Plan Overview

A Data Management Plan created using DMPTool

Title: Immunological and locomotor response of individuals of the anuran species Xenopus laevis and Xenopus allofraseri injected with lipopolysaccharide (lps) as a pro-inflammatory stimulus.

Creator: Thaysa Gomes de Oliveira -**ORCID:** 0000-0001-5280-5319

Affiliation: Universidade de São Paulo (www5.usp.br)

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Project abstract:

Anurans display different types of locomotion that are related to their ecology and habitat use and are regarded as crucial for survival. Locomotion also has a toll on energy budget, and compromise with other physiological processes such as immune response to infection. Consequently, immune responses and locomotion may be involved in trade-offs regarding the allocation of energy, therefore imposing limits on either measure of performance. If allocating energy to locomotion reduces energy for activating the immune system, a depressed immune system may free up energy for activity, as perhaps happens with highly dispersive lineages. Different types of equilibrium between energy invested in locomotion and immunity likely have evolved, with some species displaying depressed immune responses and enhanced locomotor capacity, and vice versa. The former may be the case of invasive species given that the spread of the species at their distribution edge is often associated with boldness and high dispersal capacity. The current project explores the relationship between immunological responses and locomotion comparing the invasive species (*Xenopus laevis*) and a non-invasive congeneric (*Xenopus allofraseri*), under the perspective that the former is less responsive to an immune challenge.

For this, we studied the effect of a simulated infection by injection of lipopolysaccharide from a gram-negative bacterial cell wall (LPS) on locomotor endurance, voluntary movement, and jump force in male and female frogs of the species *Xenopus laevis* and females of *Xenopus allofraseri.* We analyzed two locomotion traits (endurance and jump force) and voluntary movement, before and after LPS injection. Within the context of these variables, we hypothesized that the immune response would 1) depress locomotor performance; 2) reduce voluntary movement, and 3) differentially impact males and females of *X. laevis*; 4) invasive species *X. laevis* would be more resistant to infection than the non-invasive species *X. allofraseri*, which would have its locomotion more affected. Our results show LPS-induced differences in the locomotion of anurans both in terms of performance and behavior. Male and female *X. laevis* showed reduced locomotor endurance and jumping force and an increase in voluntary movements after LPS treatment, and patterns compared between sex. Species differed, with female *X. allofraseri* being more responsive to treatment, and displaying comparably greater reduction locomotion, in relation to females of *X. laevis.*

In our study LPS reduced fitness-relevant measures of locomotor performance, endurance capacity and jump force and for movement, voluntary movements. We interpret this data as providing indirect evidence for energy allocation trade-offs between immune response and locomotion. This is confirmed by the differential responses of *X. laevis*, an invasive species that tends to allocate more energy for locomotion and dispersion, and the non-invasive species *X. allofraseri.* This is compatible with the cost of locomotion, a behavior that not only mobilizes energy but requires energetically expensive tissue like bone, muscle, and the cardiovascular system. Similarly, the cost of mounting an immune response is high and most lineages appear to decrease activity to enhance energy support to the immune system. We report different after-treatment responses related to locomotor behavior in *X. laevis* and *X. allofraseri*, which are consistent with and infection-mediated behavioral depression. Overall, our results corroborate energy allocation *trade-offs* between immune response and locomotion that seem related to ecology natural history, with differences and even contrasts among lineages.

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Copyright information:

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Coleta de Dados

Que dados serão coletados ou criados?

Data were collected from 18 males and 18 females of *X. laevis* and 18 females of *X. allofraseri*. Being 6 animals of each species and sex for: Locomotor endurance; jumpr force; voluntary movement.

Como os dados serão coletados ou criados?

Morphometry

All subjects were weighed on an electronic balance (Cgoldenwall model CNA-383H; precision ± 0.1g) before and after LPS treatment (see Table 1).

Administration and dosage of LPS

The simulation an infection was performed by injecting LPS (gram-negative bacterial cell wall lipopolysaccharide) from the bacterium *Escherichia coli* Serotype 0111: B4 purified by phenol extraction at a dose of 2mg / Kg, injected into the dorsal lymph sac of the frog with syringe with 29-gauge needle attached (Llewellyn *et al*., 2011; Llewellyn *et al*., 2012; Olarte, 2017).

Locomotor endurance

Locomotor resistance was tested using a circular track measuring 3 meters in circumference. The track had a cork substrate and was covered with 5cm of water. Animals were placed into the track and encouraged to move until exhaustion, indicated by the lack of a righting response. Animals were tested three times with a rest interval of 48 hours between trials. After each test day the animals were fed with beef heart. We recorded the A - total time spent moving, B - total distance covered, and C - number of laps (Herrel *et al*.,2012; Herrel and Bonneaud, 2012a; Herrel and Bonneaud, 2012b; Herrel *at al*., 2014).

Voluntary movement

To quantify voluntary movement, we used an open-field test. 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). Next, animals were placed under the shelter and filmed in dorsal view for 12 hours (8:00 am - 8:00 pm) for three days, with 48 hours between subsequent days of recording. The videos were analyzed with a stopwatch and the A - total time in movement, B - total time in the refuge, C - the number of taps on the sides of the tank, D - the number of exits from the refuge, and E - the number of times the animal breached he surface for breathing were recorded.

Jump force

The animals were tested on a 20 x 10 cm piezoelectric Kistler 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 touching them lightly on the body. This test was repeated on three days, with 48-hour intervals between trials. Forces were recorded at 500 Hz during 60 second recording sessions and frogs were induced to jump multiple times per session. The three best jumps per session were analyze using the Kistler Bioware software. To do so the peak X-, Y- and Z-forces were extracted and the overall resultant forces was calculated (Herrel *et al.,* 2014).

Statistical analyses

All locomotor endurance data were log10-transformed to meet assumptions of normality and homoscedasticity. To test for the effect of LPS treatment, a multivariate analysis of covariance (MANCOVA) was used, with treatment, sex, and day as factors and body mass as co-variate. One was added to all voluntary movement data which was subsequently Log10-transformed to meet assumptions of normality and homoscedasticity. To test whether an effect of LPS treatment on voluntary movement in the open field test we ran a MANCOVA with treatment, sex, and test day as factors and body mass as our covariate. Resultant jump force was again Log10-transformed to meet assumptions of normality and homoscedasticity. We ran a multiple linear regression to test the effect of treatment, sex, test day, and animal weight on peak resultant force.

Documentação e Metadados

Que documentação e metadados acompanharão os dados?

Excel spreadsheet
Textos (MACONVAS)
Documents with tables
Graphics

Ética e Conformidade Legal

Como você administrará qualquer questão ética?

All experiments were carried out with permission and following all ethical guidelines of the country where the research was carried out.

Como você vai gerenciar os direitos autorais e os direitos de propriedade intelectual (IP / IPR)?

All copyrights and ownership are assured.

Armazenamento e Backup

Como os dados serão armazenados e terão backup durante a pesquisa?

The data will be stored in the data repository of the University of São Paulo - USP.

Como você vai gerenciar o acesso e a segurança?

The data will be opened for general consultation, but only authorized administrators will be able to access and make modifications to the data management plan and data stored in the USP repository.

Seleção e Preservação

Quais dados são de valor a longo prazo e devem ser mantidos, compartilhados e / ou preservados?
All data in spreadsheets; in text and picture format; in video.

Qual é o plano de preservação a longo prazo do conjunto de dados?

Question not answered.

Compartilhamento de Dados

Como você vai compartilhar os dados?
The data will remain in the repository of the University of São Paulo open for consultation.

Existem restrições ao compartilhamento de dados requeridos?
No.

Responsabilidades e Recursos

Quem será responsável pelo gerenciamento de dados?
Principal author: Thaysa G. Oliveira

Quais recursos você precisará para entregar seu plano?

Question not answered.

Planned Research Outputs

Spreadsheets - "Locomotor Endurance/ Voluntary Movement/ Jump Force"

Excel tables with data from the animals used in each test, data collected from each experimental stage and description of the variables used.

Text - "MANCOVA tests"

Results of multivariate statistical analysis.

Image - "Graphs"

Graphs of results.

Planned research output details

Title	Type	Anticipated release date	Initial access level	Intended repository(ies)	Anticipated file size	License	Metadata standard(s)	May contain sensitive data?	May contain PII?
Locomotor Endurance/ Voluntary Movement/ Jump Forc ...	Spreadsheets	2021-09-17	Open	None specified		None specified	None specified	No	No
MANCOVA tests	Text	2021-09-17	Open	None specified		None specified	None specified	No	No
Graphs	Image	2021-09-17	Open	None specified		None specified	None specified	No	No