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Intersexual differences in response to an immune challenge in the frog *Xenopus laevis*

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Activities such as locomotion and reproduction, as well as immune responses, require energy and metabolites, which can lead to conflicts in the allocation of these resources. Consequently, the allocation of energy and metabolites can limit immune response or activity patterns, resulting in trade-offs. In most sick animals immune responses include behavioural depression and consequently reduced activity, perhaps enhancing resource conservation and promoting activation of the immune system. These trade-offs mediated by reduction in activity may differ between males and females due to differences in energy expenditure primarily linked to reproduction. Therefore, we investigated the effect of a simulated infection on the locomotor performance and voluntary movement of males and females of the species *Xenopus laevis*. We analysed locomotor endurance, jump force and voluntary activity before and after a simulated infection through injections of LPS (endotoxins from the cell wall of gram-negative bacteria). Our results showed that locomotor performance and jump force decreased after simulated infection in both sexes. Furthermore, the magnitude of the responses differed between the sexes. Females of *X. laevis* exhibited greater reductions in performance and jump force, which may be related to differential energy allocation, including a greater investment in gamete production by females, leading individuals to be more immunocompetent than males. Unexpectedly, and independently of sex, the simulated infection had no impact on most variables pertaining to voluntary movement, suggesting that behavioural depression did not occur. Only the number of breaths increased after the infection, with this increase being more pronounced in females, suggesting a greater energy requirement in females in the face of an immune challenge.

Keywords: amphibians, behavioural depression, energetic resources, immune system, locomotion

INTRODUCTION

In most lineages of vertebrate animals, including anurans, the evolution of morphology and physiology is closely related to locomotor performance and, in turn, ecology (Taigen et al., 1982; Dickinson et al., 2000; Irschick & Garland, 2001; Nathan et al., 2008). The relationship between form and function is the result of natural selection because functional traits such as locomotion affect ecologically relevant behaviours such as foraging, reproduction, and escape from predators, directly impacting the fitness of the individual (Taigen et al., 1982; Dickinson et al., 2000). However, locomotor behaviour can require considerable use of energy and metabolic resources, which may cause conflict with other physiological processes that also require these resources, such as activation of the immune system and/or investment in reproduction (Sheldon, 1996; Herrel et al., 2009; Husak et al., 2016; Courant et al., 2017). Thus, when locomotion, immune responses, reproduction and other activities need to occur in parallel this can result in trade-offs, given that energy and metabolic resources are not unlimited (Pough et al., 1992; Sheldon, 1996). Given that, animals might fail to maximise activities and immune system simultaneously (Hart, 1988; Dantzer et al., 2000; Larson & Dunn, 2001).

The activation of the immune system can occur due to contact with bacteria and/or endotoxins such as LPS, which, when recognised by the body as pathogens, stimulate an immune response in endothermic (Dantzer et al., 2000; Zuk & Stoher, 2002) and also ectothermic animals such as anurans (Bastos et al., 2022; Ferreira et al., 2021). Immune responses can include an autonomic component and a complex group of physiological, hormonal and behavioural responses that are triggered by the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) released during the infectious process (Hart, 1988; Dantzer, 2004; 2006; Ferreira et al., 2021). The subsequent responses may include behavioural depression, resulting in reduced activity levels and voluntary movements (Hart, 1988; Aubert, 1999; Larson & Dunn, 2001; Dantzer, 2006).

Behavioural depression has been observed as a dominant behavioural response to an immune challenge in endothermic animals (Hart, 1988; Dantzer, 2004; 2006; Adelman & Martin, 2009) and has also been observed in ectothermic animals such as anurans (Llewellyn et al., 2011; Braga, 2013; Oliveira, 2022; 2024). The reduction in activity

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caused by behavioural depression may result in the saving of energy and metabolic resources that can be allocated and used by the immune system to mount an immune response (Hart, 1988; Larson & Dunn, 2001). Furthermore, behavioural depression can be viewed as an adaptive response in an ecological context, since reduced activity may lead to lower exposure to predators when the animal is sick (Lefcort & Eiger, 1993). Behavioural depression thus appears to be part of a group of functionally important and ecologically relevant responses.

In anurans, it has been observed that a simulated infectious process leads to reduced feeding and locomotion rates, which may be related to behavioural depression (Llewellyn et al., 2011; Braga, 2013; Oliveira, 2022; Oliveira et al., 2024). However, although behavioural depression is a dominant response in most animal lineages (Hart, 1988; Adelman & Martin, 2009), some previous other studies with anurans have shown that, even when faced with an immunological challenge, species characterised by high dispersal rates appear to invest less resources in immune function and maintain activities linked to dispersal (Llewellyn et al., 2012; Brown & Shine, 2014; Goetz et al., 2017; Gardner et al., 2020). Therefore, activities such as locomotion and activation of the immune system appear to be involved in physiological compensation or trade-offs (Llewellyn et al., 2011).

Trade-offs between the activation of the immune system and activity may be even more pronounced when other resource demands are involved, such as investment in reproduction (Nunn et al., 2008; Klein & Flanagan, 2016; Kelly et al., 2018). Differences in immunocompetence between male and female anurans have been observed, these differences combined with life history characteristics such as growth, metamorphosis and sexual maturation, which may differ substantially between the sexes, can lead to differential expenditure of energy resources in activities such as reproduction (e.g. Miaud et al., 2006; Bryan et al., 2023; Guarino et al., 2023; Cantekin et al., 2023). Reproduction consists of several steps for both males and females, and these steps have variable costs (Nunn et al., 2008; Courant et al., 2017; Kelly et al., 2018). Furthermore, the seasonality of energetic demands may vary. In seasonal scenarios, males present high metabolic costs during the reproductive period, for example, due to vocalisation (Bucher et al., 1982; Taigen & Pough, 1985; Taigen & Wells, 1985; Wells & Taigen, 1989), but females present higher costs in other phases of reproduction, for example, through direct investment in gametes and offspring (Wells, 1977; Grafe et al., 1992; Courant et al., 2017). As observed in previous studies, energy expenditure during egg production in females is approximately three times higher than for sperm production (Hayward & Gillooly, 2011). Furthermore, it has been observed that gamete biomass production rates are two to four orders of magnitude higher in females, leading to higher energy expenditure (Ryan et al., 1983; Grafe et al., 1992; Hayward & Gillooly, 2011). Therefore, in general, females present greater investment in reproductive activity compared to males, and in a scenario where resources may need to be allocated to activity, immune responses or reproduction this may lead to lower immunocompetence of females (Nunn et al., 2008; Klein & Flanagan, 2016).

In a previous study, we showed that after a simulated infection two species of the genus *Xenopus* presented reduced levels of locomotor performance (Oliveira et al., 2024). In the present study, we examined whether changes in locomotor performance after a simulated infection differed between males and females of *X. laevis*. We hypothesised that females would show greater reduction in locomotor performance and greater behavioural reduction compared to males, due to differences in immunocompetence related to differential investment in reproduction between the sexes. Furthermore, we predicted that, as previously demonstrated (Oliveira et al., 2024), the reduction in locomotor performance would be a dominant response in this species. To simulate an infectious process, individuals were injected with LPS. We measured the impact of simulated infection on two locomotor performance traits (endurance capacity and maximum jumping force) and on the voluntary movements of the animals.

MATERIALS & METHODS

Study animals

The animals used in this study, the African clawed frog *X. laevis* (Daudin, 1802), were collected from a single lake near the centre of the invasion in the Deux Sèvres department, France. After collection, the animals were brought back and housed at the Muséum National d'Histoire Naturelle in Paris, France (MNHN). This species is native to South Africa but, after escapes and releases from laboratories, has become invasive in several regions of the world (Lillo et al., 2005; Lobos & Jaksic, 2005; Fouquet & Measey, 2006). Animals were housed by sex in automated water recirculation systems (Xenrack, Aquatic enterprises) in a room maintained at 22 °C, considered the ideal temperature for the species (Casterlin & Reynolds, 1980; Miller, 1982), with a 12 hours light/dark cycle, and were fed a cube of beef heart twice weekly. A total of 18 males and 18 females were analysed.

Animal maintenance during testing and experimental time

The tests were carried out between March and August 2021. To carry out the tests, the animals were removed from their original tanks and kept separately in uniform tanks under the same conditions as described above. During the tests, the animals were fed two days before the test and immediately after each test day. Both sexes were tested separately and sequentially three times for three days, with resting intervals of 48 hours between test days. The animals were tested before LPS injection and, after an interval of one week, the animals were injected and tested again.

Administration and dosage of LPS and saline

The infection simulation was carried out through the injection of LPS from the bacterium *Escherichia coli* Serotype 0111:B4 purified by phenol extraction at a dose of 2 mg/kg diluted in saline solution to the specific dose for each animal according to its weight. This solution was injected into the frog's dorsal lymphatic sac with a syringe with a 29-gauge needle (Bicego & Branco, 2002; Llewellyn et al., 2011; Olarte, 2017). Injections were always performed at 07:00 h and the animals were tested one hour later. The control group was injected with phosphate-buffered saline (Sigma Aldrich, pH 7.4) at a dose of 2 mg/kg, following the same injection protocol.

Experimental groups

The 18 individuals of each sex were randomly distributed into three groups of equal size. Specifically, we tested six animals of each sex for endurance, six of each sex for jump force, and six animals of each sex for voluntary movement. Given the limited number of animals at our disposal, we first analysed each individual before and after LPS injection. Since re-injection of LPS in animals already treated was not desirable, we opted for using different groups of animals for each test. Furthermore, LPS has a finite action, leading to a short window of maximum effects and the tests should therefore be performed within this window at standardised post-injection times. After the LPS injection experiments, the animals were euthanised for ethical reasons. Subsequently, a control test with a different group of animals injected with saline solution only was performed. This demonstrated that the handling and injection did not bias our results. Therefore, the analyses performed on the individuals before treatment with LPS correctly represent control analyses for comparison with animals injected with LPS. All experiments were performed in accordance with local regulations and approved by the institutional Ethics Committee (Comité Cuvier).

Experimental set-up

All subjects were weighed on an electronic balance (Cgoldenwall candel CNA-383H; precision ± 0.1 g) before and after the tests.

Locomotor endurance. This performance trait was tested using a circular track measuring 3 m in circumference. The track was filled with 10 cm of water. For testing, animals were placed into the track and encouraged to swim until exhaustion with soft touches on the back. We recorded the total time spent moving (min), using a stopwatch, and the total distance travelled (m) (see Herrel et al., 2012; Herrel & Bonneaud, 2012a, 2012b; Herrel at al., 2014).

Jump force. This variable was tested on a 20 x 10 cm piezoelectric force platform connected to a Kistler charge amplifier (see Herrel et al., 2014 for a detailed description of the set-up). 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. Forces were recorded at 500 Hz during 60 second 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 when present, and calculated the final resultant force in newtons unit (N). We ran analyses based on the three best jumps per session (Herrel et al., 2014).

Voluntary movement. We used an open-field test to quantify voluntary movement. Animals were placed in a large tank (80 x 50 x 40 cm) filled with 20 cm of water and a shelter (Videlier et al., 2015). At the beginning of the test, the animals were placed under the shelter and left in the tank, being filmed in dorsal view for 12 hours (08:00–20:00 h) with a Sony Handycam (HDR-CX740). The videos were analysed manually and the total time spent moving (min), the total time in the shelter (min), and the number of times the animal went to the surface to breathe (count) were recorded using a stopwatch.

Statistical analysis

First, we used a Shapiro-Wilk test to check for normality. Next, the data were $\log_{10}$ transformed to ensure homoscedasticity assumptions were met.

To analyse whether there were differences in responses between the sexes that could be explained by body mass, a one-way ANCOVA test was performed first comparing sexes, using sex as a factor and $\log_{10}$ body mass as a covariate, and then comparing each sex before and after LPS treatment, using treatment as a factor and $\log_{10}$ body mass as a covariate. The test was applied for each variable analysed. The influence of test days was analysed in the same way. However, there were no differences in responses observed between the three test days (all P > 0.05), so test days were not further explored or included in the results.

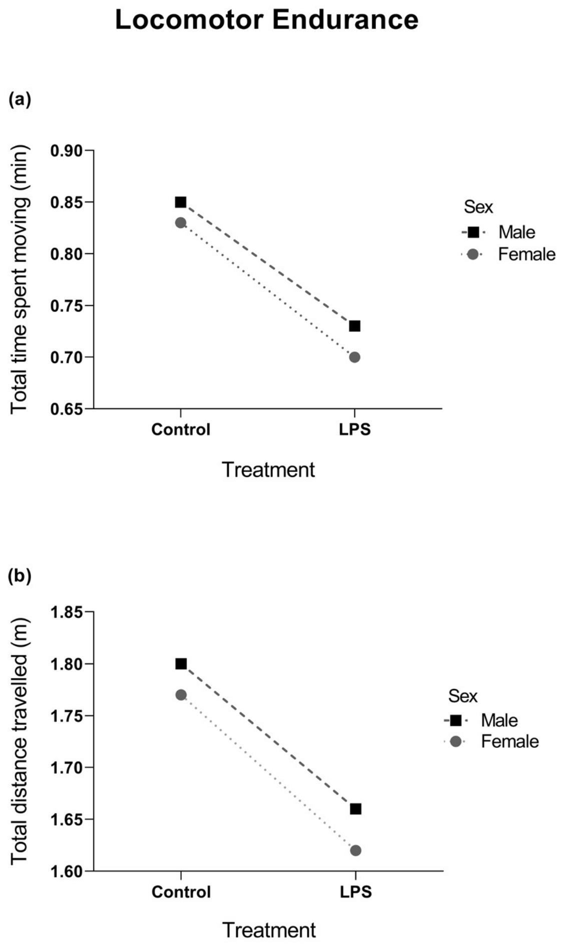

Figure 1. Locomotor endurance in males and females of *Xenopus laevis* before and after LPS injection- **(a)** Total time spent moving (min), **(b)** Total distance travelled (m).

Table 1. Two-way ANOVA test comparing treatment (before/after LPS injection) and sex and their interaction in *Xenopus laevis* for each variable tested. Each variable was tested separately.

Variable	Comparison	Sum of Squares	d.f	Mean square	F	P
Locomotor endurance						
Total distance travelled (m)	Sex	0.0162	1	0.0162	0.55	0.460
	Treatment	0.2312	1	0.2312	7.87	0.007
	Sex * Treatment	9.39e-4	1	9.39e-4	0.03	0.859
Total time in moving (min)	Sex	0.1970	1	0.1970	4.88	0.073
	Treatment	0.5601	1	0.5601	10.9	0.001
	Sex * Treatment	1.25e-5	1	1.25e-5	2.45e-4	0.988
Jump force (N)	Sex	0.1340	1	0.1340	2.07	0.155
	Treatment	0.2420	1	0.2420	1.72	0.038
	Sex * Treatment	2.3870	1	2.3870	36.76	**<0.001***
Voluntary movement						
Total time in moving (min)	Sex	0.3016	1	0.3016	1.03	0.314
	Treatment	0.0068	1	0.0068	0.02	0.879
	Sex * Treatment	0.9614	1	0.9614	3.28	0.074
Total time in shelter (min)	Sex	0.0026	1	0.0026	0.13	0.715
	Treatment	0.0150	1	0.0150	0.75	0.389
	Sex * Treatment	8.00e-4	1	8.00e-4	0.04	0.842
Number of breaths (count)	Sex	0.0691	1	0.0691	0.98	0.324
	Treatment	0.2415	1	0.2415	6.45	0.037
	Sex * Treatment	0.5530	1	0.5530	7.97	**0.006***

Note: Comparisons based on estimated marginal means; d.f = degrees of freedom.

To analyse the differences between sexes (males vs. females), we employed a two-way ANOVA with one between-subjects factor (sex) and one within-subjects factor (treatment - before vs. after LPS injection) as well as sex by treatment interaction effects. Next, a Tukey post-hoc test was performed for each variable to identify significant differences between treatment groups and sexes. To analyse the differences before and after LPS injections in males and females separately for each variable, a repeated measures ANOVA test was used. To analyse the differences before and after saline injection in males and females for each variable tested, a repeated measures ANOVA test was used. Analyses were performed using the open-source R-based statistical software Jamovi (Jamovi Open Statistical Software).

RESULTS

Influence of the body mass

The body mass had no influence on locomotor endurance and voluntary movement (all P > 0.05; see Table S1 in supplementary material). However, for jump force the body mass differed significantly between sexes and had an influence on this variable, both before (N = 12; $F_{1,11}$ = 4.88; P > 0.001) and after LPS injection (N = 12; $F_{1,11}$ = 5.85; P = 0.021). Males used for the jumping trials were 43% larger than females (see Table S1 in supplementary material). The effect of body mass was, however, not significant when testing the effect of treatment (i.e. before or after LPS injection) for each sex separately (all P > 0.05; see Table S1 in supplementary material).

Control group – treatment with saline solution

Injection of saline solution into animals did not affect any of the performance or behavioural traits examined, demonstrating that the effect of manipulation and injection did not affect the behaviour of the animals (all P > 0.005; see Table S2 in supplementary material).

Locomotor endurance

The two locomotor endurance variables analysed, 'total time spent moving (min)' and 'total distance travelled (m)', did not show differences between sexes as demonstrated by the two-way ANOVA, neither before nor after LPS injection (all P > 0.005; see Tables 1 and 2). However, when comparing individuals of each sex separately, before and after LPS injection, significant differences were observed. For the 'total time spent moving (min)', males showed a 17% reduction after LPS injection (N = 6; $F_{1,11}$ = 5.31; P = 0.036), and females a 21% reduction (N = 6; $F_{1,11}$ = 13.3; P = 0.001; Table 3; Fig. 1a). For the variable 'total distance travelled (m)' there were also differences, with a reduction of 58% in males (N = 6; $F_{1,11}$ = 8.95; P = 0.009) and 63% in females (N = 6; $F_{1,11}$ = 13.6; P = 0.025; Table 3; Fig. 1b) after LPS injection.

Jump force

The interaction effect between sex and treatment was highly significant (P < 0.001; Table 1) showing that the effect of treatment was different for males and females. Moreover, there were differences between sexes as demonstrated by the two-way ANOVA (N = 12; $F_{1.23}$ = 36.7; P < 0.001; see Table 1), both before (N = 12; $t_{4,48}$ = 3.27; P = 0.009), and after LPS injection (N = 12; $t_{4,48}$ = 5.30; P < 0.001; see Table 2). Jump force was 27% lower in females before and 45% lower after LPS injection compared to males (Fig. 2). Maximum jump force also showed significant differences when comparing individuals of each sex before

Table 2. Tukey post-hoc test applied after two-way ANOVA to identify significant differences between treatment groups (control: before injection; LPS: after LPS injection) and sexes of *Xenopus laevis* for each variable tested. Each variable was tested separately.

Variable	Sex	Treatment	Sex	Treatment	Mean Difference	S.E	d.f	t	P tukey
Locomotor endurance									
Total distance travelled (m)	Male	Control	Female	Control	0.0228	0.0571	44.0	0.399	0.978
	Male	Control	Female	LPS	0.1433	0.0571	44.0	2.510	0.067
	Male	LPS	Female	Control	-0.0833	0.0571	44.0	-1.459	0.468
	Male	LPS	Female	LPS	0.0372	0.0571	44.0	0.652	0.915
Total time in moving (min)	Male	Control	Female	Control	0.1039	0.0752	44.0	1.381	0.516
	Male	Control	Female	LPS	0.2811	0.0752	44.0	0.737	0.620
	Male	LPS	Female	Control	-0.0717	0.0752	44.0	-0.953	0.777
	Male	LPS	Female	LPS	0.1056	0.0752	44.0	1.403	0.502
Jump force (N)	Male	Control	Female	Control	0.2778	0.0849	44.0	3.270	**0.009***
	Male	Control	Female	LPS	0.4800	0.0849	44.0	5.651	**< .001***
	Male	LPS	Female	Control	0.2483	0.0849	44.0	2.924	**0.024***
	Male	LPS	Female	LPS	0.4506	0.0849	44.0	5.305	**< .001***
Voluntary movement									
Total time in moving (min)	Male	Control	Female	Control	-0.102	0.180	44.0	-0.564	0.942
	Male	Control	Female	LPS	0.110	0.180	44.0	0.610	0.929
	Male	LPS	Female	Control	0.149	0.180	44.0	0.825	0.842
	Male	LPS	Female	LPS	0.361	0.180	44.0	1.999	0.199
Total time in shelter (min)	Male	Control	Female	Control	0.01889	0.0471	44.0	0.401	0.978
	Male	Control	Female	LPS	0.04111	0.0471	44.0	0.873	0.819
	Male	LPS	Female	Control	-0.01667	0.0471	44.0	-0.354	0.985
	Male	LPS	Female	LPS	0.00556	0.0471	44.0	0.118	0.999
Number of breaths (count)	Male	Control	Female	Control	-0.2372	0.0881	44.0	2.693	**0.043***
	Male	Control	Female	LPS	-0.1778	0.0881	44.0	2.718	0.059
	Male	LPS	Female	Control	-0.1133	0.0881	44.0	1.612	0.075
	Male	LPS	Female	LPS	-0.2911	0.0881	44.0	3.304	**0.008***

Notes: Comparisons are based on estimated marginal means for all data of each sex and each variable. Data used were $\log_{10}$ transformed. SE = Standard error; d.f = degrees of freedom.

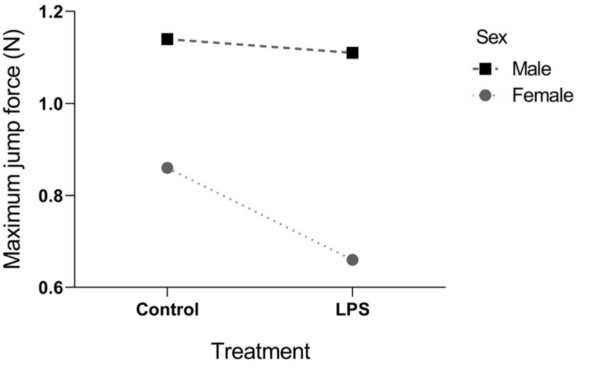

Figure 2. Jump force (N) in males and females of *Xenopus laevis* before and after LPS injection

and after LPS treatment, with a reduction of 10% in males (N = 6; $F_{1,11}$ = 6.16; P = 0.035) and 26% in females (N = 6; $F_{1,11}$ = 6.17; P = 0.025; Table 3; Fig. 2).

Voluntary movement

For voluntary movement, the only variable that presented significant interaction effects was 'the total number of breaths (count)' (P = 0.006) showing that the effect of treatment was different for males and females. Moreover, as demonstrated by two-way ANOVA (N = 12; $F_{1.23}$ = 7.97; P = 0.006; see Table 1), both before (N = 12; $t_{4,48}$ = 2.69; P = 0.043) and after LPS injection (N = 12; $t_{4,48}$ = 3.30; P = 0.008; see Table 2) differences between sexes in behaviour were apparent. Females took breaths 45% more often than males before LPS treatment and 16% more after (Fig. 3). This variable also differed within of each sex after treatment. Males showed a 40% increase (N = 6; $F_{1,11}$ = 1.50; P = 0.005) in the number of breaths, and females an increase of 16% (N = 6; $F_{1,11}$ = 1.06; P = 0.038) after LPS injection (Table 3; Fig.3). The other voluntary movement variables, 'total time spent moving (min)' and 'total time in the shelter (min)', did not show differences between sexes (all P > 0.005; see Tables 1 and 2), nor between individuals of each sex before and after LPS treatment (all P > 0.005; see Table 3).

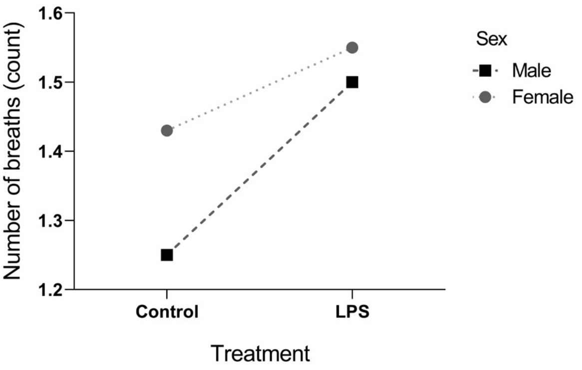

Figure 3. Number of breaths (count) in males and females of *Xenopus laevis* before and after LPS injection.

Table 3. Repeated measures ANOVA test comparing locomotor endurance, jump force and voluntary movement of *Xenopus laevis* before and after treatment with LPS for each sex separately. Each variable was tested separately.

Variable / Sex	Mean square	d.f	F	P
MALE				
Locomotor endurance				
Total time spent moving (min)	0.098	1.11	5.31	**0.036***
Total distance travelled (m)	0.176	1.11	8.95	**0.009***
Jump force (N)	0.029	1.11	6.16	**0.035***
Voluntary movement				
Total time spent moving (min)	0.251	1.5	1.16	0.111
Total time in shelter (min)	0.035	1.5	1.56	0.230
Number of breaths (count)	0.247	1.5	1.50	**0.005***
FEMALE				
Locomotor endurance				
Total time spent moving (min)	0.121	1.5	13.3	**0.001***
Total distance travelled (m)	0.177	1.5	13.6	**0.025***
Jump force (N)	0.202	1.5	6.17	**0.025***
Voluntary movement				
Total time spent moving (min)	0.212	1.5	2.15	0.163
Total time in shelter (min)	0.022	1.5	0.17	0.688
Number of breaths (count)	0.068	1.5	1.06	**0.038***

Note: d.f = degrees of freedom.

DISCUSSION

Intersexual differences before and after simulated infection

When faced with an immunological challenge, females may be more affected, presenting greater reductions in activity due to the differential investment of resources in activity, immune system responses and reproduction between the sexes (Nunn et al., 2008; Klein & Flanagan, 2016; Ducret et al., 2020). Thus, we expected that females would be more affected after a simulated infection and, therefore, would present a greater reduction in performance and reduction in voluntary movement. However, few intersexual differences were observed. Except for jump force (endurance variable) and for the number of breaths (voluntary movements variable) which both differed between sexes.

Among the performance variables, jump force was different between the sexes both before and after LPS treatment. Nevertheless, the males that were used to test this variable had greater body mass than females, so animals with greater body mass (males) exerted greater jumping forces. Despite that, after LPS injection the difference in performance between the sexes almost doubled, demonstrating a greater effect of LPS on this variable in females which showed a greater reduction in jump force. This suggests a possible energetic trade-off between immune responses, gamete production and locomotion in females.

Among the voluntary movement variables analysed, only the number of breaths differed between the sexes before and after treatment with LPS, with a marginal increase of 16% in the number of breaths after treatment in females compared to males. Contrary to what was observed in another study that demonstrated that *Xenopus* females are more tolerant to hypoxia compared to males (Rousseau et al., 2016), the females in our study swam to the surface to breathe more often than males. Thus, the marginal effect of LPS may have had caused females, which are more immunocompetent and have greater energy needs, to breathe more often.

Many anurans, include *X. laevis*, show behavioural changes after activation of the immune system, which can lead to reduced locomotor activity and performance which is related to expenditure of resources to mount an immune response (Llewellyn et al., 2011; Braga, 2013; Wu, 2023; Olarte, 2017; Oliveira, 2022; Oliveira et al., 2024). Reduction in activities may be more pronounced in females due to the stages that are part of the reproductive process, such as the maturation of the gonads, production of eggs, gamete biomass, oviposition, and direct investment in offspring, that lead to greater energy expenditure in breeding by females (Wells, 1977; Ryan et al., 1983; Grafe et al., 1992; Hayward & Gillooly, 2011). Previous studies have shown that females of this species have a higher basal metabolic rate (Louppe et al., 2018; Ducret et al., 2020) and allocate more energy to reproductive tissues (Courant et al., 2017). However, in the context of our study, there appear to be no intersexual differences in the immunocompetence of *X. laevis*, which may be related to the fact that the animals used are captive and not limited in resources, as we discuss below.

Differences in individuals before and after a simulated infection

We observed that LPS treatment reduced locomotor endurance and jump force in different species of *Xenopus*, as demonstrated previously (Oliveira et al., 2024). This is corroborated by the present study demonstrating changes in locomotor performance and behaviour in *X. laevis* after activation of the immune system with LPS, in both sexes. However, individuals injected with saline did not show any behavioural or performance differences, demonstrating that the handling of the animals or the injection itself did not affect the behaviour or performance of the frogs. Behavioural changes after the activation of the immune system by LPS are possible, as the effects of the infectious process caused by LPS in the body can be observed shortly after applying the LPS injection as they are part of the acute phase immune response (Ulevitch & Tobias, 1999) and were previously observed in other anurans (Llewellyn et al., 2011).

Reductions in locomotor endurance and jump force provided evidence for the existence of trade-offs in resource allocation for both males and females. The reduction in locomotion observed may be associated with behavioural depression (Hart, 1988), that has been interpreted as an adaptation that may be associated with the ecological costs that the environment presents (Oliveira, 2022). The consequent saving of resources could theoretically facilitate a stronger activation of the immune system (Hart, 1988; Aubert, 1999; Adelman & Martin, 2009).

However, although performance was reduced following LPS treatment, few differences in voluntary behaviour were observed, suggesting little or no evidence of behavioural depression in *X. laevis* irrespective of sex. Among the voluntary movement variables analysed, the only quantified variable that showed a notable difference was the number of breaths taken by individuals, which increased dramatically after treatment with LPS. This suggests a greater need for oxygen in animals after a simulated infection. Studies with amphibians have shown that injections of inactivated bacteria or LPS can increase the metabolic rate of anurans by around 20 and up to 100% and indicate that this increase is related to the energetic cost that the immune system imposes (Muchlinski, 1985; Sherman & Stephens, 1988; Llewellyn et al., 2011; Moretti, 2016).

The maintenance of voluntary movements after immune challenge suggests that behavioural depression does not appear to occur as a dominant behavioural response in *X. laevis*. Although it is still unclear why no greater differences were observed in voluntary movement, we believe that some factors may have influenced this. The fact that the animals used were maintained in captivity with access to unlimited resources may have played a role (Husak et al., 2016). Indeed, in captivity animals do not need to invest resources in the search for food, refuges, and habitat exploration, for example, as they are fed ad libitum. Similar tests with additional treatments of dietary restriction would be important to explore the role of differences in resource allocation in a more naturalistic context.

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Author contributions

T.O. and L.A. conducted experiments. T.O. analysed the data. T.O., A.H. and C.N. conceived the study and contributed to the writing of the manuscript.

Data accessibility

The files containing the data are stored in the Scientific Data Repository of the University of São Paulo and can be accessed on the website https://repositorio.uspdigital.usp.br/?codmnu=9980, through a search for the project title, name of the authors or subjects related to the research topic, such as "anurans, locomotion, behavioural depression, infection, intersexual, LPS, *Xenopus laevis*". The data is available for access to the general public.

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