

## LETTER

## Diversity and disease: community structure drives parasite transmission and host fitness

Pieter T. J. Johnson,<sup>1\*</sup> Richard B. Hartson,<sup>2</sup> Donald J. Larson<sup>3</sup> and Daniel R. Sutherland<sup>3</sup>

<sup>1</sup>*Ecology and Evolutionary Biology, University of Colorado, Ramaley N122, Campus Box 334, Boulder, CO 80309, USA*

<sup>2</sup>*Center for Limnology, University of Wisconsin, 680 N. Park St., Madison, WI 53706, USA*

<sup>3</sup>*Department of Biology and River Studies Center, University of Wisconsin, 1725 State St., La Crosse, WI 54601, USA*

\*Correspondence: E-mail: pieter.johnson@colorado.edu

### Abstract

Changes in host diversity and community structure have been linked to disease, but the mechanisms underlying such relationships and their applicability to non-vector-borne disease systems remain conjectural. Here we experimentally investigated how changes in host community structure affected the transmission and pathology of the multi-host parasite *Ribeiroia ondatrae*, which is a widespread cause of amphibian limb deformities. We exposed larval amphibians to parasites in monospecific or heterospecific communities, and varied host number to differentiate between density- and diversity-mediated effects on transmission. In monospecific communities, exposure to *Ribeiroia* significantly increased mortality (15%), malformations (40%) and time-to-metamorphosis in toads. However, the presence of tree frogs significantly reduced infection in toads, leading to fewer malformations and higher survival than observed in monospecific communities, providing evidence of parasite-mediated facilitation. Our results suggest that interspecific variation in parasite resistance can inhibit parasite transmission in multi-species communities, reducing infection and pathology in sensitive hosts.

### Keywords

Biodiversity loss, *Bufo americanus*, decoy effect, dilution effect, emerging disease, host susceptibility, *Hyla versicolor*, *Rana clamitans*, trematode.

*Ecology Letters* (2008) 11: 1017–1026

### INTRODUCTION

Global biodiversity loss and disease emergence are two of the most challenging issues confronting science and society. Growing evidence indicates that these issues are inter-related. On one hand, emerging diseases can threaten or eliminate sensitive populations or species, thereby contributing to declines in biodiversity (Daszak *et al.* 2000; Skerratt *et al.* 2007). On the other hand, an increasing number of studies suggest that biodiversity and host community structure can influence parasite transmission (Ostfeld & Keesing 2000; Mitchell *et al.* 2002; LoGuidice *et al.* 2003; Dobson 2004; Dobson *et al.* 2006). Of particular interest is the hypothesis that higher host diversity can inhibit parasite transmission and reduce disease ('dilution effect' *sensu*; Keesing *et al.* 2006). Because many parasites infect multiple hosts, and such hosts vary in their competency to support and transmit parasites, parasite abundance depends upon the composition of the host community. In a widely cited example, Ostfeld *et al.* found that increases in wildlife host diversity can reduce tick-borne transmission of *Borrelia burgdorferi*, potentially decreasing the risk of Lyme disease in

humans (e.g. Ostfeld & Keesing 2000; LoGuidice *et al.* 2003). With increases in host diversity, the relative abundance of the most competent host decreases, leading to an increase in 'wasted' transmission events in which vectors feed on low-competency hosts. Similar relationships between host diversity and disease prevalence have been suggested to influence other vector-borne diseases, including West Nile virus, tick-borne encephalitis and bubonic plague (Ezenwa *et al.* 2006; Keesing *et al.* 2006).

Despite recent theoretical advances and a growing list of diseases that are influenced by host diversity, the ecological mechanisms responsible for these patterns remain largely theoretical (Holt *et al.* 2003; Dobson 2004; Keesing *et al.* 2006). Experimental approaches have been rare (but see Mitchell *et al.* 2002), often precluding our ability to understand whether reduced infection is due to changes in host diversity or simple changes in host density (Begon 2008). As emphasized by Begon (2008), a reduction in parasite transmission with increasing host diversity can often be explained by a decrease in the abundance of competent hosts in response to increasing competition. For example, Mitchell *et al.* (2002) found a significant inverse relationship

between plant diversity and foliar fungal pathogen infections. However, because pathogens were relatively host specific, these relationships were driven by competition-mediated reductions in host plant abundance at higher community richness. While changes in host abundance resulting from competition represent one form of the dilution effect, numerous other mechanisms have been advanced (see Keesing *et al.* 2006), and there is a growing need to examine their applicability. By allowing for simultaneous control over host density and community diversity, mechanistic experiments offer an important yet under-represented step in this process.

Thus far, most investigations of host diversity and disease have focussed on vector-borne diseases with frequency-dependent transmission, limiting our ability to broadly forecast when and for what types of pathogens host diversity can mediate disease risk. Parasites with complex life cycles involving sequential transmission among multiple host species may be promising candidates for exploring the mechanistic linkages between host diversity and parasite transmission (Chernin 1968; Combes & Moné 1987; Kopp & Jokela 2007). These life cycles, which are common among many helminths, myxozoans and fungi, often alternate between free-living infectious stages and endoparasitic reproductive forms. Free-living infectious stages (cercariae, miracidia, zoospores, etc.) are usually short-lived and under enormous selective pressure to quickly infect an appropriate host. In diverse host communities where host susceptibility varies among species, this challenge is likely magnified and could reduce the proportion of parasites successful in locating a suitable host ('encounter reduction'; Keesing *et al.* 2006). Importantly, encounter reduction could decrease transmission even when the density of competent hosts remains constant. Furthermore, if parasites are relatively pathogenic, the resulting decrease in transmission in diverse host communities could confer indirect protection to sensitive host species. This notion of 'parasite-mediated facilitation' has received little attention relative to parasite-mediated competition (Holt & Lawton 1994; Hudson & Greenman 1998), and its importance would depend on whether the benefits of reduced infection exceed the costs of interspecific competition within diverse communities. Nevertheless, given the ubiquity of infectious parasites in the environment and the broad variation in host competency, the potential benefits of parasite-mediated facilitation are substantial.

In this study, we experimentally evaluated the effects of host density and community structure on transmission of a complex life cycle parasite, *Ribeiroia ondatrae*, which causes mortality and severe limb malformations in amphibians (Johnson *et al.* 1999, 2002). *Ribeiroia* has a complex life cycle involving pulmonate snails, amphibians and amphibian-eating birds (Johnson *et al.* 2004), and we focus here on the

parasite's transmission from snails to larval amphibians. Free-living parasites (cercariae) released by infected snails have only a short temporal window (12–24 h) in which to find a suitable host. Because amphibian species vary in susceptibility to infection, community structure likely influences parasite transmission and the resulting pathology. We capitalized on this variation in host competency to assess how changes in community composition influenced both the average infection levels within competent hosts and the total parasite transmission in the community. Importantly, by independently manipulating both competent host density and community composition (monospecific vs. heterospecific), we sought to differentiate between density- and non-density-mediated mechanisms of the dilution effect. We further compared the relative benefits of decreased parasitism in diverse communities with the increased costs of competition to evaluate the relevancy of parasite-mediated competition. Experiments were conducted at two scales: laboratory experiments focussed on a simple two host species community to maximize mechanistic understanding, and a short-term outdoor experiment with three host species and four community structures. Considering the dramatic declines recently recorded in amphibians (Stuart *et al.* 2004) and the similarity of the *Ribeiroia* life cycle to important medical and veterinary pathogens, an increased understanding of the effects of community structure on complex life cycle parasites has broad significance for epidemiology and conservation.

## MATERIALS AND METHODS

### Laboratory experiment

We experimentally manipulated parasite exposure (present or absent), competent host density (high or low) and amphibian community (monospecific or heterospecific) to determine the effects of community structure on parasite transmission. We considered American toads (*Bufo americanus*) as the focal host because this species is (1) a competent host for supporting *Ribeiroia* and (2) is broadly distributed throughout central and eastern USA (Cline 2005). We used larvae of the Eastern grey treefrog (*Hyla versicolor*) as our decoy host. Grey treefrogs are a poor host for *Ribeiroia* and exhibit little infection or pathology following exposure (Johnson & Hartson, 2008). Both species are widely distributed in the Mid-western and Eastern USA, have similar breeding phenologies and often develop in the same wetlands (e.g. Cline 2005; Green 2005), suggesting that patterns of their co-occurrence have ecological relevance for transmission of *Ribeiroia*, which is also common in this region (Johnson & Hartson, 2008; Johnson *et al.* 2004).

Egg masses of *H. versicolor* and *B. americanus* were obtained from Bashett Wildlife Refuge, Missouri and the Woodruff

Fish Hatchery, Wisconsin, respectively, and allowed to hatch in the laboratory. As larvae reached Gosner's (1960) stage 28, they were transferred to 2.0-L containers and assigned randomly to a treatment condition. Larval toads were raised either individually ('*Bufo*' treatment), with another larval toad ('*Bufo* + *Bufo*' treatment), or with a larval treefrog ('*Bufo* + *Hyla*' treatment). Thus, our experiment evaluated how changes in focal host density (from 1 to 2 toad larva) and in host community composition (monospecific to heterospecific) influenced parasite transmission and host pathology. In the parasite treatment, we added 20 *Ribeiroia* cercariae to each 2.0-L container on 15 June and 17 June (total of 40 cercariae) and allowed parasites to seek out and infect larvae. These infection levels are ecologically relevant and well within the levels animals are exposed to in nature, particularly when the number of experimentally recovered parasites is compared with field studies (see *Results*; Johnson *et al.* 2002). Replicates in the unparasitized condition were given a comparable volume of water from an uninfected snail. In 10 additional replicates of each treatment (not part of the experiment), we filtered the water after an 8-h exposure period to determine what fraction of parasites failed to colonize a host. In only four instances were any cercariae recovered, and the number never exceeded two parasites (of the 20 administered), suggesting that the majority of parasites infected an amphibian larvae. Each treatment was replicated 30 times for a total of 180 containers and 300 larval amphibians. Tadpoles were fed *ad libitum* a slurry mixture of Tetramin® (Tetra Werke, Melle, Germany) and *Spirulina* fish flakes and water was changed weekly. Following parasite additions, we added a 10-cm piece of washed *Anachris* to each container to provide refuge for larval amphibians. The experiment was maintained in a randomized block design with all containers rotated among shelves weekly to avoid positional effects.

At metamorphosis (>95% tail resorption), surviving individuals were measured (Snout vent length or SVL, millimetres), weighed (wet mass, milligrams) and examined for abnormalities. We necropsied metamorphosed treefrogs and toads from parasite treatments to quantify *Ribeiroia* metacercariae. A subset of animals from unparasitized treatments ( $n = 10$  per condition) was also necropsied to verify the absence of infection. We analysed infection at two levels: total parasite abundance, measured as the sum of metacercariae among animals within the same container, and the mean abundance of parasites within toads specifically. Thus, we sought to understand how changes in host community affected both overall parasite transmission as well as infection and pathology within the focal host (*B. americanus*). In the *Bufo* + *Bufo* treatment, we averaged response variables (e.g. size, mass, infection) within a replicate container before averaging across the treatment. However, we randomly selected only one of the two

co-occurring individuals in determining the malformation response. Because malformation risk is a function of parasite exposure, a higher infection in one animal could reduce the infection in a co-occurring individual. The same approach was not necessary for survival, as mortality in one animal was followed immediately by culling of the second animal, and culled animals were omitted from the analysis.

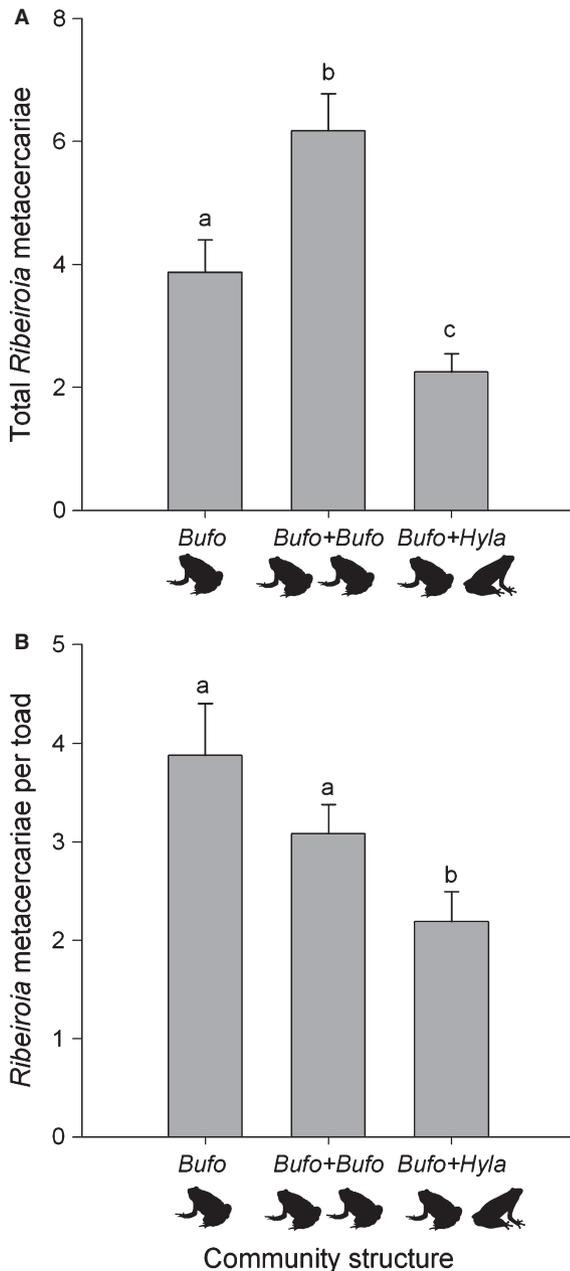
### Mesocosm experiment

To enhance the ecological realism of our experiments and explore the significance of more complex communities on transmission, we conducted short-term transmission experiments within 100-L mesocosms involving permutations of a three-species community. While a three-species community may seem depauperate with respect to plants or insects, species richness for larval amphibians in temperate wetlands generally ranges between 1 and 10, with only a subset of these occurring concurrently (e.g. Werner *et al.* 2007). Mesocosms contained a total of 30 tadpoles in one of four configurations: 30 *B. americanus* (one species, '*Bufo*'), 15 *B. americanus* and 15 *H. versicolor* (two species, '*Bufo* + *Hyla*'), 15 *B. americanus* and 15 *Rana clamitans* (two species, '*Bufo* + *Rana*'), or 10 *B. americanus*, 10 *H. versicolor* and 10 *R. clamitans* (three species, '*Bufo* + *Hyla* + *Rana*'). Because transmission of free-swimming parasites is likely a function of host mass, we carefully selected larvae such that individuals of different species were of comparable sizes and masses (mean total length  $\pm 1$  SE: *R. clamitans*: 2.40  $\pm$  0.04 cm; *B. americanus*: 2.21  $\pm$  0.02 cm; *H. versicolor* 2.71  $\pm$  0.06 cm). This necessitated that the species varied in developmental stage (Gosner stages: *R. clamitans*: 26–28; *B. americanus* and *H. versicolor*: 31–32). Thus, unlike in the laboratory experiment, we held the total density and biomass of hosts constant, varying only the composition of the community. We added 500 cercariae on 12 July and 14 July for a total of 1000 parasites. Each treatment was replicated four times. After 8 days, all surviving larvae were enumerated and examined for infection. We evaluated the effects of community composition on the average level of *Ribeiroia* infection in toads (i.e. the focal hosts) using a mixed model analysis, with the presence of *Hyla* and *Rana* as independent factors and toad infection nested within mesocosms.

## RESULTS

### Parasite transmission

Community structure significantly affected both the average and total number of parasites recovered in metamorphosing amphibians. The total number of *Ribeiroia* metacercariae ( $\log_{10}$ -transformed) increased with larval toad density, as expected (Fig. 1A). However, the presence of *H. versicolor*



**Figure 1** Effect of amphibian community structure on transmission of *Ribeiroia*. (A) The total number of metacercariae recovered, calculated as sum of metacercariae among individuals in the same replicate, varied significantly as a function of community structure. (B) Mean number of metacercariae recovered per metamorphic toad (+1 SE) as a function of whether toads were raised alone (*Bufo*), with a conspecific (*Bufo* + *Bufo*) or with a larval grey treefrog (*Bufo* + *Hyla*). For both panels, differences in letter groupings (a, b or c) indicate a pairwise significant difference ( $P < 0.05$ ).

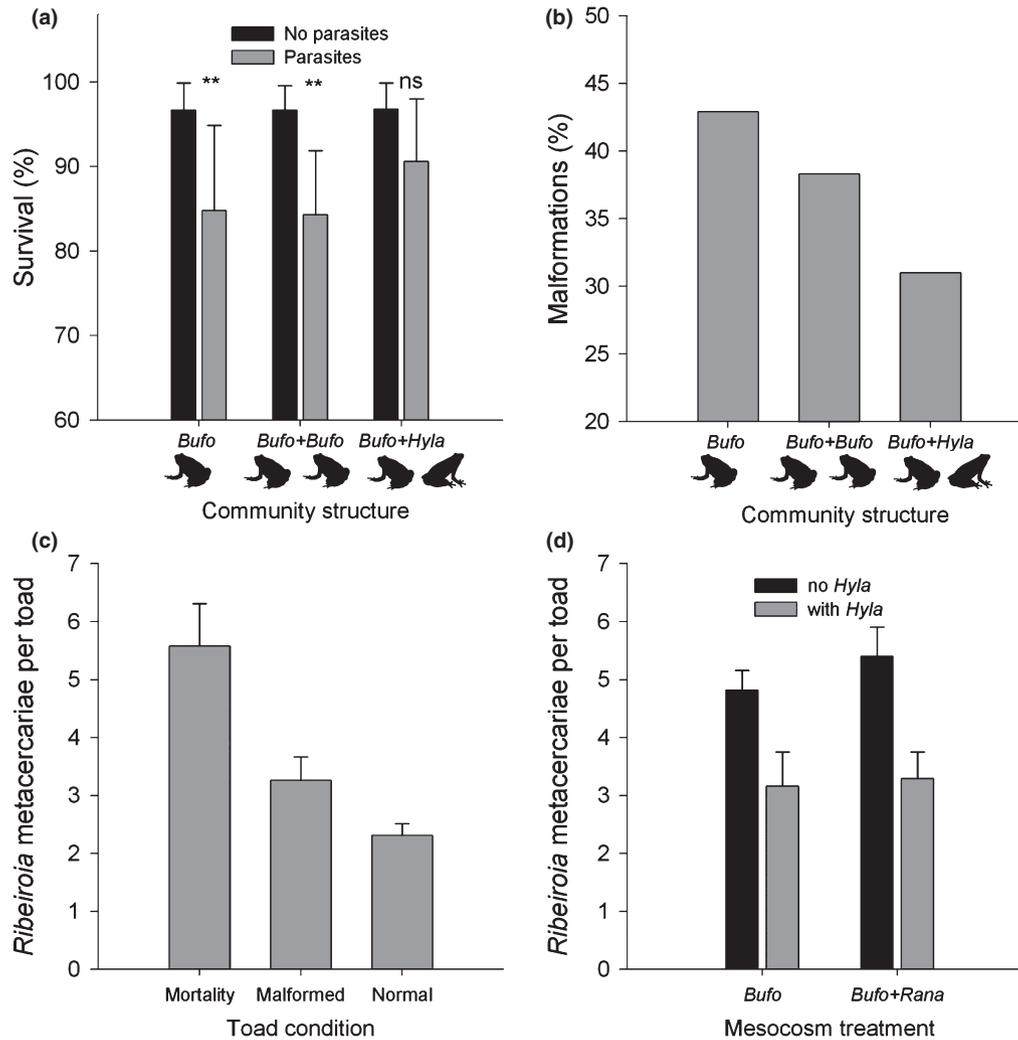
significantly reduced parasite recovery relative to the other two treatments (ANOVA:  $F_{[2,96]} = 14.85$ ,  $P < 0.0001$ ; Fig. 1A). In part, this result was due to the low infection

levels in *H. versicolor*; while metacercariae were recovered from 88% of toads in parasite treatments (mean infection  $\pm 1$  SE =  $3.07 \pm 0.21$ ,  $n = 134$ ), only 3.3% of exposed *H. versicolor* supported *Ribeiroia*, for an average of  $< 1$  metacercariae per frog. However, toads raised with *H. versicolor* also exhibited lower average infections ( $\log_{10}$ -transformed) than those raised alone or with a conspecific (ANOVA:  $F_{[2,97]} = 4.84$ ,  $P = 0.01$ ; Fig. 1B). Thus, while addition of a second competent host increased parasite transmission, the presence of a less competent host (e.g. *H. versicolor*) reduced total infection and the average infection within co-occurring competent hosts (*B. americanus*).

### Host pathology

Exposure to *Ribeiroia* increased mortality and malformations in toads (Fig. 2a). Among parasitized conditions, 14.1% of toads died prior to metamorphosis ( $n = 135$ ) (logistic regression of parasite exposure on toad survival:  $R^2_N = 0.127$ ,  $\chi^2 = 12.84$ ,  $P < 0.0001$ , d.f. = 1). Of those surviving, 37.5% exhibited one or more limb malformations [logistic regression:  $\chi^2 = 51.02$ , d.f. = 1,  $P < 0.0001$ ;  $R^2_N = 0.43$ ,  $B$  (unstandardized coefficient) = 20.69, odds ratio =  $9.7E+8$ ; Fig. 2b]. Malformations primarily affected the hind limbs (87.5%) and were dominated by skin webbings (26.6%), bony triangles (28%, Fig. 3) and extra limbs or limb elements (34.4%; Table 1), similar to field observations with *Ribeiroia* infection (Johnson & Hartson, 2008). In the absence of parasites, toads developed normally and exhibited high survival ( $> 96\%$ ). Treefrogs exhibited high survival and normal development, regardless of parasite exposure (logistic regression:  $\chi^2 = 1.31$ , d.f. = 1,  $P = 0.25$ ).

Community structure also influenced patterns of infection and pathology. Among parasite-exposed toads, survival was 30% higher in the *Bufo* + *Hyla* treatment relative to *Bufo* alone or *Bufo* + *Bufo* (Fig. 2a). Parasite infection interacted significantly with community composition, such that *Hyla* presence reduced toad mortality only in the parasitized treatments (logistic regression:  $\chi^2 = 15.97$ , d.f. = 2,  $P < 0.0001$ ;  $R^2_N = 0.16$ ; parasite  $B = 2.20$ , odds ratio = 9.03; *Hyla*-by-parasite  $B = -1.09$ , odds ratio = 0.34). In the presence of *H. versicolor*, parasite exposure did not affect toad survival (logistic regression:  $\chi^2 = 1.048$ , d.f. = 1,  $P = 0.306$ ) (Fig. 2a). Similarly, toads raised with treefrogs had 10% fewer malformations than those that developed alone and 7% fewer than those raised with another toad. This trend was not statistically significant due to the limited sample size (i.e. increased mortality in parasite-exposed toads reduced statistical power), but malformed toads supported 50% more parasites than did normal individuals, and toads that died prior to metamorphosis had twice as many parasites as those that survived (Fig. 2c). In addition, among malformed toads, the average malformation severity in the *Bufo* + *Hyla* treatment



**Figure 2** Results of laboratory and mesocosm-based experiments. (a) Effect of parasite exposure and community structure on survival of American toads (*Bufo americanus*) to metamorphosis. Asterisks indicate whether there was a significant difference in survival within a treatment due to parasite exposure. (b) Influence of community structure on the frequency of malformations in metamorphosing toads. No malformations were observed among grey treefrogs, or among toads not exposed to *Ribeiroia* cercariae. (c) Recovery of *Ribeiroia* metacercariae among toads that died prior to metamorphosis ('mortality') and, among those surviving, within normal and malformed individuals. Toads that died prior to metamorphosis supported significantly more parasites than those that survived [*t*-test:  $t = 3.86$  (equal variance not assumed), d.f. = 20.49,  $P = 0.001$ ]. Malformed toads also supported more parasites than those that developed normally [*t*-test:  $t = 2.12$  (equal variance not assumed), d.f. = 56.42,  $P = 0.039$ ]. (d) The presence of *Hyla versicolor* – but not of *Rana clamitans* – significantly reduced recovery of *Ribeiroia* metacercariae in toads in an outdoor mesocosm experiment.

was lower than in the monospecific communities (Table 1). These trends support the notion that the infection level, which was significantly affected by host community structure (see above), influenced the risk of pathology (mortality and malformation), but more data are needed to conclusively evaluate this pattern.

Both parasite exposure and community structure significantly affected toad size and development time (MANOVA: parasite, Wilks'  $\lambda = 0.94$ ,  $F_{[3,159]} = 3.416$ ,  $P = 0.019$ ; treatment, Wilks'  $\lambda = 0.412$ ,  $F_{[6,318]} = 29.61$ ,  $P < 0.0001$ ).

Although the addition of a second toad had no effect, the addition of a treefrog caused a delay in time-to-metamorphosis and a decrease in toad size- and mass-at-metamorphosis, regardless of parasite exposure level (Fig. 4). *Ribeiroia* exposure also delayed toad development (albeit less sharply), but increased toad size- and mass-at-metamorphosis (Fig. 4). This increase in toad size was likely due to the delay in metamorphosis, and inclusion of days-to-metamorphosis as a covariate eliminated the parasite's effect on toad size and mass. There was no interaction between infection

**Table 1** Composition of limb malformations in *Bufo americanus*

Abnormality type	<i>Bufo</i>	<i>Bufo</i> + <i>Bufo</i>	<i>Bufo</i> + <i>Hyla</i>	Total
Fore limb				
Ectrodactyly (missing digit)	1 (4.0)	0 (0.0)	0 (0.0)	1 (1.6)
Polydactyly (extra digit)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hemi- and ectromelia (missing limb)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Polymelia and polypody (extra limb or foot)	1 (4.0)	3 (10.3)	1 (10.0)	5 (7.9)
Cutaneous fusion (skin webbings)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Taumelia (bony triangle)	1 (4.0)	1 (3.5)	0 (0.0)	2 (3.1)
Hind limb				
Ectrodactyly (missing digit)	0 (0.0)	0 (0.0)	1 (10.0)	1 (1.6)
Polydactyly (extra digit)	0 (0.0)	2 (6.9)	1 (10.0)	3 (4.7)
Hemi- and ectromelia (missing limb or foot)	0 (0.0)	1 (3.5)	0 (0.0)	1 (1.6)
Polymelia and polypody (extra limb or foot)	3 (12.0)	4 (13.8)	0 (0.0)	7 (11.0)
Femoral projection (digit-like outgrowth)	2 (8.0)	2 (6.9)	3 (30.0)	7 (10.9)
Cutaneous fusion (skin webbing)	8 (32.0)	6 (20.7)	3 (30.0)	17 (26.6)
Taumelia (bony triangle)	7 (28.0)	8 (27.6)	1 (10.0)	16 (25.0)
Micromelia (small or shrunken limb)	2 (8.0)	1 (3.5)	0 (0.0)	3 (4.7)
Other malformed	0 (0.0)	1 (3.5)	0 (0.0)	1 (1.6)
No. abnormal	12	18	9	39
No. inspected	28	46	29	103
No. abnormalities per abnormal frog	2.08 ± 0.6	1.61 ± 0.2	1.11 ± 0.1	1.64 ± 0.2

Presented are the numbers (and relative percentages) of each abnormality type from toads in each treatment condition. Only animals from the parasitized treatments are included. Malformation terminology and classification follows Johnson *et al.* (2001). Because each animal can exhibit > 1 abnormality, the total number of abnormalities can exceed the number of abnormal individuals. The mean number of abnormalities per abnormal toad, an index of malformation severity, was significantly lower in the *Bufo* + *Hyla* treatment than in the combined monospecific communities (*t*-test: *t* = 2.472, d.f. = 36.3, *P* = 0.018).

and community structure, however, and parasite infection had no effect on treefrog size or growth rate within the heterospecific condition (MANOVA: Wilks'  $\lambda$  = 0.98,  $F_{[3,54]} = 0.363$ , *P* = 0.78).

### Mesocosm experiment

Results of the outdoor mesocosm experiment supported the importance of community structure on parasite transmission. The average number of *Ribeiroia* recovered per toad was greatest in the *Bufo* only condition and in the *Bufo* + *Rana* condition (Fig. 2d). Based on a mixed model analysis that nested tadpoles within mesocosms, the presence of *Hyla* (but not of *Rana* or their interaction) significantly reduced infection in toads (*Hyla*  $F_{[1,11.56]} = 10.84$ , *P* = 0.007). This indicates that the presence of *H. versicolor*

specifically, rather than the presence of any additional amphibian species, was responsible for inhibiting *Ribeiroia* transmission.

### DISCUSSION

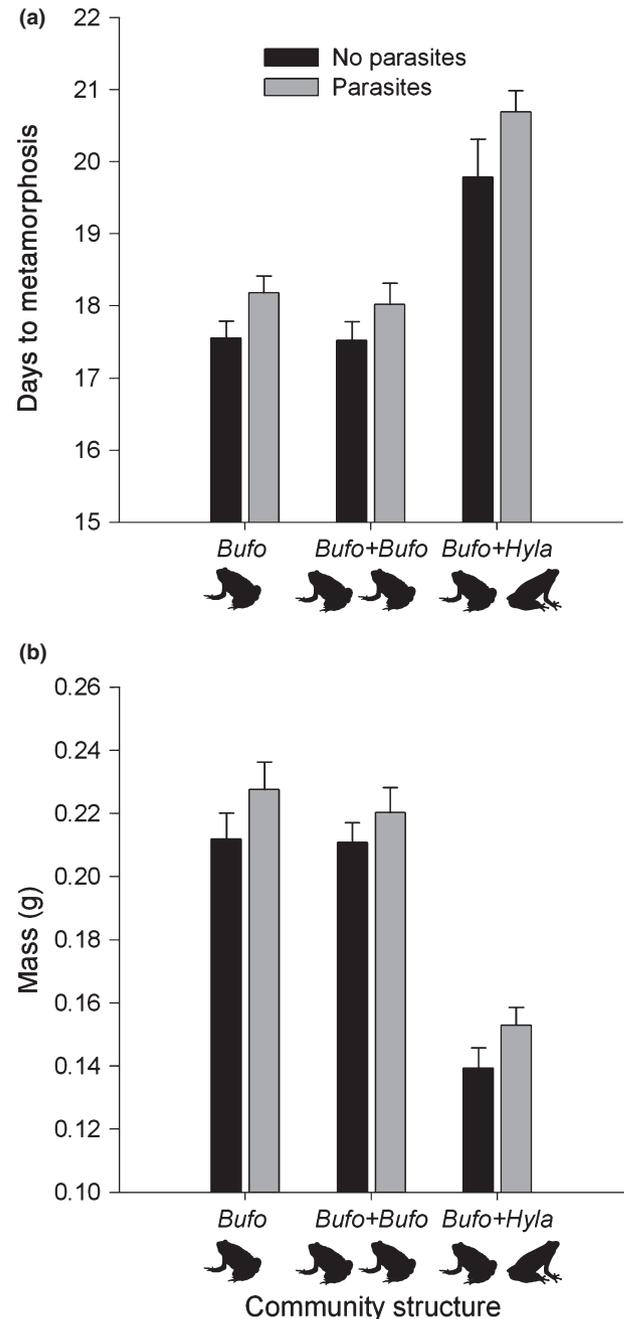
Our results underscore the importance of host community composition in determining parasite transmission and the resulting pathology in sensitive species. According to the dilution effect, more diverse host communities are hypothesized to inhibit parasite transmission as a result of variation in host competency (Ostfeld & Keesing 2000; Keesing *et al.* 2006). Thus far, however, experimental research on the mechanisms underpinning the dilution effect has been limited, and what data are available suggest that changes in competent host density – rather than in host diversity –



**Figure 3** Representative malformation caused by *Ribeiroia* infection. American toad (*Bufo americanus*) with taumelia of the left hind limb.

often explain observed patterns (Mitchell *et al.* 2002; Begon 2008). By decoupling the effects of host density from those of community composition, our experiment identified the independent effects of both density and diversity on transmission of a complex life-cycle pathogen. While an increase in the number of competent hosts (*B. americanus*) increased transmission and total parasite abundance, the addition of a low-competency host (*H. versicolor*) significantly reduced the abundance of *Ribeiroia* metacercariae. Parasite recovery in the heterospecific treatments (*Bufo* + *Hyla*) was 42% lower than in the monospecific low-density condition and 64% lower than in the *Bufo* + *Bufo* condition (Fig. 1). This pattern resulted from two inter-related processes: first, because treefrogs supported few metacercariae at metamorphosis, they contributed little to the total parasites available for transmission to definitive hosts (e.g. birds). Second, toads raised alongside treefrogs also had 37% fewer parasites than those raised alone or with a conspecific, further depressing the total level of parasitism. Although treefrogs can become infected with *Ribeiroia*, the parasites often fail to establish successfully (Johnson & Hartson, 2008) and are therefore 'lost' prior to metamorphosis ('wasted transmission'), presumably owing to innate differences in immune function. In this manner, treefrogs reduced total parasite survival while also providing an indirect benefit to toads. Keesing *et al.* (2006) have termed this dilution mechanism 'encounter reduction'.

The outdoor mesocosm experiment further highlights the importance of community composition for parasite transmission while providing insights about more complex community structures. While the laboratory experiment considered only variations of a simple two-host community, the mesocosm study incorporated four permutations of three species larval amphibian community, which approximates the average richness in temperate wetlands



**Figure 4** Effect of parasite exposure and community structure on toad mass and developmental rate. (a) *Ribeiroia* infection and especially the presence of *Hyla versicolor* both delayed the days required for toads to reach metamorphosis (parasite on days:  $F_{[1,161]} = 6.60$ ,  $P = 0.011$ ; community structure on days:  $F_{[2,161]} = 37.86$ ,  $P < 0.0001$ ). (b) *Ribeiroia* infection increased the mass of metamorphosing toads (parasite on mass:  $F_{[1,161]} = 4.18$ ,  $P < 0.05$ ), whereas competition from *H. versicolor* caused a reduction in toad mass (community structure on mass:  $F_{[2,161]} = 63.72$ ,  $P < 0.0001$ ). The presence of a second larval toad (*Bufo* + *Bufo*) did not significantly alter toad mass or developmental rate relative to toads raised individually.

(Werner *et al.* 2007). Importantly, while verifying the results of the simpler laboratory study, this experiment revealed that the reduction in parasite transmission was specifically due to the presence of a low-competency host (*H. versicolor*), rather than to a generic increase in host species richness. The addition of green frogs (*R. clamitans*) did not elicit the same diluting properties on infection as found for treefrogs; toads raised in a heterospecific community with green frog larvae exhibited comparable infection levels to the monospecific treatments. Only the presence of treefrogs – alone or alongside green frogs – caused a significant reduction in focal host infection. Thus, the important determinant of parasite transmission was not total host diversity, but the specific composition of the host community (e.g. LoGuidice *et al.* 2003). More data are needed to assess whether other amphibian species have similar diluting properties and how empirical patterns of amphibian community assembly influence the extent to which dilution occurs in natural environments (see Ostfeld & LoGuidice 2003).

The mechanism through which treefrogs inhibited *Ribeiroia* transmission likely involves innate immune defences. In a previous study, Johnson and Hartson (2008) suggested that larval *H. versicolor* act as 'immunological sponges' for *Ribeiroia*, such that larvae become infected but are able to eliminate most metacercariae via innate immune defences. As a result, treefrogs exhibited limited pathology and no malformations following exposure (Johnson & Hartson, 2008). However, this does not explain how treefrog larvae reduced infection in toads. We suggest this pattern resulted from variation in larval mass. Because larval treefrogs are *c.* 30% larger than larval toads of the same developmental stage, cercariae may be more likely to initially encounter and colonize a larval treefrog, wherein they are subsequently eliminated by immune defences. This might explain how treefrogs effectively 'shielded' toads against cercariae, leading to lower infection in toads that were raised alongside treefrogs. However, it is also possible that tree frogs either actively consumed cercariae or caused changes in larval toad behaviour (e.g. increased swimming activity) that indirectly led to reduced infection. Nevertheless, all of these pathways still represent manifestations of encounter reduction.

In this manner, low-competency hosts may have important consequences for more sensitive host species in the same community. While many studies have focussed on the role of parasites in mediating the outcome of competition (e.g. Schmitz & Nudds 1994; Hudson & Greenman 1998), few have examined how competitors influence the outcome of parasite transmission, or what we term 'parasite-mediated facilitation'. By reducing the abundance of *Ribeiroia* cercariae, larval treefrogs provided an indirect benefit to toads, such that toads raised with treefrogs had higher survival and

fewer malformations than those raised alone or with other toads. However, to understand the net benefit of the dilution effect, we must also identify the costs associated with increasing diversity (e.g. interspecific competition). The addition of treefrogs (a competitor) had pronounced negative effects on toad size and developmental rate, consistent with previous studies on anuran competition (Alford & Wilbur 1985; Morin 1987). This same effect was not observed with the addition of a second toad (intraspecific competition), which had no detectable effects on host growth or development. Overall, it is likely that the beneficial effects of reduced infection – namely higher survival and fewer malformations – outweigh the competitive costs associated with heterospecific communities. Nevertheless, even small changes in developmental timing can have significant effects on amphibian survival (e.g. Berven 1990), and additional field work is needed to assess the net consequences of heterospecific communities on host fitness under natural conditions.

We suggest that parasite-mediated facilitation may be a common corollary to the dilution effect. Kopp & Jokela (2007), for example, found that introduced snails, which are refractory to native trematode infections, indirectly protected native snail hosts from infection and thereby reduced their likelihood of becoming castrated. Similarly, Johnson *et al.* (in preparation) found that heterospecific freshwater snail communities reduced the frequency of successful infections by *Schistosoma mansoni*, the causative agent of human schistosomiasis. Not only did mixed species communities produce 60–80% fewer cercariae, but also host snails (*Biomphalaria*) in heterospecific treatments exhibited a doubling in fecundity relative to monospecific communities when parasites were present. In a vector-borne disease system, Woodworth *et al.* (2005) suggested that non-native birds in Hawai'i could have comparable effects for avian malaria. Because of resistance to *Plasmodium*, some introduced birds represent an epidemiological 'dead end' for the parasites and may help reduce infection. In a striking example similar to the current study, Lane *et al.* (2006) reported that the presence of western fence lizards (*Sceloporus occidentalis*) can influence transmission of the spirochaete responsible for Lyme disease. Interestingly, lizards not only have low susceptibility to infection following the bite of an infected tick, but also properties of their blood can eliminate infection within the tick itself, thereby reducing the percentage of infected vectors in the community.

While our experiments involved simple artificial communities, we expect that patterns of community composition in nature are likely to be important in influencing infection and pathology for the following reasons. First, trematode cercariae survive for only a short time (< 24 h) following emergence from infected snails, and infection of the 'wrong' host represents a loss term in the abundance of infectious

parasites. Based on an examination of the total number of parasites recovered per treatment, a community dominated by *B. americanus* would likely support a higher total abundance of metacercariae than would a heterospecific community, even after accounting for the increased mortality in toads raised in monospecific communities. Second, pathology due to macroparasites is intensity-dependent, such that the severity of a host's disease depends on the number of parasites it supports. Thus, the risk of malformation (or mortality) in a host is a function of parasite exposure (Johnson *et al.* 1999, 2002), suggesting that community structures with higher infections will also support more pathology. Third, parasite transmission to definitive hosts (e.g. via predation of infected amphibians) may depend on the level of pathology in an amphibian host. Malformed frogs are hypothesized to be more vulnerable to bird predators than normal conspecifics, thereby enhancing *Ribeiroia* transmission. Because malformed amphibians also support more metacercariae, communities that produce more malformed individuals could enhance overall parasite transmission and the likelihood the life cycle is completed. We recognize, however, that the importance of community structure for transmission is likely to vary with the force of infection; extremely high levels of cercarial production could, in theory, overwhelm the protective effects of diluting hosts such as *H. versicolor*. Moreover, patterns of community composition and diversity will almost certainly be dynamic over time, thereby influencing – and possibly being influenced by – the levels of parasitism. Amphibian or snail community structures may therefore vary over time as a function of infection, potentially creating dynamic cycling of host–parasite interactions.

Finally, these results have important implications for species conservation and human health. Emerging infectious diseases are a growing threat to humans and wildlife alike, whereas ongoing biodiversity losses associated with habitat destruction and degradation likely have significant ecological as well as economic consequences (Daszak *et al.* 2000). The intersection between these issues – namely the effects of community structure on disease patterns – has not been broadly considered. The free-living infectious stages produced by *Ribeiroia* are similar to those of a broad variety of parasites infectious to humans and wildlife, including other trematodes (e.g. *Schistosoma*), acanthocephalans, chytridiomycetes, myxozoans, fungi and others (Kagami *et al.* 2004). A greater understanding of the roles of individual host species, non-host species (e.g. predators and decoys) and of specific community structures in affecting the establishment and transmission of parasites is therefore an important priority in disease ecology (Lafferty *et al.* 2006; Schotthoefter *et al.* 2007; Thielgtges *et al.* 2008). While generic losses in biodiversity may be associated with increases in disease, it is much more likely that the addition or

elimination of select species to a community will have the largest impacts. Thus, in the face of growing disease threats, interspecific competition may not be the greatest enemy, emphasizing the importance of identifying species that disproportionately reduce or enhance parasite infection (e.g. dilution vs. amplification hosts; Begon 2008). Considering the dramatic declines and extinctions recently recorded in amphibians, an increased understanding of the relationship between host diversity and disease transmission also has important implications for conservation.

## ACKNOWLEDGEMENTS

We are grateful to Tim Kratz and the University of Wisconsin's Trout Lake Research station for logistical support. We thank Christine Britton-Bridges for providing amphibian egg masses and Kira Langree and Katie Dosch for help with data collection. Brett Melbourne provided feedback helpful in improving the manuscript and Elizabeth Daly assisted with artwork. For funding, we acknowledge the support of NSF award DEB-0411760 (to PTJJ) and a Summer Research Fellowship from the Juday Family (to RBH).

## REFERENCES

- Alford, R.A. & Wilbur, H.M. (1985). Priority effects in experimental pond communities: competition between *Bufo* and *Rana*. *Ecology*, 66, 1097–1105.
- Begon, M. (2008). Effects of host diversity on disease dynamics. In: *Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems* (eds Ostfeld, R.S., Keesing, F. & Eviner, V.T.). Princeton University Press, Princeton, NJ, pp. 12–29.
- Berven, K.A. (1990). Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology*, 71, 1599–1608.
- Chernin, E. (1968). Interference with the capacity of *Schistosoma mansoni* miracidia to infect the molluscan host. *J. Parasitol.*, 54, 509–516.
- Cline, G.R. (2005). *Hyla versicolor*. In: *Amphibian Declines: the Conservation Status of United States Species* (ed. Lannoo, M.J.). University of California Press, Berkeley, CA, pp. 458–461.
- Combes, C. & Moné, H. (1987). Possible mechanisms of the decoy effect in *Schistosoma mansoni* transmission. *Int. J. Parasitol.*, 17, 971–975.
- Daszak, P., Cunningham, A.A. & Hyatt, A.D. (2000). Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science*, 287, 443–449.
- Dobson, A.P. (2004). Population dynamics of pathogens with multiple host species. *Am. Nat.*, 164, S64–S78.
- Dobson, A., Cattadori, I., Holt, R.D., Ostfeld, R.S., Keesing, F., Krichbaum, K. *et al.* (2006). Sacred cows and sympathetic squirrels: the importance of biological diversity to human health. *PLoS Medicine*, 3, 714–718.
- Ezenwa, V.O., Godsey, M.S., King, R.J. & Guptill, S.C. (2006). Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. *Proc. R. Soc. Lond., B, Biol. Sci.*, 273, 109–117.

- Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16, 183–190.
- Green, D.M. (2005). *Bufo americanus*. In: *Amphibian Declines: the Conservation Status of United States Species* (ed. Lannoo, M.J.). University of California Press, Berkeley, CA, pp. 692–704.
- Holt, R.D. & Lawton, J.H. (1994). The ecological consequences of shared natural enemies. *Annu. Rev. Ecol. Syst.*, 25, 495–520.
- Holt, R.D., Dobson, A.P., Begon, M., Bowers, R.G. & Schaubert, E.M. (2003). Parasite establishment in host communities. *Ecol. Lett.*, 6, 837–842.
- Hudson, P.J. & Greenman, J.V. (1998). Parasite mediated competition. Biological and theoretical progress. *Trends in Ecol. & Evol.*, 13, 387–390.
- Johnson, P.T.J., Lunde, K.B., Ritchie, E.G. & Launer, A.E. (1999). The effect of trematode infection on amphibian limb development and survivorship. *Science*, 284, 802–804.
- Johnson, P.T.J., Lunde, K.B., Ritchie, E.G., Reaser, J.K. & Launer, A.E. (2001). Morphological abnormality patterns in a California amphibian community. *Herpetologica*, 57, 336–352.
- Johnson, P.T.J., Lunde, K.B., Thurman, E.M., Ritchie, E.G., Wray, S.W., Sutherland, D.R. *et al.* (2002). Parasite infection linked to amphibian malformations in the western United States. *Ecol. Monogr.*, 72, 151–168.
- Johnson, P.T.J., Sutherland, D.R., Kinsella, J.M. & Lunde, K.B. (2004). Review of the trematode genus *Ribeiroia* (Psilostomidae): ecology, life history, and pathogenesis with special emphasis on the amphibian malformation problem. *Adv. Parasitol.*, 57, 191–253.
- Johnson, P.T.J. & Hartson, R.B. (2008). All host are not equal: explaining differential patterns of malformations in an amphibian community. *Journal of Animal Ecol.*, (in press).
- Kagami, M., Van Donk, E., de Bruin, A., Rijkeboer, M. & Ibelings, B.W. (2004). *Daphnia* can protect diatoms from fungal parasitism. *Limnol. Oceanogr.*, 49, 680–685.
- Keesing, F., Holt, R.D. & Ostfeld, R.S. (2006). Effects of species diversity on disease risk. *Ecol. Lett.*, 9, 485–498.
- Kopp, K. & Jokela, J. (2007). Resistant invaders can convey benefits to native species. *Oikos*, 116, 295–301.
- Lafferty, K.D., Dobson, A.P. & Kuris, A.M. (2006). Parasites dominate food web links. *Proc. Natl Acad. Sci. U.S.A.*, 103, 11211–11216.
- Lane, R.S., Mun, J., Eisen, L. & Eisen, R.J. (2006). Refractoriness of the western fence lizard (*Sceloporus occidentalis*) to the Lyme disease group spirochete *Borrelia bissettii*. *J. Parasitol.*, 92, 691–696.
- LoGuidice, K., Ostfeld, R.S., Schmidt, K.A. & Keesing, F. (2003). The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proc. Natl Acad. Sci. U.S.A.*, 100, 567–571.
- Mitchell, C.E., Tilman, D. & Groth, J.V. (2002). Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology*, 83, 1713–1726.
- Morin, P.J. (1987). Predation, breeding asynchrony, and the outcome of competition among treefrog tadpoles. *Ecology*, 68, 675–683.
- Ostfeld, R.S. & Keesing, F. (2000). Biodiversity and disease risk: the case of Lyme disease. *Conserv. Biol.*, 14, 722–728.
- Ostfeld, R.S. & LoGuidice, K. (2003). Community disassembly, biodiversity loss, and the erosion of an ecosystem service. *Ecology*, 84, 1421–1427.
- Schmitz, O.J. & Nudds, T.D. (1994). Parasite-mediated competition in deer and moose – how strong is the effect of meningeal worms on moose? *Ecol. Appl.*, 4, 91–103.
- Schotthoefer, A.M., Labak, K.M. & Beasley, V.R. (2007). *Ribeiroia ondatrae* cercariae are consumed by aquatic invertebrate predators. *J. Parasitol.*, 93, 1240–1243.
- Skerratt, L.F., Berger, L., Speare, R., Cashins, S., McDonald, K.R., Phillott, A.D. *et al.* (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *Eco-Health*, 4, 125–134.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L. *et al.* (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, 306, 1783–1786.
- Thieltges, D.W., Jensen, K.T. & Poulin, R. (2008). The role of biotic factors in the transmission of free-living endohelminth stages. *Parasitology*, 135, 407–426.
- Werner, E.E., Skelly, D.K., Relyea, R.A. & Yruewicz, K.L. (2007). Amphibian species richness across environmental gradients. *Oikos*, 116, 1697–1712.
- Woodworth, B.L., Atkinson, C.T., LaPointe, D.A., Hart, P.J., Spiegel, C.S., Tweed, E.J. *et al.* (2005). Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. *Proc. Natl Acad. Sci. U.S.A.*, 102, 1531–1536.

Editor, Gabriele Sorci

Manuscript received 10 March 2008

First decision made 9 April 2008

Manuscript accepted 13 May 2008