

Article

Invasive Cuban Treefrogs (*Osteopilus septentrionalis*) Have More Robust Locomotor Performance Than Two Native Treefrogs (*Hyla* spp.) in Florida, USA, in Response to Temperature and Parasitic Infections

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Abstract: Introduced species pose a threat to biodiversity, and ecological and physiological factors are important in determining whether an introduced species becomes successfully established in a new region. Locomotor performance is one such factor that can influence the abundance and distribution of an introduced species. We investigated the effects of temperature and parasitism by the intestinal nematode *Aplectana hamatospicula* on the maximum jump distance and endurance in one invasive and two native treefrogs in Florida, USA. We collected frogs from the wild, estimated their parasite loads, and tested their locomotor performance at three temperatures. Contrary to expectations, invasive Cuban treefrogs (*Osteopilus septentrionalis*), which are adapted to a warmer climate in the Caribbean, outperformed pinewoods treefrogs (*Hyla femoralis*) and squirrel treefrogs (*H. squirella*) at each temperature, even when controlling for body size differences. In all three species, maximum jump distance was positively related to temperature, and this relationship was stronger for larger frogs. Parasites influenced both the maximum jump distance and endurance of frogs. In all three species, larger frogs jumped farther maximum distances than smaller frogs, but this relationship was stronger when frogs had lower, rather than higher, parasite loads. Parasitism had little effect on endurance in invasive frogs, but it tended to decrease the endurance of native frogs at high temperatures. Furthermore, at low temperatures, the lengths of consecutive jumps of infected native frogs tended to increase, suggesting that parasites limited the distances of initial jumps. Effects of temperature and parasites on the locomotor performance of frogs could influence their abilities to forage, escape predators, and disperse. The tremendous locomotor performance of *O. septentrionalis*, which is maintained across temperatures and parasite loads, likely contributes to the invasion success of this species.

Keywords: amphibian; endurance; introduced species; jump distance; locomotion; parasitism

1. Introduction

The establishment of an introduced species in a new region can be influenced by many ecological factors, including climate, disease, and their interaction [1–4]. Locomotor performance is one mechanism by which ecological factors can influence the abundance and distribution of introduced species [5]. Decreased locomotor ability can impair food intake or predator escape, which can reduce fitness by influencing survival rates, growth rates, body size, time to sexual maturity, and reproductive success [6–9]. Locomotor performance can also influence patterns of dispersal in introduced species, and therefore

rates of invasion into new areas. For example, the accelerating rate of the spread of invasive *Rhinella marina* (cane toads) in Australia is related to the increased endurance of toads at the invasion front [10].

An important determinant of locomotor performance in ectothermic vertebrates is temperature; performance typically increases with temperature within an optimal temperature range, but decreases strongly outside this range [11–13]. Ectotherms can maintain their activity levels and maximize their performance by regulating their body temperatures behaviorally, but they are limited by the temperatures available in the environment [14,15]. Locomotor performance should be maximized at the temperatures to which a species is adapted [14], so introduced species might perform poorly when they experience temperatures outside of those that they are adapted to in their native range, thus limiting their invasion success and ability to compete effectively with native species. Furthermore, populations may disappear or fail to establish in areas where the species's thermal limits are exceeded or where individuals cannot maintain performance to complete critical tasks, such as foraging and reproducing [16,17]. Therefore, invaders are often most successful when the climate in introduced locations matches that in their native range [1,2].

Parasites can also affect the locomotor performance of ectothermic vertebrates. Reduced sprint speed and endurance have been observed in parasitized, compared to non-parasitized, amphibians [18–21] and reptiles [22,23]. The adverse effects of parasites on locomotor performance can be caused by a range of mechanisms, including reduced energy stores available for locomotion resulting from energy lost to tissue repair and immune responses [24]. These deleterious effects of parasitism can be compounded at higher temperatures, as the metabolic rate of both the host and parasite exponentially increases with temperature, rapidly depleting oxygen and energy stores [25,26]. Diminished locomotor performance in parasitized animals can thus lead to reduced foraging efficiency, predator escape, and consequently, reduced fitness [6–9].

Locomotor performance is a useful trait for assessing the effects of temperature and parasites on ectothermic animals because it is closely tied to fitness and can be strongly affected by these factors [6,8,11,18]. Jumping is the primary mode of locomotion in frogs, and work output of muscles during jumping is temperature-dependent, with greater work being produced at higher temperatures [11,27,28]. However, parasites may limit the work output of muscles as metabolic demands increase exponentially with temperature, and less oxygen and energy are available to maintain locomotion [25]. Adaptations to local temperatures might also be crucial to maintaining jumping performance, which could limit the success of tropical animals introduced into subtropical and temperate regions.

The goal of our study was to determine the effects of temperature and parasitism by the nematode *Aplectana hamatospicula* on the jumping performance of invasive and native treefrogs in Florida, USA. Likely introduced to Florida, this parasite can reduce growth and survival rates of invasive and native frogs [24,29,30]. However, effects on host locomotor performance or other physiological or behavioral traits are unknown. We predicted that the maximum jump distance and endurance would be negatively correlated with parasite load, and that the effect of parasitism on locomotor performance would be strongest at high temperatures. We expected that frogs would jump longer distances at higher temperatures, and that introduced *Osteopilus septentrionalis* (Cuban treefrogs), which are native to Caribbean islands and adapted to a tropical climate, would perform more poorly at lower temperatures than native *Hyla femoralis* (pinewoods treefrogs) and native *H. squirella* (squirrel treefrogs) that are adapted to the subtropical climate of central Florida.

2. Materials and Methods

2.1. Frog Background

Hyla femoralis and *H. squirella* are native to Florida, USA, and distributed throughout the southeastern USA. *Osteopilus septentrionalis* is native to several Caribbean island groups and is considered an invasive species in Florida [31]. This species was first reported from the Florida Keys in 1931, and from mainland Florida in 1952 [32,33]. This species has

been spreading north throughout the Florida peninsula since then, and was first reported in the Tampa area (our study area) in 1996 [34]. Phylogenetic analyses indicate that *O. septentrionalis* in Florida descended from at least two source populations in Cuba and was likely introduced to Florida by humans [35]. A comparison of climate data [36] for our study area near Tampa, Florida and the site of origin in western Cuba shows that the average maximum temperature of the warmest month is similar for Florida and Cuba (33.9 °C and 32.1 °C, respectively), but the average minimum temperature of the coldest month is lower for Florida (9.5 °C) than for Cuba (17.1 °C).

2.2. Parasite Background

Aplectana hamatospicula is widespread in Cuba [37–39] and was likely introduced to Florida with *O. septentrionalis* (although see suspect record in Florida predating *O. septentrionalis*; [37]). It has also been recorded in Mexico [37] and Oklahoma, USA [40], where it is presumably native. This nematode parasitizes the large intestine of a broad taxonomic range of frogs (bufonids, eleutherodactylids, hylids, microhylids; [37–42]). It has a direct life cycle and ovoviviparous reproduction [43]. Adults reside in the large intestine and produce juveniles, which are shed through feces. When juveniles reach the infective stage, they penetrate the skin of amphibians and migrate to the large intestine, where they mature and complete their life cycle. A common parasite at our study site, *A. hamatospicula* has been found in 50% of *O. septentrionalis* examined [42], but this is the first study to examine the prevalence of infection in native frogs in Florida. Little is known about the effects of *A. hamatospicula* on viability, physiology, and behavior of amphibian hosts. This parasite can reduce growth and survival rates of *O. septentrionalis* and *Hyla* spp. native to Florida [24,29,30]. Furthermore, native species may be more adversely affected by the parasite than invasive species. Experimental infection with *A. hamatospicula* caused a greater loss in body mass of *H. femoralis* than *O. septentrionalis* despite *H. femoralis* shedding fewer total worms in their feces than *O. septentrionalis* [30]. In addition, resistance and tolerance to the parasite are affected by host diet and microbiota [43,44].

2.3. Frog Collection and Husbandry

This study was conducted under permit LSSC-15-00014 issued by the Florida Fish and Wildlife Conservation Commission, and adhered to protocol WIS00000587 approved by the Institutional Animal Care and Use Committee at the University of South Florida. We collected 45 *O. septentrionalis*, 30 *H. femoralis*, and 30 *H. squirella* (all postmetamorphic frogs) in February 2016 from Flatwoods Wilderness Park, near Tampa, Florida, USA. During the week before capture, the average daily minimum temperature was 13.4 °C (range: 4.0–21.0 °C) and daily maximum temperature was 22.9 °C (range: 14.5–29.5 °C). Frogs were collected from polyvinyl chloride (PVC) pipe refugia that were installed at six wetlands [29]. We collected frogs that represented the full range of postmetamorphic body sizes possible for each species. All frogs were measured and weighed at capture. The mean snout-vent length (SVL) (range) was 40.3 mm (23–66 mm) for *O. septentrionalis*, 25.3 mm (22–35 mm) for *H. femoralis* and 26.3 mm (23–34 mm) for *H. squirella*. Frogs were transported to the laboratory and maintained individually in round plastic containers (12 cm diameter × 14 cm height) with moist paper towels. Containers were changed weekly and frogs were fed vitamin-dusted crickets *ad libitum* twice each week. All frogs were captured within three days, maintained in captivity for three days at 25 °C, and then tested over three consecutive days (at one temperature per day), thereby minimizing any differences in acclimation temperatures among frogs. At the end of the study, all frogs were released into the PVC pipes from which they were captured.

2.4. Parasite Presence and Abundance

We determined the presence and abundance of *A. hamatospicula* in each frog by examining fecal samples. After frogs were transported to the laboratory, we checked for feces every 12 h. We collected the first fecal sample produced by each frog and examined it imme-

diately. The sample was placed in a petri dish with 1 mL of deionized water and examined under a microscope to quantify juvenile *A. hamatospicula* present in the sample. Juvenile *A. hamatospicula* have not been formally described in the literature, so we identified them by comparing their morphology and behavior to *A. hamatospicula* juveniles that were produced by positively identified adults (using the distinct morphology of the spicules; [45]) in petri dishes [46]. This nematode is a common parasite at our study site that is often found in 50% of *O. septentrionalis* examined [42], and no other Cosmocercid nematodes have been found at our study site in the 330 frogs examined in Ortega et al. [42] or 369 frogs examined in Roznik et al. [29]. No other gastrointestinal parasites were observed in our samples.

To examine the accuracy of our technique of using the number of *A. hamatospicula* juveniles present in fecal samples as an estimate of the number of adult *A. hamatospicula* present within the frog's large intestine, we performed a separate experiment. We exposed 76 *O. septentrionalis* each to 30 juvenile *A. hamatospicula* in 2 mL deionized water for 24 h. After four weeks, we collected one fecal sample from each frog and quantified juvenile worms, as described above. We then euthanized frogs using a topical application of 20% benzocaine, and quantified the number of adult worms present in their large intestines. We used a negative binomial generalized linear model (using the *glm.nb* function in package MASS in R statistical software) to test whether the number of juvenile worms (independent variable; non-zero values were $\log_{10}$ transformed) is related to the number of adult worms (dependent variable), when controlling for the SVL and mass of frogs. We calculated the P-value using the *Anova* function in the *car* package [47]. We found that there was a significant, positive relationship between these two life stages ($\chi^2 = 15.7015$, $p < 0.0001$, Figure 1), but that the percentage of false negatives was high (71%). These results suggest that this technique can accurately predict parasite loads when there are worms present in feces, but it may result in false negatives when worms are absent during examination of fecal samples. This experiment was based on a one-time exposure to parasites in the lab, rather than multiple exposures and continual infections that wild frogs experience (in part due to reinfection), which are more likely to reduce the occurrence of false negatives because frogs are more likely to have mature parasites that are reproducing.

2.5. Locomotor Performance

Locomotor performance trials took place on three consecutive days. The performance of each frog was measured at three temperatures: 14 °C, 22 °C, and 30 °C. Each frog was tested at each temperature in a random order. Frogs were held at their assigned temperature in temperature-controlled rooms for eight hours prior to jumping trials. A roll of paper was cut to 5 m and taped down to the floor in the hallway (22 °C) adjacent to the temperature-controlled rooms. Frogs were then taken out of the room one at a time and dipped into water of their assigned temperature to help mark the locations of their jumps and prevent them from sticking to the paper. Each frog was allowed to jump five times; most frogs jumped readily, but some were more reluctant to jump, so we gently prodded those frogs on the urostyle to encourage them to jump. The test for each frog lasted less than 10 s, limiting any possible changes in body temperature. We marked the location of each jump on the paper using nontoxic markers (a different color for each frog). After five jumps were completed, the length of each jump was measured to the nearest 0.5 cm.

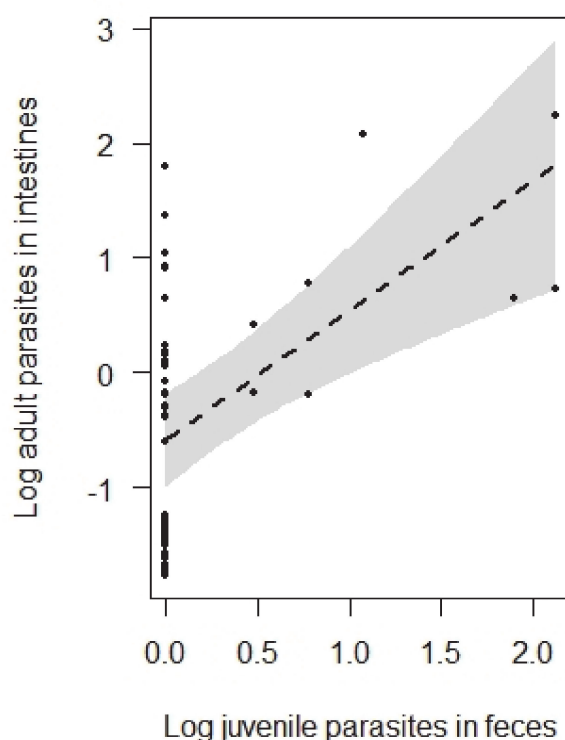


Figure 1. Relationship between $\log_{10}$ juvenile (found in fecal samples) and $\log_{10}$ adult (found in intestines) *A. hamatospicula* in frog hosts (*O. septentrionalis*). This plot is a conditional plot with fitted regression line (dotted line), partial residuals (dots), and a 95% confidence band (shaded) that controls for all factors in the statistical model that are not shown in the figure (frog snout-vent length and mass).

2.6. Statistical Analyses

We first quantified the maximum distance of the five consecutive jumps, and then we calculated the change in jumping performance across the five jumps (slope of jump distance against jump number) for each frog as a measure of endurance. We used linear mixed models (*lme* function in package *nlme* [48] in R statistical software) to test for the main and interactive effects of SVL, species, temperature (as a continuous predictor), and number of parasites (non-zero values were $\log_{10}$ transformed) on the maximum distance and the change in jumping performance across consecutive jumps. To help clarify patterns in our endurance metric, particularly when slopes were positive, we also examined the distance of the last (fifth) jump using a linear mixed model that included the main and interactive effects of SVL, species, temperature, and number of parasites. We treated the identification number of individual frogs as a random effect in all models. P-values were calculated using the *Anova* function in the *car* package.

To test the alternative hypothesis that any differences in locomotor performance were caused by poor body condition, rather than direct effects of parasitism, we compared the body condition of frogs that were and were not parasitized. We predicted the length-adjusted mass for each frog using the scaled mass index, according to the methods of Peig and Green [49]; scaling exponents were 3.3 for *O. septentrionalis*, 3.1 for *H. femoralis*, and 2.9 for *H. squirella*. We then compared the difference in predicted and actual mass between parasitized and non-parasitized frogs using a separate ANOVA for each species. If parasitized frogs did not have significantly lower size-adjusted mass than non-parasitized frogs, then any reductions in the jumping performance of parasitized frogs were likely caused by the infection, rather than by their body condition pre-infection.

3. Results

We found that parasitized frogs were not in significantly poorer body condition than non-parasitized frogs for *O. septentrionalis* ($F_{1,43} = 0.0202$, $p = 0.8877$), *H. femoralis* ($F_{1,28} = 4.1195$, $p = 0.0520$), and *H. squirella* ($F_{1,28} = 3.5773$, $p = 0.0690$). Marginally significant results for the two native species suggest that infected frogs actually had slightly larger body masses than predicted (by an average of 0.14 g for *H. femoralis* and 0.07 g for *H. squirella*). These results suggest that any differences in the locomotor performance of parasitized frogs were caused by parasitic infections and not by pre-existing poor body condition or an indirect effect of parasitism on locomotor performance via the parasite's effect on body condition.

In all three species, maximum jump distance was positively related to temperature and SVL (Table 1, Figures 2 and 3). The maximum jump distance of larger frogs was more strongly affected by temperature (i.e., had a more positive slope) than that of smaller frogs (SVL $\times$ temperature; Table 1, Figure 3A), indicating that there was a greater increase in jumping distance as temperatures increased for larger than smaller frogs. The invasive species outperformed the native species at each temperature, having longer maximum jumps than the native species, even when controlling for body length differences (Table 1, Figure 2). The relationship between maximum jump distance and temperature was similar in all three species (e.g., similar slopes), indicating that lower temperatures did not decrease the maximum jumping ability of *O. septentrionalis* more than the native species (Table 1, Figure 2).

Table 1. Main and interactive effects of snout-vent length (SVL), temperature, species, and $\log_{10}$ parasite load on the maximum jumping distance of frogs. These statistical results are from a linear mixed model that also included the identification number of individual frogs as a random effect.

Predictors	χ^2	DF	p
SVL	25.9379	1	<0.0000
Temperature	93.6099	1	<0.0000
Species	32.0013	2	<0.0000
Parasites	0.6059	1	0.4363
SVL $\times$ Temperature	10.3155	1	0.0013
SVL $\times$ Species	1.6159	2	0.4458
Temperature $\times$ Species	1.6722	2	0.4334
SVL $\times$ Parasites	7.8877	1	0.0050
Temperature $\times$ Parasites	0.6931	1	0.4051
Species $\times$ Parasites	2.4599	2	0.2923
SVL $\times$ Temperature $\times$ Species	2.5219	2	0.2834
SVL $\times$ Temperature $\times$ Parasites	1.5764	1	0.2093
SVL $\times$ Species $\times$ Parasites	1.4525	2	0.4837
Temperature $\times$ Species $\times$ Parasites	1.2773	2	0.5280
SVL $\times$ Temperature $\times$ Species $\times$ Parasites	1.1283	2	0.5688

Of the 105 frogs examined in our study, 33 frogs (31%) were parasitized by *A. hamatospicula*, including 24 *O. septentrionalis* (53%), 6 *H. femoralis* (20%), and 3 *H. squirella* (10%). The number of juvenile parasites in fecal samples ranged from 0–210 parasites (*O. septentrionalis*: 0–180 parasites; *H. femoralis*: 0–210 parasites; *H. squirella*: 0–118 parasites). Our results for the effects of parasitism on *H. femoralis* and *H. squirella* should be considered with caution because of the small sample size of parasitized individuals. The maximum jump distance of frogs of all three species was significantly affected by the interaction between parasite load and SVL (Table 1, Figure 3B). The relationship between SVL and maximum jumping distance was more positive for frogs with lower than higher parasite loads. This indicates that jumping performance was compromised by increasing parasite loads, and larger frogs were more negatively affected by parasites than smaller frogs (Figure 3B).

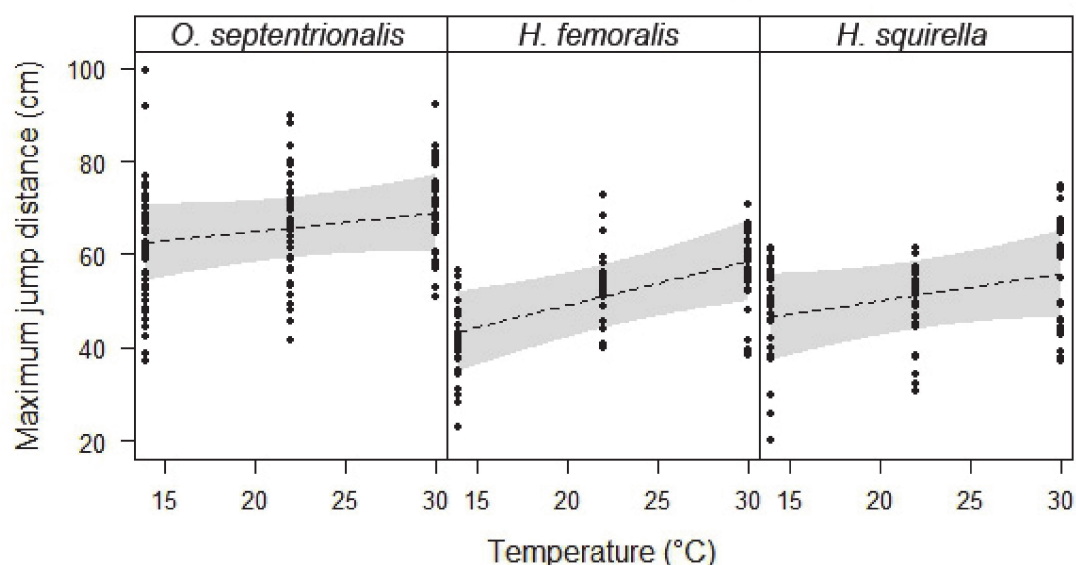


Figure 2. Maximum jumping distance of *O. septentrionalis*, *H. femoralis*, and *H. squirella* across temperatures. These plots are conditional plots with fitted regression line (dotted line), partial residuals (dots), and 95% confidence bands (shaded) that control for all factors in the statistical model that are not shown in the figure (see Table 1).

The change in locomotor performance across five consecutive jumps (slope of jump distance against jump number) was used as a measure of endurance. Frogs had high endurance if their jump distance was maintained across the five repeated jumps, but if jump distance declined steeply across the repeated jumps, they had low endurance. Endurance was significantly affected by the interaction among species, SVL, temperature, and parasite load (Table 2, Figure 4). Overall, across body sizes, temperatures, and parasite loads, the invasive *O. septentrionalis* was able to maintain locomotor performance across repeated jumps and thus its endurance was mostly independent of these factors (Figure 4). In contrast, our measure of endurance was affected by all three of these factors in the native species (Figure 4). Slopes appeared to be positively and negatively associated with parasite load at low and high temperatures, respectively (Figure 4). Among parasitized frogs, consecutive jump lengths of large native frogs appeared to decrease at high temperatures, but consecutive jump lengths of large native frogs at low temperatures appeared to increase. In addition, jump lengths of small *H. femoralis* also increased, whereas jump lengths of *H. squirella* showed no change or a slight decrease. When controlling for the effects of SVL, temperature, and species, the distance of the last (fifth) jump was not affected by the main effect of parasite load or any interactive effects (all $p > 0.12$), suggesting that the increases in consecutive jump lengths that we observed do not indicate an increase in endurance, but rather shorter initial jumps.

Table 2. Main and interactive effects of snout-vent length (SVL), temperature, species, and $\log_{10}$ parasite load on the endurance of frogs (slope of jump distance against jump number). These statistical results are from a linear mixed model that also included the identification number of individual frogs as a random effect.

Predictors	χ^2	DF	p
SVL	0.1923	1	0.6610
Temperature	0.5299	1	0.4666
Species	15.8867	2	0.0004
Parasites	1.1357	1	0.2866
SVL $\times$ Temperature	2.0081	1	0.1565
SVL $\times$ Species	11.2456	2	0.0036
Temperature $\times$ Species	0.0490	2	0.9758

Table 2. Cont.

Predictors	χ^2	DF	<i>p</i>
SVL $\times$ Parasites	0.1826	1	0.6692
Temperature $\times$ Parasites	0.9905	1	0.3196
Species $\times$ Parasites	9.8773	2	0.0072
SVL $\times$ Temperature $\times$ Species	11.7538	2	0.0028
SVL $\times$ Temperature $\times$ Parasites	0.0103	1	0.9192
SVL $\times$ Species $\times$ Parasites	1.4758	2	0.4781
Temperature $\times$ Species $\times$ Parasites	17.1816	2	0.0002
SVL $\times$ Temperature $\times$ Species $\times$ Parasites	7.8832	2	0.0194

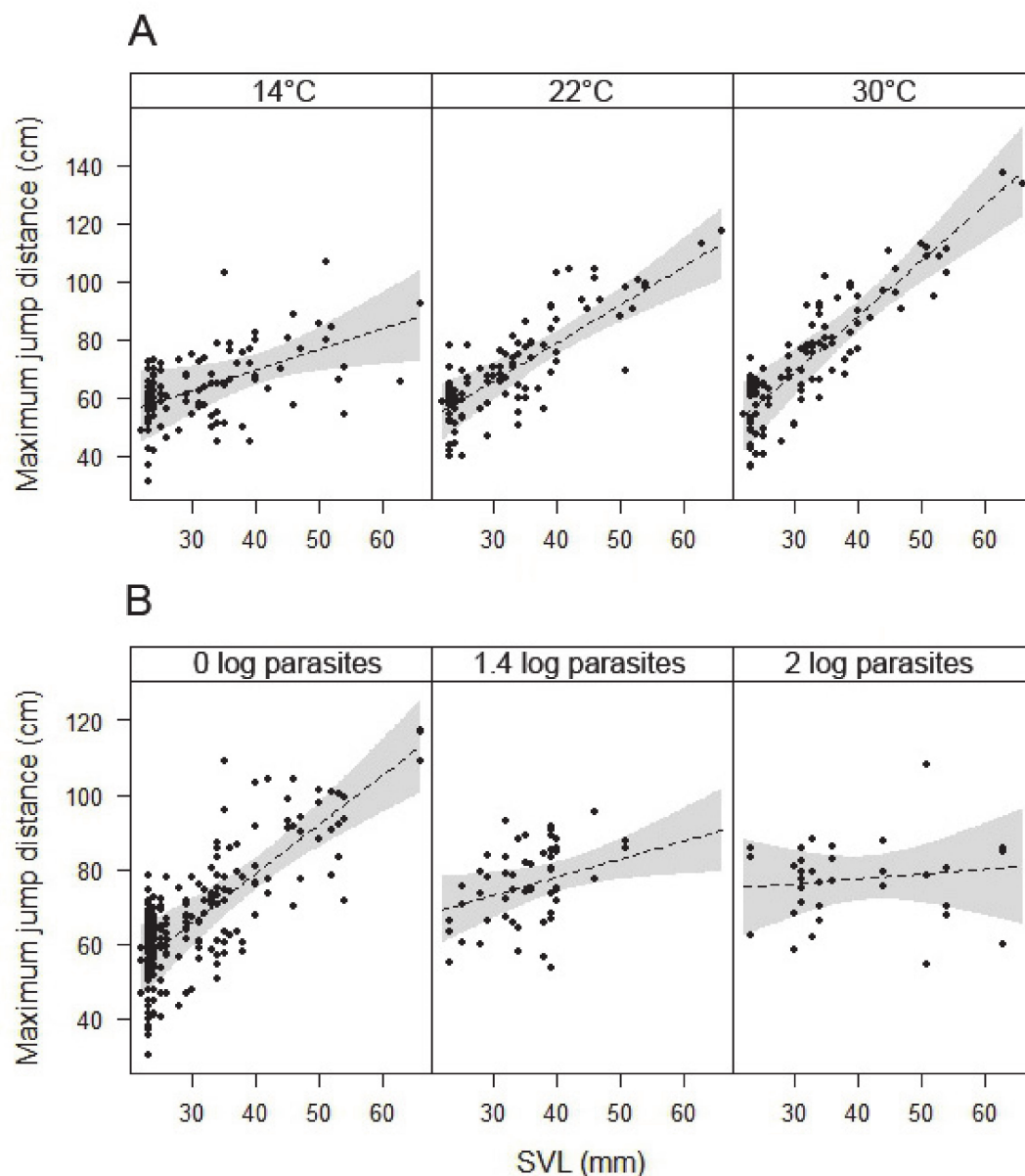


Figure 3. Interactive effects of (A) snout-vent length (SVL) and temperature and (B) SVL and log₁₀ parasite load on the maximum jumping distance of frogs. These plots are conditional plots with fitted regression line (dotted line), partial residuals (dots), and 95% confidence bands (shaded) that control for all factors in the statistical model that are not shown in the figure (see Table 1).

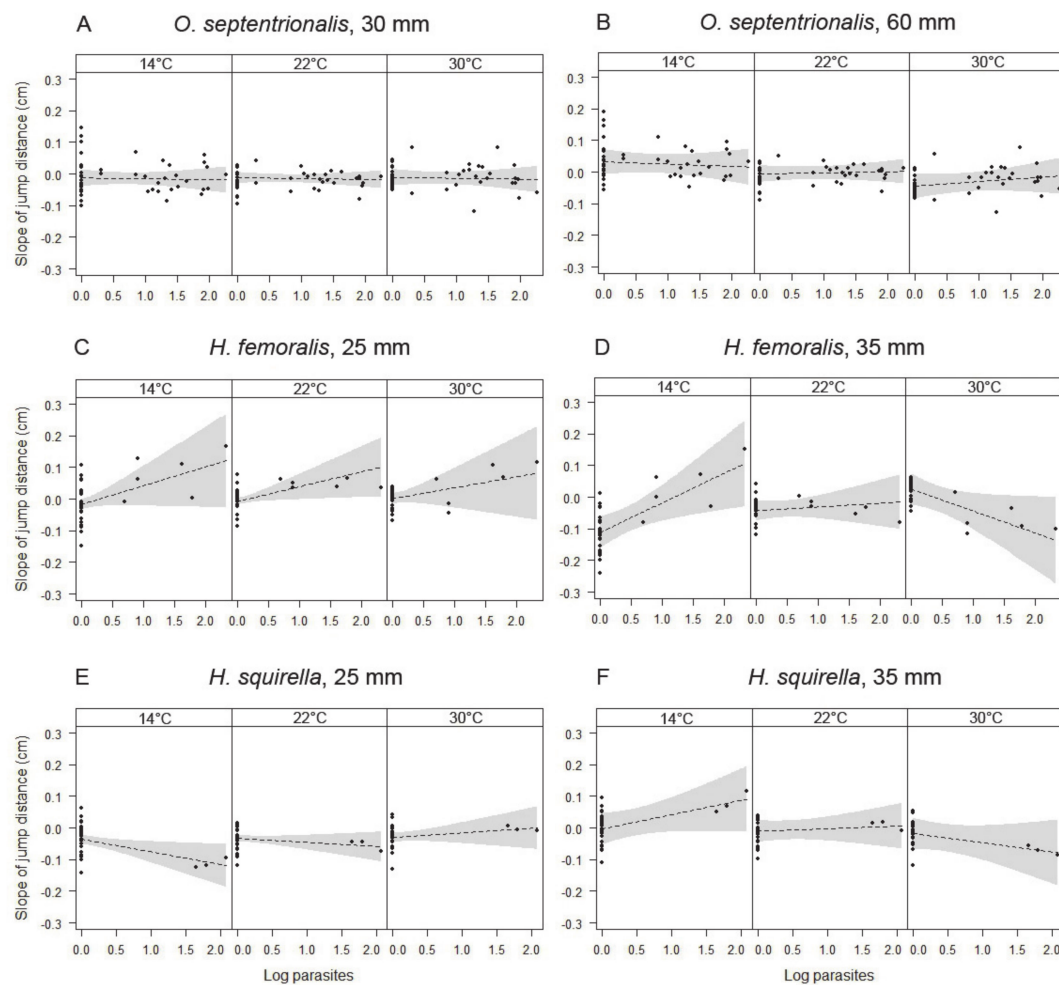


Figure 4. Interactive effects of species, snout-vent length (SVL), temperature, and $\log_{10}$ parasite load on the endurance of frogs (slope of jump distance against jump number) for (A,B) *O. septentrionalis*, (C,D) *H. femoralis*, and (E,F) *H. squirella*. For each species, relationships are shown for frogs with small and large body sizes (20th and 80th percentiles). These plots are conditional plots with fitted regression line (dotted line), partial residuals (dots), and 95% confidence bands (shaded) that control for all factors in the statistical model that are not shown in the figure (see Table 2).

4. Discussion

We examined factors influencing the locomotor performance of introduced (*O. septentrionalis*) and native (*H. femoralis* and *H. squirella*) treefrogs in Florida, USA, and found strong effects of species, body size, temperature, and parasitism. Overall, we found that the invasive species had robust locomotor performance and outperformed the two native species across temperatures and parasite loads, which likely contributes to its invasion success. In all three species, maximum jump distance was positively related to temperature and body size, and these relationships were stronger for larger frogs (Figures 2 and 3). This was expected because frogs with longer limbs and larger muscles can jump farther [50] and work output of frog muscles during jumping is greater at higher temperatures [11,27,28]. Locomotor performance is typically maximized at the temperatures to which a species is adapted [15], so it was not surprising that our three study species performed the best at the highest temperature (30 °C), which is common in Florida and the Caribbean. We predicted that invasive *O. septentrionalis* would perform more poorly than the native treefrogs at the coolest temperature, relative to their size, because they are adapted to a warmer climate with higher minimum temperatures. However, we did not find support for this hypothesis; lower temperatures did not decrease the maximum jumping ability of invasive *O. septentrionalis* more so than the native frogs (Figure 2), demonstrating that these invasive frogs

either have a broad thermal breadth (before their introduction) or have possibly adapted to the cooler climate present in Florida than in their native tropical range. The invasive species had longer maximum jumps than the native species at all temperatures, even when controlling for body size differences (Figure 2), likely due to longer leg length and/or muscle mass relative to body length. These anatomical traits are tightly linked with muscle contractile properties associated with muscle speed (e.g., time to peak tetanus, maximum shortening speed, peak isotonic power), which are correlated with jumping performance in frogs [50].

Individuals of all three species were parasitized by the intestinal parasite *A. hamatospicula*, which was likely introduced to Florida with *O. septentrionalis*. The reported prevalence of infection by this parasite in *O. septentrionalis* at our study site is 50% [42] and our present study is the first to examine the prevalence of infection in frogs that are native to Florida. We found that the infection prevalence was much higher in invasive (53%) than native frogs (*H. femoralis*: 20%, *H. squirella*: 10%). Despite this high infection rate, the invasive species seems to tolerate the parasite better than the native frogs, perhaps because of a longer shared evolutionary history. Tolerance of a parasite often requires less energetic resources than costly immune functions that resist infections [51,52]. Therefore, by tolerating infections, more resources may be allocated towards processes that improve host fitness, such as increased growth, reproduction, or locomotor performance [52,53]. Hosts that do not share an evolutionary history with a parasite, such as is the case for native treefrogs and *A. hamatospicula*, could lack evolved tolerance and thus might be spending costly resources on resistance to infections. Another possible explanation for low observed infection rates in native frogs in our study is that infected native frogs quickly succumb to the parasite and are therefore rarely sampled. This explanation is, however, unlikely because our three study species have similar survival rates at our study site [29]. Although the small number of infected native frogs in our study means any conclusion regarding the effect of *A. hamatospicula* on these species should be taken with caution, the patterns observed will help form hypotheses for additional work on how this parasite affects invasive and native species.

Parasitic infections affected both the maximum jumping distance (Figure 3B) and endurance (Figure 4) of frogs. Parasitism decreased locomotor performance in native frogs, especially in large individuals and/or at high temperatures. This may be explained by reduced energy stores available for locomotion resulting from energy lost to tissue repair and immune responses [24], combined with increases in metabolic rate as temperature and body size increase [25,26]. Metabolic rates in ectotherms increase exponentially with temperature [25], so high temperatures can be particularly costly to hosts and can rapidly deplete oxygen and stored energy. Parasites decreased the maximum jump distance of large individuals of all species, and affected our measure of endurance of both native species, but not the invasive species. In both native species, parasites appeared to decrease lengths of consecutive jumps of large frogs at high temperatures, but increase consecutive jump lengths of large frogs at low temperatures and small frogs at all temperatures (Figure 4). Because the distances of final recorded jumps of parasitized and non-parasitized were similar, increases in jump distances over time were caused by short starting jumps, rather than an increase in endurance. Therefore, it appears that parasites limited the initial jumps of large, native frogs at low temperatures, but that this limitation was reduced as frogs continued jumping. Among ectotherms, parasitized individuals often have decreased locomotor performance than non-parasitized individuals. Numerous examples exist for amphibians [18–21] and reptiles [22,23]. Importantly, we found that parasitized frogs were not in poorer body condition than non-parasitized frogs, suggesting that decreases in the locomotor performance of parasitized frogs were caused by other factors.

The interactions among parasite load, temperature, and body size that we observed are likely to affect the fitness and competitive ability of frogs. Reduced jump distances and endurance in parasitized animals can compromise foraging efficiency and predator escape abilities, and consequently, can reduce fitness [6–9]. Frogs that can jump farther may

be more likely to escape from predators encountered at breeding ponds or in terrestrial habitats, such as snakes and wading birds. Higher-performing frogs may also be more successful at capturing invertebrate prey. Parasitism by *A. hamatospicula* also may affect competitive and predator-prey interactions among treefrog species. Invasive treefrogs compete with native treefrogs for food and space and they also prey on native treefrogs directly [31,54–56]. The superior locomotor performance of this invasive species, which is maintained across temperatures and parasitic infection loads, is one possible mechanism by which this species is able to outcompete and prey on native species, which are more sensitive to these factors.

The success of introduced species can be influenced by many ecological factors, including climate, disease, and their interaction. Investigating the factors influencing the locomotor performance of *O. septentrionalis* can provide useful information for understanding and predicting its success and its impacts on native species. This invader grows to be a large frog that can reach high population densities and negatively impact native frogs through competition and direct predation [31,54–56]. We demonstrate that *O. septentrionalis* also have longer maximum jumps than the native species, even when controlling for body size differences (Figure 2), which may give them a competitive advantage. The tremendous jumping performance of *O. septentrionalis*, which is maintained under low temperatures and infection by *A. hamatospicula*, likely contributes to the invasion success of this species. Future studies investigating the effects of temperature and *A. hamatospicula* on other physiological or behavioral traits of *O. septentrionalis* and native amphibians would enable a more comprehensive understanding of how climate and biotic interactions (including parasitism) affect the success and impact of an important invasive species.

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