Impacts of a Simulated Infection on the Locomotor Behavior of Invasive and Noninvasive Species of Congeneric Anurans

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ABSTRACT

Locomotion is essential for survival, but it requires resources such as energy and metabolites and therefore may conflict with other physiological processes that also demand resources, particularly expensive processes such as immunological responses. This possible trade-off may impose limits on either the magnitude of immune responses or the patterns of activity and performance. Previous studies have shown that invasive species may have a depressed immune response, allowing them to maintain locomotor function and reproduction even when sick. This may contribute to the ecological success of invasive species in colonization and dispersal. In contrast, noninvasive species tend to reduce activity as a response to infection. Here, we studied the impact of a simulated infection on locomotor performance and voluntary movement in the anurans *Xenopus laevis* (a globally invasive species) and *Xenopus allofraseri* (a noninvasive congeneric). We found that a simulated infection reduces locomotor performance in both species, with an accentuated effect on *X. allofraseri*. Voluntary movement was marginally different between species. Our data suggest that a simulated infection leads to behavioral depression and reduced locomotor performance in anurans and show that this effect is limited in the invasive *X. laevis*. Contrasting responses to an immune challenge have been reported in the few amphibian taxa analyzed to date and suggest relationships between ecology and immunology that deserve further investigation. Specifically, a depressed immune response may underlie a propension to invasion in some species. Whether this is a general trend for invasive species remains to be tested, but our data add to the growing body of work documenting depressed immune systems in invasive species.

Keywords: anurans, behavioral depression, dispersal, locomotion, immune system, invasive species.

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Introduction

Locomotion is related to fitness and in many motile species is fundamental for feeding, escaping predators, and searching for reproductive partners (Dickinson et al. 2000; Gomes et al. 2009). In parallel, locomotion is costly and sensitive to energy and resource availability, including key metabolites, and therefore is affected by reproductive status, health, and overall physiological condition (Dickinson et al. 2000; Nathan et al. 2008; Pough et al. 2008; Lowe 2009). Depending on the ecology of a species, locomotion may be a dominant activity and, in some species, may consume a considerable portion of the daily energy budget (Taigen et al. 1982; Dickinson et al. 2000). In these species, locomotion may occur in parallel with other activities or processes that also require significant resources (e.g., vocalization, immune responses, reproduction), sometimes leading to trade-offs (e.g., Pough et al. 1992; Konuma and Chiba 2007; Herrel et al. 2009).

The need for the activation of the immune system can emerge at any time along the life of an individual animal, for example, because of pathogenesis. Examples include microorganismal infection vehiculated by bacteria, which may elicit cascades of responses from inflammatory processes (Ader et al. 1987; Hart 1988; Aubert 1999; Larson and Dunn 2001; Adelman and Martin 2009) to complex autonomic responses resulting in significant physiological, hormonal, and behavioral reactions (Ader et al. 1987; Hart 1988; Sherman and Stephens 1998; Aubert 1999; Dantzer et al. 2000). These responses are typically triggered by

proinflammatory cytokines (IL-1, IL-6, and TNF-α), leading to behavioral depression, a state characterized mainly by reduced activity (Hart 1988; Lefcort and Eiger 1993; Dantzer et al. 2000; Dantzer 2004, 2006). Behavioral depression is best characterized in endothermic vertebrates (Hart 1988; Lefcort and Eiger 1993; Dantzer et al. 2000; Larson and Dunn 2001; Dantzer 2004, 2006; Adelman and Martin 2009) but has been observed in ectothermic animals such as anurans (Llewellyn et al. 2011; Braga 2013; Olarte 2017). In these animals, behavioral depression may involve reduced locomotion (Llewellyn et al. 2011; Braga 2013; Olarte 2017), a pattern compatible with a resource trade-off and the channeling of energy to activation of the immune system (Hart 1988; Sherman et al. 1991; Lefcort and Eiger 1993). Behavioral depression may thus be functionally important and ecologically critical in terms of facing immunological challenges (Hart 1988; Dantzer 2006; Adelman and Martin 2009).

The idea of a locomotion–immune system trade-off is sound and has some empirical support but does not seem universal for anurans (Llewellyn et al. 2011, 2012; Braga 2013; Brown and Sine 2014; Goetz et al. 2018; Gardner et al. 2020). The data available remain scarce and phylogenetically restricted and support the notion of substantial diversity in the degree of basal activation of the immune system. Facing a comparable immune challenge, some anuran species present a strong immune response paired with substantial behavioral (locomotion) depression (Llewellyn et al. 2011; Braga 2013; Olarte 2017), but other species maintain typical performance and locomotion, which has been interpreted as the result of a comparatively depressed immune response (Llewellyn et al. 2012; Brown and Sine 2014; Goetz et al. 2018; Gardner et al. 2020). It is possible, therefore, that anuran lineages display divergent energetic and metabolic costs of locomotion and immune responses, so that some, but not all, species maintain a highly activated immune system despite the metabolic expenses involved.

Anuran amphibians thus appear situated along a continuum between two extreme immune response strategies. Species positioned at one extreme would be characterized by a permanent and expensive state of immune alertness (Llewellyn et al. 2011; Braga 2013; Olarte 2017), whereas species at the other end of the spectrum would display immune depression as a reversible basal state to be activated only if necessary (Llewellyn et al. 2012; Brown and Sine 2014; Goetz et al. 2018; Gardner et al. 2020). The latter case may not be exclusive for anurans but a characteristic of different lineages, including both endothermic and ectothermic animals, especially those with a high capacity for dispersal (Lee et al. 2005; Llewellyn et al. 2012; White and Perkins 2012; Brown and Shine 2014; Goetz et al. 2018; Gardner et al. 2020).

It has been proposed that the above divergence in basal activation of the immune system may be related to invasion capacity. Indeed, some invasive anuran species maintain the ability to move and continue to explore new environments even if infected (Lee and Klasing 2004; Brown and Shine 2014; Gardner et al. 2020). A possible explanation is that in expanding populations near the edge of the range, natural selection and spatial sorting lead to an enhanced allocation of resources to dispersal, growth, and re-

production at a cost to immune function (Brown and Shine 2014; Gardner et al. 2020). Therefore, a depressed immune response could confer an ecological advantage to invasive species compared to noninvasive species (Lee and Klasing 2004; Brown and Shine 2014; Goetz et al. 2018; Gardner et al. 2020).

In this study, we ask whether a simulated infection reduces locomotor behavior and performance in two congeneric anuran species, the invasive *Xenopus laevis* and the noninvasive *Xenopus allofraseri*. We further ask whether the two species studied demonstrate differences in the observed responses. We simulated an infection by injecting lipopolysaccharide (LPS) in individuals of both species. LPS is derived from the cell wall of gram-negative bacteria, which are recognized as endotoxins by the anuran immune system (Sherman et al. 1991; Sherman and Stephens 1998; Bicego and Branco 2002; Ferreira et al. 2021; Junior et al. 2021; Bastos et al. 2022). LPS triggers an acute-phase immune response by promoting the mobilization of cytokines. In turn, these molecules act as endogenous pyrogens and may induce behavioral changes through humoral and neuronal pathways (Hart 1988; Dantzer 2006; Ferreira et al. 2021; Junior et al. 2021; Bastos et al. 2022). Examples include reduction of activity and thus of locomotion (Hart 1988; Aubert 1999; Dantzer 2006; Llewellyn et al. 2011).

After treatment with LPS, we analyzed two locomotor performance traits (endurance and jump force) and evaluated voluntary movement. We predicted that the two species would present differences in the observed responses and, according to the literature, that the reduction in activity would be less pronounced in the invasive species (Llewellyn et al. 2012; Brown and Shine 2014) whereas the noninvasive species would display behavioral depression as a dominant response. Accordingly, our key hypotheses are that a simulated infection reduces locomotor performance and voluntary movement and that these responses are less prominent in the invasive species (*X. laevis*).

Material and Methods

Study Animals

This study includes two species in the genus *Xenopus*: the globally invasive African clawed frog (*Xenopus laevis*, first described by François Daudin in 1802) and the noninvasive congeneric false Fraser's clawed frog (*Xenopus allofraseri*, first described by George Boulenger in 1905 and most recently described by Ben Evans in 2015). The *X. laevis* frogs were collected from a single pond near the center of the invasive range in the Département de Deux Sèvres in France in 2019. This species is mostly aquatic yet able to move over land when dispersing, capturing prey, or fleeing from predators (Measey et al. 2012; Measey 2016; Rodder et al. 2017; Courant et al. 2019). This species is native to Southern Africa but, after escapes and illegal releases from laboratories and breeding facilities, became invasive in several regions of the world. The pattern of invasion suggests resistance to environmental conditions that are generally considered adverse for anurans, a generalist diet, and rapid reproduction. Today, its trading is prohibited in the European Union and the United States (Measey et al. 2012). The noninvasive species, *X. allofraseri*, is

uncommon in laboratories. The individuals used in this study were collected in Cameroon in 2009 and then maintained in the lab in Paris (Herrel et al. 2012; Ducret et al. 2021). Little is known about the ecology and natural history of the species, but no invasive populations are known. This species is predominantly aquatic and lives in small ponds in primary forest in tropical areas of West Africa (Rödel 2000; Ducret et al. 2021). This species also uses terrestrial locomotion, particularly during heavy rains (Rödel 2000; Herrel and Bonneaud 2012b).

Animals

We worked with female frogs in the nonreproductive state ($N = 18$ females of *X. laevis*, $N = 18$ females of *X. allofraseri*) that were housed at the Muséum National d'Histoire Naturelle in Paris. We used only females, as sexual dimorphism is prominent in these species. Furthermore, given the strong investment in reproduction by females, we expected the impact of a simulated infection to be stronger. Animals were kept in uniform tanks under the same temperature and food conditions before and during the tests. During testing, the animals were kept separately in individual aquariums. Both species were tested (endurance, jump force, voluntary movement) separately and sequentially before and after LPS treatment. All tests were completed at 22°C, which is considered the optimal temperature for the species (Casterlin and Reynolds 1980). Animals were fed beef heart twice weekly.

Administration and Dosage of Lipopolysaccharide

The simulation of an infection was performed by injecting LPS (gram-negative bacterial cell wall LPS) from the bacterium *Escherichia coli* serotype 0111:B4 purified by phenol extraction at a dose of 2 mg/kg, injected into the dorsal lymph sac of the frog with a syringe with a 29-G needle attached (Bicego and Branco 2002; Llewellyn et al. 2011; Olarte 2017).

Experimental Groups

The 18 individuals available for each species were randomly distributed into three groups of equal size consisting of six individuals each, which were tested for performance (locomotor endurance and jump force) and used to quantify voluntary movement. The animals were first tested without treatment (control group) and then tested after treatment (LPS injection). For *X. laevis*, we performed a second control, where a different group of animals was injected with only a saline solution, permitting the impact of manipulation and injection on performance and behavior to be tested. However, this control was possible only for *X. laevis*, as not enough female *X. allofraseri* were available for testing.

Reinjection of LPS for animals already used in one test was not possible. Moreover, LPS has a finite action leading to a short window of maximum effects, and tests must be accomplished within this window at standardized postinjection times. Consequently, each individual was allocated to only one of the three tests (endurance, jump force, or voluntary movement). After the LPS

injection, animals were euthanized. All experiments were performed in accordance with local regulations and approved by the local ethics committee (Comité Cuvier).

Experimental Setup

Locomotor Endurance. Locomotor endurance was tested using a circular track measuring 3 m in circumference. The substrate was cork, and the track was filled with about 10 cm of water. For testing, animals were placed into the track and encouraged to swim until exhaustion, indicated by the lack of a righting response. Each individual was tested three times, with resting intervals of 48 h, and were fed after each test day. We recorded the time spent moving and the distance traveled (Herrel et al. 2012, 2014; Herrel and Bonneaud 2012a, 2012b).

Jump Force. The animals were tested on a 20 × 10-cm piezoelectric force platform connected to a Kistler charge amplifier (for a detailed description of the setup, see Herrel et al. 2014). Individuals were placed on the platform one at a time and left to rest for a few seconds. Next, they were encouraged to jump by gentle touching on the body. This test was repeated on 3 d at 48-h intervals. Forces were recorded at 500 Hz during 60-s recording sessions, and frogs were induced to jump multiple times per session. We used the Kistler BioWare software to extract x-, y-, and z-forces for each jump, corrected the signals for drift, and calculated the final resultant force in newtons (N; Herrel et al. 2014). We ran analyses based on the three best jumps per session.

Voluntary Movement. To quantify voluntary movement, we used an open-field test. Animals were placed in a large tank (80 cm × 50 cm × 40 cm) filled with 20 cm of water and a shelter (Videlier et al. 2015). Next, animals were placed under the shelter and filmed in dorsal view for 12 h (8:00 a.m.–8:00 p.m.) for 3 d with 48 h between subsequent days of recording. Videos were analyzed with a stopwatch. We recorded the total time spent moving, the total time spent in the shelter, and the number of times the animal went to the surface to breathe.

Statistical Analysis

First, we $\log_{10}$ transformed data to ensure normality and applied distribution-based tests. To test differences between animals of each species before and after LPS injections, we applied a two-factor repeated-measures ANOVA test. This test is adequate for paired data such as ours. To test the differences between the two species (*Xenopus laevis* vs. *Xenopus allofraseri*) for each variable analyzed before and after LPS injections, we applied an independent *t*-test suitable for independent data. To scrutinize the effects of saline injections in *X. laevis* (control group), we employed an ANOVA. This test compared individuals before and after the injection of saline solution to test whether an injection by itself could impact performance and behavior.

We explored the importance of body mass and test day as confounding variables. However, body mass did not affect endurance (*X. laevis*: $P = 0.910$; *X. allofraseri*: $P = 0.374$),

jump force (*X. laevis*: $P = 0.057$; *X. allofraseri*: $P = 0.059$), or voluntary movement (*X. laevis*: $P = 0.134$; *X. allofraseri*: $P = 0.119$; see tables S1, S2; tables S1–S3 are available online). The same was observed for the test day (all $P > 0.05$). Therefore, these two variables were not analyzed further or included in the results. To carry out the analyses, we used the R-based and open-source statistical software Jamovi (https://www.jamovi.org).

Results

Locomotor Endurance

The variable "time spent moving until exhaustion" was similar between control animals before and after injection with saline, according to the ANOVA performed (*Xenopus laevis*: $N = 5$ individuals, $F_{1,4} = 0.88$, $P = 0.788$; table S3). In contrast, LPS injection did have an impact on the time moved until exhaustion. LPS-treated frogs showed a 23% reduction in endurance relative to the before-treatment endurance in the species *Xenopus allofraseri* ($N = 6$ individuals, $F_{1,5} = 13.2$, $P = 0.002$) and about a 21% reduction in *X. laevis* ($N = 6$ individuals, $F_{1,5} = 13.6$, $P = 0.001$; table 1; fig. 1). Species differed, as demonstrated by the *t*-test, where females of *X. allofraseri* moved 23% less until exhaustion before LPS injection than females of *X. laevis* ($N = 12$ individuals, $t_{2,10} = 6.71$, $P < 0.001$; table 2; fig. 1). After LPS injection, the difference between species doubled, with *X. allofraseri* showing 47% less endurance than *X. laevis* ($N = 12$ individuals, $t_{2,10} = 18.7$, $P < 0.001$; table 2; fig. 1).

The distance traveled until exhaustion remained the same in the control group after saline injection (*X. laevis*: ANOVA,

$N = 5$ individuals, $F_{1,4} = 10.4$, $P = 0.320$; table S3). However, after LPS injection, the distance moved until exhaustion decreased by 19% in *X. laevis* ($N = 6$ individuals, $F_{1,5} = 6.0$, $P = 0.025$). A similar pattern was observed for *X. allofraseri*, with a reduction of 31% in the distance moved before exhaustion after LPS injection ($N = 6$ individuals, $F_{1,5} = 12.0$, $P = 0.002$; table 2; fig. 1). Regarding the differences between species before LPS injection, female *X. allofraseri* traveled 37% less distance than female *X. laevis* ($N = 12$ individuals, $t_{2,10} = 13.6$, $P < 0.001$). Effects of LPS injection were greater for *X. allofraseri*, so that LPS-injected females of *X. allofraseri* covered 46% less distance than those of *X. laevis* ($N = 12$ individuals, $t_{2,10} = 13.9$, $P < 0.001$; table 2; fig. 1).

Jump Force

For the individuals from the control group injected with saline, there were no differences in jump force before or after injection (*X. laevis*: $N = 5$ individuals, $F_{1,4} = 4.59$, $P = 0.095$; table S3). However, for both species, the jump force was reduced after LPS treatment. Force decreased by about 26% in *X. laevis* ($N = 6$ individuals, $F_{1,5} = 6.1$, $P = 0.025$) and 28% in *X. allofraseri* ($N = 6$ individuals, $F_{1,5} = 4.7$, $P = 0.027$; table 1; fig. 2) when we compared individuals before and after LPS treatment. Between species, as demonstrated by the *t*-test, this variable was also different in both animals before treatment ($N = 12$ individuals, $t_{2,10} = 2.82$, $P < 0.001$), with *X. allofraseri* producing 28% less force than *X. laevis*. After LPS injection, the difference between species was 30% ($N = 12$ individuals, $t_{2,10} = 3.46$, $P < 0.001$; table 2; fig. 2).

Table 1: Two-factor repeated-measures ANOVA comparing animals before and after injection with lipopolysaccharide for both species

| Species, variable	MS	df	F	P
Xenopus allofraseri:				
Locomotion endurance:				
Total time on track	.1156	1, 5	13.2	.002
Total distance covered	.0283	1, 5	12.3	.002
Jump force	.1534	1, 5	4.72	.027
Voluntary movement:				
Time in moving	.7425	1, 5	7.91	.013
Time in shelter	.0756	1, 5	.48	.538
Number of breaths	.0841	1, 5	1.91	.186
Xenopus laevis:				
Locomotion endurance:				
Total time on track	.1308	1, 5	13.3	.001
Total distance covered	.2827	1, 5	13.6	.025
Jump force	.3680	1, 5	6.17	.025
Voluntary movement:				
Time in moving	.4031	1, 5	2.15	.163
Time in shelter	.0444	1, 5	.17	.688
Number of breaths	.0841	1, 5	2.46	.138
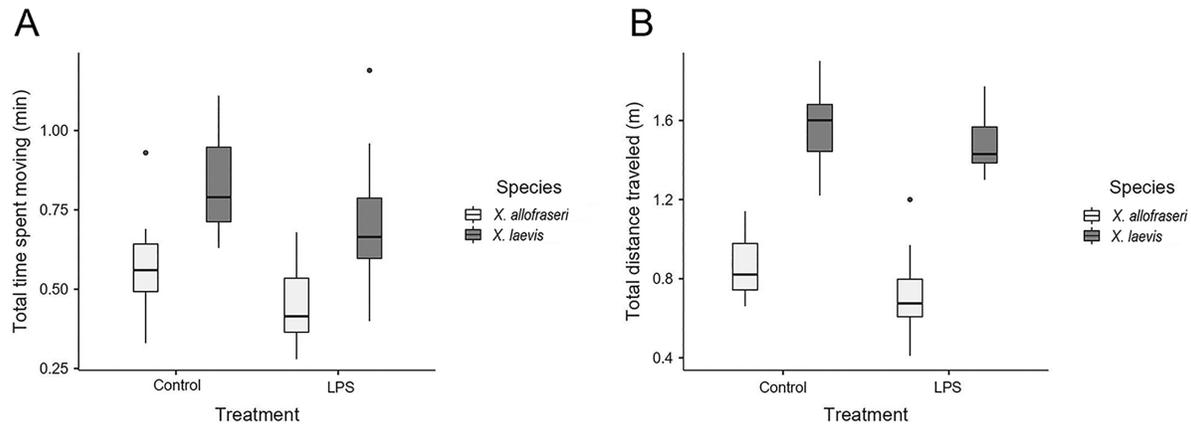
Figure 1. Endurance capacity in *Xenopus laevis* and *Xenopus allofraseri* females before versus after being injected with lipopolysaccharide (LPS) and comparison between the species. *A*, Total time spent moving until exhaustion. *B*, Total distance traveled until exhaustion.

Voluntary Movement

Individuals of *X. laevis* from the control group did not show differences before and after injection with saline in all variables analyzed (*X. laevis*: $N = 5$ individuals, all $F_{1,4} > 2.0$, all $P > 0.005$; table S3). As demonstrated by the ANOVA, the time spent moving reduced by about 24% in *X. allofraseri* after LPS injection ($N = 6$ individuals, $F_{1,5} = 8.0$, $P = 0.013$), but this pattern was not replicated in *X. laevis* ($N = 6$ individuals, $F_{1,5} = 2.1$, $P = 0.163$; table 1; fig. 3), which showed no difference in the total time spent moving. Differences existed between species, with *X. allofraseri* spending 30% less time moving than *X. laevis* before treatment ($N = 12$ individuals, $t_{2,10} = 1.83$, $P = 0.017$). The difference remained at 31% after LPS injection ($N = 12$ individuals, $t_{2,10} = 1.68$, $P = 0.019$; table 2; fig. 3).

The time spent in the shelter remained stable after LPS injection for both *X. laevis* ($N = 6$ individuals, $F_{1,5} = 0.16$, $P = 0.688$) and *X. allofraseri* ($N = 6$ individuals, $F_{1,5} = 1.9$, $P = 0.538$; table 1; fig. 3), yet the two species differed when comparing behavior before treatment (12% difference; $N = 12$ individuals, $t_{2,10} = 2.22$, $P = 0.028$) and after treatment (16% difference; $N = 12$ individuals, $t_{2,10} = 2.86$, $P = 0.017$). *Xenopus allofraseri* spent less time in the shelter than *X. laevis* (table 2; fig. 3).

The variable "number of breaths" remained similar when comparing control females (before treatment) and females injected with LPS in both species (*X. laevis*: $N = 6$ individuals, $F_{1,5} = 2.4$, $P = 0.138$; *X. allofraseri*: $N = 6$ individuals, $F_{1,5} = 1.9$, $P = 0.186$; table 1; fig. 3). Species differed in the number of breaths, being 24% greater in *X. allofraseri* before

Table 2: Independent *t*-test comparing species before and after lipopolysaccharide (LPS) injection

Variable	*t*	df	*P*
Locomotion endurance:			
Total time on track before LPS	6.71	2, 10	<.001
Total time on track after LPS	18.7	2, 10	<.001
Total distance covered before LPS	13.6	2, 10	<.001
Total distance covered after LPS	13.9	2, 10	<.001
Jump force:			
Jump force before LPS	2.82	2, 10	<.001
Jump force after LPS	3.46	2, 10	<.001
Voluntary movement:			
Time in moving before LPS	1.83	2, 10	.017
Time in moving after LPS	1.68	2, 10	.019
Time in shelter before LPS	2.22	2, 10	.028
Time in shelter after LPS	2.86	2, 10	.017
Number of breaths before LPS	2.73	2, 10	.002
Number of breaths after LPS	1.74	2, 10	.010

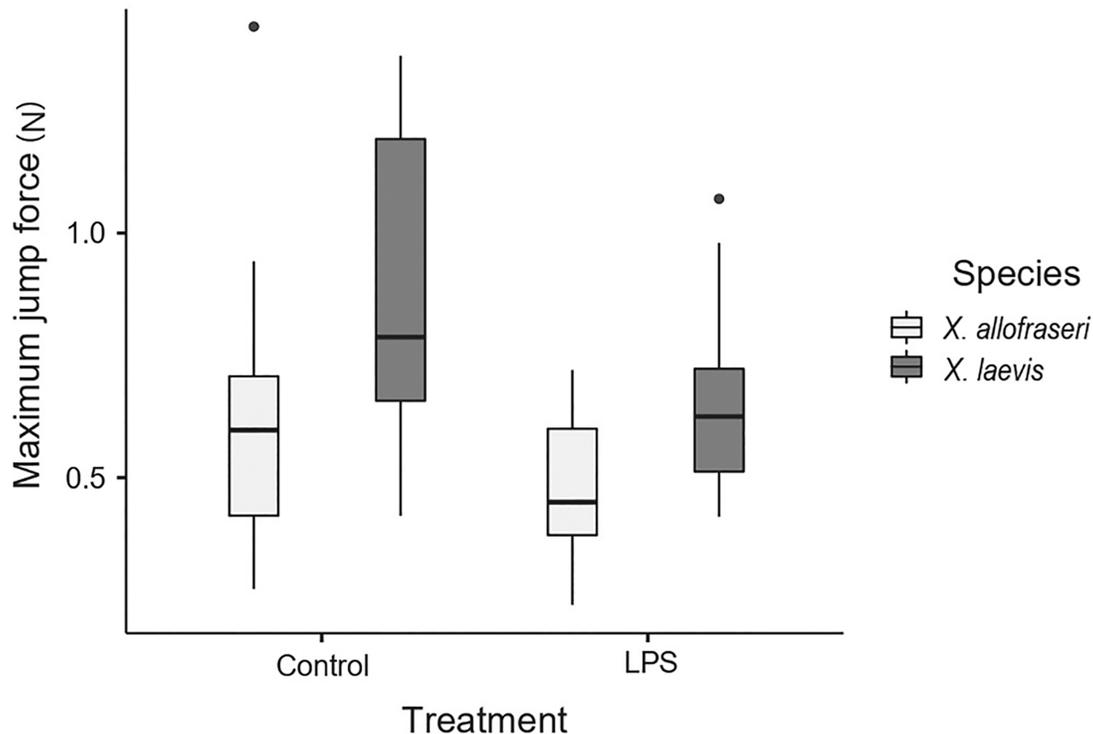Note. Species differences are significant both before and after LPS injection.

Figure 2. Analysis of the maximum jump force in *Xenopus laevis* and *Xenopus allofraseri* females before versus after being injected with lipopolysaccharide (LPS) and comparison between the species.

treatment ($N = 12$ individuals, $t_{2, 10} = 2.73$, $P = 0.002$), and this difference (22%) remained after LPS injection ($N = 12$ individuals, $t_{2, 10} = 1.74$, $P = 0.010$; table 2; fig. 3).

Discussion

Treatment with LPS altered the locomotor performance in both species studied, with trends that were similar in direction but different in magnitude. The results for the noninvasive *Xenopus allofraseri* matched expectations based on its natural history, given that it is common for anuran species, after an immune challenge, to show behavioral depression that results in a reduction of activity as well as performance (Llewellyn et al. 2011; Braga 2013). In contrast, the species with greater potential for dispersal, *Xenopus laevis* (according to its present distribution; Rodder et al. 2017; Courant et al. 2019), exhibited milder impacts of LPS injection, thus corroborating the findings of previous studies on invasive species (Lee et al. 2005; Llewellyn et al. 2012; White and Perkins 2012; Brown and Shine 2014; Goetz et al. 2018; Gardner et al. 2020). Indeed, even after an immune challenge, some anuran species may preserve the ability to carry out activities such as locomotion. This characteristic seems linked to a comparatively depressed immune system. These species thus maintain their activity and locomotion even when mildly sick, a trait of possible importance that may be correlated to dispersal and the colonization of new habitats (Lee et al. 2005; Llewellyn et al. 2012; White

and Perkins 2012; Brown and Shine 2014; Goetz et al. 2018; Gardner et al. 2020).

The differential impact of an immunological challenge on locomotor performance and voluntary movement is interesting and requires analyzing the two independent components that Fry (1947, p. 6) described as follows: "These are, in everyday language, how the animal works and what the animal does." We analyzed voluntary movement, which is a directive factor (Fry 1947) or, in other words, a behavioral response related to a neurophysiological decision to move. This response may have multiple goals, including exploration of the environment, escape from predators, search for partners for reproduction, search for water sources, and search for food (Nielsen-Schmidt 1975; Pough 2008), and therefore can affect the organism's energy balance (Zug 1972; Bennett and Licht 1973; Duellman and Trueb 1986; Stehouwer 1992; Dickinson et al. 2000; Gomes et al. 2009). In contrast, we interpret measures of maximal locomotor performance as being limited by metabolic output. In other words, this is the maximum possible performance that an individual can produce under a given physiological state, for example, the maximum an animal can move or perform while facing an infection (Zug 1972; Bennett and Licht 1973; Stehouwer 1992; Dickinson et al. 2000).

In our study, locomotor performance was greatly reduced, suggesting a direct energetic trade-off between the cost of mounting an immune response and the locomotor performance. In other words, the energy allocated to mounting an immune
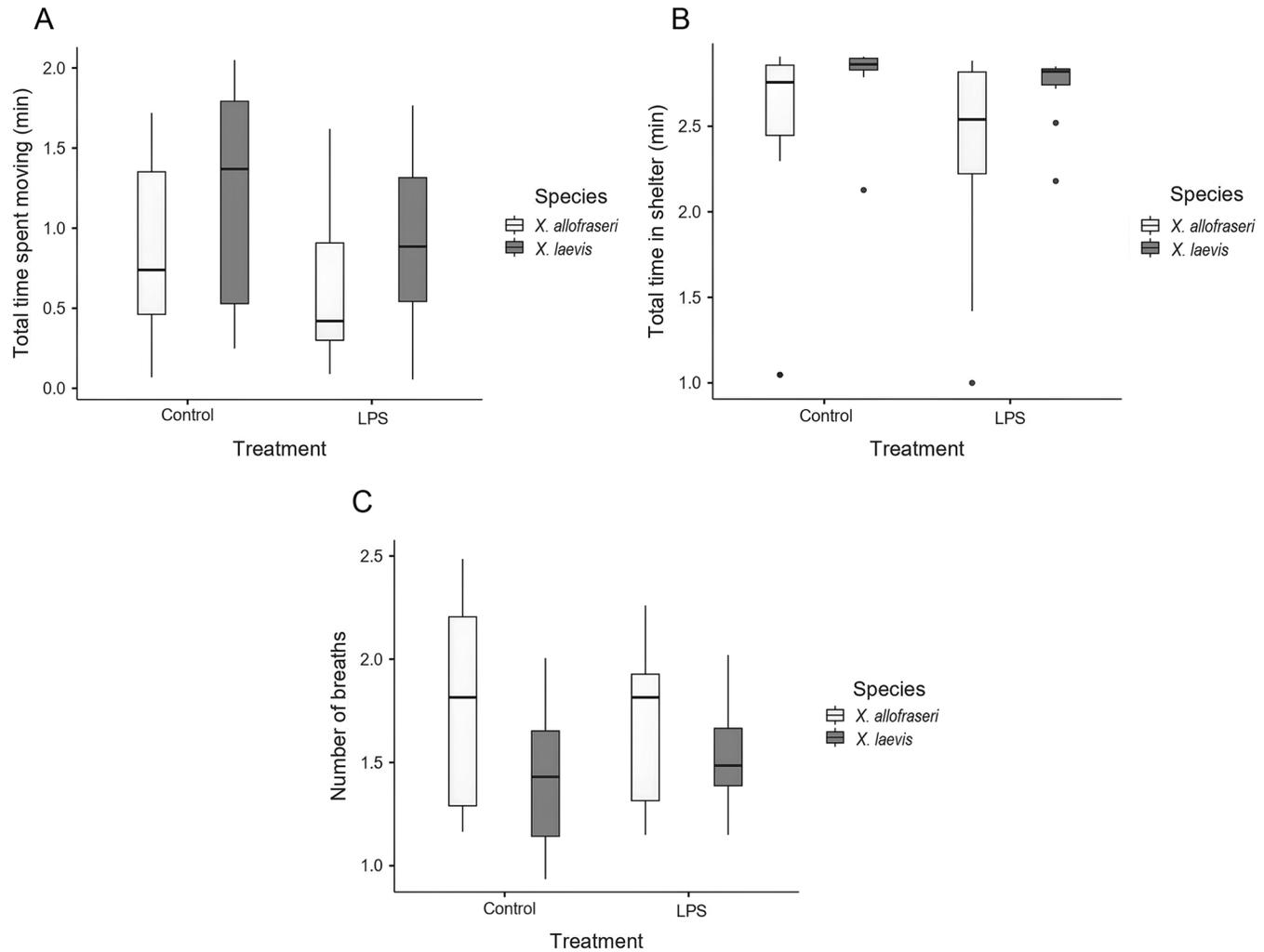
Figure 3. Analysis of voluntary movement in *Xenopus laevis* and *Xenopus allofraseri* females before versus after being injected with lipopolysaccharide (LPS) and comparison between the species. *A*, Total time spent moving. *B*, Total time in the shelter. *C*, Number of breaths.

response may no longer be available for locomotion, causing a reduction in maximal performance. However, the effect of LPS injection on voluntary movement showed a behavioral depression in the noninvasive species yet little to no impact on the invasive species. This result suggests that the invasive species chooses to move even when less energy is available for movement, likely mirroring fundamental differences in the behavioral drive for movement in the two species.

The reduction in endurance after LPS injection could be a broad pattern, as it has been observed in other anurans and in some cases is linked to behavioral depression (Llewellyn et al. 2011; Braga 2013). In our study, performance in *X. allofraseri* was more affected, and a reduction in the total time of voluntary movement was also observed, illustrating a response pattern with both trade-offs and behavioral depression. These observations correspond to studies carried out in other noninvasive anuran species, which also showed reduced overall locomotor behavior after a simulated infection (Llewellyn et al. 2011; Braga 2013; Olarte 2017). These species may thus rely on partial behavioral

depression to free up energetic and metabolic resources to mount a stronger immune response (Llewellyn et al. 2011; Braga 2013).

Contrary to what was observed in the noninvasive species, individuals of *X. laevis* showed a smaller reduction in locomotor performance (endurance and maximal force) and their voluntary movement was not affected, suggesting no behavioral depression. This shows how, in this species, a simulated infection does lead to trade-offs (reduced performance) but does not affect neurophysiological processes linked to voluntary movement (i.e., no behavioral depression). Possibly, invasive anuran species that have a depressed immune system allocate more energy to activities related to dispersal (Lee and Klasing 2004; Lee et al. 2005; Llewellyn et al. 2012; White and Perkins 2012; Brown and Shine 2014; Goetz et al. 2018; Gardner et al. 2020).

Overall, *X. allofraseri* presents behavioral depression and energetic trade-offs resulting in reduced performance, although it is not as pronounced as that observed in some other species (Llewellyn et al. 2011; Braga 2013). Our study contributes to the view that invasive frog species may have a comparatively

depressed immune system, allowing them to maintain relatively higher levels of locomotor capacity. This is likely related to the importance of dispersal into new environments, allowing animals to access new resources and environments free from competition (Llewellyn et al. 2012; Brown and Shine 2014; Goetz et al. 2018; Gardner et al. 2020).

Whereas we acknowledge the limitations of a single study based on two species (Garland and Adolph 1994), we advocate that cumulative information on pairs of congeneric species, invasive and noninvasive, will illuminate the relationship between the immune system and ecology in amphibians, particularly in the context of invasive biology. Repeated patterns across genera, using pairs of species of contrasting ecology, may corroborate evolutionary convergence, particularly if phylogenetic diversity exists in the global pool represented. This diversity is necessary because invasive and noninvasive species may differ in various aspects of ecology whose link to invasion biology remains to be understood. As such, our data add to a growing body of work highlighting differences in the impact of a simulated infection in invasive species. The two species studied here share overall habits and natural history, yet *X. allofraseri* lives in small ponds in the middle of tropical forests (Rödel 2000; Videlier et al. 2015; Herrel and Bonneaud 2012*b*), whereas *X. laevis* lives in a great diversity of generally more open habitats (Measey et al. 2012; Measey 2016; Rodder et al. 2016). Possibly, differences in the native habitats may drive part of the divergence in the natural history of dispersal (e.g., more chances for ponds to dry up in open habitats). This factor may have favored, evolutionarily, the responses to infection, facilitating *X. laevis* to be an excellent invader.

Our study shows that two species of the genus *Xenopus*, which differ in natural history from the point of view of invasion biology, show reduced locomotor performance after a simulated infection. However, only the noninvasive species showed a reduction in voluntary movement. These results are consistent with the view that some animals, such as *X. allofraseri*, exhibit behavioral depression following an immunological challenge in addition to a reduction in peak performance as a result of trade-offs in energy allocation. Our data also corroborate that invasive species, such as *X. laevis*, make different behavioral decisions and may be less affected after a simulated infection. These findings suggest that invasive species have a depressed immune system and maintain locomotor activity related to dispersal, but additional studies on other genera are clearly needed. Our data show that even congeneric species of anurans may differ substantially in their response to the same immune challenge, a factor that possibly relates to contrasts in ecological resilience among amphibians.

Acknowledgments

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