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The costs of parasite infection: Effects of removing lungworms on performance, growth and survival of free-ranging cane toads

Patrick B. Finnerty | Richard Shine | Gregory P. Brown

School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia

Correspondence
Patrick B. Finnerty
E-mail: pfin1253@uni.sydney.edu.au

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Abstract

1. Most research on the effects of parasites on their hosts has focused on the parasites of mammals or birds (especially, domesticated taxa) rather than systems in which the hosts are ectothermic wildlife species.
2. We used experimental methods (antihelminthic drugs) to quantify the effects of lungworms (*Rhabdias pseudosphaerocephala*) on their anuran hosts, the invasive cane toad (*Rhinella marina*).
3. In captivity, eradicating lungworms enhanced toad activity (measures of boldness and level of spontaneous activity), performance (locomotor speed, climbing ability) and foraging success (feeding rate).
4. In free-ranging toads (n = 123) at a site in tropical Australia, eradicating lungworm infection increased rates of host survival by 8%, movement by 20%, growth by 28% and elaboration of male secondary sexual characteristics by 30%. The presence of the lungworm thus has a substantial negative effect on fitness-related traits of the host.
5. Given their long shared evolutionary history and the mild inflammatory and immune response elicited by the parasite in the host, the magnitude of the effects of parasite removal were surprising. Parasites may impose hidden costs, related to modification of host behaviour or metabolism. Experimental removal of parasites can be a useful means of quantifying costs of infection.

KEYWORDS
anthelmintic, *Bufo marinus*, host–parasite system, pathogenicity

1 | INTRODUCTION

Parasites can affect a host's growth and performance, across multiple taxa and life stages and at all levels from individual host performance to community function and structure (Barber, Hoare, & Krause, 2000; Craig, Jones, Pilkington, & Pemberton, 2009; Fenner & Bull, 2008; Hatcher, Dick, & Dunn, 2012; Poulin, 2011; Worsley-Tonks & Ezenwa, 2015). Nonetheless, most research into the effects of parasitism has come from laboratory studies conducted under artificial conditions, or correlative field surveys from which it is not possible to unambiguously identify causal effects (Crump & Pounds, 1985; Kelehear, Brown, & Shine, 2011). In nature, parasite–host interactions are influenced by factors such as host sex, weather, life stage and habitat (Minchella & Scott, 1991). Thus, quantifying the effects of parasitic infection on host growth and performance in the wild is difficult, which may be why it is often avoided. Integrated manipulative laboratory and field studies can provide a more in-depth understanding of parasite effects on host performance (Heise-Pavlov, Paleologo, & Glenny, 2014; Kelehear et al., 2011). By using methods better-suited to identifying causal effects, such as manipulative field experiments combined with

laboratory investigations, we could more effectively clarify the natural dynamics of infection, mechanisms of parasite transfer and the extent to which parasites affect their hosts.

Understanding parasite–host relationships can contribute to applied science as well as basic knowledge, in the context of the conservation of native species, management of threatened ecosystems and the potential control of invasive species (Blaustein & Kiesecker, 2002; Hatcher et al., 2012; Holmes, 1996; Kelehear, Webb, & Shine, 2009; Messing & Wright, 2006; Pizzatto & Shine, 2011; Prenter, MacNeil, Dick, & Dunn, 2004). For example, global amphibian declines have stimulated investigations into the biology of microparasites potentially driving such declines; an iridovirus, an oomycete, ranaviruses and a zoosporic fungus *Batrachochytrium dendrobatidis* (Blaustein & Kiesecker, 2002; Collins, 2010; Dang, Searle, & Blaustein, 2017; Kelehear et al., 2009). Infection by macroparasites also has negative effects on amphibians of many species (Koprivnikar et al., 2012) although overall, the effects of parasites on their hosts may have been underestimated (Dobson & Hudson, 1992; Minchella & Scott, 1991; Worsley-Tonks & Ezenwa, 2015).

Parasites can have negative impacts on their host through several pathways. By feeding on host cells or products, parasites can appropriate energy or materials otherwise available to the host. The physical presence of parasites can occlude airways, alimentary tracts and blood or lymphatic vessels and reduce their functionality. If the parasite damages host tissue through feeding, attachment or migration, it can stimulate pathological and immune responses which can also impose energetic and functionality costs to the host. Thus, even in the absence of overt disease symptoms, the accumulation of subtle sublethal effects caused by parasites can have important fitness consequences over a host's lifetime (Pizzatto, Kelehear, Dubey, Barton, & Shine, 2012; Tompkins & Begon, 1999).

Quantifying the impacts of parasites and pathogens is important for conservation efforts not just when host populations are declining (Collins, 2010; Dang et al., 2017), but also when host populations are increasing, if the host is an invasive species that poses ecological threats (Stiling & Cornelissen, 2005). Cane toads (*Rhinella marina* Linnaeus 1758) in Australia are a notorious example of an introduced species that imperils native ecosystems (Shine, 2010). As such, their parasites and pathogens have been intensively studied (Barton, 1997; Hartigan et al., 2011; Kelehear, Brown, & Shine, 2012b; Speare, 1990). Although cane toads lost many native-range parasites during their translocation from South America to Australia (Selechnik, Rollins, Brown, Kelehear, & Shine, 2017), they retained the native-range lungworm *Rhabdias pseudosphaerocephala* Kuzmin et al. 2007 (Barton, 1997; Dubey & Shine, 2008). Because this nematode rarely, if ever, infects Australian native frogs (Pizzatto, Shilton, & Shine, 2010), it has been suggested as a potentially useful component of an integrated pest management strategy for cane toads (Kelehear et al., 2009, 2011; Phillips et al., 2010; Pizzatto & Shine, 2012a; Tingley et al., 2017).

Although many aspects of the cane toad–*Rhabdias* system, such as distribution and infection dynamics, are well understood (Kelehear, Brown, & Shine, 2012a; Kelehear et al., 2011; Pizzatto et al., 2010, 2012), considerable ambiguity remains regarding the effect of the parasite on host fitness. Previous experimental studies have focused on metamorphs rather than adult toads (Kelehear et al., 2009; Pizzatto et al., 2010), or have been based on captive or correlational studies (Kelehear et al., 2011). Despite interest in the role of *Rhabdias* as a potential biocontrol (Pizzatto & Shine, 2012b), we lack a clear understanding of the magnitude of impact of the lungworm on free-ranging toads. In this study, we experimentally manipulated parasite infection levels to measure the effects of parasites on toads both in the wild and in captivity. Commercially available antihelminthic drugs can effectively kill nematodes (including *Rhabdias*) in infected hosts (Lind & Christensson, 2009; Rehbein & Visser, 2002; Wright, 2001), generating parasite-free animals for experimental purposes. Fitness-related traits (e.g. behaviour, activity, growth, survival) of these individuals can then be compared to those of appropriate controls (individuals with maintained infections or naturally uninfected). This experimental approach has been used to clarify parasite impacts on wild mammals (Arneberg, Folstad, & Karter, 1996; Craig et al., 2009; Folstad, Nilssen, Halvorsen, & Andersen, 1991; Newey, Thirgood, & Hudson, 2004), and here we apply it to investigate the fitness consequences of *Rhabdias* infection in cane toads, under both captive and free-living settings.

2 | MATERIALS AND METHODS

2.1 | Host–parasite system

Cane toads (*Rhinella marina*, formerly *Bufo marinus*) are large bufonid anurans native to Central and South America. Since their introduction into Australia in 1935, cane toads have dispersed rapidly (Phillips, Brown, Webb, & Shine, 2006; Urban, Phillips, Skelly, & Shine, 2008), causing population declines of endemic predators (Shine, 2010).

The lung nematode *Rhabdias pseudosphaerocephala* occurs through most of the cane toad's Australian range (Dubey & Shine, 2008), but lags behind the expanding invasion front (Phillips et al., 2010). *Rhabdias* nematodes have a direct life cycle with hermaphroditic adults feeding on blood inside the toad's lungs. Eggs enter the toad's digestive tract and hatch into first-stage male and female free-living forms. Following defecation, the free-living larvae in toad faeces mate to produce infective third stage larvae (L3). L3 develop inside free-living females before breaking free and entering the soil (Baker, 1979). When an L3 locates an anuran host, it penetrates the host and migrates into the lungs (Pizzatto et al., 2010). Prevalence of this parasite in cane toads can exceed 80% (Barton, 1998), with intensity of up to 282 adult worms per host (Pizzatto, Kelehear, & Shine, 2013).

2.2 | Study site

Leaning Tree Lagoon (12°71′33″S, 131°41′96″W) in the Adelaide River floodplain, Northern Territory, Australia, is a 6-ha billabong 80 km south-east of Darwin. The area experiences a wet-dry tropical climate, and our study took place over the dry season (May–November). Average maximum air temperature exceeded 35°C each month and the mean monthly minimum temperature between August and November was 21°C (BOM—Bureau of Meteorology, 2016). Cane

toads arrived in the area (Leaning Tree Lagoon) late in 2005, and lungworms were first recorded in toads in the area in 2008 (Phillips et al., 2010).

2.3 | Methods for studying captive toads

We captured 49 toads and weighed, measured and individually marked them, then housed them individually in 300 × 200 × 200 mm plastic boxes for the next 4 months. To quantify infection status, toad faeces were viewed under a dissecting microscope for *Rhabdias* larvae. Non-infected and infected toads were randomly assigned to receive two doses (once at capture, and again 2 weeks later) either of a deworming solution (Ivomec©, 0.02 mg/100 g toad) or a control dose of Amphibian Ringer's solution, to generate four treatment groups henceforth referred to as ID (infected and dewormed, $n = 11$ toads), IC (infected and Amphibian Ringer's control, $n = 13$), ND (not infected and dewormed, $n = 13$), and NC (not infected and Amphibian Ringer's control, $n = 12$). The solutions were administered by injection into the dorsal lymph sac of each toad using a 1.0 ml surgical syringe with a 16 mm 25G gauge needle. A 1-g faecal sample was viewed each week following this procedure to check infection status. After trials, all toads were dissected and lungworm infection intensity counted. All assays conducted were blind to host treatment.

2.4 | Behavioural attributes of toads

2.4.1 | Activity level

We scored the time captive toads took to emerge from a shelter, a measure of "boldness" (González-Bernal, Brown, & Shine, 2014). We filmed the arena for 30 min to score: (1) the time elapsed until the first emergence of the toad's head from the shelter; and (2) spontaneous activity level, defined as the number of 100 × 100 mm^2 (drawn onto the bottom of each arena) which the toad entered whilst moving around the area.

2.4.2 | Performance

For captive toads, we measured locomotor performance on a rectangular raceway (150 mm wide, 180 mm high, 2,500 mm long) at 28.2–33.6°C. We tapped the toad gently on the urostyle whenever it stopped running; trials concluded when five consecutive taps failed to induce a response. The fastest time to cover 250 mm was used as a measure of sprint time. Total distances travelled and total running times were used as endurance measures (Kelehear et al., 2009).

Climbing performance of captive toads was assessed in open-topped trellis mesh cylinders (500 mm height, 150 mm diameter) at 28.2–33.6°C and filmed for 1 hr. We scored the time before climbing commenced, time to reach the top of the cylinder and a binary measure (Yes/No) of escape success.

2.4.3 | Foraging success

Captive toads were fasted for 2 days and placed in individual arenas (300 × 200 × 200 mm) at 28.2–33.6°C. We recorded the number of crickets (out of 10 provided) that were eaten in 10 min (Kelehear et al., 2009). Four foraging trials were conducted for each toad monthly.

2.4.4 | Infection intensity

All captive toads were euthanised and dissected, and the number of adult lungworms per toad was recorded.

2.5 | Methods for studying free-ranging toads

We captured 454 free-ranging toads at Leaning Tree Lagoon (average of 25 per night) in five collection bouts, each 5 days in duration and with 10 days between successive bouts. We divided the area into fourteen 125-m transects surrounding the lagoon, walked transects between 19.30 and 21.00 hr, and collected any toads within 1 m of the transect line. In the laboratory, toads were held overnight to obtain faecal samples (and also weighed, measured, and marked). For male toads, we scored secondary sexual characteristics (release call, blackness of nuptial pads, skin colour and skin rugosity; these scores were summed to give an overall index of male secondary sexual character development).

About 123 toads provided a faecal sample at their first capture event (inspected to assess infection status), and became the focal animals for the experimental deworming protocol. Approximately, half of the infected and uninfected toads were treated with anthelmintic deworming solution and the remainder given a control dose of Amphibian Ringer's solution. This design resulted in the same four treatment groups as in the captive study (ID: $n = 31$, IC: $n = 33$, ND: $n = 24$, NC: $n = 35$). Toads were released at their capture location within 24 hr. Each time a toad was recaptured it was re-measured and re-dosed with the same treatment it received originally. Using these mark-recapture data, we quantified rates of survival, growth and movement of toads from all treatments. Assays conducted were blind to host treatment.

2.6 | Ecological attributes of toads

2.6.1 | Rates of movement

From the field trials, we measured displacements of toads over both the short-term (radiotracking) and longer-term (mark-recapture). For the radiotracking trials, toads from two of the experimental groups (infected control IC, and infected dewormed ID) were fitted with a 3-g radiotransmitter, released at their point of capture the following morning, and re-located for four consecutive mornings. From these data, we calculated total displacements per unit time (see above).

2.6.2 | Rates of growth and survival

To compare field-based growth rates, we recorded mass, snout-urostyle length (SUL), right tibia length, and head width each time a toad was captured. For growth rates of captive toads, we measured the same traits initially (at capture) and at 2-week intervals thereafter. Body condition was quantified as the residual score from the general linear regression of log mass against log SUL.

Based on the data collected during our 12-week mark-recapture study, we fitted 16 Cormack–Jolly–Seber (CJS) models in which survival (phi) and recapture rates (p) were either held constant, varied between treatment groups, varied over time, or varied over time in a different manner for each group. We assessed the fit of the 16 CJS models by comparing their Akaike information criterion (AIC_c) values (White & Burnham, 1999; see Appendix S1 for detailed materials and methods).

2.7 | Statistical analyses

We used linear mixed effects models fit by restricted maximum likelihood estimation to investigate the main effects of *Rhabdias pseudosphaerocephala* infection on host growth and performance. Toad ID and capture number (for free-ranging toads) were included as random factors. Where necessary, data were transformed to meet the assumptions of parametric analyses, or nonparametric analysis was used. For analyses where group had a significant effect, we conducted Tukey HSD post hoc tests to locate significant differences (see Appendix S2 for detailed information on analyses).

3 | RESULTS

3.1 | Effectiveness of toad faecal examination and experimental manipulation of infection status

Faecal examination of captive toads had a 100% detection efficiency for *Rhabdias* infection, but counts of larvae per gram of faeces were not correlated with the number of adult worms a toad had in its lungs. The average L3 count over the nine faecal samples for each toad was not significantly correlated with lungworm numbers (Spearman $r = .12$, $p = .77$). Faecal examination thus accurately determined infection status (Yes vs. No), but not intensity of infection.

In captivity, toads from the two "already infected" groups had similar faecal larvae counts prior to treatment (IC: $n = 13$, $M \pm SE = 92 \pm 14$ larvae/g faecal matter, vs. ID: $n = 11$, $M \pm SE = 97 \pm 16$ larvae/g faecal matter; $F_{1,23} = 0.02$, $p = .87$). Treatment with the Ivomec dewormer induced a rapid decline in larvae/g of faecal matter in captive toads (100% larvae-free in 4 weeks; ID: $n = 11$; $F_{1,186} = 8.74$, $p < .01$), whereas larval numbers did not change through time in control-injected toads (ND: $n = 13$, NC: $n = 12$; $F_{1,186} = 0.28$, $p = .91$; Figure 1a). Similarly, initial counts of larvae/g faecal matter were similar in the two "already infected" experimental groups of free-ranging toads (prior to treatment: respective larvae/g faecal matter $M \pm SE = 121 \pm 16$ in toads that received dewormer, $n = 55$, vs. 110 ± 12 in toads that received Amphibian Ringer's control solution, $n = 68$; $F_{1,26} = 0.01$, $p = .93$).

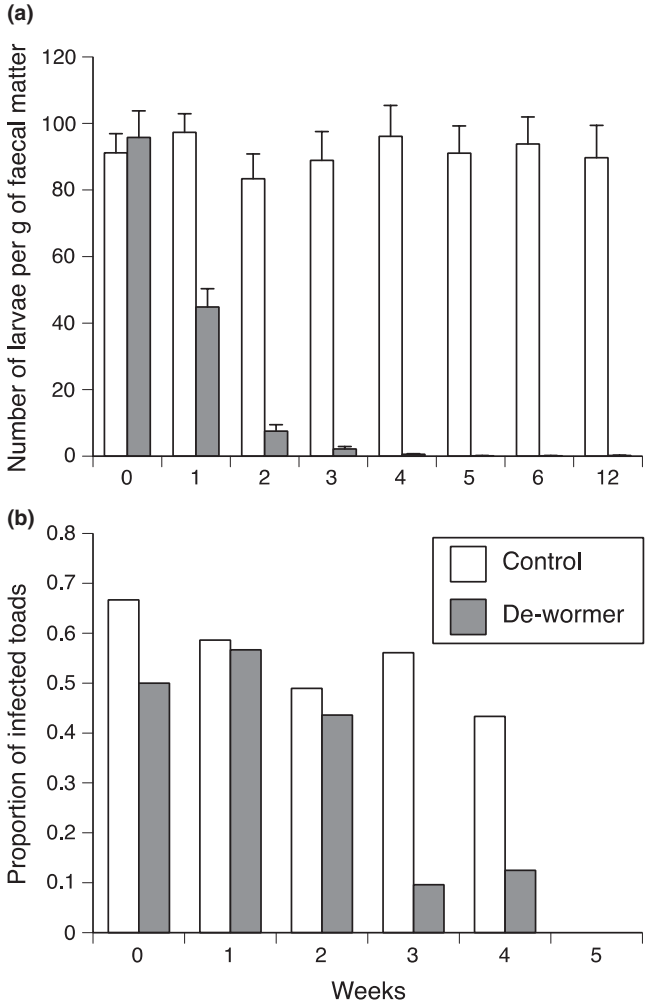

FIGURE 1 Changes in (a) faecal larvae counts ($M \pm SE$) from captive toads, and (b) infection status among free-ranging toads, as a function experimental deworming. At week 0, prior to treatment, all toads had similar levels of infection. Toads subsequently treated with anthelmintic exhibited a rapid decline in the number of faecal larvae counts (captive toads) and prevalence of infection (free-ranging toads) compared to "control" conspecifics treated with Amphibian Ringer's solution

During subsequent captures over the 12-week mark-recapture period, free-ranging toads injected with Ivomec were less likely to be infected when retested ($n = 31$, $\chi^2 = 23.53$, $df = 1$, $p < .01$), whereas toads that were not given Ivomec did not show any shift in parasite counts ($n = 33$, $\chi^2 = 1.58$, $df = 1$, $p = .21$; Figure 1b).

3.2 | Behavioural attributes of toads

3.2.1 | Activity level

Toads that remained infected (IC: $n = 13$) were less likely to emerge from shelters than were non-infected toads (ID: $n = 11$, ND: $n = 13$, NC: $n = 12$; 54.4% vs. 81.2% emerged, $\chi^2 = 19.06$, $df = 3$, $p < .01$; post hoc tests show that IC < ID = NC = ND). Of the toads that emerged during the boldness trials, infected toads also took longer to emerge

from their shelters (mean time ± *SE* = 4.94 ± 1.11 min longer; toads that did not emerge were excluded from analysis [Table 1, Figure 2a]; post hoc tests show that IC > ID = NC = ND), and had lower levels of spontaneous activity (Table 1, Figure 2b; post hoc tests show that IC < ID = NC = ND). In analyses using the actual number of lungworms per toad (and omitting data for non-infected animals), anurans carrying more lungworms were less active (n = 13, $F_{1,31}$ = 10.97, p < .01), but their time to emergence was not significantly affected by their parasite burden ($F_{1,31}$ = 0.78, p = .38).

3.2.2 | Performance

Analysis revealed a significant interaction between treatment and trial number on the sprint times of adult cane toads (Table 2, Figure 3). Previously infected toads that were dewormed (ID: n = 11) showed enhanced sprint speed over four trial periods spanning 2 months, whereas toads that remained infected (IC: n = 13) remained slow (35% slower than ID). Captive cane toads that remained infected (IC) ran for less time (58% less; Table 2, Figure 4a; post hoc tests show that IC < ID = NC = ND) and over a shorter distance (43% shorter; Table 2, Figure 4b; post hoc tests show that IC < ID = NC = ND) than did toads that had been dewormed or toads that were never infected (ID: n = 11, ND: n = 13, NC: n = 12). More heavily infected toads ran for a shorter period of time (Table 3, Figure 4c) and did not run as far (Table 3, Figure 4d), but moved at similar speeds as less heavily infected animals (Table 3).

During climbing trials, infected toads (IC: n = 13) did not escape from the cylinder as often as did non-infected toads (ID: n = 11, ND: n = 13, NC: n = 12; 63.6% vs. 11.3% did not escape, χ^2 = 22.0, df = 3, p < .01). The infected toads that managed to escape took longer to begin climbing, to climb the enclosure and to successfully escape (Table 3, Figure 5; post hoc tests show that IC > ID = NC = ND). Infection intensity (number of lungworms per host) was related to time to initiate an escape attempt (Table 3, Figure 6a) and the probability of successful escape (χ^2 = 9.54, df = 1, p < .01; Figure 6b), but not to climbing time or time to escape (Table 3).

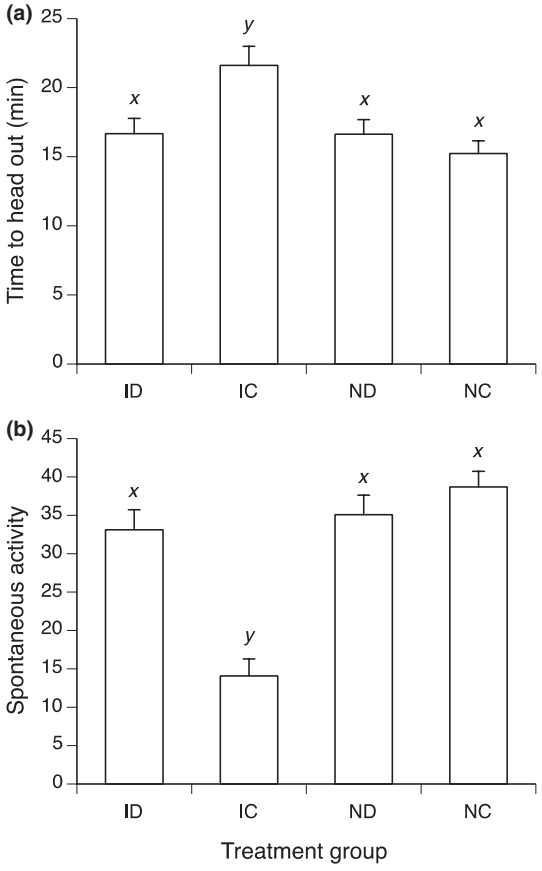

FIGURE 2 Comparison of behaviour of cane toads in standardised activity trials as a function of treatment group. The upper left panel (a) shows latency time for a toad's head to emerge from shelter, and the lower left panel (b) shows the average number of 100 × 100 mm lines crossed during the 30 min trial. Experimental groups are as follows: ID = infected and dewormed (n = 11), IC = infected and Amphibian Ringer's control (n = 13), ND = not infected and dewormed (n = 13), NC = not infected and Amphibian Ringer's control (n = 12). Each individual was trialled four times over 2 months. Bars with the same alphabetical superscript do not differ significantly from each other (p > .05). Graphs show mean values ±1 *SE*

3.2.3 | Foraging success

The number of crickets eaten increased in all groups over the four trials that were carried out monthly, but infected toads (IC: n = 13) ate fewer crickets than did non-infected toads (ID: n = 11, ND: n = 13, NC: n = 12; 43% less, treatment × trial number, $F_{1,190}$ = 44.56, p < .01; Figure 7a). Higher levels of infection intensity (number of lungworms) were associated with lower feeding rates ($F_{1,27}$ = 14.27, p < .01; Figure 7b).

Boldness variable	Effect	*df*	*F*	*p*
Time to head out	Trial	3,133	0.13	.85
	Infection group	3,133	7.89	**.02**
	Trial × infection group	3,133	0.29	.82
Spontaneous activity	Trial	3,133	0.59	.44
	Infection group	3,133	15.46	**<.01**
	Trial × infection group	3,133	0.35	.78

Significant values (p < .05) are shown in boldface font. See text for details of infection groups.

TABLE 1 Results of mixed model statistical analyses (infection group and trial number as fixed factors, toad ID as a random factor) for boldness behaviour and spontaneous activity trials for 49 captive adult toads (*Rhinella marina*) over four trial periods

TABLE 2 Results of statistical analyses using a multiple mixed model (infection group and trial number as fixed factors, toad ID as a random factor) to examine data from locomotor behaviour trials for 49 captive adult toads (*Rhinella marina*) over four trial periods

Locomotor variable	Effect	*df*	*F*	*p*
Sprint time	Trial	3,185	10.44	**<.01**
	Infection group	3,185	5.95	**<.01**
	Trial × infection group	3,185	3.13	**.03**
	Infection intensity	1,45	2.64	.11
Total running time	Trial	3,185	0.06	.79
	Infection group	3,185	7.94	**<.01**
	Trial × infection group	3,185	0.41	.74
	Infection intensity	1,45	10.89	**<.01**
Total running distance	Trial	3,185	0.05	.84
	Infection group	3,185	11.08	**<.01**
	Trial × infection group	3,185	1.32	.26
	Infection intensity	1,45	9.16	**<.01**

Significant values ($p < .05$) are shown in boldface font. See text for details of infection groups.

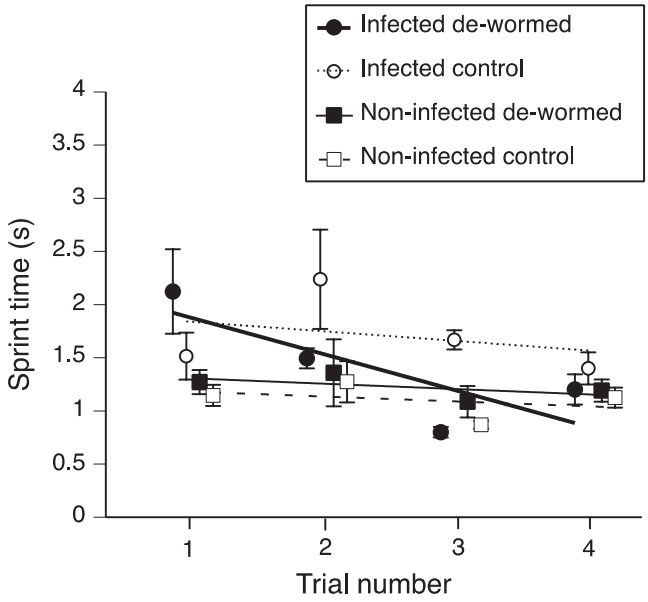

FIGURE 3 Changes in cane toad locomotor performance (sprint speed) as a function of treatment group. The figure illustrates a significant interaction between treatment group and trial number. Each individual was trialled four times over 2 months following deworming vs. Amphibian Ringer's control treatment. Experimental groups are as follows: ID = infected and dewormed ($n = 11$), IC = infected and Amphibian Ringer's control ($n = 13$), ND = not infected and dewormed ($n = 13$), NC = not infected and Amphibian Ringer's control ($n = 12$). Points are mean group values ±1 *SE* for each trial

3.3 | Ecological attributes of toads

3.3.1 | Rates of movement

We radiotracked infected ($n = 12$) and non-infected ($n = 11$) toads over 4 days, and recorded usage of 141 separate daily refugia. Overall, infected toads did not move as far (average distance ± *SE* = 41.46 ± 9.18 m) between daily refugia as did infected toads that had been dewormed (258.53 ± 80.64 m; $F_{1,11} = 7.21$, $p = .02$). Over longer-term mark-recapture trials, infected toads that were injected with dewormer were recaptured further from their previous capture site (average distance ± *SE* = 114.31 ± 6.13 m) than were infected toads that were dosed with Amphibian Ringer's control solution (95.28 ± 5.89 m; $F_{1,183} = 5.04$, $p = .03$).

3.3.2 | Rates of growth

Compared to non-infected conspecifics (ID: $n = 31$, ND: $n = 24$, NC: $n = 35$), free-ranging infected toads (IC: $n = 33$) exhibited lower daily rates of growth in SUL (30% lower), right tibia length (23% lower), and mass (54% lower; Table 4, Figure 8a–c; post hoc tests show that IC < ID = NC = ND in all cases). Change in head width per day (Figure 8d) showed no significant treatment effect, but exhibited a similar trend to the other three morphological variables (Table 4).

In captive toads, daily rates of growth in SUL (27% lower), right tibia length (34% lower), head width (29% lower), and mass (29% lower) were lower in infected toads (IC: $n = 13$) than in non-infected toads (ID: $n = 11$, ND: $n = 13$, NC: $n = 12$; Table 5, Figure 9a–d; post hoc tests show that IC < ID = NC = ND in all cases). Higher infection intensities (number of lungworms per host) were associated with lower rates of growth in SUL, right tibia length, head width, and mass (Figure 9e–h), with a significant interaction between group and date for body condition trajectories over the 12-week period of captivity (Table 5). Infected toads lost body condition throughout the trials, whereas non-infected and dewormed conspecifics retained or increased body condition over the same period ($F_{1,106} = 7.74$, $p < .01$; Figure 10). During the captive trial period, three infected toads did not survive and data for these animals were not included in the analysis.

3.3.3 | Male sexual characteristics

Scores for secondary sexual characteristics in free-ranging male toads decreased in infected toads (IC: $n = 18$; 0.01 score decrease per day) over the 12-week mark-recapture session, whereas scores

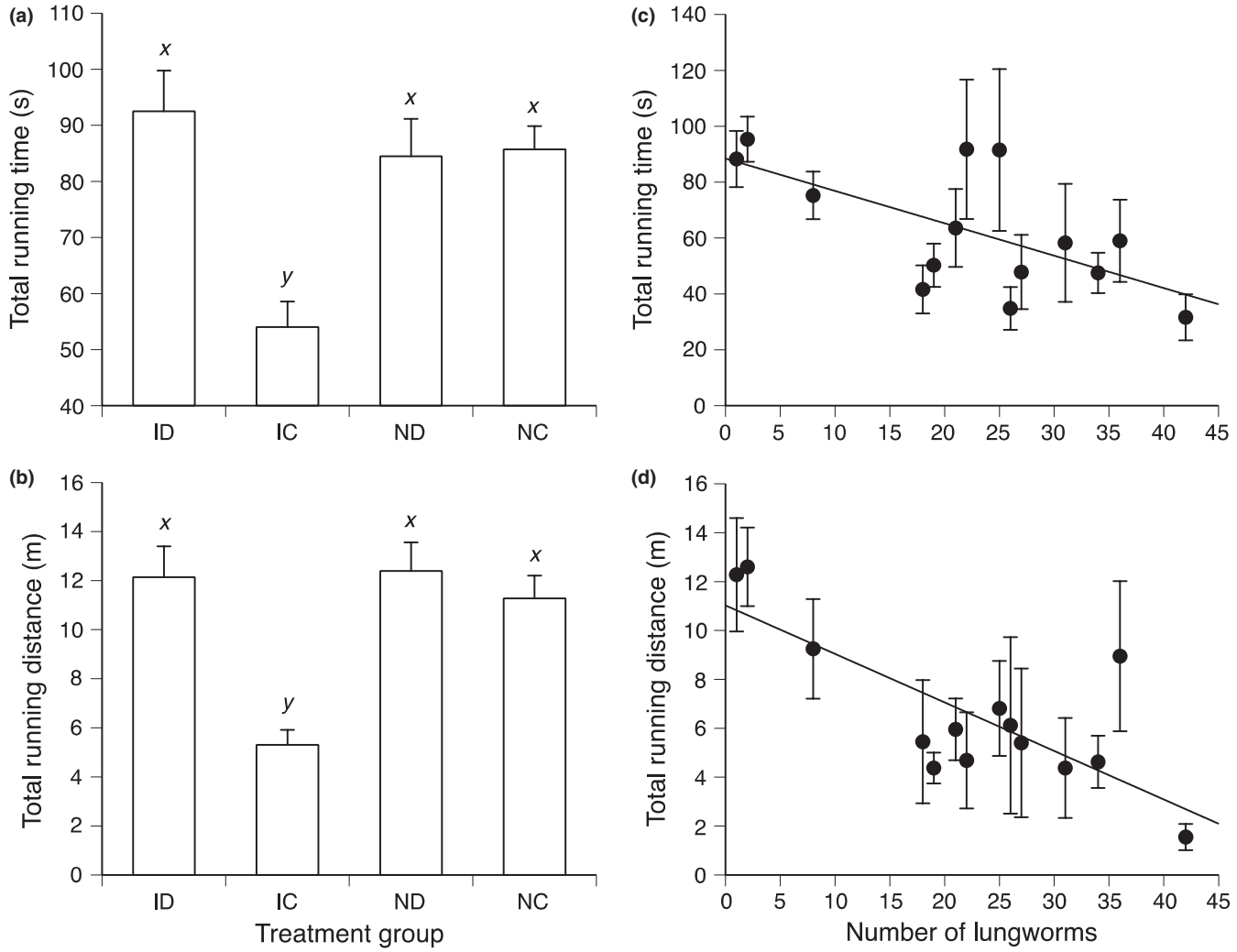

FIGURE 4 Effects of *Rhabdias* infection on locomotor performance of cane toads. Each individual was trialled four times over 2 months after a deworming or control injection. (a) total running duration and (b) total distance covered as a function of treatment group; ID = infected and dewormed (n = 11), IC = infected and Amphibian Ringer's control (n = 13), ND = not infected and dewormed (n = 13), NC = not infected and Amphibian Ringer's control (n = 12). Among the 13 infected toads (IC) subsequently dissected, panels (c) total running duration and (d) total distance covered, compare performance to the intensity of *Rhabdias* infection. Bars with the same alphabetical superscript are not significantly different from one another (p > .05). Graphs show mean values ±1 *SE*

increased in most toads that were given the dewormer (n = 9; 0.04 score increase per day) or that were never infected (n = 9; 0.07 score increase per day, $F_{3,22}$ = 35.16, p = .01; post hoc tests show that IC < ID = NC = ND).

3.3.4 | Survival

The single best-fitting model was one in which survival rates of free-ranging toads varied among the four groups and recapture probability was time-dependent. Toads that remained infected (IC: n = 33) had a 92% chance of surviving the day compared to infected and dewormed conspecifics that had a 98% chance (ID: n = 31, post hoc tests show that IC < ID = NC = ND). The next most highly ranked model had substantially less support (difference in AIC_c = 3.09) and contained survival and recapture parameters that were both time-dependent.

4 | DISCUSSION

Infection with the native-range lungworm *Rhabdias pseudosphaerocephala* is common in cane toads within their invasive range in Australia; in our study population, about half of the adult toads we tested contained this parasite. Rates of lungworm infection in Australian cane toads vary geographically and seasonally, and depend upon host body size, but similarly high infection rates are common (Barton, 1998; Phillips et al., 2010; Pizzatto et al., 2013). In this context, the dramatic effects of these lungworms on cane toad behaviour and ecology, as revealed by our study, may influence the invasive anuran's abundance and spread in tropical Australia.

The methodology we adopted worked well; injection of Ivomec consistently eliminated lungworms from infected toads and did not induce artefacts in uninfected animals (based on procedural control toads, ND vs. NC). Thus, our methodology allows us to draw robust

TABLE 3 Results of statistical analyses using a multiple mixed model (infection group and trial number as fixed factors, toad ID as a random factor) for the climbing behaviour of 49 captive adult toads (*Rhinella marina*) over four trial periods spanning 2 months

Climbing variable	Effect	*df*	*F*	*p*
Time to feet off the ground	Trial	3,36	0.10	.75
	Infection group	3,36	10.20	**<.01**
	Trial × infection group	3,36	0.21	.65
	Infection intensity	1,15	5.13	**.04**
Climbing time	Trial	3,36	3.04	.09
	Infection group	3,36	4.32	**.01**
	Trial × infection group	3,36	1.12	.12
	Infection intensity	1,15	0.67	.45
Time to escape	Trial	3,36	0.12	.72
	Infection group	3,36	11.38	**<.01**
	Trial × infection group	3,36	0.11	.89
	Infection intensity	1,15	4.19	.06

Significant values ($p < .05$) are shown in boldface font. See text for details of infection groups.

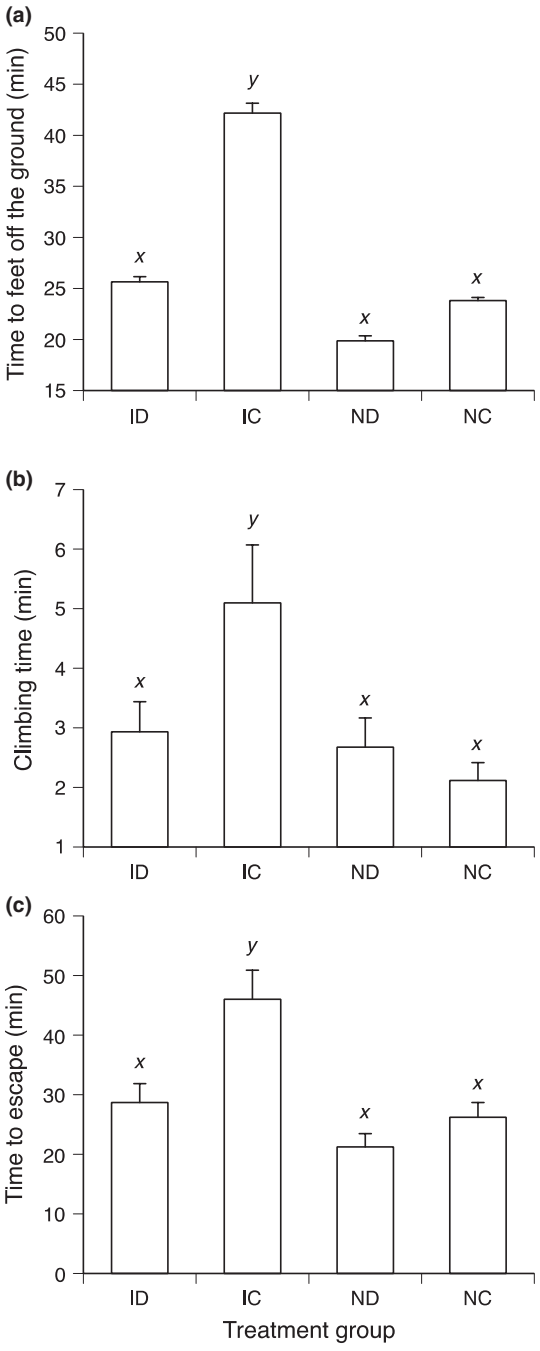

FIGURE 5 Climbing performance of cane toads as a function of treatment group. (a) latency to toad taking both hind feet off the ground, (b) total climbing time, (c) total escape time. Treatment groups are as follows: ID = infected and dewormed ($n = 11$), IC = infected and Amphibian Ringer's control ($n = 13$), ND = not infected and dewormed ($n = 13$), NC = not infected and Amphibian Ringer's control ($n = 12$). Each individual was trialled four times over 2 months after a deworming or Amphibian Ringer's control injection. Bars with the same alphabetical superscript are not significantly different from one another ($p > .05$). Graphs show mean values ±1 *SE*

conclusions regarding the effects of the parasite *R. pseudosphaerocephala* on its cane toad host. *Rhabdias* infection strongly reduced toad growth and performance and affected the behaviour of cane toads both in captivity and (importantly) in the wild. The effect sizes of infection on toads were substantial: for example, free-ranging infected toads on average travelled only 17% as far as uninfected animals, grew 28% as much, and had a daily survival probability 8% lower than that of uninfected conspecifics. Those effects were apparent very soon after experimental deworming (in <3 weeks) and remained evident for at least 2 months.

In virtually every trait that we examined, lungworm infection was associated with a significant shift in host behaviour and ecology. Compared to uninfected conspecifics, infected toads in captivity were less active, slower, less capable of climbing, and ate less. In the wild, experimental deworming enhanced toad dispersal rates, growth rates, elaboration of secondary sexual characteristics in males and rates of survival. Our procedural controls (uninfected toads injected with Ivomec) showed that these enhanced levels of biologically significant processes, such as dispersal, growth and survival, were not direct effects of the drug itself; those effects were apparent only in toads that were initially infected, and experimentally dewormed. Overall, the clear pattern from our results is that the single treatment group that maintained lungworm infections (IC; infected toads given Amphibian Ringer's solution) exhibited lower values on all performance and growth variables than did any of the other groups, all of which either lacked lungworms throughout the study (NC, ND) or were experimentally dewormed (ID).

Lungworm infection appeared to exert a profound and generalised effect on toad vigour. Captive animals that remained infected showed reduced activity levels, locomotor performance, climbing ability, and feeding rates than did their non-infected conspecifics. For some but not all of these traits, comparisons within the subset of infected toads revealed significant correlations between performance and the

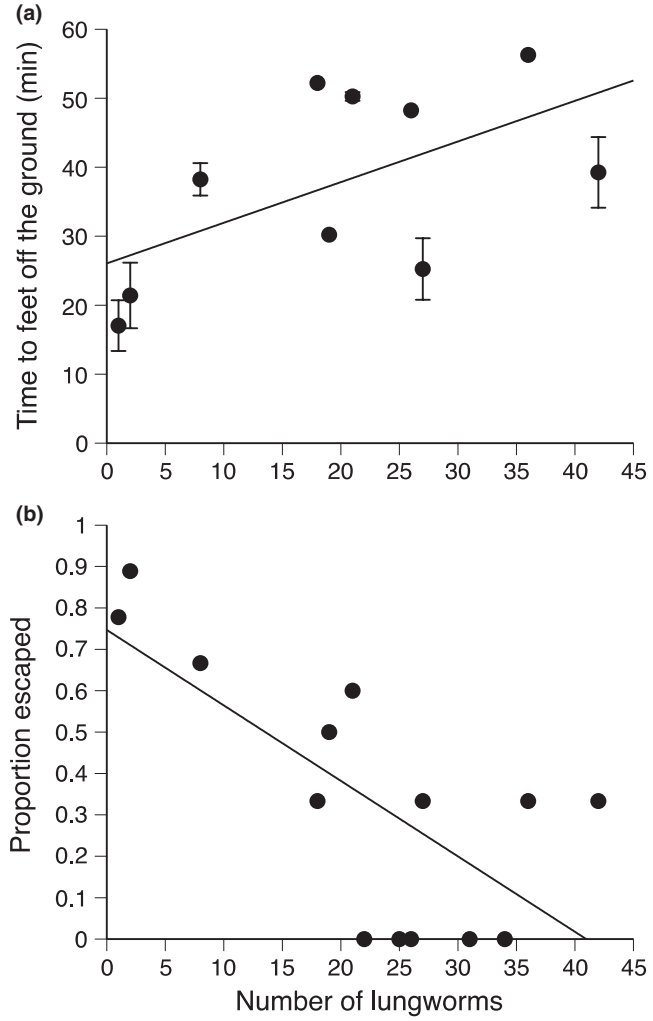

FIGURE 6 Effects of *Rhabdias* infection intensity on climbing performance of 13 cane toads. (a) latency to taking both hind feet off the ground, and (b) escape success (proportion of four trials spanning 2 months)

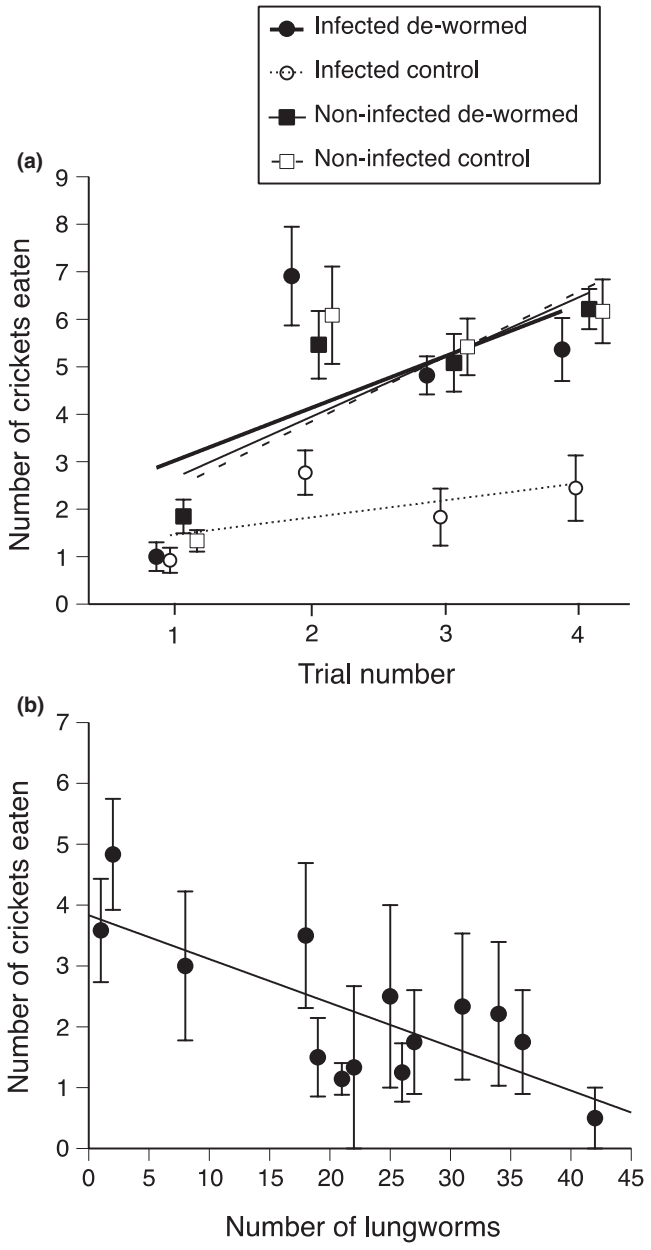

FIGURE 7 Changes in feeding rates of cane toads as a function of infection with lungworms (as determined by treatment group) over trials spanning 2 months. Panel (a) shows the significant interactive effect of treatment group and trial number on number of crickets eaten. Treatment groups were: ID = infected and dewormed (n = 11), IC = infected and Amphibian Ringer's control (n = 13), ND = not infected and dewormed (n = 13), NC = not infected and Amphibian Ringer's control (n = 12). Points are mean group values ±1 *SE*. Panel (b) shows the effect of *Rhabdias* infection intensity on feeding performance of 13 toads (IC group) that were subsequently dissected. Points are mean values ±1 *SE*

parasite load (number of worms per host), as expected if the presence of the parasites was a causal influence on the viability decrement. Although those results are correlative only and could, for example, be due to less-viable toads being more vulnerable to lungworm infection, the comparison between treatment groups provides direct experimental evidence that lungworm infection has devastating consequences for adult cane toads.

These findings indicate a stronger and more consistent negative effect of lungworm infection on host growth and performance than has been shown by earlier studies on this system and on similar amphibian host–parasite interactions. Although the effects of *R. pseudosphaerocephala* on the performance of cane toads have attracted considerable research, different methodologies and life stages have made it difficult to form a clear consensus on the strength of impact (Brown, Kelehear, Pizzatto, & Shine, 2016; Kelehear et al., 2009; Pizzatto & Shine, 2012a). Other research on the influence of *Rhabdias* lungworms on bufonid anurans have similarly documented significant but variable effects (Goater, Semlitsch, & Bernasconi, 1993; Goater & Ward, 1992).

This study provides robust and ecologically relevant measures of the parasite's effects, reinforcing the value of experimental deworming manipulations for studies on other ectotherm host–parasite systems.

The effects we documented seem likely to influence toad fitness in an evolutionary sense, with infected toads growing slower, dying sooner, and investing less into the elaboration of sexually dimorphic

TABLE 4 Results of statistical analyses, using multiple mixed models (infection group and initial snout-urostyle length [SUL], right tibia length, head width and mass as fixed factors, respectively, and toad ID as a random factor) on growth and mass change per day over a 12-week mark-recapture period for 125 free-ranging adult toads (*Rhinella marina*)

Growth variable	Effect	$F_{3,22}$	*p*
SUL growth/day	Infection group	3.79	**.03**
Right tibia growth/day	Infection group	4.09	**.02**
Head width growth/day	Infection group	2.26	.12
Mass change/day	Infection group	23.15	**<.01**

Significant values ($p < .05$) are shown in boldface font. See text for details of infection groups.

TABLE 5 Results of statistical analyses using a multiple mixed model (infection group and infection intensity as fixed factors, and toad ID as a random factor) on growth and mass change per day over a 12-week sampling period for 56 captive adult toads (*Rhinella marina*)

Growth variable	Effect	*df*	*F*	*p*
SUL growth/day	Infection group	3,31	13.06	**<.01**
	Infection intensity	1,8	7.84	**.02**
Right tibia growth/day	Infection group	3,31	4.95	**.01**
	Infection intensity	1,8	6.49	**.04**
Head width growth/day	Infection group	3,31	7.78	**<.01**
	Infection intensity	1,8	5.70	**.04**
Mass change/day	Infection group	3,31	13.28	**<.01**
	Infection intensity	1,8	7.66	**.01**

Significant values ($p < .05$) are shown in boldface font. See text for details of infection groups.
SUL, snout-urostyle length.

traits, which in turn influence male mating success (Bowcock, Brown, & Shine, 2008; Narayan, Christi, Morley, & Trevenen, 2008). Thus, this parasite-host relationship may have ecological importance,

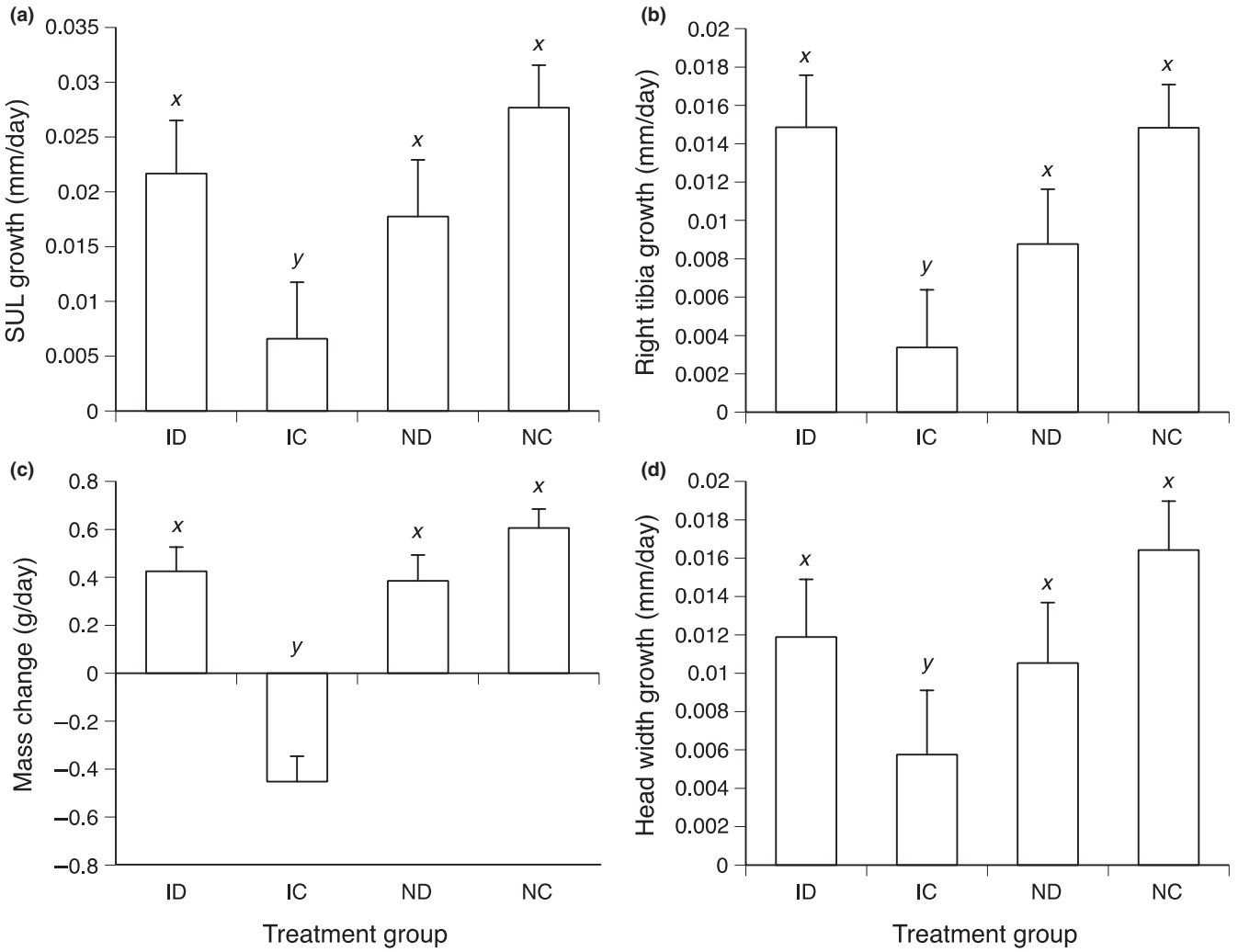

FIGURE 8 Effects of the nematode *Rhabdias* infection on the daily rates of growth of free-ranging adult cane toads (*Rhinella marina*). Panels show (a) snout-urostyle length growth per day, (b) right tibia growth per day, (c) mass change per day, and (d) increase in head width per day. ID = infected and dewormed ($n = 31$), IC = infected and Amphibian Ringer's control ($n = 33$), ND = not infected and dewormed ($n = 24$), NC = not infected and Amphibian Ringer's control ($n = 35$). Bars with the same alphabetical superscript are not significantly different from one another ($p > .05$). Graph shows mean values ±1 *SE*

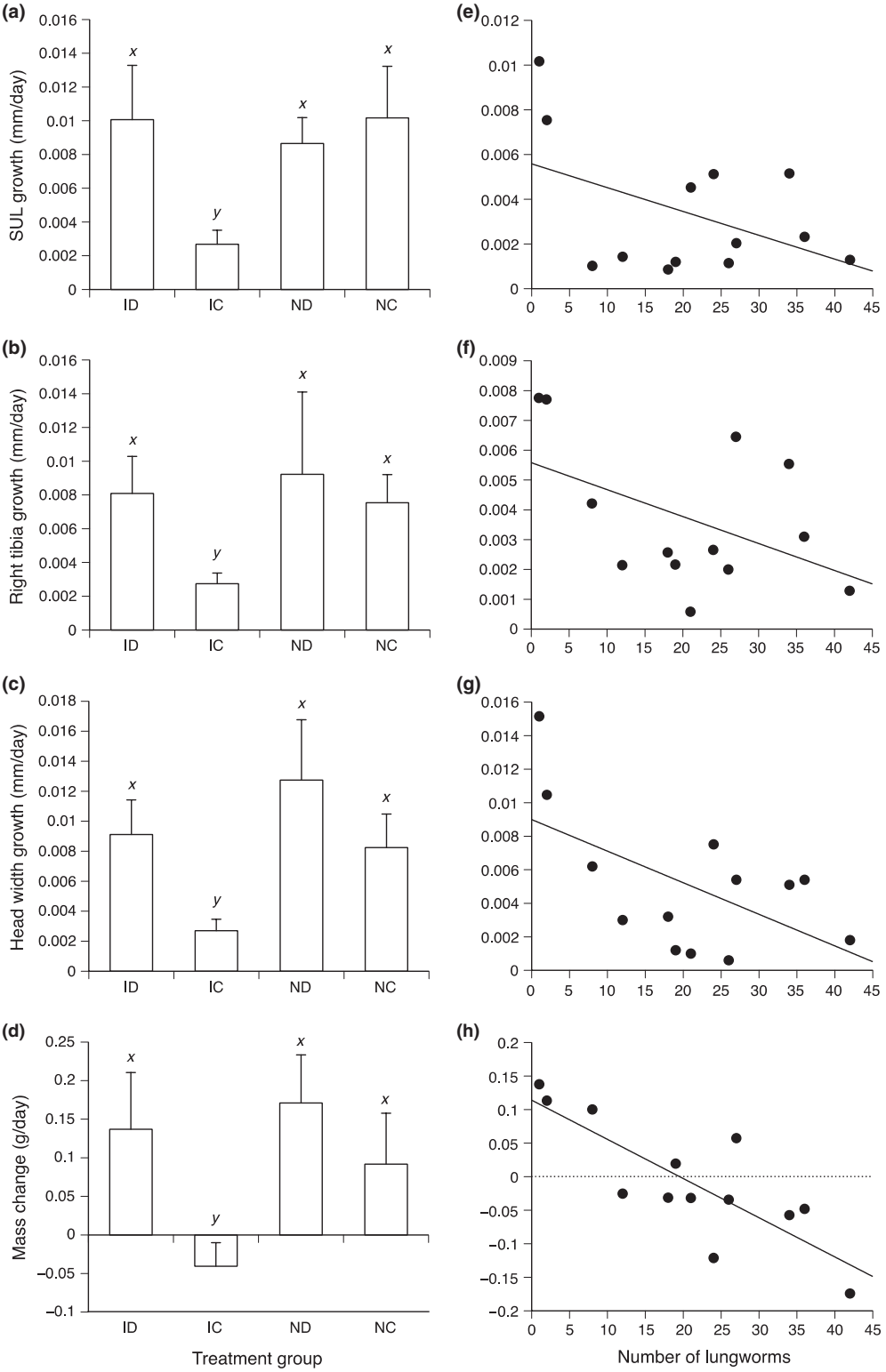

FIGURE 9 Effects of the nematode *Rhabdias* infection on the daily rates of growth of captive adult cane toads (*Rhinella marina*). Panels show (a) snout-urostyle length growth per day, (b) right tibia length growth per day, (c) head width growth per day, and (d) mass change per day. ID = infected and dewormed (n = 11), IC = infected and Amphibian Ringer's control (n = 13), ND = not infected and dewormed (n = 13), NC = not infected and Amphibian Ringer's control (n = 12). Each individual was measured at 2-week intervals over a 12-week period once before and then after a deworming or Amphibian Ringer's control injection. Bars with the same alphabetical superscript are not significantly different from one another (p > .05). Graphs show mean values ±1 *SE*. The other panels show relationships between intensity of *Rhabdias* infection (as measured at later dissection) and growth in (e) snout-urostyle length, (f) right tibia length, (g) head width, and (h) mass

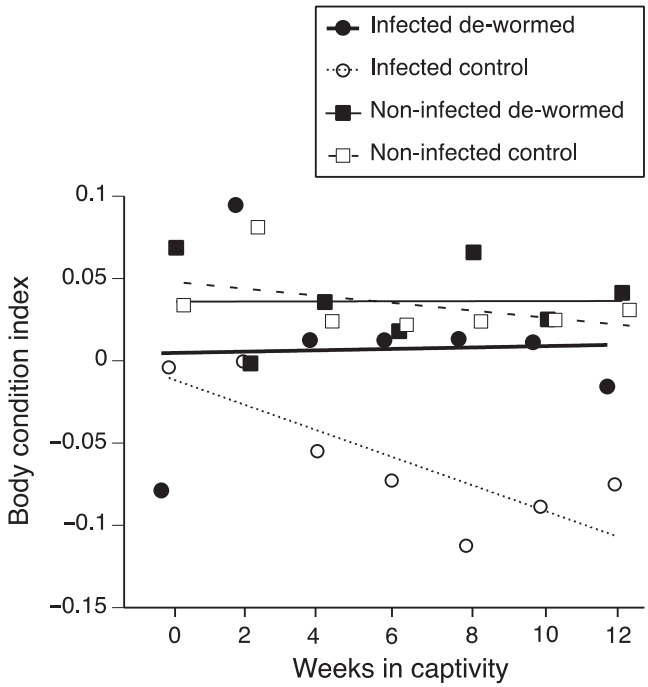

FIGURE 10 Effects of infection with the nematode *Rhabdias pseudosphaerocephala* on the body condition of adult cane toads (*Rhinella marina*). Each individual was measured at 2-weekly intervals over a 12-week period once before and then after a deworming or Amphibian Ringer's control injection. Points represent mean group values

influencing broad demographic features of toad populations, potentially reducing toad abundance and/or rates of dispersal (Brown et al., 2016; Ebert, Lipsitch, & Mangin, 2000; Hudson, Dobson, & Newborn, 1998; Phillips et al., 2010). Given the destructive effect of the invasive cane toad on native Australian fauna, and the toad's still-advancing invasion front (Shine, 2010), our data demonstrating a negative influence of parasite infection in free-ranging hosts are encouraging for the potential use of *R. pseudosphaerocephala* as a component of a biological control approach for toads in Australia (Tingley et al., 2017).

Nonetheless, it is important to note that *Rhabdias* clearly do not eliminate cane toad populations. The host and parasite share a long evolutionary history and toads occur at high abundance in many areas where the parasite is prevalent. However, our results suggest that the parasites do have strong suppressive effects on their hosts, and hence, densities of toads would plausibly be higher in the absence of lungworms. Toad populations reach maximal levels within 3–4 years of initial establishment and then decline precipitously (Brown & Shine, 2016) as do many invader populations (Simberloff & Gibbons, 2004). Although *Rhabdias* has not been directly implicated in these declines, the timing does coincide with the arrival of the parasites (which lag behind the toad invasion by 1–3 years: Phillips et al., 2010) and thus they may contribute to the decreased toad abundance to some extent.

Cane toads cease dispersing during the dry-season and accumulate at high densities around dwindling sources of moisture, circumstances that are ideal for pathogen transmission (Freeland, 1986). Additionally, the lack of lungworms at the toad invasion front means that there may have been no selection to tolerate and/or resist lungworm infection for many generations in invasion-vanguard lineages of toads (Phillips et al., 2010). That escape from pathogens and parasites might explain some of the distinctive modifications of immune-system function that have evolved in Australian cane toads at the invasion front (Brown, Phillips, Dubey, & Shine, 2015; Llewellyn, Brown, Thompson, & Shine, 2011). If so, the effects of lungworms may be more devastating for toads at the invasion front than is the case in the already-colonised areas in which we studied these interactions. By the same token, virulence of the parasite may have diminished at the invasion front (Kelehear et al., 2012a; Phillips et al., 2010). Thus, exposing naïve invasion-front toads to more virulent parasites from long-established populations may result in even greater declines in host fitness than we document here. In combination with other methods (see Tingley et al., 2017), strategic deployment of *Rhabdias*-infected toads could play a useful role in local population control.

The inflammation and immune reaction elicited by *Rhabdias* in their toad hosts are relatively mild (Brown et al., 2016; Pizzatto et al., 2010; Santos et al., 2016), and presumably shaped by their long co-evolutionary history. Thus, the dramatic effects of removing the parasites are surprising. Adult parasites feeding off capillary networks in the lungs no doubt deplete blood products to a small extent (Barton, 1995), but removing this minor drain on resources alone is unlikely to explain the magnitude of deworming effects. Parasites and pathogens can also produce compounds that affect host metabolism and behaviour (Adamo, 2003; Libersat, Delago, & Gal, 2009). In addition, parasites can stimulate the host to produce chemicals that alter its own metabolism and behaviour in ways that enable it to resist the parasite, or to tolerate its presence (Ashley & Wingfield, 2011; Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Demas & Carlton, 2015; Sears, Rohr, Allen, & Martin, 2011). Conceivably, removal of parasites could quickly eliminate any neuro- or immuno-modulatory compounds that alter host metabolism or behaviour and allow rapid improvement in performance. Documenting a causal pathway such as this would require identifying and quantifying effector chemical produced by both parasite and host.

In summary, the deworming methodology we adopted has great promise for quantifying the effects of nematode parasites on host populations. Although this technique worked remarkably well on Australian cane toads (whose single major parasite is a nematode lungworm), it may also be a useful experimental manipulation for hosts that harbour more diverse parasite communities. More generally, a manipulative approach that includes both field-based and laboratory-based studies holds great promise for extending our understanding of the dynamic interaction between ectothermic hosts and their parasites.

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AUTHORS' CONTRIBUTIONS

P.F., G.B. and R.S. conceived the ideas and designed methodology; P.F. and G.B. collected and analysed the data; P.F., G.B. and R.S. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.fr8n0 (Finnerty, Shine, & Brown, 2017).

ORCID

Patrick B. Finnerty http://orcid.org/0000-0001-5762-6272
Richard Shine http://orcid.org/0000-0001-7529-5657
Gregory P. Brown http://orcid.org/0000-0002-2924-9040

REFERENCES

Adamo, S. A. (2003). Modulating the modulators: Parasites, neuromodulators and host behavioral change. *Brain, Behavior and Evolution*, *60*, 370–377.

Arneberg, P., Folstad, I., & Karter, A. (1996). Gastrointestinal nematodes depress food intake in naturally infected reindeer. *Parasitology*, *112*, 213–219.

Ashley, N. T., & Wingfield, J. C. (2011). Sickness behavior in vertebrates. In G. E. Demas & R. J. Nelson (Eds.), *Ecoimmunology* (pp. 45–91). New York, NY: Oxford University Press.

Baker, M. (1979). The free-living and parasitic development of *Rhabdias* spp. (Nematoda: Rhabdiasidae) in amphibians. *Canadian Journal of Zoology*, *57*, 161–178.

Barber, I., Hoare, D., & Krause, J. (2000). Effects of parasites on fish behaviour: A review and evolutionary perspective. *Reviews in Fish Biology and Fisheries*, *10*, 131–165.

Barton, D. P. (1995) *The cane toad: A new host for helminth parasites in Australia*. PhD Thesis, James Cook University, Townsville, Qld.

Barton, D. P. (1997). Introduced animals and their parasites: The cane toad, *Bufo marinus*, in Australia. *Australian Journal of Ecology*, *22*, 316–324.

Barton, D. P. (1998). Dynamics of natural infections of *Rhabdias* cf. *hylae* (Nematoda) in *Bufo marinus* (Amphibia) in Australia. *Parasitology*, *117*, 505–513.

Blaustein, A. R., & Kiesecker, J. M. (2002). Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecology Letters*, *5*, 597–608.

BOM—Bureau of Meteorology. (2016). *Climate data online*. Retrieved from http://www.bom.gov.au/climate/data/

Bowcock, H., Brown, G. P., & Shine, R. (2008). Sexual communication in cane toads, *Chaunus marinus*: What cues influence the duration of amplexus? *Animal Behaviour*, *75*, 1571–1579.

Brown, G. P., Kelehear, C., Pizzatto, L., & Shine, R. (2016). The impact of lungworm parasites on rates of dispersal of their anuran host, the invasive cane toad. *Biological Invasions*, *18*, 103–114.

Brown, G. P., Phillips, B. L., Dubey, S., & Shine, R. (2015). Invader immunology: Invasion history alters immune system function in cane toads (*Rhinella marina*) in tropical Australia. *Ecology Letters*, *18*, 57–65.

Brown, G. P., & Shine, R. (2016). Frogs in the spotlight: A 16-year survey of native frogs and invasive toads on a floodplain in tropical Australia. *Ecology and Evolution*, *6*, 4445–4457.

Collins, J. P. (2010). Amphibian decline and extinction: What we know and what we need to learn. *Diseases of Aquatic Organisms*, *92*, 93–99.

Craig, B., Jones, O., Pilkington, J., & Pemberton, J. (2009). Re-establishment of nematode infra-community and host survivorship in wild Soay sheep following anthelmintic treatment. *Veterinary Parasitology*, *161*, 47–52.

Crump, M. L., & Pounds, J. A. (1985). Lethal parasitism of an aposematic anuran (*Atelopus varius*) by *Notochaeta bufonivora* (Diptera: Sarcophagidae). *Journal of Parasitology*, *71*, 588–591.

Dang, T. D., Searle, C. L., & Blaustein, A. R. (2017). Virulence variation among strains of the emerging infectious fungus *Batrachochytrium dendrobatidis* (Bd) in multiple amphibian host species. *Diseases of Aquatic Organisms*, *124*, 233–239.

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., & Kelley, K. W. (2008). From inflammation to sickness and depression: When the immune system subjugates the brain. *Nature Reviews Neuroscience*, *9*, 46–56.

Demas, G. E., & Carlton, E. D. (2015). Ecoimmunology for psychoneuroimmunologists: Considering context in neuroendocrine–immune–behavior interactions. *Brain, Behavior and Immunity*, *44*, 9–16.

Dobson, A. P., & Hudson, P. J. (1992). Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse. II. Population models. *Journal of Animal Ecology*, *61*, 487–498.

Dubey, S., & Shine, R. (2008). Origin of the parasites of an invading species, the Australian cane toad (*Bufo marinus*): Are the lungworms Australian or American? *Molecular Ecology*, *17*, 4418–4424.

Ebert, D., Lipsitch, M., & Mangin, K. L. (2000). The effect of parasites on host population density and extinction: Experimental epidemiology with *Daphnia* and six microparasites. *American Naturalist*, *156*, 459–477.

Fenner, A. L., & Bull, C. M. (2008). The impact of nematode parasites on the behaviour of an Australian lizard, the gidgee skink *Egernia stokesii*. *Ecological Research*, *23*, 897–903.

Finnerty, P. F., Shine, R., & Brown, G. P. (2017) Data from: The cost of parasite infection: Removing lungworms improves performance, growth and survival of cane toads. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.fr8n0

Folstad, I., Nilssen, A. C., Halvorsen, O., & Andersen, J. (1991). Parasite avoidance: The cause of post-calving migrations in *Rangifer*? *Canadian Journal of Zoology*, *69*, 2423–2429.

Freeland, W. J. (1986). Populations of cane toad *Bufo marinus* in relation to time since colonization. *Australian Wildlife Research*, *13*, 321–330.

Goater, C. P., Semlitsch, R. D., & Bernasconi, M. V. (1993). Effects of body size and parasite infection on the locomotory performance of juvenile toads, *Bufo bufo*. *Oikos*, *66*, 129–136.

Goater, C. P., & Ward, P. I. (1992). Negative effects of *Rhabdias bufonis* (Nematoda) on the growth and survival of toads (*Bufo bufo*). *Oecologia*, *89*, 161–165.

González-Bernal, E., Brown, G. P., & Shine, R. (2014). Invasive cane toads: Social facilitation depends upon an individual's personality. *PLoS ONE*, *9*, e102880.

Hartigan, A., Fiala, I., Dyková, I., Jirků, M., Okimoto, B., Rose, K., … Šlapeta, J. (2011). A suspected parasite spill-back of two novel *Myxidium* spp. (Myxosporea) causing disease in Australian endemic frogs found in the invasive cane toad. *PLoS ONE*, *6*, e18871.

Hatcher, M. J., Dick, J. T. A., & Dunn, A. M. (2012). Diverse effects of parasites in ecosystems: Linking interdependent processes. *Frontiers in Ecology and the Environment*, *10*, 186–194.

Heise-Pavlov, S. R., Paleologo, K., & Glenny, W. (2014). Effect of *Rhabdias pseudosphaerocephala* on prey consumption of free-ranging cane toads (*Rhinella marina*) during Australian tropical wet seasons. *Journal of Pest Science*, *87*, 89–97.

Holmes, J. C. (1996). Parasites as threats to biodiversity in shrinking ecosystems. *Biodiversity and Conservation*, *5*, 975–983.

Hudson, P. J., Dobson, A. P., & Newborn, D. (1998). Prevention of population cycles by parasite removal. *Science*, *282*, 2256–2258.
Kelehear, C., Brown, G. P., & Shine, R. (2011). Influence of lung parasites on the growth rates of free-ranging and captive adult cane toads. *Oecologia*, *165*, 585–592.
Kelehear, C., Brown, G. P., & Shine, R. (2012a). Rapid evolution of parasite life history traits on an expanding range edge. *Ecology Letters*, *15*, 329–337.
Kelehear, C., Brown, G. P., & Shine, R. (2012b). Size and sex matter: Infection dynamics of an invading parasite (the pentastome *Raillietiella frenatus*) in an invading host (the cane toad *Rhinella marina*). *Parasitology*, *139*, 1596–1604.
Kelehear, C., Webb, J. K., & Shine, R. (2009). *Rhabdias pseudosphaerocephala* infection in *Bufo marinus*: Lung nematodes reduce viability of metamorph cane toads. *Parasitology*, *136*, 919–927.
Koprivnikar, J., Marcogliese, D. J., Rohr, J. R., Orlofske, S. A., Raffel, T. R., & Johnson, P. T. J. (2012). Macroparasite infections of amphibians: What can they tell us? *EcoHealth*, *9*, 342–360.
Libersat, F., Delago, A., & Gal, R. (2009). Manipulation of host behavior by parasitic insects and insect parasites. *Annual Review of Entomology*, *54*, 189–207.
Lind, E. O., & Christensson, D. (2009). Anthelmintic efficacy on *Parascaris equorum* in foals on Swedish studs. *Acta Veterinaria Scandinavica*, *51*, 45.
Llewellyn, D., Brown, G. P., Thompson, M. B., & Shine, R. (2011). Behavioral responses to immune system activation in an anuran (the cane toad, *Bufo marinus*): Field and laboratory studies. *Physiological and Biochemical Zoology*, *84*, 77–86.
Messing, R. H., & Wright, M. G. (2006). Biological control of invasive species: Solution or pollution? *Frontiers in Ecology and the Environment*, *4*, 132–140.
Minchella, D. J., & Scott, M. E. (1991). Parasitism: A cryptic determinant of animal community structure. *Trends in Ecology and Evolution*, *6*, 250–254.
Narayan, E., Christi, K., Morley, C., & Trevenen, P. (2008). Sexual dimorphism in the cane toad *Bufo marinus*: A quantitative comparison of visual inspection methods for sexing individuals. *Herpetological Journal*, *18*, 63–65.
Newey, S., Thirgood, S. J., & Hudson, P. J. (2004). Do parasite burdens in spring influence condition and fecundity of female mountain hares *Lepus timidus*? *Wildlife Biology*, *10*, 171–176.
Phillips, B. L., Brown, G. P., Webb, J. K., & Shine, R. (2006). Invasion and the evolution of speed in toads. *Nature*, *439*, 803.
Phillips, B. L., Kelehear, C., Pizzatto, L., Brown, G. P., Barton, D., & Shine, R. (2010). Parasites and pathogens lag behind their host during periods of host range advance. *Ecology*, *91*, 872–881.
Pizzatto, L., Kelehear, C., Dubey, S., Barton, D., & Shine, R. (2012). Host-parasite relationships during a biologic invasion: 75 years postinvasion, cane toads and sympatric Australian frogs retain separate lungworm faunas. *Journal of Wildlife Diseases*, *48*, 951–961.
Pizzatto, L., Kelehear, C., & Shine, R. (2013). Seasonal dynamics of the lungworm, *Rhabdias pseudosphaerocephala*, in recently colonised cane toad (*Rhinella marina*) populations in tropical Australia. *International Journal for Parasitology*, *43*, 753–761.
Pizzatto, L., Shilton, C. M., & Shine, R. (2010). Infection dynamics of the lungworm *Rhabdias pseudosphaerocephala* in its natural host, the cane toad (*Bufo marinus*), and in novel hosts (native Australian frogs). *Journal of Wildlife Diseases*, *46*, 1152–1164.
Pizzatto, L., & Shine, R. (2011). Ecological impacts of invading species: Do parasites of the cane toad imperil Australian frogs? *Austral Ecology*, *36*, 954–963.
Pizzatto, L., & Shine, R. (2012a). Lungworm infection modifies cardiac response to exercise in cane toads. *Journal of Zoology*, *287*, 150–155.
Pizzatto, L., & Shine, R. (2012b). New methods in the battle against cane toads: When should we move from research to implementation? *Animal Conservation*, *15*, 557–559.
Poulin, R. (2011). *Evolutionary ecology of parasites*. Princeton, NJ: Princeton University Press.
Prenter, J., MacNeil, C., Dick, J. T. A., & Dunn, A. M. (2004). Roles of parasites in animal invasions. *Trends in Ecology and Evolution*, *19*, 385–390.
Rehbein, S., & Visser, M. (2002). Efficacy of ivermectin delivered via a controlled-release capsule against small lungworms (Protostrongylidae) in sheep. *Journal of Veterinary Medicine, Series B*, *49*, 313–316.
Santos, J. N., da Silva, D. C., Feitosa, L. A., Furtado, A. P., Giese, E. G., & de Vasconcelos Melo, F. T. (2016). *Rhinella marina* (Amphibia: Bufonidae) versus *Rhabdias paraensis* (Nematoda: Rhabdiasidae): Expanding the view on a natural infection. *Journal of Parasitology*, *102*, 349–355.
Sears, B. F., Rohr, J. R., Allen, J. E., & Martin, L. B. (2011). The economy of inflammation: When is less more? *Trends in Parasitology*, *27*, 382–387.
Selechnik, D., Rollins, L. A., Brown, G. P., Kelehear, C., & Shine, R. (2017). The things they carried: The pathogenic effects of old and new parasites following the intercontinental invasion of the Australian cane toad (*Rhinella marina*). *International Journal for Parasitology: Parasites and Wildlife*. https://doi.org/10.1016/j.ijppaw.2016.12.001
Shine, R. (2010). The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *Quarterly Review of Biology*, *85*, 253–291.
Simberloff, D., & Gibbons, L. (2004). Now you see them, now you don't! Population crashes of established introduced species. *Biological Invasions*, *6*, 161–172.
Speare, R. (1990). A review of the diseases of the cane toad, *Bufo marinus*, with comments on biological control. *Australian Wildlife Research*, *17*, 387–410.
Stiling, P., & Cornelissen, T. (2005). What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control*, *34*, 236–246.
Tingley, R., Ward-Fear, G., Greenlees, M., Schwarzkop, L., Phillips, B., Brown, G., … Shine, R. (2017). New weapons in the Toad Toolkit: A review of methods to control and mitigate the biodiversity impacts of invasive cane toads (*Rhinella marina*). *Quarterly Review of Biology*, *92*, 123–149.
Tompkins, D., & Begon, M. (1999). Parasites can regulate wildlife populations. *Parasitology Today*, *15*, 311–313.
Urban, M. C., Phillips, B. L., Skelly, D. K., & Shine, R. (2008). A toad more traveled: The heterogeneous invasion dynamics of cane toads in Australia. *American Naturalist*, *171*, E134–E148.
White, G. C., & Burnham, K. P. (1999). Program MARK: Survival estimation from populations of marked animals. *Bird Study*, *46*, S120–S139.
Worsley-Tonks, K. E. L., & Ezenwa, V. O. (2015). Anthelmintic treatment affects behavioural time allocation in a free-ranging ungulate. *Animal Behaviour*, *108*, 47–54.
Wright, C. W. (2001) *Artemisia. Medicinal and aromatic plants – Industrial profiles*. Boca Raton, FL: CRC Press.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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