75	40.015	-105.271	172300	-
San Mateo County, CA	16	5	3.12	2.06	37.434	-122.401	1673354	Goldberg & Bursey 2002
Santa Clara County, CA	15	7	4.675	1.3	37.294	-121.72	1640975	Goldberg & Bursey 2002
Central East Texas*	69	12	-	-	29.76	-95.37	150671	Hollis 1972
Arizona**	25	5	-	0.64	35.513	-109.378	1437240	Goldberg <i>et al.</i> 1998
Cuba***	116	11	-	0.686	21.522	-77.781	922166	Martínez <i>et al.</i> 1982
Taiwan	17	2	89	0.118	23.698	120.961	13249971	-
Native Sites:								
Big Hill Bayou, TX	45	8	-	1.83	29.849	-94.026	0	Yoder <i>et al.</i> 2007
Pawnee Lake, NE	27	8	3.075	1.406	40.842	-96.87	0	Mata-Lopez <i>et al.</i> 2010
Bishops Mills, Ontario	26	19	-	1.423	44.872	-75.701	0	Dare & Forbes 2012
Fulton, NY	23	5	7.84	2.048	43.323	-76.417	0	-
Coshocton County, OH	16	5	2.74	0.12	40.274	-81.953	0	Bursey <i>et al.</i> 1998
LaRue Pine Hills, IL	31	7	6.3	0.835	37.563	-89.443	0	Anderws <i>et al.</i> 1992
New Brunswick, Canada	268	21	-	-	45.951	-66.618	0	McAlpine 1997
Turkey Marsh, MI	127	12	5.37	1.0233	44.315	-85.602	0	Muzzal 1991
Cherokee County, KS	50	6	-	-	37.114	-94.811	0	Jinks & Johnson 1970
Glendale, IN	20	7	-	2.75	38.568	-87.078	0	Lank 1971
Crosley Lake, IN	20	7	-	2.05	39.957	-85.589	0	Lank 1971
Willow Slough, IN	16	7	-	2.0375	39.002	-87.187	0	Lank 1971
Tri-County, IN	16	8	-	2.3125	41.428	-85.355	0	Lank 1971
Louisiana	20	9	2.64	1.525	31.245	-92.145	0	-

Table 2. Site-specific information of parasites collected from all 22 sample sites from firsthand collection through dissections and a literature search. References to articles are provided for all parasite information obtained from the literature review. Note that sites are separated into native and invasive ranges.

*Angelina, Cherokee, Houston, Nacogdoches counties

**Apache, Cochise, Pima, Santa Cruz counties

***Guines, Paso Real de San Diego, Sierra del Rosario, Santiago, & Palacios

Model	Variables	AIC	Δ AIC	R ²	p	Direction of Effect
1	Latitude, Distance from native range (m)	204.371	0	0.4411	0.0071	Latitude: positive Distance: negative
2	Latitude	205.392	1.021	0.3109	0.0106	Positive
3	Latitude, Distance from native range (m), Range (native/invasive)	205.904	1.533	0.4964	0.0103	Latitude: positive Distance: negative
4	Latitude, Range (native/invasive)	207.833	3.462	0.3354	0.031	Latitude: positive
5	Range (native/invasive)	210.526	6.155	0.1092	0.1548	-
6	Distance from native range (m)	210.724	6.353	0.1003	0.1737	Negative
7	Distance from native range (m), Range (native/invasive)	213.434	9.063	0.1206	0.3354	Distance: negative

Table 3. A General Linear Model analysis for the predictor variables latitude, distance from native range, and range (native/invasive) with parasite species richness as the response variable. The best model shown in bold (lowest AIC value) demonstrates that latitude and distance from the native range together account for 44% of variation in bullfrog parasite species richness across all sites. Models 2 and 3 have Δ AIC values of less than 2. This indicates that these models would also serve as good approximating models for the data.

Model	Variables	AIC	ΔAIC	R	p	Direction of Effect
1	Latitude	89.706	0	0.0518	0.3965	Negative
2	Distance from native range (m)	90.405	0.699	0.0095	0.7195	Negative
3	Range (native/invasive)	90.547	0.841	0.0007	0.9236	-
4	Latitude, Distance from native range (m)	92.922	3.216	0.0764	0.5965	Latitude: negative Distance: negative
5	Latitude, Range (native/invasive)	93.246	3.54	0.0575	0.6808	Latitude: negative
6	Distance from native range (m), Range (native/invasive)	93.808	4.102	0.0237	0.158	Distance: negative
7	Latitude, Distance from native range (m), Range (native/invasive)	97.037	7.331	0.0907	0.7563	Latitude: negative Distance: negative

Table 4. A General Linear Model analysis for the predictor variables latitude, distance from native range, and range (native/invasive) with summed prevalence as the response variable. Note that the best model (shown in bold) is not significant, and accounts for very little of the variation in species richness across all sites. For this reason, the effects that these biogeographic factors had on sum prevalence were not discussed.

References

- Aliabadi, B. W. and S. A. Juliano. 2002. Escape from gregarine parasites affects the competitive interactions of an invasive mosquito. *Biological Invasions* **4**:283-297.
- Andrews, K. D., R. L. Lampley, M. A. Gillman, D. T. Corey, S. R. Ballard, M. J. Blasczyk, and W. G. Dyer. 1992. Helminths of *Rana catesbeiana* in Southern Illinois with a checklist of helminthes in bullfrogs in North America. *Transactions of the Illinois State Academy of Science* **85**:147-172.
- Burnham, K. P. and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. 2nd edition. Springer-Verlag New York Inc., New York, NY.
- Bursey, C. R., and W. F. DeWolf II. 1998. Helminths of the frogs, *Rana catesbeiana*, *Rana clamitans*, and *Rana palustris*, from Coshocton County, Ohio. *Ohio Journal of Science* **98**:28-29.
- Cain, M. L., W. D. Bowman, and S. D. Hacker. 2011. *Ecology*, Second Edition. Sinauer Associates.
- Calvo-Ugarteburu, G. and C. D. McQuaid. 1998. Parasitism and introduced species: epidemiology of trematodes in the intertidal mussels *Perna perna* and *Mytilus galloprovincialis*. *Journal of Experimental Marine Biology and Ecology* **220**:47-65.
- Cassey, P., T. M. Blackburn, R. P. Duncan, and S. L. Chown. 2005. Concerning invasive species: reply to Brown and Sax. *Austral Ecology* **30**:475-480.
- Colautti R. I., A. Ricciardi, I. A. Grigorovich, and H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? *Ecology Letters* **7**:721-733.
- Cushman, J. H., J. H. Lawton, and B. F. J. Manly. 1993. Latitudinal patterns in European ant assemblages-variation in species richness and body-size. *Oecologia* **95**:30-37.
- Dare, O. K., M. R. Forbes. 2012. Do invasive bullfrogs in Victoria, British Columbia, Canada, show evidence of parasite release? *Journal of Helminthology* 1-8
- Densmore, C. L. and D. E. Green. 2007. *Diseases of Amphibians*. Institute for Laboratory Animal Research Journal **48**:235-254.
- Di Castri, F. 1989. History of biological invasions with special emphasis on the old world. *Biological Invasions: a Global Perspective*, Chapter 1.
- Dove, A. D. M. 2000. Richness patterns in the parasite communities of exotic poeciliid fishes. *Parasitology* **120**:609-623.
- Ficetola, G. F., W. Thuiller and C. Miaud. 2007. Prediction and validation of the potential global distribution of a problematic alien invasive species—the American bullfrog. *Diversity and Distributions* **13**:476-485.
- Ficetola, G. F., C. Coic, M. Detaint, M. Berroneau, O. Lorvelec, and C. Miaud. 2006. Pattern of distribution of the American bullfrog, *Rana catesbeiana*, in Europe. *Biological Invasions* **9**:767-772.
- Goldberg, S. R., and C. R. Bursey. 2002. Helminths of the bullfrog, *Rana catesbeiana* (Ranidae), in California with revisions to the California anuran helminth list. *Bulletin (Southern California Academy of Sciences)* **101**.
- Goldberg, S. R., C. R. Bursey, and H. Cheam. 1998. Helminths of two native frog species (*Rana chiricahuensis*, *Rana yavapaiensis*) and one introduced frog species (*Rana catesbeiana*) (Ranidae) from Arizona. *Journal of Parasitology* **84**:175-177.

- Govindarajulu, P., W. S. Price, and B. R. Anholt. 2006. Introduced bullfrogs (*Rana catesbeiana*) in western Canada: Has their ecology diverged? *Journal of Herpetology* **40**:249-260.
- Graves, B. M., and S. H. Anderson. Habitat suitability index models: bullfrog." *United States Geological Survey. US Fish and Wildlife Service Biological Report* 82 1987.
- Guernier, V., M. E. Hochberg, and J. Guégan. 2004. Ecology drives the worldwide distribution of human diseases. *PLoS Biology* **2**:0740-0764.
- Hammerson, G. A. 1999. Amphibians and reptiles in Colorado: A Colorado field guide. University Press of Colorado & Colorado Division of Wildlife 2nd edition.
- Hollis, P.D. 1972. A survey of parasites of the bullfrog, *Rana catesbeiana* Shaw, in Central East Texas. *The Southwestern Naturalist* **17**:198-200.
- "Invasive Species: Laws and Regulations - Executive Order 13112." *National Invasive Species Information Center*. United States Department of Agriculture, 3 Feb. 1999. Web.
- Jinks, J. L., and J. C. Johnson, Jr. 1970. Trematodes of *Rana catesbeiana* from three strip-mine lakes in Southeast Kansas. *Transactions of the Kansas Academy of Sciences* **73**:519-520.
- Kaufman, D. M. and M. R. Willig. 1998. Latitudinal patterns of mammalian species richness in the New World: the effects of sampling method and faunal group. *Journal of Biogeography* **25**:795-805.
- Keane, R. M. and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* **17**:164-170.
- Lank, D. R., Jr. 1971. Parasites of the bullfrog in Indiana. *The Proceedings of the Indiana Academy of Science* **81**:359-364.
- Laufer, G., A. Canavero, D. Nunez, and R. Maneyro. 2008. Bullfrog (*Lithobates catesbeianus*) invasion in Uruguay. *Biological Invasions* **10**:1183-1189.
- Lindenfors, P., C. L. Nunn, K. E. Jones, A. A. Cunningham, W. Sechrest, and J. L. Gittleman. 2007. Parasite species richness in carnivores: effects of host body mass, latitude, geographical range, and population density. *Global Ecology and Biogeography* **16**:495-509.
- Lowe, S., M. Browne, S. Boudjelas and M. De Poorter. "100 of the world's worst invasive alien species a selection from the Global Invasive Species Database." *The Invasive Species Specialist Group. Aliens* 12 Dec. 2000. *Invasive Species Specialist Group Online* Nov. 2004.
2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Auckland, New Zealand: Invasive Species Specialist Group.
- Lueng, B., D. M. Lodge, D. Finnoff, J. F. Shogren, M. A. Lewis, and G. Lamberti. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proceedings of the Royal Society* **269**:2407-2413.
- Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences and control. *Issues in Ecology* Number 5.
- Martínez J. J., A. Coy Otero, and L. Ventosa. 1982. Helminths of *Rana catesbeiana* Shaw (Ranidae) en Cuba. *Poeyana* **243**:2-10.

- Mata-Lopez, R., L. Garcia-Prieto, and V. Leon-Regagnon. 2010. Helminths of the American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), from Pawnee Lake, Lancaster, Nebraska, USA with a checklist of its helminth parasites. *Zootaxa*:1-53.
- McAlpine, D. F. 1997. Helminth communities in bullfrogs (*Rana catesbeiana*), green frogs (*Rana clamitans*), and leopard frogs (*Rana pipiens*) from New Brunswick, Canada. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **75**:1883-1890.
- McCoy, E. D. and E. F. Connor. 1980. Latitudinal gradients in the species-diversity of North-American mammals. *Evolution* **34**:193-203.
- McKercher, L. and Gregorie D. R. 2013. *Lithobates catesbeianus*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL.
<http://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=71>
- Mitchell, C. E. and A. G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* **421**:625-627.
- Moyle, P. B. 1973. Effects of introduced bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin Valley, California. *Copeia* **1**:18-22.
- Muzzall, P. M. 1991. Helminth infracommunities of the frogs *Rana catesbeiana* and *Rana clamitans* from Turkey Marsh, Michigan. *Journal of Parasitology* **77**:366-371.
- National Invasive Species Council. 2008. 2008-2012 National invasive species management plan. 35 pp.
- Novak, C. W. & Goater, T. M. 2013. Introduced bullfrogs and their parasites: *Haematoloechus longiplexus* (Trematoda) exploits diverse damselfly intermediate hosts on Vancouver Island.
- Nunn, C. L., S. M. Altizer, W. Sechrest, and A. A. Cunningham. 2005. Latitudinal gradients of parasite species richness in primates. *Diversity and Distributions* **11**:249-256.
- Parker, G. A., J. C. Chubb, M. A. Ball and G. N. Roberts. 2003. Evolution of complex life cycles in helminth parasites. *Nature* **425**:480-484.
- Pearl, C. A., M. J. Adams, R. B. Bury, and B. McCreary. 2004. Asymmetrical effects of introduced Bullfrogs (*Rana catesbeiana*) on native ranid frogs in Oregon. *Copeia* **1**:11-20.
- Pimentel, D., R. Zuniga, D. Morrison. 2004. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**:273-288.
- Ricciardi, A., R. J. Neves and J. B. Rasmussen. 1998. Impending extinctions of North American freshwater muscels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology* **67**:613-619.
- Ritter, M. E. 2006. The Physical Environment: an Introduction to Physical Geography.
- Roberts L. S. and J. Janovy Jr. 2008. Gerald D. Schmidt & Larry S. Roberts' foundations in parasitology. McGraw-Hill Education, 8th edition.
- Rohde, K. 1992. Latitudinal gradients in species diversity: the search for the primary cause. *Oikos* **65**:514-527.

- Rohde, K. and M. Heap. 1998. Latitudinal differences in species and community richness and in community structure of metazoan endo- and ectoparasites of marine teleost fish. *International Journal for Parasitology* **28**:461-474.
- Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, S. G. Weller. 2001. The population biology of invasive species. *Annual Review of Ecological Systems* **32**:305-332.
- Schmidt, G. D. and Roberts, L. S. (1989). *Foundations of Parasitology: Fourth Edition*. St. Louis: Times Mirror/Mosby College Publishing.
- Schloegel, L. M., C. M. Ferreira, T. Y. James, M. Hipolito, J. E. Longcore, A. D. Hyatt, M. Yabsley, A. Martins, R. Mazzoni, A. J. Davies, and P. Daszak. 2010. The North American bullfrog as a reservoir for the spread of *Batrachochytrium dendrobatidis* in Brazil. *Animal Conservation* **13**:53-61.
- Schloegel, L. M., A. M. Picco, A. M. Kilpatrick, A. J. Davies, A. D. Hyatt, and P. Daszak. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). *Biological Conservation* **142**:1420-1426.
- Schotthoefer, A. M., R. Cole and V. R. Beasley. 2003. Relationship of tadpole stage to location of echinostome cercariae encystment and the consequences for tadpole survival. *Journal of Parasitology* **89**:475-482.
- Snow, N. P., Witmer, G. 2010. American bullfrogs as invasive species: a review of the introduction, subsequent problems, management options, and future directions. *Wildlife Damage Control Proceedings* 86-89.
- Stevens, R. D., and M. R. Willig. 2002. Geographical ecology at the community level: perspectives on the diversity of New World bats. *Ecology* **83**:545-560.
- Tompkins, D. M., A. R. White and M. Boots. 2003. Ecological replacement of native red squirrels by invasive greys driven by disease. *Ecology Letters* **6**:189-196.
- Torchin, M. 2004. Introduced species and their missing parasites: What's gained by their loss? *Ecological Society of America Annual Meeting Abstracts* **89**:509.
- Torchin, M. E., C. E. Mitchell. 2004. Parasites, pathogens, and invasions by plants and animals. *Frontiers in Ecology* **2**:183-190.
- Torchin, M. E., K. D. Lafferty and A. M. Kuris. 2001. Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions* **3**:333-345.
- Vila, M., J. L. Maron, and L. Marco. 2004. Evidence for the enemy release hypothesis in *Hypericum perforatum*. *Oecologia* **142**:474-479.
- Wake D. B. and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences* **105**:suppl. 1.
- Wang, Y., Z. Guo, C. A. Pearl, and Y. Li. 2007. Body size affects the predatory interactions between introduced American Bullfrogs (*Rana catesbeiana*) and native anurans in China: an experimental study. *Journal of Herpetology* **41**:5514-520.
- Williams, J. D. and G. K. Meffe. 1998. Nonindigenous species. *Status and Trends of the Nation's Biological Resources*, US Geological Survey 117-129.

Yoder, H. R. and G. W. Gomez. 2007. Helminth parasite assemblages in bullfrogs (*Rana catesbeiana*) from southeast Texas. The Texas Journal of Science **59**:33-38.