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Seasonal differences in parasite load in a short-lived lizard

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ABSTRACT

Parasite load can vary with seasonality, but this is rarely quantified. The garden skink (*Lampropholis guichenoti*) is host to multiple species of endoparasite. To measure seasonal effects of parasite transmission we established three captive groups of wild-caught individuals in which 2 of 16 individuals (12.5%) were initially infected with nematodes. We collected three faecal samples from each lizard, a sample at the beginning and at the end of the non-activity season and at the end of the following activity season. We measured parasite load (ascarid group) by counting parasite eggs per gram of faeces using a microscope. We found that parasite load was significantly higher in the activity season than in the non-activity season. The prevalence of parasites increased from 15.9% in the non-activity season to 72.5% in the activity season. The activity season is characterised by greater host activity and warmer ambient temperatures, which promote parasite egg survival in the environment as well as egg development. Taken together, this facilitates parasite transmission and could ultimately explain the higher parasite load during the activity season.

Keywords: Ascaridae, endoparasite, *Lampropholis*, life history, nematode, parasitism, reptile, roundworm.

Introduction

Individual differences in physiology and behaviour can lead to variation in parasite infection within host populations. One physiological difference is immune response (Huyghe *et al.* 2010; Cousineau and Alizon 2014), which is an effective defence against pathogens and parasites. However, when environmental conditions are challenging (e.g. limited resources and high predation), host individuals may prioritise growth and survival over immunity (Cotter *et al.* 2011; Korfel *et al.* 2015; Tieleman 2018), which can lead to seasonal variation in parasite infection. Furthermore, behavioural differences among host individuals can result in divergent risks of becoming infected with parasites as well as then transmitting them further, once infected. For example, individuals that engage in frequent social interactions or that interact with more individuals tend to have higher parasite loads (Godfrey *et al.* 2009, 2010).

Social contact rates, or space use patterns can differ between seasons (Leu *et al.* 2010; Spiegel *et al.* 2015), which may result in seasonal transmission patterns for directly, as well as indirectly, transmitted parasites. For instance, how frequently host individuals encounter infective stages of an indirectly transmitted parasite that has been deposited in the environment (e.g. faecal oral transmission) can differ based on seasonal host activity. During the activity season when hosts have larger home ranges or move greater distances, they are more likely to encounter parasites (e.g. infectious stages of gastrointestinal parasites) deposited in the environment (Benavides *et al.* 2012). This is especially true for parasites that have an immobile infective stage that is indirectly transmitted. In this case, the infection depends on whether a susceptible host moves through a certain habitat area or uses certain resources (Kerr and Bull 2006) where parasites have been deposited (Leu *et al.* 2011). Other parasites are directly transmitted between two host individuals during social interactions. If the frequency of social interactions is seasonal, for instance higher during the mating season, this can lead to

seasonal differences in parasite infection levels. For example, some lizard species show different social interaction patterns during the mating season (high frequencies of interaction) compared to after mating (Leu *et al.* 2010). Other factors include seasonal differences in individual immune function (Altizer *et al.* 2006) or demographic changes in host populations (Gorsich *et al.* 2014). The external environment that is favourable to the parasite may also facilitate parasite transmission. For example, parasites are more prevalent in ungulate hosts (*Equus quagga*, *Antidorcas marsupialis*, *Connochaetes taurinus*, *Oryx gazella*) during the wet season than during the dry season (Turner and Getz 2010).

The common garden skink (*Lampropholis guichenoti*) is host to several gastrointestinal parasites, including a coccidian parasite (*Eimeria lampropholidus*), a tapeworm (*Baerietta hickmani*), and a nematode (*Hedruris wogwogensis*) (Cannon 1967; Jones 1985; Jones and Resasco 2016). These parasites are transmitted between hosts by ingesting infectious stages either through foraging or drinking from contaminated sources or through tongue-flicking when lizards sample chemical cues in their environment. Consequently, sharing space with infected lizards facilitates the transmission of indirectly transmitted parasites. Male garden skinks are sometimes aggressive during the activity season and may exhibit some aggression during the non-activity season (Torr and Shine 1996). When agonistic behaviours occur, large males tend to exclude smaller males from certain areas, including from shelters (Torr and Shine 1996). This spatial exclusion and potential avoidance of aggressive individuals can affect the disease transmission processes.

We asked whether parasite load differed between the activity and non-activity seasons in male garden skinks. We predicted a higher parasite load during the activity season than during the non-activity season because lizards are expected to have higher encounter rates with infected individuals and/or eggs of parasites then.

Materials and methods

Animal collection and husbandry

We collected lizards from suburban Sydney during October–November 2019 and March 2020. Lizards captured in 2019 were kept for a longer period in our facility so we could make sure these lizards were free from parasites. We captured them using mealworm fishing or by hand (Michelangeli *et al.* 2016). We measured snout–vent length (SVL) to the nearest 0.01 mm using callipers and weighed each lizard to the nearest 0.001 g using a digital scale. We sexed each lizard based on the presence/absence of hemipenes. Each lizard was then given an individual ID by toe-clipping. We housed each lizard individually in well-sealed containers (200 mm × 135 mm × 70 mm) in our animal room to prevent cross infection with parasites among individuals.

We provided UV lighting above each lizard, and a heating cable underneath one end of the tub, which created a thermal gradient for the lizard. We set the room temperature at approximately 22°C, and the daily photoperiod from 0800 to 2000 hours (12 h). We fed the lizards with crickets three times a week, as well as vitamin supplements and calcium powder once per week. Water was available *ad libitum*.

Seasonal effects of parasite load

To test the effect of seasonality on parasite infection, we allocated 48 lizards to three groups. Each group consisted of 16 male lizards, two infected and 14 uninfected (initial infection rate was 12.5%). All lizards were housed outdoors in their groups in tubs (3.2 m diameter) in a netted, bird-proof enclosure at Macquarie University (refer to Supplementary material). Each tub had a layer of crushed rock in the bottom for drainage followed by a layer of soil and bark chips on the surface. We provided seven stacks of two roofing tiles in each tub as shelters for the lizards. These shelters created more space for the lizards, helping reduce social conflict. The setup was identical for all three tubs. In the outdoor enclosures, we fed the lizards with crickets once a week, and provided water *ad libitum*. In addition to the crickets, the lizards could forage for naturally occurring invertebrates in the tub. The study was approved by the Animal Ethics Committee of Macquarie University (2019/026-4).

Collection of faecal samples

To measure parasite loads, we collected faecal samples from March 2020 to January 2021. The start of the experiment was on 30 March, when we transferred the lizards to the outdoor tubs. The parasite load at the start was measured prior to the transfer. To assess parasite loads at the end of the non-activity season, we recaptured the lizards from their tubs during 24–29 August 2020 and collected faecal samples while they were housed individually indoors during a 7-day period. On the day of capture, we collected the first faecal sample by gently pressing the abdomen of the lizard. If the lizard did not defaecate on the day of capture, we collected the faecal sample the next morning either by picking up moist scats naturally deposited in the tub or by pressing the abdomen again. We then repeatedly collected faecal samples every second day (usually the day after feeding). The faecal samples were preserved with DESS in a microtube and stored in 4°C. DESS is a solution developed to preserve specimens in an adequate condition for morphological analyses and also allows for subsequent molecular work if needed (Yoder *et al.* 2006). We prepared 2 L of DESS solution by mixing 0.5 M disodium EDTA (1 L), dimethyl sulphoxide (DMSO; 400 mL), and distilled water (600 mL) in a beaker on a magnetic stirring plate. We then added NaCl (~300 g) to the solution and then repeatedly added a little more until

salt could no longer be dissolved in the solution. Finally, we filtered out the excess salt with filter paper and stored the solution in a 2 L serum bottle at ambient temperature. Following faecal collection, we released the lizards back into their outdoor tubs. We repeated this process at the end of the activity season, during 4–9 January 2021. These two sampling points represented the outcome of the transmission processes during the non-activity season and activity season, respectively.

Examination of faecal samples

We weighed the faecal samples using a digital scale to the nearest 0.001 g. To maximise parasite detection, we pooled three faecal samples from the same lizard. We examined the faecal samples using saline floatation (Mehlhorn 2016) with Epsom salt solution. The Epsom salt solution was made up by dissolving 400 g of Epsom salt in 1 L of distilled water. We floated the faecal sample in 1 mL of Epsom salt solution and gently shook the microtube to break the big fragments in the faecal sample. Then, a total of 650 μ L supernatant were transferred into a McMaster chamber. We counted parasite eggs in the McMaster chamber using a microscope (Olympus BX50). We then calculated the eggs per gram of the faecal sample as a measure of individual parasite load (Fenner and Bull 2008). The parasites were identified to the lowest taxonomic resolution possible (usually genus).

Statistical analyses

We calculated the means and ranges of infected lizards (excluding individuals that have zero parasite eggs) so our parasite load can be easily compared to those reported in other studies. To test for the effect of seasonality on parasite load, we used a generalised linear mixed effect model with a Poisson distribution. We included all lizards in this analysis to investigate the variation of parasite load between seasons. The fixed effect of season had three levels: the start, the non-activity season, and the activity season. We included lizard ID and tub number as random effects to account for the repeated measure design of the experiment using the model: $\text{parasite load} \sim \text{season} + (1|\text{ID}) + (1|\text{tub})$. We then analysed the effect of season on parasite prevalence. We used a generalised linear model with a binomial distribution: $\text{prevalence} \sim \text{season} + (1|\text{ID}) + (1|\text{tub})$. The analyses were conducted using the *lme4* package in R (Bates *et al.* 2015).

Results

The nematode eggs belonged to two different groups (Fig. 1): the trichinelloid group, and the ascarid group (roundworms). Trichinelloid eggs occurred in only five lizards and during the non-activity season only. We excluded them from the analysis because of their low prevalence. Parasite loads varied among

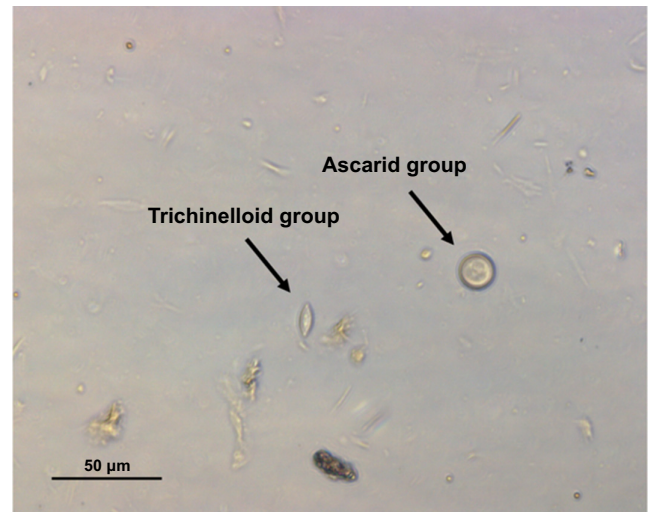


Fig. 1. Eggs of two different parasites found in the lizard. The oval egg is from parasites of the trichinelloid group. The round egg is from parasites of the ascarid group, also known as roundworms. The photo was taken at $\times 200$ magnification using an image capture (14MP Aptima COMS, RisingCam) connected to an Olympus BX50 microscope.

individuals within each tub (Table 1). However, the parasite load was significantly greater in the activity season than in the non-activity season (Wald chi-square = 69.7, d.f. = 1, $P < 0.001$). The mean parasite load of infected lizards was 21 245 eggs per gram faeces (111–74 692) at the start of the experiment but only 1923 egg per gram (125–6539) in the non-activity season (Fig. 2). Finally, in the activity season, the mean parasite load of infected lizards was 7263 eggs per gram (74–30 120) (Fig. 2).

The experiment started with 48 lizards. Some natural deaths occurred, and the number of lizards decreased to 38 in the non-activity season (after 4.5 months in outdoor tubs) and then to 30 lizards in the activity season (after 9 months in outdoor tubs) (Fig. 3). At the start of the experiment 12.5% (2/16) of lizards were infected with roundworms in each tub. The prevalence of roundworms did not differ between the start of the experiment and the non-activity season (mean = 15.9%, coefficient = 0.27, $P = 0.66$). However, the prevalence significantly increased in the activity season (mean = 72.5%, coefficient = 2.96, $P < 0.001$) compared to the start of the experiment.

Discussion

We experimentally showed that parasite load in garden skinks varied across seasons. Parasite load was significantly higher at the end of the activity season (austral spring and summer) than at the end of the non-activity season (austral autumn and winter). Several factors influence host parasite

Table 1. Mean parasite loads of infected lizards and standard deviations of each group in the non-activity season and in the activity season.

	Tub	Infection rate	Mean eggs per gram of faeces of infected lizards (s.d.)	Mean eggs per gram of faeces of all lizards (s.d.)	Range
Non-activity season (30.iii.–29.viii.2020)	M1	2/14	1.31×10^3 (1.18×10^3)	1.87×10^2 (5.77×10^2)	0– 2.14×10^3
	M2	1/12	1.43×10^2 (NA)	12 (41)	0– 1.43×10^2
	M3	3/12	2.92×10^3 (3.28×10^3)	7.31×10^2 (1.93×10^3)	0– 6.54×10^3
Activity season (30.viii.2020–09.i.2021)	M1	9/11	1.67×10^3 (2.44×10^3)	1.37×10^3 (2.29×10^3)	0– 7.44×10^3
	M2	8/10	1.36×10^4 (9.30×10^3)	1.09×10^4 (1.00×10^4)	0– 2.93×10^4
	M3	5/9	7.21×10^3 (1.28×10^4)	4.00×10^3 (9.84×10^3)	0– 3.01×10^4

Due to the zero-inflated nature of parasite loads, we reported both the means of infected lizards (excluding individuals having zero parasite eggs) and the means of all lizards.

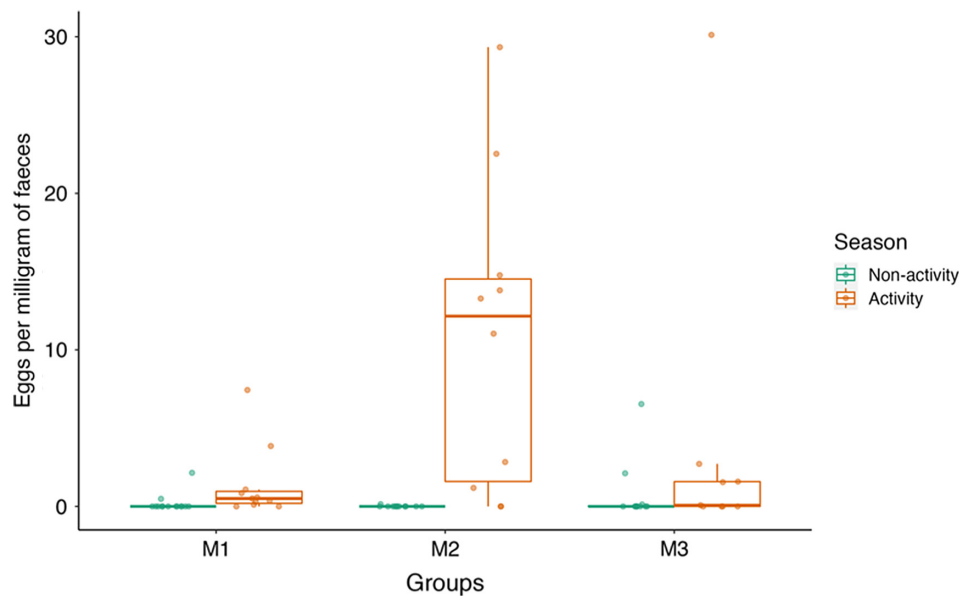


Fig. 2. Parasite load was significantly higher in the activity season than in the non-activity season. The parasite load was measured as eggs per milligram of faeces. M1, M2, and M3 represent three tubs of males. Several lizards were uninfected, i.e. had egg counts per milligram equal to zero. The unit (milligram) was used for visualisation purposes.

loads. The environmental condition of the host's habitat is important for parasites that have an environmental stage and are indirectly transmitted (Carbayo *et al.* 2019). During periods of suitable environmental conditions parasites can persist longer in the environment, increasing the probability of being transmitted to the next host individual (Turner *et al.* 2021). These conditions may include humidity, temperature, and UV level (Pietroock and Marcogliese 2003). For instance, in xeric shrubland, helminth species abundance was high during the wet season, whereas in broadleaf forest it was high during the dry season (Filho *et al.* 2017). In terms of the humidity, our result is similar to parasite infections of a Neotropical lizard (*Tropidurus montanus*), which has a higher prevalence of helminth during the dry season than the wet season (Václav *et al.* 2017).

In the garden skink, the higher nematode load can be explained by two non-mutually exclusive factors. The warm and dry environmental conditions during the activity season may be more suitable for the nematodes by increasing the time the eggs remain viable in deposited faeces. Temperature and humidity are important factors that can affect endoparasite egg development outside the host (Anderson 2000; Poulin 2006) as well as growth rates inside the host (Griffin 1993). Hence, nematode infections often increase with ambient temperature (Griffiths *et al.* 1998; Lettoof *et al.* 2020). At the same time, garden skinks are more active during the warmer months, which is their activity season (September–December). Thus, garden skinks may encounter more areas that contain faeces and hence are more likely to ingest the infective stages of these parasites

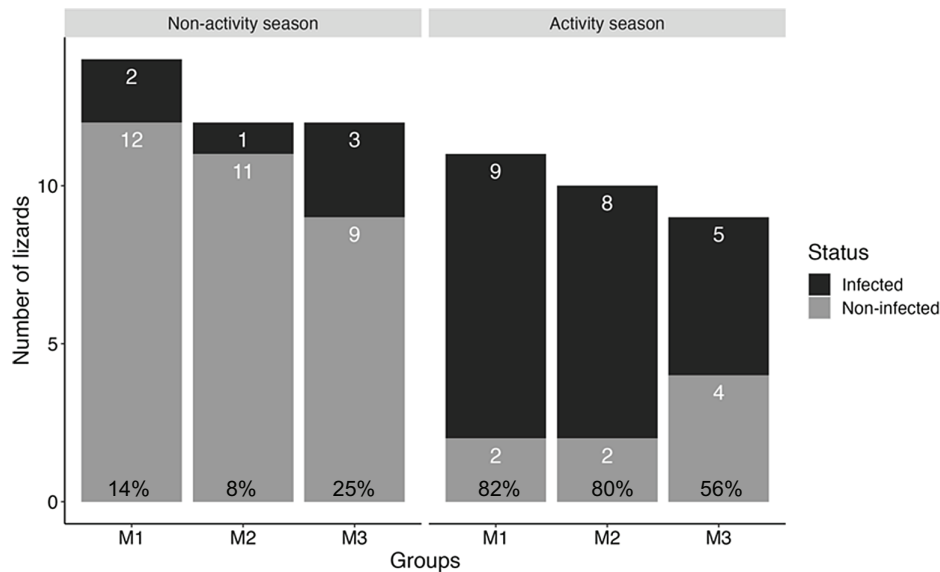


Fig. 3. Parasite prevalence (percentage at bottom of bar) was significantly higher in the activity season than in the non-activity season. Lizards were recaptured and their parasite load measured in the non-activity ($N = 38$) and activity ($N = 30$) season.

from their environment and become infected. A similar result was found in lava lizards (*Tropidurus hispidus* and *Tropidurus semitaeniatus*), which had a higher parasite abundance when they expanded their foraging area (Brito et al. 2014).

In conclusion, we have shown a seasonal difference in parasite load in the garden skink, with higher loads during the warmer months of the activity season of the host. We suggest that if the environmental conditions are favourable for both the host (increased activity) and the parasite (improved survival and development), the seasonal effect may be particularly pronounced.

Supplementary material

Supplementary material is available [online](#).

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Data availability. The data are available at OSF (https://osf.io/vwkn7/?view_only=a8741604335343f8991618461d4bdccb).

Conflicts of interest. The authors declare no conflicts of interest.

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