International Journal for Parasitology: Parasites and Wildlife 4 (2015) 295–300

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Helpful invaders: Can cane toads reduce the parasite burdens of native frogs?

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ARTICLE INFO

Article history:
Received 8 April 2015
Received in revised form
20 May 2015
Accepted 22 May 2015

Keywords:
Bufo marinus
Invasion
Nematode
Sink hypothesis

ABSTRACT

Many invading species have brought devastating parasites and diseases to their new homes, thereby imperiling native taxa. Potentially, though, invaders might have the opposite effect. If they take up parasites that otherwise would infect native taxa, but those parasites fail to develop in the invader, the introduced species might reduce parasite burdens of the native fauna. Similarly, earlier exposure to the other taxon's parasites might 'prime' an anuran's immune system such that it is then able to reject subsequent infection by its own parasite species. Field surveys suggest that lungworm counts in native Australian frogs decrease after the arrival of invasive cane toads (*Rhinella marina*), and laboratory studies confirm that native lungworm larvae enter, but do not survive in, the toads. In laboratory trials, we confirmed that the presence of anurans (either frogs or toads) in an experimental arena reduced uptake rates of lungworm larvae by anurans that were later added to the same arena. However, experimental exposure to lungworms from native frogs did not enhance a toad's ability to reject subsequent infection by its own lungworm species.

1. Introduction

Biological invasions can disrupt many aspects of ecosystem function. Much research has focused on mechanisms such as competition (e.g. Levine et al., 2003) and predation (e.g. Short et al., 2002) but disruption of host-parasite dynamics may be an important additional route of impact (Thieltges et al., 2009; Hartigan et al., 2011). Invaders often exhibit reduced parasite levels (the enemy release hypothesis: Marr et al., 2008), but novel pathogens brought by invaders can still devastate native taxa by directly reducing survival or by mediating the outcome of competition between native and invasive species (e.g. Settle and Wilson, 1990; Hudson and Greenman, 1998). The reverse scenario (the transfer of native pathogens to the invader) has similar effects (Dunn, 2009; Hartigan et al., 2011; Pizzatto and Shine, 2012).

Past studies have investigated cases where invaders act to increase parasitism of native fauna by introducing a new parasite or by acting as a reservoir for native parasites (Dobson and Foufopoulos, 2001; Mastitsky and Veres, 2010; Pizzatto and Shine, 2011; Hartigan et al., 2011). However, little research has been done on the alternative scenario — cases where invaders decrease parasite loads of native animals by acting as a 'sink' for native parasites (Kelly et al., 2009a; Lettoof et al., 2013). In this instance, native parasites are taken up by the invader but fail to complete their life cycle due to a lack of co-evolutionary history. When the parasite enters a foreign host it becomes disoriented or attacked by the immune system. Invasive species can therefore act as 'resistant targets', reducing the density of parasites in the environment and thus, lowering the risk of infection for native hosts (Heimpel et al., 2003; Kelly et al., 2009a).

Because free-living stages of parasites are time-limited and exposed to threats such as predation and desiccation (Johnson and Thieltges, 2010) they are under heavy selective pressure to rapidly infect an appropriate host. Finding a host becomes more of a challenge in assemblages with a number of host species that differ in susceptibility to the parasite, such as invasive systems (Keesing et al., 2006).

The 'sink' mechanism has been investigated in only a few invasive systems (Trejo, 1992; Telfer et al., 2005; Kopp and Jokela, 2007; Thieltges et al., 2009; Paterson et al., 2011, 2013a,b) but these studies are geographically and taxonomically diverse, meaning the crossover of native parasites to invaders is likely to be common (Dunn, 2009). Thus, it is important to explore the

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http://dx.doi.org/10.1016/j.ijppaw.2015.05.004

possibility of 'sink' mechanisms in other systems, especially involving parasite and host taxa from lineages that have not been the subjects of previous research in this respect.

Here, we examine the potential disruption of host-parasite interactions caused by the invasive cane toad, *Rhinella marina*, in Australia. Recent studies have shown that cane toads and native frogs have separate nematode lungworm fauna (Pizzatto et al., 2012). The cane toad lungworm, *Rhabdias pseudosphaerocephala*, arrived in Australia with the invasive species in 1935 when it was introduced into Queensland as a biological control agent for sugar cane pests (Dubey and Shine, 2008). In contrast, *Rhabdias hylae* is exclusive to native Australian frogs. These two parasite species are virtually indistinguishable in morphology, but genetic analysis shows that they are indeed separate species (Dubey and Shine, 2008), and experimental infections demonstrate that the lungworms perform differently in cane toad and native frog hosts (Pizzatto et al., 2010; Nelson et al., 2015a,b). *R. hylae* can penetrate cane toads but is killed by a strong immune response; it becomes 'lost' inside the novel host's body and never reaches the lungs, where it would normally mature, produce eggs and complete its life cycle (Nelson, 2014; Nelson et al., 2015b). *R. pseudosphaerocephala* readily penetrates native frogs but only reaches the lungs in a small number of species, for similar reasons (Pizzatto et al., 2010; Pizzatto and Shine, 2011).

These interactions (especially the ability of each parasite species to penetrate the 'wrong' host, but not survive) support fundamental assumptions underlying the 'sink' mechanism. The only field data to support this scenario come from a recent study conducted in northeastern New South Wales. Lettoof et al. (2013) found lower rates of infection with lungworms in native frogs living in areas with cane toads, than in the same frog species living in nearby areas that lack cane toads.

Two mechanisms could plausibly explain this result. Firstly, the cane toads could be acting as 'sinks' (removing parasite larvae from the environment, and dooming those parasites to an early death). Secondly, native frogs that are exposed to the cane toad parasite might thereby develop acquired immunity to their own parasite species (i.e., an initial exposure to toad *Rhabdias* spp. may instigate production of antibodies that are also effective targeting native *Rhabdias* spp.). A similar 'priming' of the cane toad's immune system by frog *Rhabdias* spp. against its more virulent native parasite would have substantial benefits for cane toads, and might enhance their invasion success.

Immunological 'priming' is the principle behind many vaccines, which exploit the capacity of the adaptive immune system to form 'memories' in response to inert parts of pathogens (Brunham and Coombs, 1998; Oettinger et al., 1999; Hooper et al., 2004). Within a few weeks of exposure, specific antibodies are generated to defend the body against attack. Upon re-infection, the immune response is more effective at stopping the spread of disease. Amphibians, like all vertebrates, have this capacity to encode a 'memory' of previously encountered pathogens and the acquired immune response has been shown to play a role in the improved response of amphibians towards infections (Richmond et al., 2009; Tinsley et al., 2012).

Here we test the plausibility of the 'sink' mechanism as it applies to cane toads soaking up native frog parasites, and the possibility that prior exposure to the native frog lungworm 'primes' the cane toad's immune system such that it is less vulnerable to infection by its own lungworm species.

2. Materials and methods

Descriptions and details of methods for breeding and husbandry of anurans, and collection and identification of lungworm larvae used in the following experiments, appear in the Supplementary Material.

2.1. Effect of precedence on rates of parasite uptake

To measure rates of *R. hylae* uptake by anurans we exposed each of 69 native frogs (30 *Cyclorana australis* and 39 *Limnodynastes convexiusculus*) and 31 cane toads (*R. marina*) to infective lungworm larvae. Feces containing free-living adult worms were collected from adult frogs between 4 and 18 days prior to infection and stored in petri dishes with untreated bore water. After 2–4 days in these petri dishes, the free-living adult worms had produced infective third stage larvae (L3) that we used for experimental infections. 30 larvae (L3) were collected using a glass pipette under a dissecting microscope and placed in a 3.5 cm-diameter petri dish with 2 mL of water. An anuran was then placed in each dish and held with infective larvae for 1 h. We then removed the anuran and placed the dish under a dissecting microscope to count the larvae remaining. A second anuran was then added to the petri dish for 1 h. After this second 1-h infection period, the second anuran was removed and the number of remaining larvae counted once more. The combination of anurans in each petri dish was as follows: (1) cane toad ($n = 13$) followed by *L. convexiusculus* ($n = 13$), (2) *L. convexiusculus* ($n = 13$) followed by *L. convexiusculus* ($n = 13$), (3) cane toad ($n = 10$) followed by *C. australis* ($n = 10$), (4) *C. australis* ($n = 10$) followed by *C. australis* ($n = 10$) and, (5) cane toad ($n = 4$) followed by cane toad ($n = 4$).

We measured parasite uptake as the difference in number of larvae between the beginning and end of each 1 h trial.

This assumes that any missing larvae had crawled onto the anuran host and been removed along with it at the end of the trial. Metamorphs varied by a maximum of only 1.62 g, but anuran body mass was still used as a covariate in the analyses. We analysed the data from this experiment using an ANOVA model that incorporated trial "type" (the precedence combination of species 1/species 2), order of exposure of each anuran (first vs second), body mass and the order*type interaction term as independent variables and the number of larvae taken up as the dependent variable.

We carried out histological examinations to verify that a 1 h exposure to 30 L3 was sufficient to allow successful larval penetration. Five days after the exposure trials a subsample of 17 anurans (5 cane toads, 6 *C. australis*, 6 *L. convexiusculus*) were euthanised by immersion in a solution of buffered tricaine methanesulfonate (MS-222). For histological examination, five to six 5-μm serial transverse sections were made encompassing the tissue from the head to the pelvis of each anuran and stained with hematoxylin and eosin (see Pizzatto et al., 2010 for detailed methods). Slides were examined for the presence of larvae and characteristic inflammatory foci associated with degenerating larvae (Nelson et al., 2015b).

2.2. Effect of exposure to R. hylae on the subsequent establishment of R. pseudosphaerocephala in the lungs of cane toads

As part of another study, we exposed 32 metamorph cane toads to 30 infective larvae of *R. hylae* for 24 h and then measured correlates of fitness over 45 days (Nelson et al., 2015a). After this experiment had concluded 45 days post-treatment (DPT), we exposed 7 of the cane toads that had been previously exposed to *R. hylae* as part of this experiment, and 7 control toads (with no prior exposure to *R. hylae*, but otherwise identical husbandry conditions) to 30 *R. pseudosphaerocephala* larvae. This was done by placing each metamorph separately in a 3.5 cm diameter petri dish with 2 mL of water (plus the parasite larvae) for 24 h. Toads were housed and fed for a subsequent 20 days and then euthanised by