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Behavioral, physiological and morphological correlates of parasite intensity in the wild Cururu toad (*Rhinella icterica*)

Eduardo Hermógenes Moretti[*, 1], Braz Titon Jr. [1], Carla Bonetti Madelaire [1], Raquel de Arruda, Tatiana Alvarez, Fernando Ribeiro Gomes [1]

Departamento de Fisiologia, Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rua Prof. Dr. Antônio Celso Wagner Zanin, s/n, 18618-689, Botucatu, SP, Brazil

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ABSTRACT

Large numbers of parasites are found in various organs of anuran amphibians, with parasite intensities thought to modulate the host's Darwinian fitness traits. Interaction between the anuran hosts and their multiple parasites should modulate the host's phenotypic characteristic, such as those associated with high energetic demand (such as calling effort and locomotor performance), energy balance (standard metabolic rate), and morphological plasticity (as indicated by organ masses). The present study investigated the impact of parasite intensities on the behavioral, physiological, and morphological traits of wild adult male *Rhinella icterica* (Anura: Bufonidae). We tested as to whether individuals with higher parasite intensities would present: 1) lower vocal calling effort in the field, as well as poorer locomotor performance and body-condition index; and 2) higher standard metabolic rates and internal organ masses. Measurements included: calling effort in the field; standard metabolic rate; locomotor performance; parasite intensity; internal organ masses (heart, liver, kidneys, intestines, stomach, lungs, hind limb muscle, and spleen); and the body-condition index. Results showed a negative association of parasite intensities with locomotor performance, and standard metabolic rate of *R. icterica*. A positive association between parasite intensities and relative organ masses (heart, intestines and kidneys) was also evident. Toads with higher pulmonary and intestinal parasites intensities also showed higher total parasite intensities.

1. Introduction

Accumulating evidence on the direct and indirect effects of infection indicate that parasitic infection should be an important determinant of the Darwinian fitness of the host (Holmes and Zohar, 1990; Goater et al., 1993; Clobert et al., 2000; Moore and Wilson, 2002; Zhong et al., 2005; Kolluru et al., 2008). The process of dynamic interactions between host and parasite can lead to host physiological and morphological changes (Johnson, 1999; Roberts et al., 1999; Tinsley et al., 2002; Kristan and Hammond, 2004; Dingemanse et al., 2009). Experimental studies with adult and young mice (*Mus musculus*) have shown that the intestinal nematode (*Heligmosomoides polygyrus*) decrease host body reserves (e.g.: fat mass), increase organ mass (e.g.: spleen, small intestine, large intestine, cecum, liver, kidney and heart) and modifies intestinal function (e.g.: glucose-uptake rate). Such data indicate processes of compensatory responses to organ damage and nutrient competition, as well as increased immune activity (Kristan and Hammond, 2000; Kristan, 2002a, 2002b). Such compensatory and immune responses are associated with increased metabolic rates in the early stage of the infection, which could compromise other energetically expensive activities, such as metabolic heat production and host reproduction (Kristan and Hammond, 2000; Lochmiller and Deerenberg, 2000; Kristan, 2002a). Changes may also occur in behavior, with alterations in how the host-parasite complex interacts with environment (Schall et al., 1982; Thompson, 1990; Kolluru et al., 2008; Moller, 2008). Furthermore,

* Corresponding author. Rua do Matão, Travessa 14, 101, 05508-900, São Paulo, SP, Brazil.
E-mail addresses: ehmoretti@ib.usp.br (E.H. Moretti), zuza.bio@gmail.com (B. Titon), cmadelaire@yahoo.com.br (C.B. Madelaire), r.arrudasantos@gmail.com (R. de Arruda), alvareztr.bio@gmail.com (T. Alvarez), fribeirogomes@gmail.com (F.R. Gomes).
[1] Present address: Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, 101, 05508-900, São Paulo, SP, Brazil.

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macroparasites of vertebrate hosts from the wild usually occur in multi-species communities, with the interactions of these multiple parasite species in the host impacting on the host-parasite fitness and dynamics (Jackson et al., 2006; Pedersen and Fenton, 2007; Johnson and Hoverman, 2012).

A large number of parasites are found in various organs of anuran amphibians, including the lungs and gastro-intestinal tract (Luque et al., 2005; Pinhão et al., 2009; Moretti et al., 2014). The presence of pulmonary nematodes in anurans should interfere with oxygen exchange processes at the diffusion barrier and impair the host's ability to maintain blood oxygenation under conditions of vigorous exercise (Goater et al., 1993; Pizzatto and Shine, 2012). Although the presence of parasites in gastrointestinal tract should not directly affect aerobic exercise, such parasitic infection can affect the host's energy balance, thereby lowing aerobic capacity (Holmes and Zohar, 1990; Kristan and Hammond, 2006). Calling activity can be the highest aerobic demand for many anuran species (Pough et al., 1992; Bevier, 1995; Ressel, 1996). Some phylogenetic groups, such as Dendrobatids and Bufonids, engage in active foraging that requires sustained locomotion, and therefore producing high rates of oxygen consumption (Pough et al., 1992). Accordingly, these anurans represent interesting models to investigate associations among parasite intensity, host's energetic balance and sustained aerobic locomotion.

Previous studies have shown parasite intensity to be associated with individual variation in organ masses and locomotor performance in *R. icterica* (Anura: Bufonidae) (Moretti et al., 2014). Negative associations between parasite intensity and sustained locomotor performance in *R. icterica* can also be present for vocal behavior, also an activity of elevated metabolic demand (Pough et al., 1992; Grafe, 1996; Madelaire et al., 2013), with possible consequences for the host's success in mating (Hamilton and Zuk, 1982; Folstad and Karter, 1992; Gerhardt, 1994; Wells, 2001). Presence of parasites can also be associated with many morphological and functional alterations in factors underlying aerobic performance and energy regulation. Investigation of individual covariation between parasite intensity and such energetic morpho-functional variables, including the body-condition index, should better clarify the association between parasites and host organization in their natural environments.

This study aimed to investigate how the intensity and diversity of parasitic infection associates with variations in behavioral, physiological and morphological traits in wild adult male *Rhinella icterica*. The variables measured included: 1) calling effort in the field; 2) standard metabolic rate; 3) locomotor performance; 4) parasite intensity; 5) organ mass (heart, liver, kidneys, intestines, stomach, lungs, hind limb muscle, and spleen); 6) body-condition index. We tested the hypotheses that adult males of *R. icterica* with higher parasite infection intensities are characterized by lower calling effort, locomotor performance and body-condition index as well as higher standard metabolic rates and organ mass.

2. Materials and methods

2.1. Locality and species studied

Rhinella icterica is a large Bufonidae from the *Rhinella marina* species group, found throughout the south and southeastern areas of the Brazilian Atlantic Rainforest, from Rio de Janeiro State to Uruguay (Pramuk et al., 2008). Twenty eight adult males of *R. icterica* were collected between 2009 (N = 22) and 2010 (N = 6) in Botucatu (SP/Brazil) (22°53′38.01″ S; 48°30′7.79″ W). Animals were collected during the reproductive season, which, in this locality, extends from July to September, with a peak of activity in August (Jim, 2002). We collected data on calling effort and temperature of activity in the field, body-condition index and helminth parasites for all individuals. For the first twenty two individuals, data were also collected on standard metabolic rates, locomotor performance and organ mass. Collections and transport of the animals were performed under authorization of the Brazilian Institute of the Environment and Renewable Natural Resources (SISBIO/IBAMA, process number 02010.003380/04–82), and laboratory procedures were performed under the approval of the Comissão de Ética na Experimentação Animal of Institute of Bioscience Botucatu - Univ Estadual Paulista (CEEA, process number 169/2009).

2.2. Field data

We located toads by visual inspection and conducted vocal behavioral observations for 30 min, including recording calling behavior and the movements of vocal males, as well as noting calling site characteristics and acoustically estimating the number of males from the pool chorus. During fieldwork, all observers used headlamps with red filters, which, based on previous field-studies, cause no disturbance to amphibians (Bevier et al., 2008; Kiss et al., 2009). As a further precaution, observers started behavioral recordings 10 min after the start of the chorus and only if vocal males maintained normal behavior. Data collection occurred between 19:00 h and 0:00 h. Given that temperature can affect the temporal parameters of anuran vocalizations (Wells et al., 1996; Navas and Bevier, 2001), we recorded field temperatures at 5 min intervals using four HOBO data loggers placed in typical micro-environments used by vocal males. These micro-environments are composed mainly of grass vegetation on the bank and aquatic vegetation inside permanent ponds. Temperature recordings with data loggers were also made for each individual, according to the site occupied and the hour of observation. For each individual, observers registered the duration of each vocalization to obtain calling effort, calculated from the number of vocalizations in 30 min multiplied by the average duration. After the end of each observation, we measured superficial body temperature with an infrared thermometer (TI-900, Instrutherm). Individuals were then hand-captured and transported to the laboratory in individual plastic containers for later additional data collection. Day and hour of behavioral observations were included in subsequent analyses.

2.3. Laboratory maintenance conditions

Toads were kept in individual plastic containers (0.40 m × 0.30 m x 0.25 m), under natural light/dark cycles and temperatures, with water and shelter freely available. Toads were fed six cockroaches twice per week. All toads consumed all cockroaches offered. Their containers were cleaned every day preventing auto-infection. For toads captured in 2009, all measurements in the laboratory were carried out between 14 and 30 days after arrival at the laboratory. This period of maintenance in captivity was included in subsequent analyses. We euthanized the toads that were collected in 2010 on the day following capture.

2.4. Standard metabolic rates

As an index of standard metabolic rates, we measured the rates of oxygen consumption ($\dot{V}O_2$std) of toads using positive-pressure, flow-through respirometry (Withers, 1977). Measures were recorded between 08:00 h to 18:00 h, a time-period of toad inactivity. During measurements, toads were maintained at 25 ± 1 °C and under constant light conditions. We placed toads that had fasted for three days into individual metabolic chambers (750 mL), which were placed inside an acclimatized room (FITOTRON 011, Eletrolab)

that was set at the test temperature for at least 2 h before the measurements were made. During this time, air was pumped through the chambers at 100 mL.min^{-1} using a Flowbar Multi-channel–8 pump system (Sable Systems; Henderson, NV, USA) to prevent hypoxia and hypercapnia. Moistened cotton was placed inside metabolic chambers in order to prevent dehydration prior to respirometry tests. A control system of air flow with multiple channels (V3-Sable Multiplexer Systems; Henderson, NV, USA) was used to sequentially direct air from metabolic chambers, on the same stream, for an oxygen analyzer (FC-10a O2 analyzer-Sable Systems; Henderson, NV, USA). Data from the O_2 analyzer was recorded on a computer that was equipped with EXPEDATA 1.1.15 software (Sable Systems), *via* an UI2 interface (Sable Systems). Water vapor and CO_2 was eliminated from chamber air *via* a column containing drierite/ascarite/drierite, prior to gas analysis. We calculated $\dot{V}$ O_2std as:

$$\dot{V}\ O_2\text{std} = \dot{V}*(FIO_2 - FEO_2)/(1 - FEO_2),$$

where $\dot{V}$ is flow rate (mL.min^{-1} STP; Standard Temperature and Pressure) and FIO_2 and FEO_2 are the fractional O_2 concentrations in incurrent and excurrent air, respectively. All of the respirometry tests were videotaped, and we repeated measurements until 20 min of continuous measurements at rest for each individual were obtained. We recorded 10-min baseline measurements using the CO_2- and H_2O-free air before and after each animal recording. We calculated $\dot{V}O_2$std using the 5 min period with the lowest continuous readings for fractional O_2 concentration. Body mass (0.01 g) was measured immediately before and after the tests, and the mean of these values was used to express $\dot{V}O_2$std as $mLO_2.g^{-1}.h^{-1}$.

2.5. Locomotor performance

We estimated locomotor performance as the total distance moved by toads during the 10 min of forced locomotion around a 1.5 m diameter circular track. Tests were performed inside an acclimatized room (FITOTRON 011, Eletrolab), maintained at 70 ± 5% relative humidity and 25 ± 1 °C. Animals were stimulated to hop around the circular track by being gently tapped. Prior to tests, we maintained toads within their individual maintenance containers for one hour in an acclimatized room that was set to the test environmental conditions. At the end of the locomotor performance tests, individual's snout-vent length (±0.01 mm) was measured by digital calipers (Three-button Digital Caliper, DIGIMESS). In order to determine length-independent locomotor performance, total distance moved was divided by the snout-vent length. Previous tests, conducted using ten males of *R. icterica* performing six 10-min batches of locomotor activity on different days, confirmed that this is a reliable method to identify individual differences in locomotor performance ($F_{9,45} = 13{,}446$; $P < 0.0001$). After 10 min of forced locomotion, animals were not fatigued. Some individuals could move for over 60 min, without any sign of exhaustion.

2.6. Parasite intensity and organ masses

After physiological data collection, toads were anesthetized by using a benzocaine solution (1 g.L^{-1}) over 20 min (Fernandes et al., 2005), and then euthanized by rupturing the spinal cord. The heart, lungs, liver, intestines, kidneys, stomach, hind limb muscle and spleen were dissected, and the parasites therein were collected and counted using a stereomicroscope (STEMI DV4, Zeiss). Organs and carcasses were maintained for 8 days in the oven (BT 316, Biothec) at 60 °C to obtain their dry mass. Body mass was considered as the standard measure of the effects of body size on dependent variables (morphological, physiological and behavioral traits). Residuals from a regression of body mass against body length ($R^2 = 0.94$; $P < 0.01$; $N = 26$) were used to estimate the body-condition index, which represents a standard method for quantifying the energy reserves of individual animals (Jakob et al., 1996; Schulte-Hostedde et al., 2005).

All helminths were processed accordingly, fixed in cold AFA (Alcohol-Formalin-Acetic Acid) solution and preserved in 70% ethanol (Andrade, 2000). For identification purposes, acanthocephalans and trematodes were stained using carmine and then cleared with eugenol, whilst nematodes were cleared with phenol. We analyzed all the processed specimens using a Leica Qwin Lite computerized system, identifying the species by morphology and morphometry according to Travassos et al. (1969), Vicente et al. (1991), and Smales (2007) (supplementary material). We deposited voucher helminthes in the Coleção Helmintológica do Instituto de Biociências de Botucatu (CHIBB 7071–7164). Compound variables associated with parasite intensity by organ were calculated: (1) pulmonary parasite intensity, which corresponds to *Rhabdias fuelleborni* intensity; (2) intestinal parasite intensity, which is the sum of the intensity of all intestinal parasites; and (3) total parasite intensity, which is the sum of the intensity of all parasite species present.

2.7. Data analyses

All variables were initially analyzed by descriptive statistics, with standard least-square linear regressions used to test if parasite intensity, as well as different behavioral, physiological and morphometric variables, scaled with body mass. All variables were transformed by $\log_{10}$, in order to increase normality prior to allometric regression analyses. When a significant relationship existed between dependent variables and body mass, the calculated residuals were used in subsequent analyses. We calculated prevalence, mean intensity of infection and mean abundance for each parasite species in *R. icterica* toads, in accordance with Bush et al. (1997). Correlations between parasite intensity of each organ and total parasites were tested by Pearson's correlation coefficient, excluding the parasites lodged in the correlating organ from the total.

In order to identify possible differences in variables measured in toads collected in 2009 and 2010, we performed independent t-tests for the environmental, behavioral, and parasite variables. To test for the dependency of behavioral variables on temperature, Pearson's correlations were performed among calling effort, body temperature and microenvironmental temperature used by the toads as sites of vocalization. Additionally, Spearman's correlations were used to test for a possible effect of time in captivity on parasite species intensities and phenotypic variables of the toads collected in 2009.

We then performed two Varimax Kaiser normalized Principal Component Analyses (PCA): one PCA with environmental and behavioral variables (day of observation, hour of observation, chorus density, calling effort, and body temperature); and a second PCA for morphological variables (heart, lung, liver, intestine, kidney, stomach, hind limb muscle, spleen and body condition index). The variable “year” was included in the PCA analyses when the phenotypic variables showed differences between the years of data collection. Principal components with eigenvalues >1.00 were considered for interpretation. Subsequently, we calculated results from each component extracted by regression and saved as compound variables. Pearson's correlations were then performed to test for relations between these compound phenotypic variables with compound variables of parasite intensity by organ. Finally, we

performed Spearman's correlations to test for possible associations between the compound variables from the all PCA analyses and intensity of parasite species. In all analyses, $P < 0.05$ was considered as significant. All statistical analyses were performed with Statistica 12.0 (Statsoft).

3. Results

3.1. Descriptive analyses, allometry and environmental variables

Descriptive analyses for all variables are presented in Table 1. All organ masses correlated positively with body mass ($0.45<\beta < 1.16$, Table 2). Body temperature was higher in the year 2009 than in the year 2010 ($t_{23} = 5.08$, $P < 0.01$). Calling effort was not related to body temperature and microenvironment temperature evident at the sites of vocalization used by the toads ($-0.18<r<0.15$, $P>0.43$).

3.2. Principal component analyses and association of parasite intensities with behavioral, physiological and morphological variables

The captive period did not influence parasite intensities and phenotypic variables of *R. icterica* toads ($-0.41<r_s<0.37$, $P>0.06$).

Principal component analysis of behavioral and environmental variables resulted in two components (Table 3). The first component explained 46.0% of the variance and was related to the year and day in one direction, as well as with body temperature in the opposite direction. The second component explained 31.0% of the variance and was related to calling effort and chorus density, in the same directions. Principal component analysis of morphological variables resulted in four components (Table 4). The first component explained 22.1% of the variance and was related to heart, intestines and kidney masses in the same direction. The second component explained 18.3% of the variance and was related to liver mass and body-condition index in the opposite direction. The third component explained 19.7% of the variance and was related to hind limb muscle and spleen masses in opposite directions. The fourth

Table 2
Regression equations for the effect of body mass on different variables for *Rhinella icterica* individuals.

Variables	Alfa ± SE	Beta ± SE	R^2	*P*
Heart	−2.35 ± 0.21	0.85 ± 0.13	0.68	**<0.001**
Lung	−1.89 ± 0.21	0.86 ± 0.13	0.68	**<0.001**
Liver	−1.91 ± 0.16	1.16 ± 0.10	0.86	**<0.001**
Intestine	−1.19 ± 0.24	0.45 ± 0.15	0.29	**0.009**
Kidney	−2.87 ± 0.31	1.15 ± 0.20	0.63	**<0.001**
Stomach	−1.3 ± 0.13	0.62 ± 0.08	0.73	**<0.001**
Hind limb muscle	−1.38 ± 0.10	1.07 ± 0.06	0.95	**<0.001**
Spleen	−3.25 ± 0.50	0.85 ± 0.31	0.31	**0.016**
Locomotor performance	2.99 ± 0.18	−0.08 ± 0.12	0.03	0.498
$\dot{V}O_2$std	−0.77 ± 0.49	−0.44 ± 0.32	0.10	0.177
Vocal effort	−1.61 ± 2.34	2.45 ± 1.53	0.15	0.130
Total parasite intensity	2.39 ± 1.59	−0.38 ± 1.02	0.71	0.712
Pulmonary parasite intensity	0.85 ± 1.54	0.16 ± 0.99	0.87	0.871
Intestinal parasite intensity	3.56 ± 1.39	−1.52 ± 0.90	0.11	0.106

Significant *P* values are in bold (<0.05). $\dot{V}O_2$std = standard rate of oxygen consumption. SE = Standard Error.

Table 3
Component score result from the principal component analysis (PCA) performed on behavioral and environment variables for *Rhinella icterica* (N = 20).

Variables	Factor 1	Factor 2
Year	**−0.33**	0.01
Day	**−0.34**	−0.01
Hour	0.10	−0.31
Vocal effort	0.02	**0.46**
Density chorus	0.15	**0.48**
Body temperature	**0.34**	0.13
Total variance (%)	0.46	0.31

The variables are expressed as $\log_{10}$ of absolute values. Traits contributing most to each component are in bold. The total amount of variance in the data that can be explained using these two principal components is 75.0%.

Table 1
Descriptive analyses of environmental, behavioral, physiological and morphological variables for *Rhinella icterica* individuals for the years of 2009 and 2010.

Variables		N	Mean ± SD	Min	Max	Confidence limit	
						−95%	+95%
2009	Body mass (g)	28	161.48 ± 58.36	59.38	315.25	138.85	184.10
	Snout-vent lenght (mm)	26	120.92 ± 13.18	89.40	148.17	115.59	126.24
	Locomotor performance(units of body length)	20	738.81 ± 115.40	537.73	905.64	684.80	792.82
	$\dot{V}$ O_2std ($mLO_2.h^{-1}.g^{-1}$)	22	0.04 ± 0.02	0.02	0.08	0.03	0.05
	Heart (g)	22	0.10 ± 0.04	0.06	0.24	0.08	0.12
	Lung (g)	21	0.30 ± 0.11	0.15	0.66	0.25	0.35
	Liver (g)	22	0.84 ± 0.41	0.40	2.19	0.66	1.02
	Intestine (g)	22	0.33 ± 0.09	0.22	0.55	0.29	0.37
	Kidney (g)	22	0.09 ± 0.05	0.03	0.22	0.07	0.11
	Stomach (g)	22	0.47 ± 0.13	0.34	0.92	0.41	0.53
	Hind limb muscle (g)	18	2.16 ± 0.88	1.11	4.93	1.73	2.60
	Spleen (g)	18	0.01 ± 0.01	0.00	0.03	0.01	0.02
	Vocal effort (s)	22	225.68 ± 226.41	0.00	673.00	174.19	323.55
	Density chorus (Un)	22	6.40 ± 5.20	0.00	20.00	4.00	7.43
	Body temperature (°C)	19	20.00 ± 2.09	14.00	23.00	18.99	21.01
	Air temperature (°C)	22	16.33 ± 1.41	14.51	18.42	1.08	2.01
	Water temperature (°C)	22	20.17 ± 2.75	16.39	23.24	2.12	3.93
	Observation hour(minutes after 18 h)	22	162.64 ± 42.68	90.00	236.00	32.84	61.00
2010	Vocal effort (s)	6	266.50 ± 152.17	127.00	561.00	94.99	373.23
	Density chorus (Un)	6	5.17 ± 0.75	4.00	6.00	0.47	1.85
	Body temperature (°C)	6	15.17 ± 2.14	13.00	19.00	12.92	17.41
	Air temperature (°C)	2	12.40 ± 0.00	12.40	12.40	0.00	0.00
	Water temperature (°C)	2	14.60 ± 0.00	14.60	14.60	0.00	0.00
	Observation hour(minutes after 18 h)	6	121.17 ± 19.63	90.00	151.00	12.25	48.15

$\dot{V}O_2$std = standard rate of oxygen consumption; SD = standard deviation; Min = minimum; Max = maximum Un = number of individual.

Table 4
Component score result from the principal component analysis (PCA) performed on morphological variables for *Rhinella icterica* (N = 16).

Variables	Factor 1	Factor 2	Factor 3	Factor 4
Heart	**0.45**	0.12	0.15	0.02
Lung	−0.08	−0.16	0.00	**0.56**
Liver	−0.09	**−0.52**	0.04	0.21
Intestine	**0.40**	0.07	−0.06	−0.06
Kidney	**0.41**	−0.21	0.02	−0.13
Stomach	−0.06	0.06	0.03	**0.55**
Hind limb muscle	0.12	0.09	**0.53**	−0.02
Spleen	0.04	0.19	**−0.54**	−0.04
Body-condition index	−0.01	**0.49**	−0.07	0.13
Total variance (%)	22.1	18.3	19.7	17.4

The heart, lung, liver, intestine, kidney, stomach, hind limb muscle, and spleen are residuals from log–log body mass regressions. Body-condition index is the residual from log–log snout–vent length. Traits contributing most to each component are in bold. The total amount of variance in the data that can be explained using these four principal components is 77.5%.

component explained 17.4% of the variance and was related to lung and stomach masses in the same direction.

Individuals with higher total, pulmonary and intestinal parasite intensities showed increased values for the morphological PC1, composed by heart, intestines and kidney masses (Table 5; Fig. 1). Individuals that had a higher intensity of *Rhabdias fuelleborni* and *Oswaldocruzia subauricularis* also showed increased values for the heart, intestines and kidney masses ($r_s = 0.73$, $P < 0.01$; $r_s = 0.55$, $P = 0.03$, respectively). Individuals with a higher total parasite intensity exhibited lower standard metabolic rates (Table 5; Fig. 2) as well as increased pulmonary and intestinal parasite intensity (Table 5). Individuals with higher pulmonary parasite intensity showed lower locomotor performance (Table 5; Fig. 3). However, individuals with higher parasite intensities did not show higher values for the behavioral and environment variables, as indicated by PC2 and comprised of vocal effort and density chorus (Table 5).

3.3. Species, prevalence, mean intensity and average abundance of parasitic infection in Rhinella icterica

The Helminth species infecting *R. icterica* individuals were comprised of: *Acanthocephalus saopaulensis* (Acanthocephala), *Physaloptera* sp. (Nematoda), *Oswaldocruzia subauricularis* (Nematoda), *Rhabdias fuelleborni* (Nematoda), unidentified species of the Cosmocercidae family (Nematoda), *Falcaustra mascula* (Nematoda), *Mesocoelium monas* (Trematoda) and an unidentified species of the Gorgoderidae family (Trematoda) (Table 6). The most prevalent species was *Rhabdias fuelleborni* and the most abundant was *Mesocoelium monas* (Table 6).

4. Discussion

Correlations of parasite intensities with relative organ masses, metabolic rates, and locomotor performance suggest possible phenotypic reorganization of *R. icterica* toads in association with the presence of parasites, although such causal associations cannot be concluded from the present study. It is possible that relations between the host's phenotype and parasite intensities reported here arise from the toad's prior body condition, the initial condition of which may determine parasite intensities, with subsequent phenotypic reorganization of the toad (Beldomenico and Begon, 2010).

Our results corroborate previous studies with *R. icterica* toads, where negative associations were observed between the intensity of pulmonary parasites and measures of the toad's sustained aerobic locomotion (Moretti et al., 2014). However, the causal

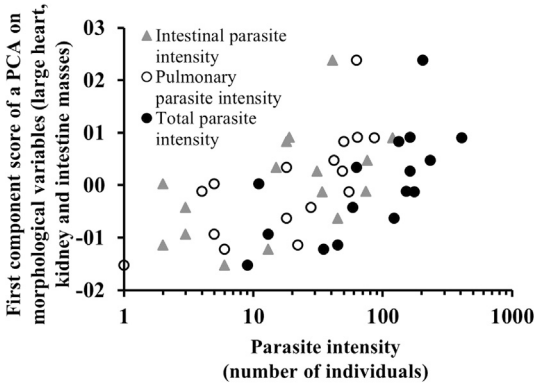

Fig. 1. Association between the score of the first component of a PCA on morphological variables (large heart, kidney and intestine masses) and parasite intensities in *Rhinella icterica* (N = 16). Full circles represent total parasite intensity (r = 0.66, $P < 0.01$), open circles represent pulmonary parasite intensity (r = 0.71, $P < 0.01$), and open triangles represent intestinal parasite intensity (r = 0.51, $P = 0.04$).

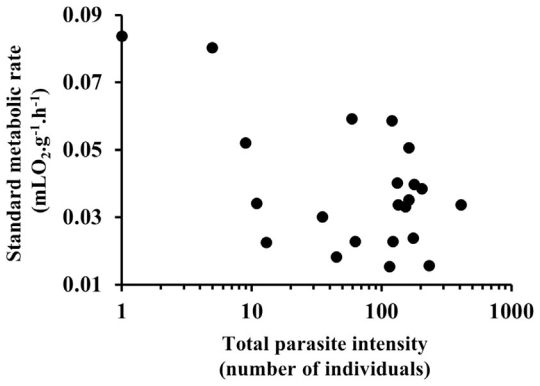

Fig. 2. Association between standard metabolic rate and total parasite intensity in *Rhinella icterica* (N = 22; r = −0.45, $P = 0.03$).

relationship of this negative association under natural conditions has still to be determined. Experimental infection of toads with L3 larvae of pulmonary parasites under laboratory conditions shows a negative impact on hosts' locomotor performance (Goater et al., 1993; Kelehear et al., 2009; Pizzatto and Shine, 2012). These studies suggest that such parasite mediated lower locomotor performance may be mediated by the level of penetration and migration of L3 larva to the target organ of the host driven by local mechanical damage and the heightened levels of the immune inflammatory response, as well as by presence of mature worms in the lungs. Such lung worms contribute not only to impaired lung functioning and the damage of lung membranes, but also to the blockage of vessels leading to the heart (Goater et al., 1993; Kelehear et al., 2009; Pizzatto et al., 2010; Pizzatto and Shine, 2012). However, the impact of pulmonary parasite intensity on sustained aerobic locomotion of toads under field conditions remains poorly understood (Brown et al., 2015). Experimental infection by pulmonary parasites, for example, did not suppress the dispersion capacity of both wild and 14-months old captivity-reared Cane toads (*R. marina*), under field conditions in Australia (Brown et al., 2015).

A negative association between the calling effort and parasite intensity in these toads was expected, given that calling activity is energetically demanding and aerobically sustained in anurans (Pough et al., 1992; Bevier, 1995; Ressel, 1996). Accordingly, a negative association between the levels of energetically demanding

Table 5
Correlation between total parasite intensity, pulmonary parasite intensity, intestinal parasite intensity, locomotor performance, $\dot{V}O_2$std, and compound dependent variables for *Rhinella icterica* (pairwise deletion of missing data).

Variables	Pulmonary parasite intensity (N = 28)	Intestinal parasite intensity (N = 28)	Locomotor performance (N = 20)	$\dot{V}O_2$std (N = 22)	Morphological factor scores (N = 16)				Behavioral (vocal effort and density chorus) and environment factor scores (N = 20)	
					PC1	PC2	PC3	PC4	PC1	PC2
Total parasite intensity[a]	**0.64***	**0.87***	−0.39	**−0.45**	**0.67***	−0.01	0.10	−0.25	−0.25	0.08
Pulmonary parasite intensity		0.30	**−0.49**	−0.40	**0.70***	−0.27	−0.16	−0.22	0.06	0.20
Intestinal parasite intensity			−0.11	−0.32	**0.53**	0.23	0.35	−0.30	−0.34	0.03

Total parasite intensity, pulmonary parasite intensity, intestinal parasite intensity, locomotor performance and $\dot{V}O_2$std are expressed as $\log_{10}$ of absolute values. Compound dependent variables factor scores results from each component extracted by regression. Significant, unadjusted correlations are indicated in bold. The absolute values of $r > 0.40$ remain significant at the 'tablewide' P of 0.05, and absolute values of $r > 0.53$ are significant to 0.01. Significant correlations after a sequential Bonferroni correction for multiple, simultaneous tests within sub-tables (Rice, 1989) are indicated with one asterisks. $\dot{V}O_2$std = standard rate of oxygen consumption; PC = Principal Component.

[a] Total parasites correspond to the total number of parasites, except those that were lodged in the correlating organ.

Fig. 3. Association between locomotor performance and pulmonary parasite intensity in *Rhinella icterica* (N = 20; r = −0.49, P = 0.03). SVL = snout-vent length.

expression of secondary sexual characters and parasite intensity is predicted by parasite-mediated models of sexual selection (Hamilton and Zuk, 1982; Folstad and Karter, 1992). Support for this prediction in anurans has been controversial. Males of *Hypsiboas prasinus*, showing higher levels of vocal calling, had a lower total parasite intensity (Madelaire et al., 2013). Furthermore, this negative association was more pronounced for smaller individuals (Madelaire et al., 2013). On the other hand, Hausfater and collaborators (1990) did not find any relationship between parasite intensity and the call duration of *Hyla versicolor* males. Males of *H. versicolor,* in amplexus, were more parasitized than individuals that were not mating (Hausfater et al., 1990). Moreover, parasitized males of *Scaphiopus couchii* were in a better body condition and displayed longer calls than unparasitized males, and unparasitized females preferred the longer calls commonly observed in parasitized males (Pfennig and Tinsley, 2002). Although calling effort was not related to parasite intensity in *R. icterica* toads, the observed negative association between parasite intensity and locomotor performance could indirectly impact on other components of sexual behavior in these toads. Sexual behavior in many species of toads is characterized by a period of active search as well as male disputes over access to a female (Wells, 1977). As such, unparasitized toads might endure longer locomotor activity, and cover a larger area in the active search for females, *versus* parasitized individuals. This will have to be determined by future research studies.

Previous studies have emphasized that parasitic deleterious effects should be prominent during periods of host high energetic demand (Holmes and Zohar, 1990; Goater et al., 1993; Pizzatto and Shine, 2012), with wild-captured *R. icterica* individuals showing no relation between parasite intensity and host performance under conditions of low energy demand (Moretti et al., 2014). However, in the present study, a negative association is observed between parasite intensity and standard metabolic rates. Lowering metabolic rates might increase host resistance to the physiological stress associated with host-parasite dynamics (Esch et al., 1975; Thompson, 1990; Parsons, 1991; Llewellyn et al., 2011). Accordingly, different vertebrates resort to hypothermia during severe infection (Deen and Hutchison, 2001; Schwanz, 2006; Liu et al., 2012). Hypothermic status decreases metabolic rates and increases host survival rate, probably by diminishing the impact of ATP demand on endogenous energetic substrates (Boutilier, 2001; Deen and Hutchison, 2001).

Previous studies have suggested that poor body-condition could be either cause and/or consequence of host parasite intensity in wild animals (Beldomenico et al., 2008; Beldomenico and Begon, 2010; Debeffe et al., 2016). However, no such

Table 6
Prevalence, mean intensity of infection, mean abundance, site of infection, and life cycle of helminth parasites in *Rhinella icterica* from Botucatu-SP.

Helminth species	Prevalence (%)	Mean intensity	Mean abundance	Site of infection	Life cycle
Acanthocephala					
Acanthocephalus saopaulensis	46.4	12.5 ± 4.4[a] (1–55)	5.8 ± 2.3[a] (0–55)	SI, LI	HETE
Nematoda					
Physaloptera sp.	7.1	1.0 ± 0.0 (1–1)	0.1 ± 0.1 (0–1)	ST, LI	HETE
Oswaldocruzia subauricularis	78.6	7.7 ± 1.5 (1–28)	6.1 ± 1.3 (0–28)	ST, SI, LI	MONO
Rhabdias fuelleborni	89.3	30.2 ± 6.5 (1–135)	26.9 ± 6.1 (0–135)	L	MONO
Cosmocercidae	64.3	31.0 ± 8.6 (1–115)	19.9 ± 6.2 (0–115)	SI, LI	
Falcaustra mascula	3.6	6.0 (6–6)	0.2 ± 0.2 (0–6)	LI	MONO
Trematoda					
Mesocoelium monas	10.7	731.7 ± 726.2 (4–2184)	78.4 ± 78.0 (0–2184)	SI	HETE
Gorgoderidae	3.6	2.0 ± 18.0 (2–58)	0.1 ± 2.1 (0–58)	BL, LI	HETE
Total parasite species	96.4	81.7 ± 412.2 (4–2206)	78.8 ± 76.8 (0–2206)	L, BL, ST, SI, LI	HETE/MONO

SI = Small intestine; LI = Large intestine. ST = Stomach; L = Lung; BL = Bladder. HETE = Heteroxenous; MONO = Monoxenous.
[a] Values represent mean ± standard error. Values between parentheses represent the range.

negative association between parasite intensities and body-condition index of wild-captured male toads was observed in the present study. A previous study investigating wild-captured *R. icterica* individuals showed parasite intensity in the field was not associated with large changes in energy stores (Moretti et al., 2014). Maintenance of energy stores may be a target of the behavioral reorganization ("sickness behavior") associated with host-parasite dynamics (Adelman and Martin, 2009). Metamorphs from Cane toads (*R. marina*), following experimental infection by lung parasites, changed their behavior from active to ambush foraging and showed reduced prey capture (Kelehear et al., 2009). Additionally, toads (*R. marina*) when injected with lipopolysaccharide, showed "sickness behavior", which was characterized by a reduction in locomotor activity and anorexia (Llewellyn et al., 2012). It is possible that characteristics associated with "sickness behavior", along with reduced basal energy expenditure, may maintain the stability of the energy reserves of infected toads. Future studies are required to test the impact of experimental nematode infection on energy sources (Lennie et al., 1995; Kyriazakis et al., 1998; Speakman and Mitchell, 2011).

As predicted, we found a positive association of relative masses of the heart, intestine, and kidney with parasite intensities in these toads. A positive association of organ masses with parasite intensities generally represents morphological plasticity of the host to parasite damage (Holmes and Zohar, 1990). This morphological plasticity may be driven by a number of factors, including compensatory mechanisms arising from the energy and nutritional drain, which could then be allocated to host structural repair; interference in host homeostasis; direct effects from the presence of parasites lodged in the organ; and secondary infection (Kristan, 2002a; Kristan and Hammond, 2003; Schwanz, 2006; Wang et al., 2008; Devevey et al., 2009). Despite evidence of morphological plasticity, the mechanisms underpinning parasite-linked organ remodeling require further investigation.

Finally, our results showed that individuals with higher intestinal or pulmonary parasite intensities also have higher total parasite intensities. This contrasts with the results of previous studies, which showed no evidence of an interspecific association between parasites in *R. icterica* toads (Luque et al., 2005; Moretti et al., 2014). Studies with amphibians suggested negative and positive interactions between parasite species within a host should impact on host-parasite fitness and dynamics (Jackson et al., 2006; Johnson and Hoverman, 2012). Species substitution or reducing the abundance of the most pathogenic species of trematode parasites lowers mortality of the amphibian host (*Pseudis regilla*) (Johnson and Hoverman, 2012). Additionally, interspecific interactions among parasites should be associated with a number of factors, including: an interaction network involving the particular organs infected; the resources utilized by parasites; competition between particular parasites; and the host's immune response to different parasites (Pedersen and Fenton, 2007; Jolles et al., 2008; Telfer et al., 2010).

5. Conclusion

Our results showed association between parasite intensities and the phenotypic characteristics of the Cururu toads. We showed a negative correlation of parasite intensities with locomotor performance, and the standard metabolic rate of *R. icterica*. Additionally, we showed positive associations of parasite intensities with relative organ masses (heart, intestine and kidney). However, the vocal effort of the toads was not associated with parasite intensities. Finally, we showed that Cururu toads with higher intestinal or pulmonary parasite intensities also have higher total parasite intensities.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijppaw.2017.06.003.

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