High infection intensities, but negligible fitness costs, suggest tolerance of gastrointestinal nematodes in a tropical snake

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Abstract

We investigated patterns of prevalence and intensity of gastrointestinal nematode infections in a tropical natricine snake, the keelback (*Tropidonophis mairii*). Ninety-eight percent of keelbacks were infected with *Tanqua anomala* (Gnathostomidae), with infection intensities of up to 243 worms per snake. Infection with *T. anomala* caused severe inflammation of stomach mucosa and submucosa at the sites of parasite attachment and encystment. Nonetheless, we did not detect detrimental effects of nematode infection on measures of fitness among wild or captive snakes. Snakes with heavier nematode infections had higher body condition scores than did less-infected individuals. De-worming captive snakes had no measurable effect on their growth rate, body condition or locomotor performance. In combination with an earlier study on blood-dwelling hepatozoons, our work suggests that keelbacks have a high tolerance to parasites. The 'fast-pace' life history and short lifespan of these snakes may make it beneficial for them to tolerate infection, rather than expend energy on resisting parasite attack.

Key words Australia, fecal flotation, inflammation, life history, resistance, tolerance

Introduction

Parasites and diseases have been identified as significant threats to wild populations of reptiles, and may have played a significant role in global decline of these animals (Gibbons *et al.* 2000). Costs of parasite infection to individual fitness, and flow-on effects to populations, have been quantified in invertebrates, amphibians, fish, birds and mammals (Barber *et al.* 2000; Poulin 2011; Schmid-Hempel 2011; Stearns and Koella 2007). There are several cross-sectional studies on the diversity and prevalence of parasites in Australian reptiles (Goldberg and Bursey 2012; Johnston and Mawson 1948; Jones 1980; Jones 2014; Mackerras 1961; Pichelin *et al.* 1999; Riley *et al.* 1985; Vilcins *et al.* 2009), and information is emerging on the effects of these infections on the individuals bearing them (Bouma *et al.* 2007; Brown *et al.* 2006; Bull and Burzacott 2006; Caudell *et al.* 2002; Fenner and Bull 2008; Madsen *et al.* 2005; Main and Bull 2000; Salkeld *et al.* 2008). The majority of these latter studies assess the effects of either ectoparasites or haemoparasites on their reptilian hosts, presumably because surveys for these types of parasites are minimally invasive. Studies that assess the impacts of helminth parasites on individual hosts are rarer, because it is more difficult to enumerate these parasites from living animals. Although parasite communities in most reptiles and amphibians are depauperate relative to those in birds and mammals (Aho 1990), individual hosts may exhibit heavy levels of infection (Brooks *et al.* 1990; Brown *et al.* 2006; Santoro *et al.* 2013; Self and Kuntz 1967).

By definition, parasites and pathogens induce costs in their hosts. In some cases, where infection results in morbidity or mortality, the costs to individuals and populations can be dramatic (e.g. chytridiomycosis (Berger *et al.* 1998)). Commonly however, natural parasite infections tend to be relatively benign, as a result of long co-evolutionary history between host and parasite; in such cases, negative impacts on any aspect of host fitness may be difficult to detect (Brown *et al.* 1994; Brown *et al.* 2006; Bull and Burzacott 2006; Caudell *et*

al. 2002). Parasite infections in wildlife are likely to remain subclinical until the individuals experience some additional stress (e.g. resource limitation, exposure to contaminants or novel pathogens, habitat degradation, climate change) (Galois *et al.* 2007; Gibbons *et al.* 2000; Schumacher 2006). Nonetheless, even subclinical pathological effects can reduce host fitness (Barber *et al.* 2000; Gibbons and Keymer 1991; Gunn and Irvine 2003; Poulin 2011).

Parasites can inflict diverse costs on their hosts (Barber *et al.* 2000; Hudson and Dobson 1995). Some of these costs result in reduced energy stores, from the parasite usurping the host's food or nutrients, and/or the energetic expenditure of mounting a sustained immune reaction (Sears *et al.* 2011). A heavy parasite burden also might decrease the host's locomotor performance (Barber *et al.* 2000) as a result of energetic, physiological or pathological effects, or even from the physical burden of carrying a large mass of foreign tissue. The mass and volume of a parasite infection might be especially costly for a limbless organism (like a snake) that moves by applying pressure against the substrate with its entire body, not just its limbs. By analogy, large food items or eggs within the abdomen of female snakes can severely decrease locomotor performance (Shine 1988). A large mass of helminths may do likewise. Decreased locomotor performance is likely to result in decreased host fitness, by compromising the host's ability to forage or escape from predators (Schwarzkopf and Shine 1992).

Here we combine correlational and experimental studies to assess the effects of gastrointestinal nematodes in a tropical snake, the keelback (*Tropidonophis mairii)*. Incidental dissections of dead keelbacks suggested they often bore heavy helminth infections and thus offered an opportunity to assess the effects of parasites under a wide range of infection intensities. Among reptiles, semi-aquatic snakes like keelbacks often bear especially high parasite burdens (Fantham and Porter 1954), possibly due to their diet, high population density or habitat conditions conducive to parasite transmission. Our goals in this study were to (1) document patterns of gastrointestinal nematode infections in keelbacks and their anuran

prey, (2) characterize populations of the nematodes infecting the snakes to elucidate factors affecting sex ratio and sexual size dimorphism of the parasite, and (3) experimentally manipulate nematode infections in captive keelbacks to assess the parasite's effect on host fitness.

Material and Methods

Correlational study

Study site and species

The study took place in the vicinity of Middle Point (12.59°S, 131.31°W) in Australia's Northern Territory. The region experiences a wet-dry tropical climate with a dry season (May - October) with almost no precipitation and a wet season (November - April) with an average accumulation of 1500 mm rain. Average maximum air temperature exceeds 31°C during all months of the year (Shine and Madsen 1996).

We assessed patterns of parasite infection in keelbacks (*Tropidonophis mairii;* S1*)*, a medium-sized natricine colubrid snake that feeds almost entirely on metamorphosed anurans (Shine 1991) and is distributed across coastal areas of northern Australia (Wilson and Swan 2013). Beginning in 2004, we collected the bodies of intact keelbacks that had been killed on roads within a 10-km radius of Middle Point. We measured body mass and snout to vent length (SVL) of each snake and determined sex by inspecting gonads. We identified prey items in the stomach and counted any oviductal eggs in female snakes. Parasites were recovered through methodical examination of the stomach and the intestine. We removed all nematodes from the digestive tract, recording their number and location, and stored them in 70% ethanol for further examination. We also measured the total weight of all nematodes in each snake, and for a subsample of snakes, we separated and weighed nematodes by sex (based on the presence of caudal alae in males: (Dewi *et al.* 2008b)).

The stomachs of two freshly run-over keelbacks were excised (S2), leaving the nematodes *in situ*, and fixed in 10% formalin. These were later sectioned and stained for histological examination to document pathological changes associated with infection. We also assessed the numbers of infective nematode larvae in frogs, the main prey item of keelbacks. Twenty-four frogs representing six species (2 *Crinia bilingua*, 1 *Limnodynastes convexiusculus*, 2 *Litoria dahlii*, 10 *L. inermis*, 7 *L. nasuta*, and 2 *L. tornieri*) were collected near Middle Point. We euthanised the frogs in a bath of tricaine methansulfonate then measured (snout-urostyle length, SUL) and weighed them and recorded the number of nematode larvae encysted on the viscera of each one.

Statistical analysis

We used a combination of correlations and linear, multiple and logistic regressions to examine relationships between parasite burden and snake body measurements, reproductive condition, sex and season. As an index of snake body condition, we used residuals from a regression of ln-transformed body mass (subtracting nematode weight) on ln-transformed snout-vent length (SVL). To correct for overdispersion and non-normal distribution of nematode numbers we used negative binomial generalized linear models GLM with a log link. We also used logistic and negative binomial GLMs to relate the presence and number of encysted nematode larvae to the body size (SUL) of frogs. All statistical analyses were performed using R (www.R-project.org) and significance was accepted at $p < 0.05$.

Experimental study

Forty adult keelbacks were collected from the wild near Middle Point (the same site where we sampled frogs (see above)) during May-June 2013 and maintained in captivity for 80 days. Snakes were sexed, weighed and measured for SVL and then individually marked by

148 scale clipping. Snakes were housed in pairs in 33 x 21 x 12 cm plastic cages lined with
149 newspaper and containing a water bowl and a plastic hide box.
150
151 *Treatment groups*
152 The growth experiment on captive keelbacks consisted of an initial 30-day growth
153 trial, a 10-day deworming period, a second 30-day growth trial and finally, a 10-day fasting
154 period. During the two 30-day growth trials, snakes were fed every five days and re-measured
155 every 10 days (see below). During the first 30-day trial, 34 snakes retained their natural
156 nematode infections. In an attempt to artificially increase the existing nematode infections in a
157 second group of six snakes, we harvested living nematodes from the stomachs of fresh road-
158 killed keelbacks, rinsed the nematodes in water and used a feeding syringe to orally
159 administer 4 to 32 worms (mean = 22.5) to the six snakes in 3 ml of water. The water and
160 worms were gently squirted down the snake's oesophagus and palpated towards the stomach.
161 Prior to the second phase of the growth experiment, we dewormed 25 of the snakes (6
162 that had had their nematode burdens increased and 19 of the 34 snakes that had retained
163 natural infections) over a six-day period using a combination of fenbendazole (Panacur 100,
164 Intervet Australia) and ivermectin (Ivomec, Merial Australia). Fenbendazole was
165 administered orally (0.05 mg/g for 6 consecutive days) and ivermectin was injected
166 intramuscularly (0.002 mg/g on day 1 and 6). After the six-day deworming period, we
167 monitored the snakes for adverse effects for a further four days and then initiated the second
168 30-day growth trial using the same protocol and regime as for the first trial. At the end of the
169 second 30-day growth trial, we maintained 34 snakes (22 dewormed, 12 with natural
170 infections) in captivity for a further 10 days without feeding them. At the end of the 10-day
171 fasting period, snakes were remeasured and released back at their capture site.
172 Because natural prey of keelbacks (frogs) are inherently variable in nutrition
173 (depending on species, size, sex, season etc.) and because of the logistical and ethical

difficulties in procuring a large number of frogs for the purposes of feeding captive snakes, we decided to maintain the snakes on a homogenized artificial diet. Tinned cat food (Mars Petcare, Australia) was supplemented with vitamin and mineral powder, and administered to the snakes using a syringe fitted with a metal feeding tube (Wright and Whitaker 2001). Every five days the snakes were fed a known mass of food in this manner and every 10 days, snakes were re-weighed and measured. To assess levels of nematode infection throughout the growth study we collected fecal samples three days after each snake had been fed. Weighed fecal samples were inspected for nematode eggs using a standardized concentration-flotation protocol (Dryden *et al.* 2005).

Locomotor trials

We measured swimming speed of each snake on four occasions; twice between day 0 and 30 and twice between day 30 and 60. Swimming trials were conducted in the afternoon between 1400 and 1700 h in a circular pool 3 m in diameter filled with water to a depth of 20 cm. We divided the circumference of the pool into quarters and used a stopwatch to record the time taken for the snake to swim each quarter for two laps. We compared both the fastest time to swim 1/4 lap and the average time per 1/4 lap among treatment groups.

Statistical analysis

We used a linear mixed model to assess the effect of treatment on mass change and body condition of the captive keelbacks. Snakes were classified into three groups: (i) natural infections throughout (N = 15), (ii) natural infection followed by deworming (N = 19), and (iii) nematodes added followed by deworming (N = 6). Because mass was recorded every 10 days, each snake contributed three data points during each of the two 30-day growth periods.

Thus, we included snake-ID as a random effect in a model that included growth period, treatment group, initial SVL and the mass of food eaten as independent variables.

We also used a linear mixed model approach to examine the effect of treatment on mean swimming speed of the keelbacks, with snake ID as a random effect. Initially, we assessed whether SVL or body condition affected swimming speed. Initial SVL was not related to swimming speed ($F_{1, 37} = 2.53$, $p = 0.120$), but snakes in better body condition swam faster ($F_{1, 38} = 6.86$, $p = 0.013$). Thus, we included body condition as covariate in the model.

Results

Correlational study

Snakes

The 93 road-killed keelbacks that were dissected to identify and enumerate parasites averaged 551 mm SVL (range 200-756mm) and 88.6 g (range 5.5-206.5g). Female keelbacks were larger than males ($F_{1, 90} = 34.9$, $p < 0.001$), but body condition did not differ between the sexes ($F_{1, 90} = 0.39$, $p = 0.53$). Thirty-four of the 93 snakes contained a total of 41 identifiable prey items (39 anurans, 2 fish) and 13 female keelbacks contained shelled eggs in their oviducts.

Nematodes

Ninety-one of the 93 keelbacks (97.9%) were infected with a total of 3162 gastrointestinal nematodes, all found in the stomach rather than the intestine. Based on their location, and the morphology of rostral bulb and caudal alae, the vast majority (>95%) of nematodes were identified as *Tanqua anomala* (Gnathostomatidae), common in natricine snakes from the Middle East, South Asia and Australasia (Al-Moussawi 2010; Dewi *et al.* 2008a; b; Farooq and Khanum 1982; Goldberg and Bursey 2011; Naidu 1978; Rao *et al.*

1977). The only other nematode species found, at low frequency (<5%), was *Abbreviata sp.* (Physalopteridae).

The number of nematodes per host ranged from 0 to 243 (mean = 35, 95% CI = 29 - 46, Fig. 1). Within each infected snake, the total mass of the nematode burden ranged from 0.002 - 3.02% of snake body mass (mean ± SE = 0.53 ± 0.05%). The 3139 individual nematodes varied a great deal in body size, ranging from 0.0005 to 0.042g (mean 0.0143 ± 0.0008). The sex of the nematodes infecting a subsample of 63 snakes was determined based on the presence of caudal alae in males (Dewi *et al.* 2008b). Overall, the sex ratio of the 1859 worms removed from these snakes did not differ from 1:1 (940 males: 919 females; $\chi^2 = 0.22$, $p = 0.64$). From a weighed sample of 382 nematodes from 16 snakes, female *T. anomala* were heavier than males (0.02 g vs 0.013 g; $t = 2.31$, $df = 27$, $p = 0.029$).

Patterns in the intensity of nematode infection

Because nematode infection was nearly ubiquitous among the 93 snakes, we could not examine factors affecting prevalence. Instead, we focused analyses on patterns of infection intensity. Larger snakes had heavier nematode infections than smaller snakes, both in terms of the number of worms ($\chi^2_{1, 90} = 7.90$, $p = 0.005$, Fig. 2a) and the total mass of worms ($\chi^2_{1, 87} = 5.03$, $p = 0.025$). Larger snakes also contained larger (heavier) individual nematodes ($F_{1, 87} = 7.61$, $t = 2.76$, $p = 0.007$, Fig. 2b). After correcting for snake body size, there was no difference in the intensity of nematode infections between male and female snakes (ANCOVA $\chi^2_{1, 89} = 0.003$, $p = 0.958$). Keelbacks were in better body condition during the wet season ($\chi^2_{1, 82} = 21.14$, $p < 0.001$). Snakes in better body condition contained significantly more nematodes ($\chi^2_{1, 90} = 7.49$, $p = 0.006$). However, a significant interaction term indicated that the relation between nematode burden and body condition differed between wet and dry seasons ($\chi^2_{1, 80} = 5.67$, $p < 0.017$). During the wet season nematode burden was positively

related to body condition but during the dry season nematode burden was independent of body condition (Fig. 2c). Multiple regression analysis revealed that after correcting for maternal body size ($F_{1, 9}$ = 1.80, p = 0.21) and body condition ($F_{1, 9}$ = 3.45, p=0.096), litter size of keelbacks was unaffected by nematode infection intensity ($F_{1, 9}$ = 3.57, p=0.091) though the trend was a positive relationship.

Pathology

The two stomachs examined histologically came from snakes with moderate *Tanqua* infections (N = 7 and 47 worms). Both stomachs exhibited extensive areas of submucosal inflammation centred around crescent-shaped granulomas, sometimes with a discernible serrated pattern (i.e., the imprint of the distinctive rostral bulb of *Tanqua*) and around encysted nematodes. Many of these granulomas, surrounding the previous site of the nematode holdfast, contained abundant bacteria, primarily colonies of small gram-negative rods. There was no indication of haemorrhage or ulceration of the stomach wall. The only ingesta observed in the nematodes consisted of proteinaceous fluid and inflammatory cells (seen in the guts of encysted worms) and clumps of bacteria (seen in the guts of intraluminal worms).

Frogs

We found encysted or free nematodes in 41.7 % (n = 10) of the 24 frogs examined, but could not assign them to families. Mean abundance of nematodes in frogs was 2.8 ± 1.3 (range, 0 - 30). Larger frogs were more likely to contain nematodes than were smaller frogs ($\chi^2_{1, 22}$ = 6.86, p = 0.008) and also tended to contain more nematodes ($\chi^2_{1, 22}$ = 3.35, p = 0.067, Fig. 3). Larval nematodes were not identified further and may not all represent *T. anomala*, although frogs are the normal route of transmission of *T. anomala* to frog-eating snakes (Goldberg and Bursey 2011; Nama 1974).

Experimental study

The average size of the 40 snakes in the growth study was 49.8 cm SVL (SE = 9.6) and 56.2 g (SE = 3.0). Over the 60-day study, the snakes were fed an average of 66.6 g (SE = 0.87) of cat food. This feeding regime approximated a maintenance diet for this sample of snakes. Eighteen of the 40 snakes increased and 22 decreased slightly in mass; average mass change over the 60 days was -2.2 g (SE = 1.1). In the wild, keelbacks in this size range would normally gain approximately 9 g in 60 days (unpublished data, 2013). Thus the experimental setting (captivity with food limitation) was one under which we might expect to see effects on the host that might not be evident under natural conditions (Mader 2005).

During the first 30-day period we detected nematode eggs in the feces of all 40 snakes used in the growth study. Egg counts ranged from 12 to 1723 per snake (47.5 - 3780.3 eggs/g feces) among the 34 individuals with unmanipulated nematode infections. Repeatability of egg counts per gram of feces for individual snakes was low (0.27), indicating substantial variability in egg counts from different samples collected from the same individual.

For the 15 snakes whose nematode infections were unmanipulated throughout the growth experiment, average egg counts did not differ between the first and second 30-day period (means of 90.7 vs 85.8 eggs/g feces, paired t-test $t = 0.20$, $df = 56.6$, $p = 0.84$). Among the 25 snakes that were de-wormed, average egg counts decreased dramatically between the first period and the second (198.2 vs 0.13 eggs/g feces, paired t-test $t = 7.25$, $df = 166.0$, $p < 0.001$). We sometimes observed dead nematodes in the feces of snakes that had undergone de-worming, which, in combination with the virtual disappearance of eggs from feces, indicates that anthelmintic treatment was successful. Our attempts to increase nematode burden were less successful, as the snakes to which we fed live worms did not have significantly more eggs in their feces than did snakes with natural nematode burdens (231.6 vs 134.1 eggs/ g

feces, t = 1.62, df = 71.9, p = 0.11). We were unable to obtain fecal samples from these snakes prior to infection and thus cannot assess whether within-individual egg counts increased following transfer of adult *T. anomala*.

Growth in mass of keelbacks over 60 days was negatively related to their initial size and positively related to the amount of food eaten (Table 1). However, growth was not affected by treatment group, time period, or the interaction between treatment group and time (Table 1, Fig. 4a). Body condition of snakes was higher during the first 30-day period (prior to de-worming) and increased with the amount of food eaten, but was unaffected by treatment group and unaffected by the interaction between period and treatment (Table 1).

Mean and minimum swimming speed were highly correlated ($r = 0.79$, $t = 11.42$, $df = 76$, $p < 0.001$). Hence, we only present the results for mean speed. During the first 30 days, mean swimming speed did not differ between snakes with natural and increased nematode infections ($F_{1, 37} = 1.62$, $p = 0.212$), but snakes in better condition swam faster ($F_{1, 38} = 5.72$, $p = 0.022$). There was also no significant difference in swimming speed between de-wormed and not de-wormed snakes during the second 30 days (after de-worming) ($F_{1, 36} = 0.29$, $p = 0.593$), nor for treatment group, time period or the interaction between group and time ($F_{1, 36} < 0.40$, $p > 0.60$) when combining both 30-day periods (Table 2, Fig. 4b).

During the 10-day fasting period that concluded the experiment, snakes lost an average of 7.8 % (±0.8) of their initial body mass. There was no significant relationship between amount of weight loss and nematode burden (scored as the egg count taken from the last fecal flotation during the growth trial) (Spearman $r=0.23$, $p=0.19$, Fig. 5).

Discussion

Infection with *T. anomala* was almost ubiquitous among our sample of road-killed keelbacks. Among the most heavily infected snakes, worm burden exceeded 2% of host body

mass. However, despite their high relative biomass and the severe gastric inflammation they cause, removing worms had no measurable effect on the host's weight change, body condition or swimming performance.

Characteristics of the infrapopulations of *T. anomola* infecting keelbacks suggest that high levels of infection may be the common condition. The sex ratio and degree of sexual size dimorphism (SSD) observed in a population of parasites can provide insight into the selective pressures acting on them. Female-biased sex ratios should be favoured at low parasite densities, either as a means of avoiding inbreeding or as a means to increase the probability of females mating when parasite abundance is low (Poulin 1997b). Furthermore, when parasite sex ratios are less female-biased, the size of males relative to females is expected to increase if physical competition among males is advantageous (Poulin 1997a). The sex ratio of *T. anomola* in keelbacks was 50:50, suggesting that inbreeding avoidance and low mating opportunities have not been strong selective forces acting on populations of this parasite. Although there was an equal sex ratio of *T. anomola*, female-biased SSD was present. However, the fact that females remain larger than males does not indicate an absence of inter-male competition. Male-male competition may affect the degree rather than the direction of SSD (Shine 1994). Thus, we would expect the relative difference in size between males and females to be even greater under a scenario of low parasite density.

The finding that larger snakes contained more and larger parasites is commonplace among reptiles and other taxa (Jones 2014; Shine *et al.* 1998). This pattern likely represents larger individuals having been exposed to (and thus, accumulating) more parasites through greater age or more feeding events. The additional observation that larger keelbacks contain larger parasites suggests that the nematodes are retained for long periods and are able to grow to larger size within a larger/older host. The positive correlation between body condition and infection intensity observed during the wet season, when amphibian prey are most abundant, is more surprising. *Tanqua* are trophically acquired parasites, with frogs acting as paratenic

hosts. Given the high incidence of adult and encysted nematodes found in frogs (42%), a successfully foraging keelback could quickly procure numerous prey and energy, but also a substantial attendant parasite exposure.

We found that the stomach walls of keelbacks exhibited severe inflammation and bacterial infection around the sites of *Tanqua* attachment, similar to findings in other taxa (Gibbons and Keymer 1991; Naidu 1978; Pflugfelder 1948). Based on the sparse gut contents of the worms, they appear to feed, at least in part, on host tissue in the form of inflammatory cells and exudate (Jones 1994; Pflugfelder 1948). No red blood cells were observed in the worms' guts and no haemorrhage was associated with attachment sites, indicating that the worms did not feed on blood. Similar severe inflammatory reactions to *Tanqua sp.* have been described in snakes and varanid lizards (Gibbons and Keymer 1991; Naidu 1978; Pflugfelder 1948), although not all gastric nematodes invoke a host inflammatory response. Encysted physalopterid larvae, for example, only appear to become infiltrated with immune cells after the larva within has died (Jones 1995).

Despite the localized inflammation of the stomach wall caused by *T. anomala*, the effect of their presence on the host appears modest. Among wild snakes, individuals with more worms exhibited better body condition than did individuals with fewer worms. Among reproducing female keelbacks, the effect of nematode burden on litter size was nonsignificant, and indeed the direction of the relationship was positive. Removal of *T. anomala* from infected keelbacks may have benefits that are more subtle or require longer than 30 days to be detected. Manual removal of *Tanqua* from the stomachs of anorexic monitor lizards resulted in improved appetite (Jacobson 2010). Our attempt to experimentally increase nematode burdens, by orally transferring adult *T. anomala* from a freshly-killed host to a live one, may not have been effective. Experimental infections are a mainstay of parasitology research and typically involve exposing host to the infective stage of the parasite under investigation. Rather than harvesting infective stages out of the snakes anuran prey, we opted to directly

transfer adult worms (because we had a source of adult worms at hand) through collecting road killed snakes. Although experimental transfer of adult nematodes is possible in reptiles (Langford *et al.* 2013), we cannot be confident that our attempt was successful.

Because parasites and their hosts often share long evolutionary histories, negative impacts of the association may not always be extensive. In some instances it may be of greater benefit to a parasite to cause as little damage to its host as possible (Poulin 2011). Thus, host responses to parasites may depend on circumstances, and can be divided into either resistance or tolerance strategies. Resistance involves the host attempting to limit the growth and reproduction of the pathogen. In contrast, a strategy of tolerance involves the host allowing the parasite to develop, rather than investing energy into mounting an immune response to repel the parasite. The level of tolerance to a parasite infection can be measured as the slope of the negative relationship between host fitness and infection intensity, with a steeper negative slope indicating lower tolerance (Raberg *et al.* 2009). If we consider the proportional mass loss during a 10-day fast as a measure of host fitness, and use the concentration of nematode eggs in feces as a proxy for infection intensity, then Figure 7 can be interpreted as a representation of keelback tolerance to *T. anomala*. Although the slope is positive, statistically it is not significantly greater than zero. Using either of these measures, keelbacks have a high tolerance to *T. anomala*. The positive correlation between body condition and nematode intensity observed among roadkilled snakes also suggests that the parasites are tolerated rather than resisted. The high prevalence of *T. anomala* infection observed among keelbacks also is consistent with a high degree of host tolerance (Miller *et al.* 2006). Although the high level of inflammation observed in the gastric tissue of infected keelbacks does not appear consistent with a strategy of tolerance (Sears *et al.* 2011), tolerance and resistance represent ends of a continuum, not exclusive categories. Thus a strategy of tolerance does not imply a total lack of inflammatory response, only a less severe response than exhibited by a resistant individual. We might predict that the level of gastric

inflammation associated with *Tanqua* infection would be much greater among snake species more likely to be resistant to nematodes (i.e., species with lower prevalence and intensity of infection). A comparative study across taxa on levels of inflammation induced by gastric nematodes could be informative.

A high prevalence and intensity of gastric nematode infections is not seen in other species of frog-eating snakes at our study site. For example, death adders (*Acanthophis praelongus*) and slatey-grey snakes (*Stegonotus cucullatus*) both have diets composed of approximately 50% frogs, and can become infected with *T.anomala* as well as other nematodes (including *Abbreviata sp, Kalicephalus sp.* and other Ascaridae). Nonetheless, prevalence of nematode infections in both species is less than 33% and maximum intensity is less than 13 worms (unpublished data, 2013). Why do these snakes remain relatively nematode-free when keelbacks do not? One explanation may be that *T. anomala* is simply better adapted to infect keelbacks than to infect other species of snake. However *T. anomala* has a wide geographic range and is capable of infecting a variety of hosts, being common in snake families as diverse as Colubridae and Achrochordidae (Al-Moussawi 2010; Dewi *et al.* 2008a; Farooq and Khanum 1982; Nama 1974). An alternative (or additional) explanation may relate to the different life history phenotypes exhibited by the different species. Keelbacks are fast-maturing and short-lived compared to other members of the local snake assemblage (Brown and Shine 2002; Brown *et al.* 2002). With such a short life expectancy, investing into self-maintenance and resistance strategies against chronic infections may be a less successful strategy than would be the case for a species with a longer life expectancy (Madsen *et al.* 2005; Sears *et al.* 2011). Consistent with that interpretation, keelbacks also exhibit high prevalence and intensity of infection with hepatozoon blood parasites, and similarly show no sign of detrimental effect of infection (Brown *et al.* 2006). Thus, these short-lived snakes may use a strategy of tolerance to multiple pathogens.

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