Hydrobiologia (2018) 817:267–277
https://doi.org/10.1007/s10750-018-3614-z

Host–pathogen dynamics among the invasive American bullfrog (*Lithobates catesbeianus*) and chytrid fungus (*Batrachochytrium dendrobatidis*)

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Received: 10 May 2017 / Revised: 27 March 2018 / Accepted: 6 April 2018 / Published online: 16 April 2018

Abstract The combination of introduced host species and emerging pathogens can result in unanticipated disease dynamics and novel host–pathogen interactions. The American bullfrog (Lithobates catesbeianus) is a successful invasive amphibian in the western U.S. that can act as a host to the emerging fungal pathogen, Batrachochytrium dendrobatidis (Bd) implicated in the decline of amphibian populations worldwide. We examined if wild-caught invasive bullfrogs were differentially susceptible to two regionally distinct isolates of Bd. Newly metamorphosed bullfrog individuals were exposed to either a Bd strain originally isolated from bullfrogs in their endemic range or a strain from the invaded range in the western U.S. We quantified initial infection load and compared mortality rates and changes in infection load after 30 days to determine strain-specific susceptibility. Wild-caught bullfrogs from the western U.S. were particularly susceptible to an eastern Bd strain. Although infection loads were not different between strains, individuals exposed to the western strain survived, suggesting the ability to reduce their infection. Our findings highlight differences between strains and response variation of an invasive species host.

Keywords Bullfrogs · Chytrid · Invasive · Strains · Pathogen dynamics

Guest editors: John E. Havel, Sidinei M. Thomaz, Lee, B. Kats, Katya E. Kovalenko & Luciano N. Santos / Aquatic Invasive Species II

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Introduction

Biodiversity loss threatens ecosystem function and ecosystem services worldwide (Naeem et al., 1999; Balvanera et al., 2006; Oliver et al., 2015). Habitat transformation, introduction of invasive species, pollution, overpopulation, and overexploitation are human activities explaining the unprecedented biodiversity loss (Brook et al., 2008; Butchart et al., 2010; Barnosky et al., 2012; Dirzo et al., 2014). Another global threat for biodiversity conservation and human health is emerging infectious diseases (EID's) (Fisher et al., 2012; Tompkins et al., 2015). EID's may have substantial ecological and economic costs (Hatcher et al., 2012). Several species have experienced

population declines and extinctions associated with EID's (Daszak et al., 2000; Frick et al., 2010; Rogers & Miller, 2013; Lorch et al., 2015). Amphibians represent one of the most threatened vertebrate groups in part, because of their susceptibility to disease (Stuart et al., 2004; Skerratt et al., 2007; Crawford et al., 2010; Olson et al., 2013).

The fungal pathogen *Batrachochytrium dendrobatidis* (Longcore, Pessier & D.K. Nichols, 1999) (Bd) is especially prominent regarding amphibian population declines, range reductions, and extinctions (Hatcher et al., 2012; Berger et al., 2016). Bd infects more than 600 amphibian species globally (Olson et al., 2013) and recent distribution models suggest shifts and potential expansion in Bd ranges under projected scenarios of climate change (Xie et al., 2016). Bd causes chytridiomycosis, which can cause excessive skin shedding, loss of reflex, lethargy, and mortality in susceptible juveniles and adults (Voyles et al., 2009). In tadpoles, chytridiomycosis affects mainly mouthpart structures (Voyles et al., 2009; Brutyn et al., 2012), although mortality can occur when larvae are exposed to Bd (Blaustein et al., 2005; Garner et al., 2009; Searle et al., 2013). Susceptibility to Bd varies across host species (Blaustein et al., 2005; Searle et al., 2011; Gahl et al., 2012; Bielby et al., 2015; Gervasi et al., 2017), population (Tobler & Schmidt, 2010; Bradley et al., 2015), life stage (Blaustein et al., 2005; Briggs et al., 2010; Ortiz-Santaliestra et al., 2013; Searle et al., 2013), and pathogen strain (Berger et al., 1998; Retallick & Miera, 2007; Doddington et al., 2013; Gervasi et al., 2013a, b; Eskew et al., 2015).

Despite extensive research since the discovery of Bd, differential impacts of Bd strain on amphibian hosts are poorly understood (Morehouse et al., 2003; Retallick & Miera, 2007; Farrer et al., 2011; Gahl et al., 2012). The response of a host to a particular strain gives us insight about the virulence of the pathogen as well as tolerance and resistance of the host. Multiple lineages of Bd have been identified using genetic and genomic information from various geographic locations (Farrer et al., 2011; Rosenblum et al., 2013). Pathogenicity of these lineages can differ according to amphibian host and/or location (Schloegel et al., 2012). Some Bd Global pandemic lineage (Bd-GPL) strains, however, have shown different virulence levels when tested in common hosts (Berger et al., 1998), highlighting the need for additional research on strain-specific interactions.

Among anuran amphibians, it has been suggested that the American bullfrog [*Lithobates catesbeianus* (Shaw, 1802)] is a relatively tolerant carrier of Bd, harboring the pathogen without signs of morbidity or mortality (Daszak et al., 2004; Garner et al., 2006). However, reports of mass mortality events in farmed American bullfrog populations suggested that exposure to Bd may cause chytridiomycosis outbreaks when bullfrogs are in captive, dense, crowded situations (Mazzoni et al., 2003). Moreover, Gervasi et al. (2013b) found differential susceptibility in juvenile bullfrogs experimentally exposed to different Bd strains. As such, American bullfrogs offer a unique opportunity to study the ecological and evolutionary relationship between an EID and its host.

Bullfrogs are endemic to the east and central regions of the United States and have established wild invasive populations in the western US (Moyle, 1973), other continents (Ficetola et al., 2007; Nori et al., 2011), and island chains around the world (Lever, 2003). In their invaded range, direct and indirect effects of bullfrogs on native amphibian species have been documented by field surveys and experimental studies (Kats & Ferrer, 2003; Bucciarelli et al., 2014). Direct effects occurred by predation (D'Amore et al., 2009) and competition (Both & Grant, 2012; Preston et al., 2012; Medeiros et al., 2017) while indirect effects involved altering the use of habitat (D'Amore et al., 2009), changes in behavior (Kiesecker et al., 2001), and changes in activity and refuge use (Kiesecker & Blaustein, 1997, 1998). Bullfrogs relationship and possible tolerance of some pathogens like Bd could be an important factor in the spread of amphibian pathogens (Garner et al., 2006; Greenspan et al., 2012).

Bd strains have been isolated from bullfrogs in their native and invasive ranges (Schloegel et al., 2012) with the earliest detection of Bd in invasive California populations reported from specimens in 1928 (Huss et al., 2013). We investigated if invasive bullfrogs in Oregon USA had differential susceptibility to Bd strains isolated from their endemic and invasive ranges. Therefore, we experimentally exposed wild-caught juveniles to Bd isolated from bullfrogs in Maine, USA (eastern strain, JEL 627) and Bd isolated from bullfrogs in Oregon, USA (western strain, JEL 630). We used wild-caught animals to estimate infection loads at the time of capture and to estimate the impact of previous exposure on strain-specific

susceptibility. While virulence typically depends on the interaction among host, pathogen, and environment (Poulin & Combes, 1999), some Bd traits such as zoosporangium size (Fisher et al., 2009), inhibition of growth in immune cells (Fites et al., 2013), and zoospores production (Langhammer et al., 2013) have been linked to virulence. We quantified the mean number of zoospores produced by Bd while in culture in agar media to identify activity differences in both Bd strains outside the host. While a high zoospore number can be linked mechanistically to a high infection rate (Briggs et al., 2010), some strains with low zoospore production can still have major impacts on their host. We hypothesized that bullfrogs from Oregon would show greater mortality when exposed to a novel Bd strain (eastern strain) due to lack of evolutionary exposure (Gervasi et al., 2013b). We also hypothesized that individuals infected at the time of capture would be more susceptible to a novel strain as constant re-exposure to Bd can compromise host immune defenses (Young et al., 2014).

Materials and methods

We collected 90 recently metamorphosed, juvenile bullfrogs at Gosner stage 45 (i.e., tail stub was still detected in the individuals Gosner, 1960), (body mass mean = 5.6 g, SD = 1.1 g) from a seasonal pond with no resident fish populations (44°24′47.0″N 123°19′38.0″W) in William L. Finley National wildlife refuge, OR (USA). Our survey followed a visual encounter survey method (VES); we used head-lamps and manual flashlights to spotlight individual frogs on the vegetation along the margin of the pond. Once an animal was detected, we hand-collected the individual wearing new nitrile-examination gloves per individual to avoid cross-contamination.

Initial infection load assessment

We handled each individual with new nitrile-examination gloves and swabbed a total of 30 strokes (10 strokes on along the ventral side, 10 strokes per hind leg with 5 strokes each on the hind foot and thigh) using one sterile swab per individual (MW113, Medical Wire & Equipment) following the standard protocol suggested by Hyatt et al. (2007).

Swabs were placed in sterile 1.5-ml microcentrifuge tubes and kept on ice in a cooler. We immediately transported the frogs in individual containers to Oregon State University and randomly assigned individuals to one of three experimental treatments: 30 to be exposed to the eastern Bd treatment (strain JEL 627), 30 to be exposed to the western Bd treatment (strain JEL 630), and 30 individuals acting as control (No Bd exposure). Individual frogs were not cleared of any fungal infection prior to the experiment. Although the use of terbinafine hydrochloride in ethanol has been effective at clearing infection in *L. catesbeianus* (Bowerman et al., 2010), we refrained from using any fungicide as a pre-treatment in this experiment. We posit that fungicide application would change immune response of the individuals to the pathogen and produce adverse reactions that could not be detected in control animals. Additionally, there is a lack of consensus regarding the minimum inhibitory concentration (MIC) appropriate to inactive zoospores in infected animals (Gold et al., 2013). As such, we chose to use wild-caught animals to obtain information about how previous exposure in wild-caught individuals affects the response to repeated Bd exposure.

Bd culture methodology

Bd strain JEL 627 is an isolate from the native range of American bullfrogs in Maine, USA; JEL 630 was isolated from bullfrogs in the local invasive range in Oregon, USA. Both strains were obtained from cultures plated by J. Longcore in May 2013 from cryogenically preserved material. Five colonies from these plates were moved under sterile conditions into tryptone broth 1% for 2 months. One ml of the broth was then plated on 1% tryptone–sterile agar and held for 6 and 8 days (JEL 627 and JEL 630, respectively) before inoculation. For control animals (n = 30) we used 1% tryptone–sterile agar plates without Bd (Searle et al., 2011; Gervasi et al., 2013a, b).

Bd growth rate methodology

To quantify the growth rate of Bd, we counted the number of zoospores produced in culture over time for both strains JEL 627 and JEL 630. We cultured both strains on the same day: 50 agar plates per strain using

1 ml of tryptone broth 1% per plate. From the bottle containing the liquid media, we aliquoted ~ 60 ml in a 100-ml beaker. Using three different drops from the aliquot, we counted the number of active zoospores in a hemocytometer. The approximate number of active zoospores for JEL 627 was 54,000 zoospores/ml and 67,333 zoospores/ml for JEL 630. After 6 days of culture in the plates, we harvested zoospores by flooding five randomly selected plates per strain with 10 ml of dechlorinated water, we scraped the surface and waited for five minutes before pooling the suspensions. From this 50 ml mix of suspensions, we counted the number of active swimming zoospores using a hemocytometer. We repeated the harvesting and counting of zoospores on days 8 through 15. We stopped our observations when the number of swimming zoospores began to decline and the counting number was lower than 15 active zoospores in the hemocytometer (Day 15), the approximate number of active zoospores at that time was 135,000 zoospores/ml for JEL 627 and 89,000 zoospores/ml for JEL 630.

Bd exposure methodology

We housed frogs individually in cell culture dishes (150 × 25 mm BD Falcon Integrid dishes) with holes in the lid and 10 ml of water covering the bottom. All units were held at 18°C and on a 12-h light:12-h dark photoperiod. Animals were acclimated for three days and then exposed to one of three treatment groups: JEL 627 (n = 30), JEL 630 (n = 30), or control (n = 30).

Individuals received 15 ml of Bd inoculum (dechlorinated water and suspended Bd zoospores) at a concentration of 1.7×10^4 zoospores/ml every week for a total of four inoculations throughout the experiment (a dose previously tested in the same species by Gervasi et al., 2013b). Bd zoospores were harvested and quantified following the same method described in the previous section (Bd growth rate methodology).

Survival of individuals was monitored twice per day for 30 days post initial treatment exposure. Individuals found dead during the experiment were immediately preserved in 95% ethanol. After 30 days, all surviving animals were euthanized (MS-222) and preserved in 95% ethanol. All animals were swabbed after preservation following the same protocol used to assess initial infection loads (strokes along the ventral side and along each thigh and rear foot using one sterile swab per individual). We quantified infection load of all animals before and after the experiment using quantitative-PCR (qPCR) (Boyle et al., 2004). All samples were analyzed in triplicate and reported as positive when replicates showed Bd DNA in at least two wells. Bd standards were prepared with transgenic *Escherichia coli* culture with Bd plasmids carrying the Bd internal transcribed spacer region with standards titration from 10^{-1} to 10^2 (USGS, https://water.usgs.gov/nrp/microbiology/resources/resources.html#Bd_std). Average number of genome equivalents per individual (infection loads) were log transformed to normalize data distribution during statistical analysis.

Statistical analysis

Using an analysis of variance (ANOVA), we evaluated if log-transformed initial infection load differed among individuals randomly assigned to the treatments. We hypothesized that some individuals would be infected with Bd upon capture, and thus we used a linear regression model [Initial infection load ~ body size (SVL) + body condition index] to determine if body size (snout-vent length-SVL) or body condition impacted initial Bd infection loads. Body condition for each individual was calculated from the residual values after fitting a linear regression of SVL on mass, which was based on the best method examined by Băncilă et al., 2010.

Since initial infection loads were at background levels, we would expect our quantification of final Bd loads to be related to both treatment level and initial Bd load. Therefore, we used an analysis of covariance (ANCOVA) to determine treatment effects on final infection loads upon death or at the termination of the experiment while accounting for the covariate of initial infection load. We then used Tukey's HSD tests to evaluate specific significant factors among groups. We evaluated if initial infection status Bd (±) may explain final Bd status or infection load using a Generalized Linear model to evaluate categorical variables (Bd status) and an ANOVA to evaluate continuous variables (infection loads).

Using Kaplan–Meier analyses, we compared survival of animals in control versus Bd-exposed treatments (JEL 627 and JEL 630). We used a Cox's proportional hazards model to statistically compare survival of each treatment group and its associated

"hazard ratio." A hazard ratio including 1 indicates that there is no difference in the probability of mortality associated with a factor, in a comparative way (a hazard ratio > 1 indicates an increase in the probability of mortality). We analyzed differences in growth rate between strains using a multiple linear regression to predict the mean number of zoospores based on strains in culture and time. We used a random slope model with a fixed intercept for both strains as we controlled inoculations having the same initial volume. While did we find a 24% difference in the number of active zoospores between strains, we assumed that the equal volume of inoculum would have equal growth potential as active zoospore counts do not represent the complete viable cell counts. Statistical analyses were performed in R (Version 1.0.143, 2009–2016).

Results

The overall prevalence of Bd in wild-caught juveniles of *L. catesbeianus* was 43%, with 39 out of 90 frogs testing positive for Bd at the time of capture (Table 1). Individuals were randomly assigned to treatment groups without a priori information on infection status. Treatment groups were significantly different in the proportion of initially infected individuals and infections loads ($F_{2,\ 87} = 4.52$, $P < 0.001$), with the treatment groups differing from the control group. A Tukey's post hoc test revealed that mean infection loads of individuals assigned to western treatment JEL 630 (0.09 ± 0.28) were similar to that of individuals assigned to eastern treatment JEL 627 (0.16 ± 0.49; $P = 0.86$) and both treatments were significantly different than the control group (0.60 ± 0.63, $P < 0.001$). The control group had a high number of individuals with higher infection loads than either exposure group (Table 1, Fig. 1). Infection loads of juveniles collected in the field were in average 11.1 genome equivalents and this initial infection load was not related to snout-vent length ($F_{1,\ 87} = 2.94$, $P = 0.09$) or body condition ($F_{1,\ 87} = 0.03$, $P = 0.86$) of the animals. Initial Bd infection status (Bd $\pm$) did not predict final Bd infection status ($\chi^2 = 0.02$, df = 2, $P = 0.88$) neither final Bd load ($F_{1,86} = 0.0035$, $P = 0.95$). Initial Bd infection load was not a significant predictor of infection load after the experiment ($F_{1,86} = 0.29$, $P = 0.59$).

At the termination of the experiment, we found reduced infection loads in the control treatment. As expected, animals exposed to Bd strains during the experiment had significantly higher infection loads than controls and infection loads after 30 days of treatment exposure were largely explained by treatment level ($F_{2,86} = 6.34$, $P = 0.0027$). A Tukey's post hoc test revealed that final mean infection loads of individuals assigned to western treatment JEL 630 (0.97 ± 0.70) were similar to that of individuals assigned to eastern treatment JEL 627 (0.80 ± 0.65; $P = 0.59$) and both treatments were significantly different than the control group (0.30 ± 0.55, $P < 0.01$) (Table 2 and Fig. 1).

After experimental exposure to Bd treatments, the rate of mortality in animals exposed to eastern treatment JEL 627 was significantly greater than the rate of mortality in control animals (Fig. 2, Cox proportional hazards model $P < 0.008$; hazard ratio = 3.6). The rate of mortality of individuals exposed to eastern treatment JEL 630 was not significantly greater than the rate of mortality in control animals (Fig. 2; Cox proportional hazards model $P = 0.55$;

Table 1 *Batrachochytrium dendrobatidis* (Bd) mean initial infection load values (raw genome equivalents GE) for all individuals upon field capture

Assigned exposure treatment ($n = 30$ per treatment)	Infection loads mean Bd raw GE (low–high)	Prevalence Bd No. Bd-positive/total no. samples (%)	Prevalence Bd CI
Control	21.8 (0–343)	26/30 (86)	69–96
JEL 627 (eastern strain)	10.7 (0–303)	8/30 (26)	12–45
JEL 630 (western strain)	0.8 (0–12)	5/30 (16)	5–34
Total	11.1 (0–343)	39/90 (43)	32–54

Bd occurrences CI 95% Clopper–Pearson binomial confidence interval for prevalence (%)

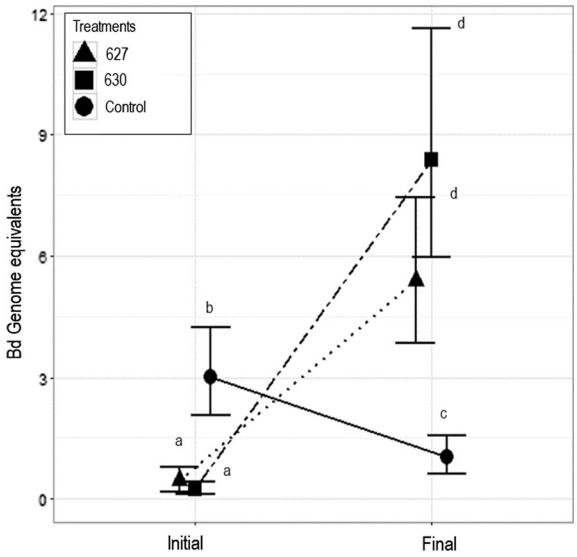

Fig. 1 Genome equivalents of Bd before and after exposure to the pathogen. Bars represent standard error range. Initial values represent infection loads of animals collected in the field. Final infection loads represent infection loads of animals after being exposed to a particular treatment. Individuals selected as controls decreased their infection loads through the experiment. Significantly different infection loads are indicated by different letters. Log genome equivalents in the y-axis are back-transformed

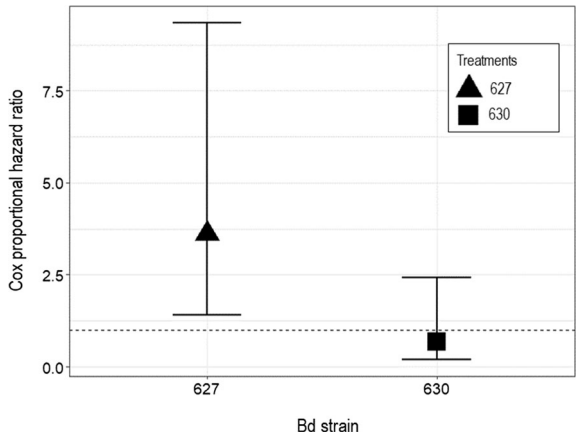

Fig. 2 Cox proportional hazard ratios for exposure treatments compared to a base level of one. Bars represent the 95% confidence interval for the hazard ratios. A hazard ratio of 1 indicates there is no difference in the probability of mortality associated with a factor, in a comparative way (a hazard ratio > 1 indicates an increase in the probability of mortality)

hazard ratio = 0.68). During the first 15 days of the experiment, 15 of 30 animals died when exposed to eastern treatment JEL 627. In comparison, only four animals died when exposed to western treatment JEL 630 in the first half of the experiment (Fig. 3). Our multiple linear regression model predicted the number of zoospores for strains in culture ($F_{2,13} = 70.42$, $P < 0.001$, $R^2 = 0.9155$) as $2.15 + 0.28$ (days) + 0.07 (strain). There was a significant interaction of strain on growth rate ($F_{1,13} = 22.73$, $P = 0.00036$, partial $R^2 = 0.6360$); JEL 627 had a 7.1% higher growth rate per day after culture than JEL 630 (CI 3.98–10.95%) (Fig. 4).

Discussion

Wild-caught American bullfrog (*L. catesbeianus*) juveniles from a population within their western USA invasion range were susceptible to a novel Bd strain. In this experiment, we found that bullfrogs exposed to a Bd strain isolated from the bullfrog's endemic range (eastern strain, JEL 627) suffered higher mortality rates compared to controls (no Bd exposure), or bullfrogs exposed to a western Bd strain (JEL 630) isolated from bullfrogs in Oregon. During

Table 2 *Batrachochytrium dendrobatidis* (Bd) mean infection load values (raw genome equivalents GE) for all individuals after being Bd-exposed to JEL 627 and JEL 630

Exposure treatment	Infection loads mean Bd raw GE (low–high)	Prevalence Bd No. Bd-positive/total no. samples (%)	Prevalence Bd CI
Control	7.3 (0–145)	11/30 (36)	19–56
JEL 627 (eastern strain)	30.7 (0–613)	28/30 (93)	77–99
JEL 630 (western strain)	76.6 (0–1721)	29/30 (96)	83–99

Bd occurrences CI 95% Clopper–Pearson binomial confidence interval for prevalence (%)

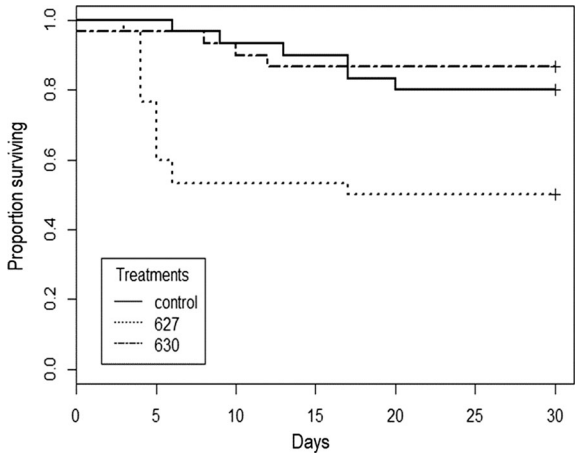

Fig. 3 Survival curves of invasive bullfrogs after exposure to amphibian chytrid fungus strains JEL 627 (dotted lines) and JEL 630 (dashed line). Survival was significantly lowered in the JEL 627 treatment group. No differences in survival occurred between control (solid line) and Bd-exposed animals in the JEL 630 treatment (dashed line)

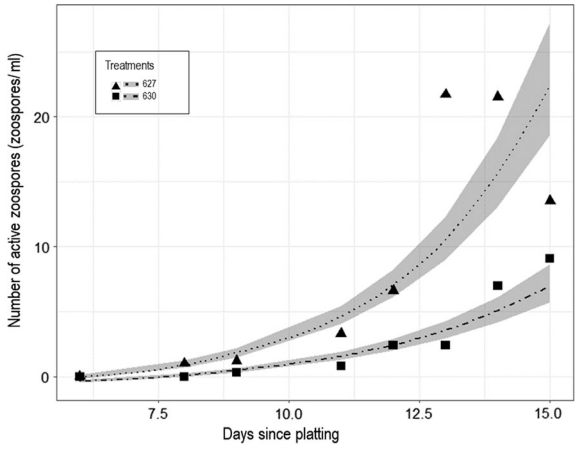

Fig. 4 Growth curves in days after culture for two Bd strains. JEL 627 represented by dotted line and triangles (top line) and JEL 630 represented by dashed line and squares (bottom line). Shadow represents the estimated standard errors per strain

the first half of the experiment, almost 50% of the individuals exposed to the eastern Bd strain died. In contrast, 86% of the individuals exposed to the western Bd strain survived suggesting strain-specific tolerance in this invasive anuran population.

We found that 43% of the bullfrog juveniles were infected with Bd prior to experimental exposure. Interestingly, 19 out of these 26 infected individuals randomly placed within the control group were able to reduce the infection over time. As such, the initial infection loads may not have reached a minimum threshold for the onset of disease (McConnell, 2007). The levels of initial Bd infection were low in terms of prevalence and intensity (mean 11.1 raw genome equivalents), and were similar to other wild-caught bullfrogs swab samples from the USA (Garner et al., 2006; Schloegel et al., 2009; Walke et al., 2015).

Infection loads can vary considerably among individuals not only due to host susceptibility but also due to pathogen virulence (Beldomenico & Begon, 2010). Although we did not characterize individual host immunity via immune response (see Gervasi et al., 2013a), we characterized the eastern and western strain growth rate while in culture as a proxy to infer virulence (Fisher et al., 2009; Langhammer et al., 2013). Our results indicate that the eastern strain had a higher growth rate relative to the western JEL 630 while in culture. A greater number of active zoospores through time could lead to an increase in the risk of mortality in animals exposed to JEL 627. However, in vitro growth rates of the pathogen are not always consistent with pathogen growth in susceptible hosts. Strains with lower in vitro growth rates can represent higher Bd loads in their hosts (Piovia-Scott et al., 2015). In this study, bullfrogs exposed to the novel eastern strain with a higher in vitro growth rate were more susceptible and died faster, despite having similar infection loads to individuals exposed to the western strain.

Individuals exposed to a novel Bd strain can experience a higher mortality risk than individuals exposed to strains isolated from conspecifics (Gervasi et al., 2013b; Eskew et al., 2015). When exposed to the native strain, individuals have high infection loads and survive which could be indicative of co-evolutionary dynamics. The host–pathogen interaction in a particular geographic distribution is expected to lead toward coexistence, with reduced susceptibility in the host and reduced pathogenicity in the pathogen (Doddington et al., 2013).

Bd is an emerging pathogen globally (Olson et al., 2013; Balaz et al., 2014; Van Rooij et al., 2015) and it is projected to spread with changes in climate (Liu et al., 2012; Xie et al., 2016). In the absence of a shared evolutionary history, the impact of a new Bd strain on the host might increase the probability of host mortality. Introductions of new Bd strains to a location could interact with local strains to create synergistic effects that are more lethal than either strain in

isolation. Invasive species capable of transporting novel strains to new geographic ranges can potentially cause chytridiomycosis outbreaks with unusual severity and magnitude (Farrer et al., 2011; Van Rooij et al., 2015). This disruption of evolved trade-offs between the host and the pathogen can be devastating to local amphibian assemblages. At least 17 different Bd strains have been isolated from *L. catesbeianus* from different geographic distributions (Schloegel et al., 2012) and archived collections from the California Academy of Sciences (CAS) reported the presence of Bd in specimens dated as far back as 1928 (Huss et al., 2013). While it is unknown which Bd strain was detected at 1928, we hypothesize that invasive bullfrogs in the western USA have coexisted with Bd, and after reaching stable pathogen–host equilibrium this strain is not virulent to its host. A similar result was found in *Taudactylus eungellensis* (Liem & Hosmer, 1973), a stream dwelling frog in Australia where populations were able to persist with endemic infections of Bd (Retallick et al., 2004).

Understanding the variation in host response to pathogens isolated from conspecifics in different distributional ranges is needed to understand how pathogen origin can mediate host response. The strains used in this experiment are part of the North American clade-Global Panzootic Lineage (Bd- GPL1). However, they are grouped within distinct clusters and thus vary in distributional range and heterozygosity (James et al., 2009; Rosenblum et al., 2013). Although the GPL contains many of the deadliest Bd isolates, our findings support that there are differences in virulence properties inside this lineage that deserve more research.

In conclusion, this study underscores the importance of experimental studies to shed light on infection dynamics and the implication of invasive species movement to different geographic locations. Translocation of an invasive species might mean the arrival of not only a potential predator and competitor but also host species harboring pathogens that affect native species. The arrival of non-native bullfrogs and their associated pathogens can represent an 'invasional meltdown' increasing their likelihood of survival and the magnitude of their ecological impacts (Simberloff & Von Holle, 1999). We need to evaluate Bd strains from different geographic locations and susceptible hosts to understand if exposure to novel strains is facilitating or precluding the onset of a disease. This may necessitate managing Bd strains as distinct entities and limiting re-exposure in locations with naturalized Bd strains. In an era of emerging diseases and globalization, understanding the impacts of a novel strain can help managers better mitigate these dangers, potentially through stronger regulation of importation of live animals, reducing the trade of species and applying informed legislation in conservation actions.

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