Rhabdias pseudosphaerocephala infection in *Bufo marinus*: lung nematodes reduce viability of metamorph cane toads

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(*Received 2 February 2009; revised 17 March 2009; accepted 28 March 2009; first published online 15 June 2009*)

SUMMARY

Cane toads (*Bufo marinus*) were introduced to Australia in 1935 and have since spread widely over the continent, generating concern regarding ecological impacts on native predators. Most Australian cane toad populations are infected with lung nematodes *Rhabdias pseudosphaerocephala*, a parasite endemic to New World (native-range) cane toad populations; presumably introduced to Australia with its toad host. Considering the high intensities and prevalence reached by this parasite in Australian toad populations, and public ardour for developing a control plan for the invasive host species, the lack of experimental studies on this host-parasite system is surprising. To investigate the extent to which this lungworm influences cane toad viability, we experimentally infected metamorph toads (the smallest and presumably most vulnerable terrestrial phase of the anuran life cycle) with the helminth. Infected toads exhibited reduced survival and growth rates, impaired locomotor performance (both speed and endurance), and reduced prey intake. In summary, *R. pseudosphaerocephala* can substantially reduce the viability of metamorph cane toads.

Key words: anuran, growth rate, helminth, locomotor performance, mortality, prey intake.

INTRODUCTION

Parasites can cause morbidity and mortality in their hosts through a diverse array of mechanisms (Poulin, 2007). The extent to which parasites exert a negative influence on their hosts remains unclear for many host-parasite systems, particularly those that include ectothermic rather than endothermic hosts. This dearth of information likely reflects a traditional research bias towards economically significant host-parasite systems. Recently, global amphibian declines have triggered a suite of studies investigating the biology of specific amphibian parasites (a chytridiomycete, an oomycete, and an iridovirus) thought to play significant roles in the apparent declines (Blaustein and Kiesecker, 2002). A consequence of this research trend is that sublethal amphibian pathogens which exert more subtle effects upon their hosts are largely disregarded in current research. Such effects may be relevant to conservation, not only where host populations are declining but also in the opposite case, where host populations are increasing so rapidly that they pose an ecological problem – as is the case in Australia with the invasive cane toad, *Bufo marinus*.

Cane toads have been introduced to more than 40 countries worldwide, for the purpose of controlling agricultural pests (Lever, 2001). Native to South and Central America, toads were introduced to Queensland in 1935, and have since spread across much of Australia (Urban *et al.* 2007), poisoning many native predators (Burnett, 1997; Webb *et al.* 2005; Doody *et al.* 2006). Australian *B. marinus* support a diverse parasitic fauna yet studies on this system have primarily focussed on identifying parasites rather than quantifying their effects on the host (Speare, 1990; Barton, 1997). Considering the noxious reputation of the host and public ardour for controlling this invasive species, the lack of research on this topic is surprising.

One notable parasite of Australian cane toads is a lung nematode previously identified as *Rhabdias* cf. *hylae*, a taxon endemic to Australia (Barton, 1997, 1998, 1999). This tentative identification was based on nematode morphology, and supported by the inference that native-range parasites were left behind during the successive translocations (Guyana to Puerto Rico to Hawaii to Queensland) of small numbers of animals prior to their release in Australia (Barton, 1997). However, recent sequence data from mitochondrial and nuclear genes unequivocally identify the parasite in Australian cane toads as *Rhabdias pseudosphaerocephala*, a helminth endemic to toad populations in Central and South America (Dubey and Shine, 2008) but not recorded from either Hawaiian toads or Australian frogs (Barton, 1994, 1997).

Most studies involving *Rhabdias* spp. have been 'new host' reports in reptiles and amphibians. Exceptions include a report that pesticide exposure can reduce the efficacy of the host immune response

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Parasitology (2009), **136**, 919–927.
doi:10.1017/S0031182009006325 Printed in the United Kingdom

to parasitism (Christin *et al*. 2003) and accelerate larval migration and maturation of *Rhabdias ranae* in the leopard frog *Rana pipiens* (Gendron *et al*. 2003); and a report of higher intensities and larger mature worms in male *vs* female wood frogs *Rana sylvatica* infected with *R. ranae* (Dare and Forbes, 2008). Experimental studies examining specific effects of *Rhabdias* spp. on anuran viability primarily have focused on the European toad *Bufo bufo*. *Rhabdias bufonis* reduced rates of feeding, growth and survival in juvenile *B. bufo* (Goater and Ward, 1992), and compromised toad endurance (Goater *et al*. 1993). The host species (*Bufo bufo*) studied by Goater *et al*. (1993) is congeneric with *B. marinus* (but see Frost *et al*. 2006 for suggested generic re-allocation of *B. marinus*), and the parasite studied (*Rhabdias bufonis*) is congeneric with *R. pseudosphaerocephala*. This lung nematode achieves a high prevalence and intensity across a wide range of toad life-history stages and consequently is likely to influence its host's ecology (Barton, 1998).

We conducted laboratory experiments to measure the extent to which the parasite *R. pseudosphaerocephala* exerts negative effects upon its host *B. marinus*. By carrying out our experiments under laboratory conditions, we are able to control for confounding effects of other ailments the toads may suffer in the field. We focused on the metamorph stage of the life cycle, immediately after the toads have transformed from the aquatic (tadpole) stage. Metamorphosis is a vulnerable stage in the amphibian life cycle, when toads may be particularly susceptible to parasitic infection (Rollins-Smith, 1998; Todd, 2007).

MATERIALS AND METHODS

The host-parasite system

Adult *B. marinus* can grow to 230 mm snout-urostyle length [SUL], but metamorphosis occurs as small as 11 mm SUL (Zug and Zug, 1979). Infective *Rhabdias* spp. larvae (L3) invade anurans through the skin or alimentary tract, and migrate to the lungs where they feed directly on host capillaries and mature into protandrous hermaphroditic adults. Eggs released in the lungs pass into the small intestine where they hatch into larvae. After the toad defecates, larvae undergo a free-living sexual stage before producing L3 (Baker, 1979).

Toad breeding and husbandry

To obtain parasite-free metamorphs, we collected 4 adult cane toads from Townsville, Queensland (19°15′S, 146°48′E), and 4 from Fogg Dam, Northern Territory (12°56′S, 131°31′E) in July 2006. The toads were housed in pairs in slanted plastic enclosures (35 × 65 × 88 cm) partially filled with water and kept at 26–32 °C with a 12 h light : 12 h dark cycle (also used for eggs, tadpoles and metamorphs). To stimulate spawning, pairs were given subcutaneous injections of the gonadotropin-releasing hormone (GnRH) agonist: leuprorelin acetate (Lucrin; Abbott Australasia, Kurnell, Australia) diluted at 1 : 20 with amphibian Ringer's solution (0·5 ml for males, 0·75 ml for females), and administered at dusk. GnRH is known to stimulate reproductive activity in amphibians (Propper and Dixon, 1997). Following fertilization, parents were removed from laying enclosures and eggs were transferred to fresh, aged tap water. When tadpoles were 1 week old, we divided the 4 clutches into 24 groups of 100 (keeping clutches separate to allow detection of clutch effects), placed them into 24 plastic tubs (27 × 37 × 59 cm) filled with 40 L of aged tap water, and fed them boiled lettuce *ad libitum*. The first metamorphs for each clutch (Gosner stage 46: Gosner, 1960) emerged 39–59 days after egg deposition and were housed individually in plastic enclosures (55 × 130 × 187 mm) with a hydric gradient. Metamorphs were randomly assigned to the control or treatment group, and fed crickets every third day.

Culturing nematodes

Twenty-seven adult cane toads from Townsville were euthanized and all *R. pseudosphaerocephala* were removed from their lungs. Three gravid female *R. pseudosphaerocephala* were placed into each of 60 sterile Petri dishes (14 × 87 mm) and lacerated to release their eggs, which were then smeared with toad faeces. After 4–7 days at 24 °C, L3 were collected with a glass pipette.

Experimental infections

Thirty L3 were transferred to glass Petri dishes (14 × 55 mm) lined with moist filter paper (8 μm Grade 2: Whatman International, Brentford, Middlesex). Metamorphs from 4 clutches were placed into individual Petri dishes with distilled water ($n = 44$ control) or distilled water plus 30 L3 ($n = 46$ treatment) at 24 °C. After 18 h, toads were removed and the remaining L3 were counted.

Metamorph growth rates

At the time of treatment, 14 (± 1) days post-treatment (p.t.), and at the time of euthanasia (mean ± S.E. = 53 ± 3 days p.t), all surviving metamorphs ($n = 34$ control, $n = 35$ treatment) were weighed to 0·0001 g to calculate daily growth rates.

Locomotor performance of metamorphs

All toads surviving to 14 (± 1) days p.t. were assessed for locomotor performance ($n = 34$ control, $n = 34$ treatment) on a square raceway (4 cm wide, 13 cm

https://doi.org/10.1017/S0031182009006325 Published online by Cambridge University Press

high, 418 cm perimeter) between 10·30 h and 16·00 h at 28·8 °C (range: 27·2–30·8 °C) and 48·5% relative humidity (range: 36–58%). During each trial, one of us (C.K.) tapped the toad on the urostyle with a paintbrush whenever it stopped running; trials concluded when 5 taps failed to induce a response. The fastest 25 cm of the first 50 cm was used as a measure of sprint speed. Total distance travelled and total running times were used as endurance measures. Times were recorded (by J.K.W.) with a stopwatch. Data for 1 control and 7 treatment toads were removed from the final analysis because of a thermal malfunction at the time of testing.

Unforced activity levels

Control ($n=22$) and treatment ($n=21$) metamorphs (selected randomly) were fasted for 2 days, and videotaped within their enclosures for 90 min at 24–88 days p.t. (mean $\pm$ S.E. $=39\pm3$ days p.t.; temperature: 28·0–30·5 °C; relative humidity: 41–58%). The numbers of unforced movements made by each toad in 3 successive 30-min periods were scored. For analysis, the data were averaged to yield mean values over 30-min.

Feeding performance

Control ($n=15$) and treatment ($n=15$) metamorphs (selected randomly) were fasted for 2 days, and placed in individual arenas ($55\times130\times187$ mm). After a 10-min acclimation period 15 small crickets were released in the centre of the arena and the numbers that were eaten in the ensuing 10 min were recorded. The numbers of movements made by the toad were counted and how far it moved was measured using a 1 cm grid on the arena floor. The toad was classified as an ambush forager if it moved $\leqslant5$ cm during a trial, and as an active forager if it moved >5 cm. Trials were carried out 50 (±10) days p.t., between 12·00 and 15·00 h at 28·2–31·6 °C and 35–55% relative humidity. Control and treatment toads were trialled alternately.

Metamorph mortality

All metamorphs were checked daily and any dead toads were dissected to count *R. pseudosphaerocephala*. For each dead toad the number of days it had survived since oviposition, metamorphosis, and treatment was recorded. Four 1 ml samples of water were taken from each toad's enclosure (with a transfer pipette) and examined for larval *R. pseudosphaerocephala* which often exit dying hosts (C.K., unpublished observations).

Post-mortem examination

At the conclusion of our experiments, we euthanized all surviving toads and examined them immediately for parasites. Upon detection, all parasites were removed and placed into separate Petri dishes and the toad's body was flushed with distilled water, left for 24 h, and examined again for any previously undetected larvae that may have since exited the body.

Data analysis

Prior to statistical analysis, we checked that our data met the assumptions of parametric analyses. Where necessary, we transformed data to meet these assumptions. In experiments with 1 or 2 dependent variables, single factor analysis of variance (ANOVA) was used to test for differences between treatments. If more than 2 response variables were measured, multivariate analysis of variance (MANOVA) was used. If the MANOVA was significant, separate ANOVAs were then used to determine which variables differed significantly between treatments. All statistical analyses were performed using JMP 5.0 (SAS, 2002) with alpha set at $<0·05$. Preliminary analyses revealed no significant effect of clutch identity on any response variables and thus, clutch was excluded as a factor from final analyses.

RESULTS

Infection success

L3 penetrated all 46 treatment toads; however, only 19 toads contained *R. pseudosphaerocephala* at the time of death. Of these 19 toads, 11 contained mature worms and the remaining 8 toads contained immature worms only. No other parasites were found in tissues post-mortem. The number of infective *R. pseudosphaerocephala* that penetrated the treatment toads (mean $\pm$ S.E. $=23·37\pm1·12$) was not significantly correlated with the total number of *R. pseudosphaerocephala* remaining in the toad at death (mean $\pm$ S.E. $=7·30\pm3·15$; range $=1$–100 immature worms, 1–4 mature worms; $r^2=0·005$, $n=46$, $P=0·65$), nor with the toad's age at metamorphosis ($r^2=0·036$, $n=46$, $P=0·11$), body mass at treatment ($r^2=0·031$, $n=46$, $P=0·24$), or post-metamorphic age at treatment ($r^2=0·012$, $n=46$, $P=0·47$).

Metamorph growth rates

Control and treatment toads did not differ significantly in initial mass ($F_{1,67}=0·19$, $P=0·67$), but infection reduced daily growth rates of metamorphs over the first 14 days ($F_{1,67}=7·75$, $P=0·007$) and over the entire experiment ($F_{1,67}=5·74$, $P=0·02$; Fig. 1).

Locomotor performance of metamorphs

Control and treatment toads were similar in mean body mass (0·183 and 0·181 g respectively, $F_{1,58}=0·02$, $P=0·88$), but treatment toads exhibited

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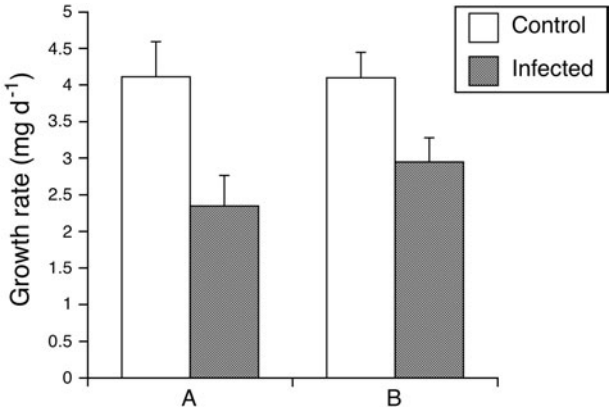

Fig. 1. Effects of the nematode *Rhabdias pseudosphaerocephala* on daily growth rates of metamorph cane toads (A) over the first 2 weeks post-treatment, and (B) from the time of treatment until death. Blank bars represent control toads ($n=34$), grey bars represent treatment toads ($n=35$). Graphs show mean values ± 1 S.E.

reduced locomotor performance (Fig. 2). To examine the relationship between body mass and sprint speed in control *vs* treatment toads, we used ANCOVA with body mass as the covariate, treatment as the factor, and sprint speed as the dependent variable. At the same body mass, control toads ran faster than treatment toads ($F_{1,57}=9{\cdot}30$, $P<0{\cdot}004$; slopes homogeneous, $F_{1,56}=0{\cdot}29$, $P=0{\cdot}59$; Fig. 2). Control toads also travelled further (ANCOVA, $F_{1,56}=15{\cdot}39$, $P=0{\cdot}0002$; Fig. 2), but the slopes of the relationship between body mass and the total distance travelled differed between the two treatments (interaction, $F_{1,56}=4{\cdot}77$, $P=0{\cdot}03$) because the decrement in endurance due to parasitic infection was more pronounced among larger toads. Control toads also sustained activity for longer than did treatment toads ($F_{1,57}=14{\cdot}60$, $P=0{\cdot}003$; slopes homogeneous $F_{1,56}=3{\cdot}41$, $P=0{\cdot}07$; Fig. 2).

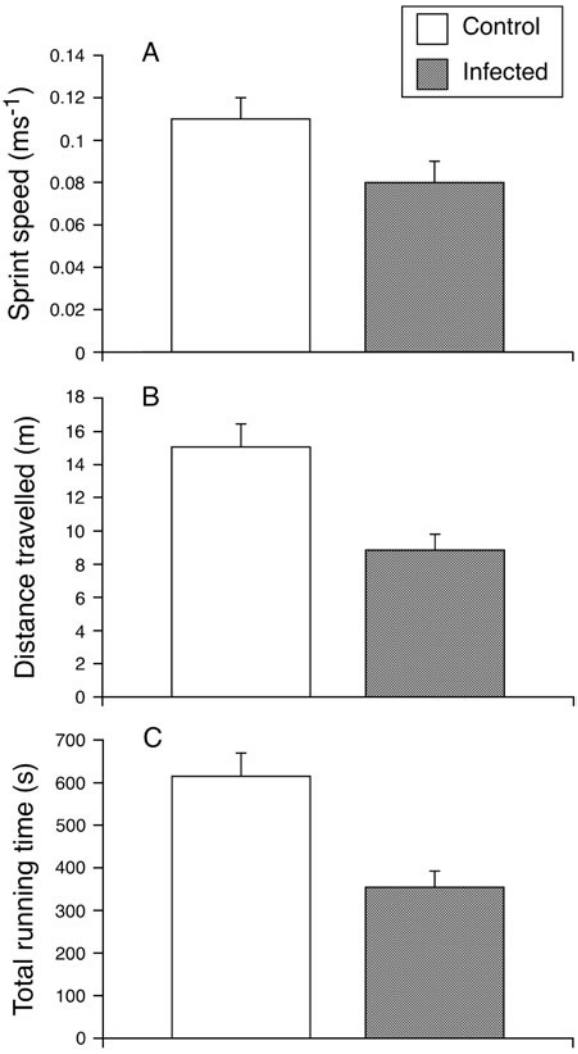

Fig. 2. Effects of the nematode *Rhabdias pseudosphaerocephala* on locomotor performance of metamorph cane toads. (A) Maximum speed over 25 cm; (B) total distance travelled; (C) total running time. Blank bars represent control toads ($n=34$), grey bars represent treatment toads ($n=34$). Graphs show mean values ± 1 S.E.

Unforced activity

Unforced activity levels (number of movements) were similar in control *vs* treatment toads (ANOVA, $F_{1,42}=0{\cdot}50$, $P=0{\cdot}48$; Fig. 3).

Feeding performance

Mean body mass did not differ between control and treatment toads ($F_{1,28}=0{\cdot}50$, $P=0{\cdot}48$). A toad's body mass was not significantly correlated with the number of movements it made during a trial ($r^2=0{\cdot}07$, $n=30$, $P=0{\cdot}17$), nor with the number of crickets that it ate ($r^2=0{\cdot}02$, $n=30$, $P=0{\cdot}42$). However, treatment toads made fewer movements ($F_{1,28}=4{\cdot}24$, $P=0{\cdot}049$) and ate fewer crickets ($F_{1,28}=4{\cdot}94$, $P=0{\cdot}03$; Fig. 4) than did controls. Foraging mode was also influenced by infection (contingency table analysis; Likelihood ratio, $\chi^2=4{\cdot}144$, $n=30$, $P=0{\cdot}04$). About half of the control toads employed active foraging ($n=7$ active, $n=8$ ambush), whereas treatment toads mostly employed ambush foraging ($n=2$ active, $n=13$ ambush).

Metamorph mortality

Helminth infection reduced toad survival (Likelihood ratio, $\chi^2=4{\cdot}14$, $n=90$, $P=0{\cdot}04$; Fig. 5A) with 11·4% of control toads dying *vs* 28·3% of treatment toads. Mean metamorph mass at treatment did not differ between treatments (two-factor ANOVA, $F_{1,86}=0{\cdot}02$, $P=0{\cdot}88$), but metamorphs that died were smaller at the time of treatment than those which lived ($F_{1,86}=41{\cdot}48$, $P<0{\cdot}0001$; interaction, $F_{1,86}=0{\cdot}76$, $P=0{\cdot}38$; Fig. 5B).

Our multiple logistic regression to identify predictors of toad survival excluded 'age at treatment'

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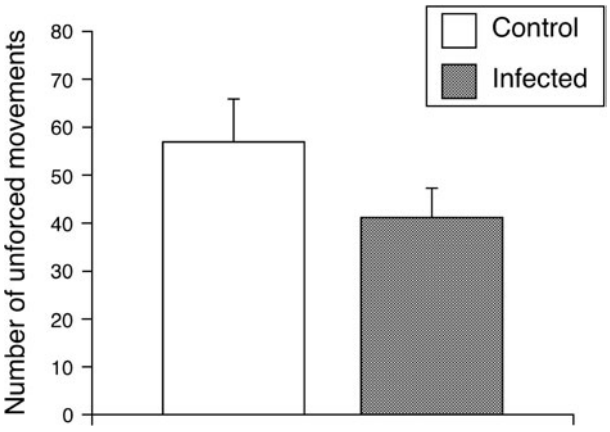

Fig. 3. Effects of the nematode *Rhabdias pseudosphaerocephala* on unforced activity levels (total number of unforced movements made during a 30-min period of metamorph cane toads). Blank bars represent control toads ($n=22$), grey bars represent treatment toads ($n=21$). Graphs show mean values ± 1 S.E.

because it was correlated with metamorph mass ($r^2=0{\cdot}66$, $n=90$, $P<0{\cdot}0001$). The final model was significant ($\chi^2=69{\cdot}33$, $n=90$, $P<0{\cdot}0001$) and showed no evidence of lack of fit ($\chi^2=20{\cdot}71$, $n=90$, $P=1{\cdot}0$). Only treatment ($\chi^2=4{\cdot}10$, $n=90$, $P=0{\cdot}04$) and body mass (likelihood ratio, $\chi^2=10{\cdot}26$, $n=90$, $P<0{\cdot}001$) were significant predictors of toad survival. Thus, both helminth infection and small body mass decreased survivorship.

DISCUSSION

Under laboratory conditions, infection with the helminth *Rhabdias pseudosphaerocephala* reduced survival, growth rates, locomotor capacity, and feeding rates of metamorph cane toads. These effects were (a) strong, with infection producing a 2-fold difference in mean values of several response variables, (b) evident soon after infection (e.g. growth rates fell in <14 days), possibly even before the worms had matured in the lungs (see Goater, 1992; Goater *et al*. 1993) and (c) still apparent 32–95 days post-treatment. In total, these nematodes reduce the viability of metamorph cane toads.

The discussion below focuses on 2 major issues. First, what is the mechanistic basis of viability reduction? By understanding the pathways by which the nematode damages the toad, we may better predict the consequences of nematode infection. Second, what are the likely consequences of these infections in nature? That is, how confidently can we extrapolate these laboratory findings to the field?

First, what are the pathways by which *R. pseudosphaerocephala* reduces toad viability? The processes likely involve direct mechanical damage caused by L3 burrowing through the toad's body en route to the lungs, subsequent damage inflicted on the lung by these large haematophagous nematodes, and/or

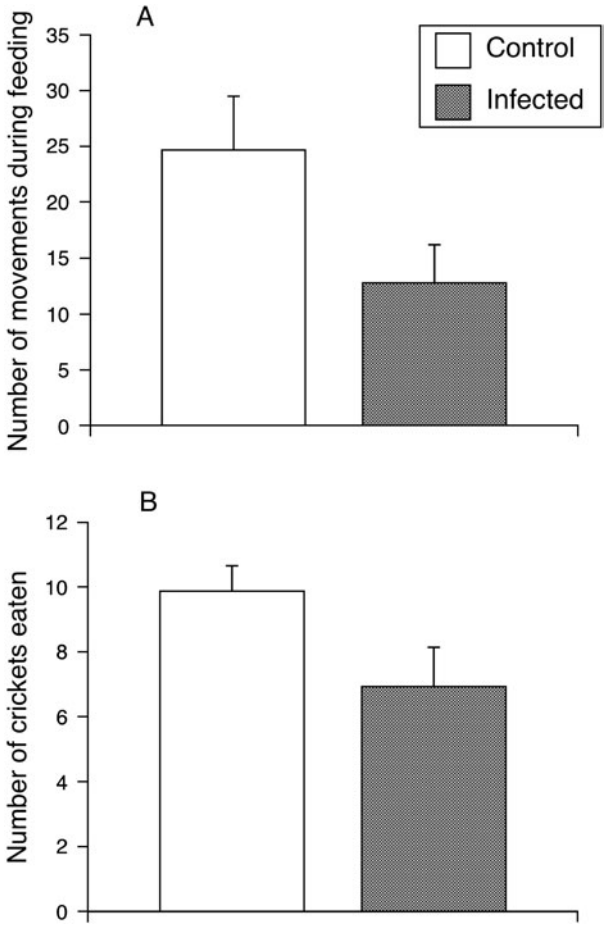

Fig. 4. Effects of the nematode *Rhabdias pseudosphaerocephala* on foraging behaviour and prey intake in metamorph cane toads. (A) Total number of movements made; (B) total number of crickets eaten in a 10-min feeding period. Blank bars represent control toads ($n=15$), grey bars represent treatment toads ($n=15$). Graphs show mean values ± 1 S.E.

increased energy demands associated with feeding the nematodes, executing an immune response, and repairing associated damage.

The decreased growth rates of infected toads may be due to one or more of several processes. Most simply, infected toads ate less and therefore would be expected to grow at a reduced rate (e.g. Goater and Ward, 1992). Considering that infected toads reduced their prey intake, we would expect them to suffer an energy deficit if they did not compensate in some way (Munger and Karasov, 1989). One method of compensation could involve reduced energy expenditure during foraging – this might explain the tendency for infected toads to ambush their prey rather than actively pursue them, but it is difficult to disentangle cause and effect. Infection may have suppressed the toad's appetite (as found by Goater and Ward, 1992 in a similar host-parasite system), or impaired the toad's foraging via reduced locomotor performance or utilizable energy stores. In keeping with this latter hypothesis, locomotor capacity correlates with prey intake in another bufonid

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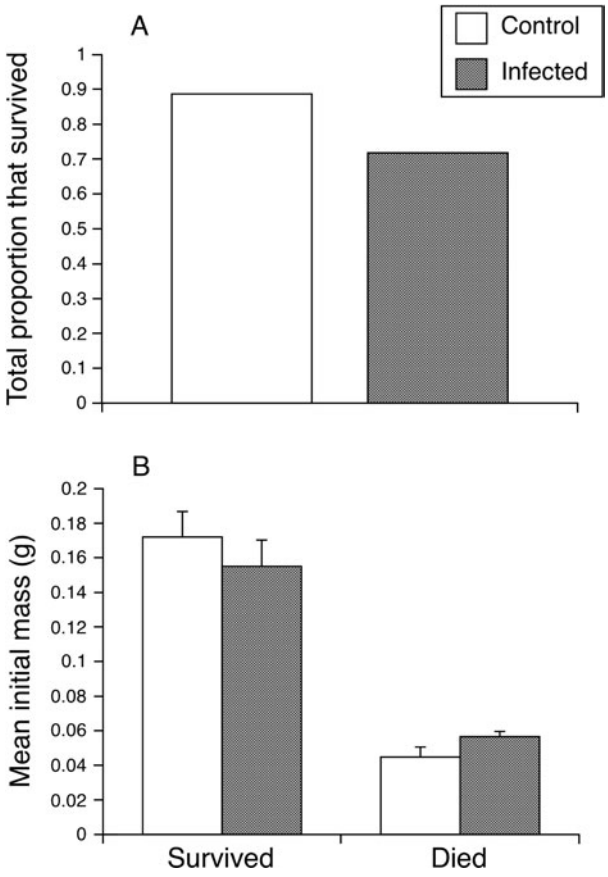

Fig. 5. Effects of the nematode *Rhabdias pseudosphaerocephala* on survival rates of metamorph cane toads. (A) Survival rates of all toads: (B) influence of initial metamorph mass (at time of infection) on subsequent survival. Blank bars represent control toads ($n=44$), grey bars represent treatment toads ($n=46$). Graphs show proportional data (A) and mean values ± 1 S.E. (B).

(frequently hopping *Bufo woodhousei fowleri* that moved long distances captured more prey items than did conspecifics that hopped less and moved shorter distances: Walton, 1988). However, the similarity in levels of unforced activity between infected and uninfected toads argues against this interpretation, and suggests that infection directly suppresses appetite. In order to compensate for reduced prey intake, toads could reduce allocation of nutrients to growth and development or increase utilization of tissue stores (Munger and Karasov, 1989). Alternatively, ongoing costs associated with mounting an immune response, repairing mechanical damage and replacing nutrients lost to the haematophagous parasite may indirectly reduce growth rates. In a congeneric host-parasite system (*Bufo bufo – Rhabdias bufonis*), decrements in growth rates were more pronounced 14 days p.t. in toads exposed to 80 L3 than in toads exposed to 10 L3, signifying dose-dependent damage inflicted by L3. Importantly, decrements in growth may have been realised even before the worms had reached the lungs, implying that mechanical damage and associated costs (repair and immune response) were largely responsible for reduced growth rates (Goater *et al*. 1993), at least in the early stages of infection. In the current study, growth rates were impaired for up to 95 days p.t., implying long-term costs of infection. Further, this decrement in growth rate held true for all treatment toads even though many (58·7%) were found to have lost their infections by the time of dissection, implying that exposure to L3 must induce some lasting effects and that energy lost directly through parasitic consumption is not the most important cause of the observed growth decrements.

Infection also reduced sprint speed and endurance in metamorph *B. marinus*. Haematophagous parasites have been found to reduce sprint speed in certain lizards (*Lacerta vivipara*: Oppliger *et al*. 1996) and endurance in toads (*B. bufo*: Goater *et al*. 1993), and other lizards (*Tiliqua rugosa*: Main and Bull, 2000; *Sceloporus occidentalis*: Schall *et al*. 1982). However, haematophagous parasites did not affect sprint speed in *B. bufo* (Goater *et al*. 1993), *S. occidentalis* (Schall *et al*. 1982), or certain snakes (*Tropidonophis mairii*: Brown *et al*. 2006), perhaps because sprinting relies on anaerobic capacity whereas endurance traditionally relies on aerobic capacity, which may be considerably more impaired by haematophagous and blood parasites (Dunlap and Mathies, 1993; Kumaraguru *et al*. 1995). Why, then, do some parasitized animals (including cane toads in the present study) show decreased sprinting ability as well as endurance? The major pathway likely involves mechanical damage due to migration of L3 and subsequent energy lost to the immune and repair response resulting in a lack of energy available to fuel locomotion. In the present study, negative effects on locomotion were realised at an early stage in infection likely before resources lost to a haematophagous parasite would become significant, indicating exposure to L3 must be responsible for significant damage. Additionally, toads have a low anaerobic capacity (Hutchinson and Miller, 1979; Putnam, 1979) and are thought to rely on aerobic rather than anaerobic metabolism which is traditionally employed to sustain sprinting (Bennett and Licht, 1974). Aerobic capacity may be sensitive to parasitic infections that influence host haematology or respiratory function, such as the nematode in the current study. Unfortunately, we were unable to extract adequate volumes of blood from the small toads (mass $<0{\cdot}7$ g) for haematological processing to quantify parasite-related changes to host haematology. However, large haematophagous *R. pseudosphaerocephala* (spanning $\sim 2/3$ the length of a metamorph lung: C.K., unpublished observations) may plausibly cause significant physical obstruction of the lung lumen, decreasing lung tidal volume, pulmonary diffusing capacity and consequently, aerobic capacity. Hence, the negative effects of helminth infection on locomotor performance may reflect diminished

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aerobic capacity, possibly combined with muscle damage and reduced utilizable energy.

We turn now to the second major issue raised above: the consequences of *R. pseudosphaerocephala* infections in wild toads. We believe that our laboratory results are relevant to field conditions but that the effects may be amplified when hosts are placed under natural stressors. First, the prevalence (24%) and intensity ($\leqslant$4 mature worms) of mature infections induced in our trials are lower than those reported in the field. For example, Barton (1998) recorded mature infections in 73% of juvenile (22–60 mm SUL) field-collected toads, with up to 68 mature worms per toad. Second, the negative effects observed in infected toads were realised under benign laboratory conditions where hosts are subjected to single rather than multiple infections and are protected from additive stresses of resource limitation, competition, predation, dehydration, and thermal extremes. In natural host-parasite systems, the deleterious effects of infection may be amplified by such additive stresses on the host (Candolin and Voigt, 2001; Kiesecker, 2002; Jokela *et al.* 2005; Coors and De Meester, 2008). Thus, infection may suppress the viability of toads even more severely under natural conditions.

The increased mortality rate is our only unambiguous evidence for a fitness decrement; however it is not clear whether this will translate to a population effect, because the death of even a high proportion of metamorphs might have little influence on adult recruitment. Our additional line of evidence for reduced metamorph fitness is more subtle and involves effects on growth rates. In metamorphosing anurans, body size is an important determinant of fitness, with smaller metamorphs having lower survival rates and reproductive success (Smith, 1987; Semlitsch *et al.* 1988; Scott, 1994). Our study suggests that a further cost of small size at metamorphosis is increased risk of death from parasitic infection (since smaller metamorphs had higher mortality rates than larger metamorphs), a result reflected in *Bufo bufo* metamorphs infected with *Rhabdias bufonis* (Goater and Ward, 1992). A more subtle effect lies in the fact that *R. pseudosphaerocephala* reduces toad growth rates, keeping them smaller for longer, thus increasing the duration of their vulnerability to desiccation (Tracy, 1976), predation (Wassersug and Sperry, 1977), cannibalism (Pizzatto and Shine, 2008), and competition (Freeland and Kerin, 1991; Van Beurden, 1980).

Reduced locomotor performance comprises a further disadvantage of small size at metamorphosis: smaller cane toads did not move as fast or as far as larger toads. Importantly, however, *R. pseudosphaerocephala* infection reduced both sprint speed and endurance even after the effects of body size were removed. That is, the locomotor advantage of increased body size typically seen in toad metamorphs (Goater *et al.* 1993; Beck and Congdon, 2000) was not realised in larger cane toad metamorphs infected with *R. pseudosphaerocephala*. Reduced locomotor performance of infected toads could impair their abilities to feed, evade predators, and disperse. Amphibian sprint capacity may influence the outcome of a predation event (Wassersug and Sperry, 1977) both when the toad is a predator, and when it is a prey item – as in a cannibalism event. Cannibalism is common in *B. marinus*, and smaller metamorphs are preferentially cannibalised (Pizzatto and Shine, 2008). Hence parasitized metamorphs may be more vulnerable due to both reduced size and reduced locomotor ability. Future work could usefully explore whether parasites can be transmitted via cannibalism in this system, and whether the deleterious effects that were observed in metamorph toads carry over to adult toads.

In summary, infection with lungworms substantially reduced the viability of metamorph cane toads in the laboratory, and is likely to do so in the field as well. This conclusion is encouraging for the prospects of utilizing this nematode as a biological control for Australian populations of cane toads, although it represents only the first step in such a process.

We thank Mattias Hagman for his expertise in husbandry, Gregory Brown for editorial suggestions, and the Australian Research Council for financial support. All procedures were approved by the University of Sydney Animal Care and Ethics Committee.

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