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Experimental habitat fragmentation disrupts host–parasite interaction over decades via life-cycle bottlenecks

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Abstract

Habitat loss and fragmentation are likely to seriously impact parasites, a less studied but critical component of ecosystems, yet we lack long-term experimental evidence. Parasites structure communities, increase connectivity in food webs, and account for a large proportion of an ecosystem's total biomass. Food web models predict that parasites with multiple obligate hosts are at greater risk of extinction because the local extinction, or reduction in abundance, of any host will result in a life-cycle bottleneck for the parasite. We examine the response of a parasite and its multiple hosts to forest fragmentation over 26 years in the Wog Wog Habitat Fragmentation Experiment in southeastern Australia. The parasite is the nematode *Hedruris wogwogensis*, its intermediate host is the amphipod, *Arcitalitrus sylvaticus*, and its definitive host is the skink, *Lampropholis guichenoti*. In the first decade after fragmentation, nematodes completely disappeared from the matrix (plantation forestry) and all but disappeared from their definitive host (skinks) in fragments, and by the third decade after fragmentation had not appreciably recovered anywhere in the fragmented landscape compared to continuous forest. The low prevalence of the nematode in the fragmented landscape was associated with the low abundance of one or the other host in different decades: low abundance of the intermediate host (amphipod) in the first decade and low abundance of the definitive host (skink) in the third decade. In turn, the low abundance of each host was associated with changes to the abiotic environment over time due largely to the dynamically changing matrix as the plantation trees grew. Our study provides rare long-term experimental evidence of how disturbance can cause local extinction in parasites with life cycles dependent on more than one host species through population bottlenecks at any life stage. Mismatches in the abundance of multiple hosts over time are likely to be common following disturbance, thus causing parasites with complex life cycles to be particularly susceptible to habitat fragmentation and other disturbances. The integrity of food webs, communities, and ecosystems in fragmented landscapes may be more compromised than presently appreciated due to the sensitivity of parasites to habitat fragmentation.

INTRODUCTION

Habitat loss and fragmentation are likely to have serious impacts on parasites, a less studied but critical component of ecosystems (Gottdenker et al., 2014). Parasites structure communities by modifying competitive and trophic interactions (Hudson et al., 2006), increase the connectivity of food webs (Lafferty et al., 2008), account for a large proportion of an ecosystem's biomass (Kuris et al., 2008), and impact food web stability when lost (Dobson et al., 2008). Clearly, the loss of parasite species could have far-reaching impacts within fragmented ecosystems.

While many studies have examined the effects of habitat fragmentation on parasites, their findings are mixed (Gottdenker et al., 2014). Fragmentation has been associated with increased parasite prevalence (Allan et al., 2003; Gillespie & Chapman, 2008), reduced prevalence (Jousimo et al., 2014; Resasco et al., 2019), and no change in prevalence (Hodder & Chapman, 2012; Püttker et al., 2008). This lack of consensus among studies that vary in their parasite species and landscape context is not surprising given the complexity of potential relationships between biodiversity loss and parasite prevalence (Faust et al., 2017; Wood et al., 2016).

However, one parasite trait likely to consistently cause a negative effect of habitat fragmentation on prevalence is a complex life cycle. Theory illustrates how parasites with complex life cycles are at greater risk of extinction because of the increased risk that at least one obligate host goes extinct (Lafferty, 2012) or is reduced below a critical threshold (Bolker & Grenfell, 1996). Therefore, the varied effects of habitat fragmentation on multiple hosts should increase the probability of a life cycle bottleneck leading to lower parasite prevalence or extinction.

Here we focus on a parasite with a complex life cycle. In the controlled, replicated Wog Wog Habitat Fragmentation Experiment in southeastern Australia, the transmission of the trophically transmitted nematode, *Hedruris wogwogensis*, was disrupted via changes in the distribution and abundance of the intermediate host in the first 12 years after experimental habitat fragmentation (Resasco et al., 2019). The intermediate host amphipod, *Arcitalitrus sylvaticus*, went locally extinct in the young pine plantation matrix and decreased in remnant *Eucalyptus* fragments embedded within the matrix. Due to the loss and decrease of the intermediate host in the matrix and fragments, respectively, the definitive host skink, *Lampropholis guichenoti*, had no nematode infections in the matrix and decreased nematode prevalence in fragments relative to nearby continuous forest.

Theory predicts that the nematode's prevalence in any part of the landscape (matrix, fragments, continuous forest) is determined by biotic and abiotic constraints and dispersal. The strongest potential biotic filters are the presence and abundance of the amphipod (intermediate host) and skink (definitive host) since the nematode is dependent on the presence of both intermediate and definitive hosts for resources and to complete its life cycle (Choisy et al., 2003). Intermediate and definitive host population sizes may also be critical due to a persistence threshold, a host-density threshold that must be exceeded to sustain a parasite population (Bolker & Grenfell, 1996; Lopez, 2005). In fragmented landscapes, intermediate and definitive host presence and abundance depend on both biotic factors (e.g., dispersal, trophic interactions, competition; Hanski et al., 2000; Didham et al., 2012) and abiotic factors through fragmentation-driven changes to the environment (e.g., fluxes of solar radiation, water; Tuff et al., 2016; Reider et al., 2018). Parasite presence may also be determined by abiotic conditions where external life stages are affected. Finally, the parasite's recovery in parts of the landscapes where it has been driven locally extinct depends on its ability to disperse there via host species (Boulinier et al., 2016). Critically, all of these biotic and abiotic factors that can ultimately determine the nematode's persistence are connected through the nematode's complex life cycle (Figure 1a), where a bottleneck at any life stage could result in the local extinction of the nematode.

Further, there is potential for all of the changing biotic and abiotic influences of the fragmented ecosystem to cause the nematode and its host's prevalence/abundance to change in time, especially because here the matrix is dynamic (i.e., fast-growing trees). Many fragmented landscapes have a dynamic matrix. Few studies have followed host–parasite interactions in fragmented landscapes over long time scales (Gottdenker et al., 2014), so the implications of a dynamic matrix are relatively unknown. However, studies of nonparasitic species have shown that species' responses to habitat fragmentation are dynamic in time when the matrix is dynamic (Bitters, Hicks, et al., 2022; Evans et al., 2017; Smith et al., 2010). In this system, the presence and abundance of the host species are likely to change as the matrix changes, ultimately determining the nematode's presence. Finally, in the presence of a
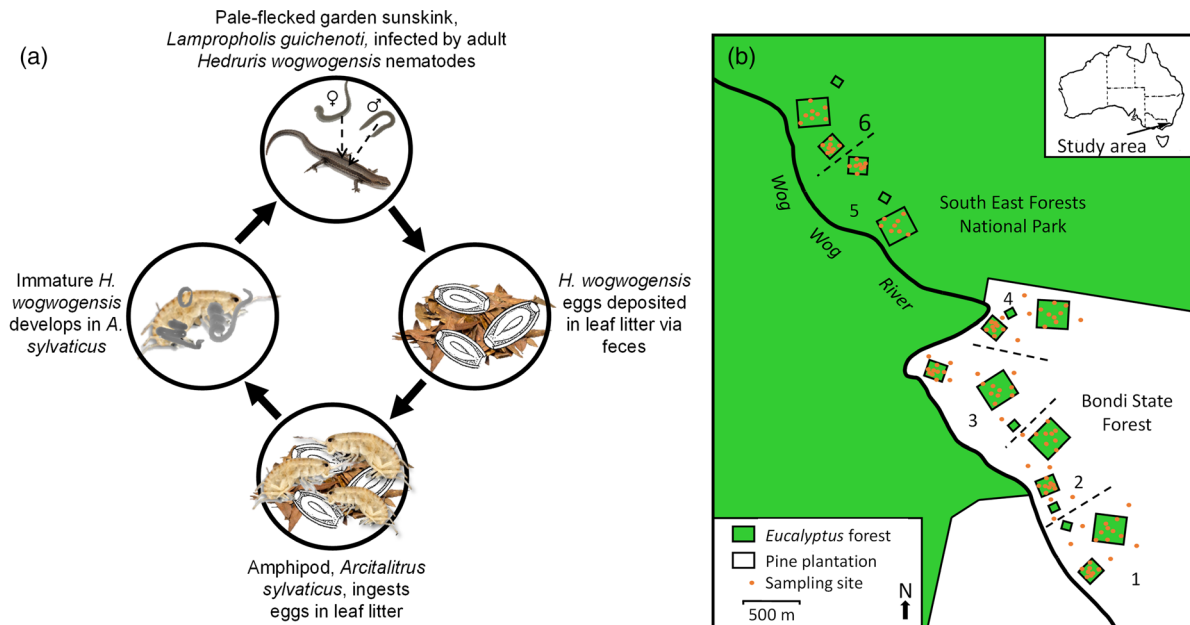
FIGURE 1 (a) Map of Wog Wog Habitat Fragmentation Experiment, New South Wales, Australia. Light green areas represent continuous *Eucalyptus* forest, white areas represent nonnative *Pinus radiata* plantation matrix, and light green boxes within the matrix represent remnant *Eucalyptus* forest fragments. Red dots represent pitfall sampling sites. Sampling sites are not shown in small fragments because of space constraints. Example fragments from Replicate 1 show how sampling sites are delineated by topography and distance to fragment edge. Image taken with permission from Evans et al. (2017). (b) Life cycle depiction of the nematode, *Hedruris wogwogensis* and its definitive host, *Lampropholis guichenoti*, and its intermediate host, *Arcitalitrus sylvaticus*. Image credits: skink (Fir0002/Flagstaffotos), amphipod (invertbase.org), nematode eggs (Hugh Jones illustration from Jones & Resasco, 2016), and adult and immature nematode (M. Bitters).

dynamic matrix and complex life cycle, we may not expect short-term responses to fragmentation of host–parasite systems to predict their long-term responses.

Here, we report a long-term follow up to Resasco et al. (2019) in the third decade of the Wog Wog experiment (years 23–26 post-fragmentation) to determine how the eventual recovery of the intermediate host amphipod in matrix and fragment habitat affected the skink–amphipod–nematode interaction (Figure 1a,b). We ask, with the recovery of intermediate host amphipod abundance, did the skink–amphipod–nematode interaction also re-establish? To determine whether nematode reinfection occurred in the third decade of the experiment where skink and amphipod distributions now overlap, we examine (1) skink and amphipod abundance in fragments, matrix, and continuous forest, (2) the presence of amphipods in skink guts in fragments, matrix, and continuous forest, and (3) nematode prevalence in skinks (percentage of skinks infected) in fragments, matrix, and continuous forest. Further, to confirm a relationship between the potential numbers of amphipods consumed by skinks and nematode infection in skinks, we examine nematode presence/absence in skinks in pitfall traps as a function of amphipod abundance in the same pitfall trap. We predict that nematode reinfection will be determined by

intermediate and definitive host presence and population size in fragments and the matrix, compared to continuous forest, so that recovery of host populations in fragments and the matrix will lead to the recovery of nematode infections in skinks to continuous forest levels. Amphipod and skink population size may be impacted by the direct impacts of fragmentation (e.g., isolating small populations on fragments) or indirectly through changes to the environment that drive changes in population size. Changed environmental conditions may also directly impact nematode prevalence in skinks in fragments by, for example, affecting egg desiccation rate. Understanding the mechanisms that determine the persistence of host–parasite interactions in fragmented landscapes has broad implications for understanding how fragmentation impacts food webs, communities, and ecosystem processes.

METHODS

Lampropholis guichenoti, "the pale-flecked garden sunskink," are small diurnal lizards commonly found throughout southeastern Australia and members of the Scincidae family, the largest and most diverse reptile

family in Australia (Cogger, 2014). They are opportunistic and generalist feeders of invertebrates as well as habitat generalists (Lunney et al., 1989; Resasco et al., 2018). The average adult size is 40 mm in snout–vent length and an additional 60–70 mm in tail length (Torr & Shine, 1993). They have a relatively small home range of ~20–30 m² (Anderson & Burgin, 2002), and their average lifespan is 2–3 years (Cogger, 2014).

Arcitalitrus sylvaticus, "lawn shrimp," are a species of amphipod in the family Talitridae and are found throughout southeastern Australia. These terrestrial crustaceans live in, and feed on, the top layer of leaf litter and must remain moist to avoid desiccation (Margules et al., 1994). At Wog Wog, they are most abundant in forested habitat with an understory dominated by *Lomandra longifolia*, an evergreen perennial herb that occupies drainage lines (Margules et al., 1994). They can occur in vast numbers. Adult *A. sylvaticus* range in size from 5 to 20 mm.

Hedruris wogwogensis are parasitic nematodes in the family Hedruridae. Species in the genus Hedruris use trophic transmission to parasitize fish, amphibians, and reptiles; the intermediate hosts of Hedruris are crustaceans (Anderson, 2000; Casalins et al., 2015). Two crustacean species, an amphipod and isopod, are trapped at Wog Wog. The amphipod is likely the main intermediate host as isopods were rarely detected in skink guts (Resasco et al., 2018). In contrast, the amphipod is abundant and frequently detected in skink guts. While one other Hedruris species has been known to infect multiple definitive hosts, some host specificity must exist because parasite prevalence, abundance, and intensity varied greatly between potential hosts (Luque et al., 2010). At Wog Wog, *L. guichenoti* occurs in vastly greater abundances than any other reptilian species, so even if *H. wogwogensis* uses multiple definitive hosts, their effect is likely small.

Study site

The Wog Wog experiment is located in New South Wales, Australia (37°04′30″ S, 149°28′00″ E; Figure 1b; Appendix S1: Figure S1) and was established in 1985 to assess the effects of large-scale habitat fragmentation on biodiversity (Margules, 1992). Experimental fragmentation of the forest occurred in 1987 when native *Eucalyptus* forest was cleared for a Monterrey Pine (*Pinus radiata*) plantation matrix for later timber harvest. The experiment consists of four replicates each with three remnant *Eucalyptus* forest fragments of different sizes, 0.25 ha (small), 0.875 ha (medium), and 3.062 ha (large), within the pine plantation matrix. Two additional replicates of the same layout are located in adjacent undisturbed, native *Eucalyptus* forest in South East Forests National Park and serve as controls.

There are eight sampling sites within each fragment, stratified by topography and distance to the edge. An additional 44 sampling sites are located in the pine matrix and are also stratified by topography. Each sampling site consists of two 90 mm diameter pitfall traps that are 5–10 m apart. Traps are set with a 1:3 glycol to ethanol pitfall trap solution. The two pitfall traps from each site are combined after collection to form one sample. Amphipod and skink specimens were stored in 75% ethanol at the University of Colorado Boulder (amphipods) and CSIRO Australian National Wildlife Collection (skinks).

Data collection

Skinks and amphipods were sampled using pitfall traps four times per year (spring, summer, fall, and winter) from 1985 to 1999 (this included 2 years prior to fragmentation) and three times per year (every season except winter) from 2010 to 2013. In addition, amphipods were sampled via pitfall trap once each summer in 2015, 2016, and 2018. Fragmentation took place in 1987. Thus, we refer to samples collected during 1987–1999 as the first decade of the experiment (years 0–12 post-fragmentation) and samples collected during 2010–2013 as the third decade of the experiment (years 23–26 post-fragmentation). Amphipods collected from 2015 to 2018 were dissected to confirm that *H. wogwogensis* uses the amphipod as the intermediate host to infect the skink, but they were not included in the analyses under *Data analysis*. After 2014, skinks were prevented from being captured in pitfall traps using skink ladders because of permitting requirements. Appendix S1: Table S1 describes the different types of data collected and how they were analyzed.

In the first decade of the experiment, 2424 skinks were collected in pitfall traps, and a subset of 186 skinks that encompassed all experimental treatments and most sampling sites were dissected and examined for the presence of nematodes and amphipods in their stomach and gut. In the third decade after fragmentation, only 142 skinks were captured, so all individuals were dissected and examined for the presence of nematodes and amphipods. In the first decade of the experiment, 36,006 amphipods were collected from pitfall traps. Physical specimens from the first decade were not kept by researchers and so could not be dissected. It was not feasible to dissect all individual amphipods from the third decade, so in traps that had more than 20 individuals, every tenth individual was dissected. We dissected 12,110 amphipods from the third decade after fragmentation to look for the presence of the nematode. An additional 3994 amphipods were collected by litter sifting and pitfall trapping from 2015 to 2018 to further confirm the suspected details of the life cycle of the

nematode, but these data were not analyzed because we did not have accompanying skink and nematode data from these years.

Nematodes collected from skinks from the first decade of the experiment were identified as a new species and described as *H. wogwogensis* in Jones and Resasco (2016). Nematodes collected from skinks from the third decade after fragmentation were determined to be *H. wogwogensis* using male and female distinguishing characteristics (Jones & Resasco, 2016). Nematodes collected from amphipods from the third decade were of various life stages ranging from cyst to immature adult. Morphological characteristics were used to confirm that they were *H. wogwogensis* when possible.

Data analysis

Nematodes and amphipods went extinct in the matrix in the first decade after fragmentation, while in fragments, amphipods declined in abundance and nematode infections in skinks became rare. We ask, with the recovery of intermediate host amphipod abundance in the third decade, did the skink–amphipod–nematode interaction also re-establish? We tested for an effect of experimental habitat fragmentation on four response variables: abundance of (1) skinks and (2) amphipods, (3) presence/absence of amphipods in skink guts, and (4) prevalence of nematodes in skink guts (percentage of skinks infected). Further, to confirm a relationship between the potential numbers of amphipods consumed by skinks and nematode infection in skinks, we examined nematode presence/absence in skinks in pitfall traps associated with amphipod abundance in the same pitfall trap. Finally, we used a structural equation model (SEM) to compare the relative effects of different factors on nematode presence in skinks. Appendix S1: Table S1 lists and compares all analyses. Details follow.

First, we analyzed the effect of habitat fragmentation on skink and amphipod abundance in the first and third decades after fragmentation. We used generalized linear mixed models with a Poisson distribution and log link function, as is typical for count data (lme4 R package, version 1.1.18.1; Bates et al., 2015). *Fragmentation* was a fixed effect with three levels: fragments, matrix, and continuous forest. *Replicate* and *patch* were included as random effects. The significance of fixed effects was assessed using likelihood ratio tests. We calculated effect sizes as the difference in natural logarithm of abundance in fragments or matrix compared to continuous forest and calculated 95% confidence intervals using likelihood profiles. In these models, sampling sites are nested within patches/fragments, which are nested within replicates.

Second, we tested for an effect of habitat fragmentation on the presence/absence of amphipods in skink guts in the first and third decades after fragmentation. Here, we used only presence/absence because it was not possible to delineate amphipod individuals in skink guts. We used a generalized linear mixed model and specified a binomial distribution with logit link function, as is typical for presence/absence data. Fragmentation was a fixed effect with three levels: fragments, matrix, and continuous forest. Replicate, patch, and site were included as random effects. The significance of fixed effects was assessed using likelihood ratio tests. Compared to the abundance analysis above, this analysis included one additional spatial scale of random effect (site), with skinks as sampling units nested within sampling sites, nested within patches/fragments, nested within replicates.

Third, we tested for an effect of habitat fragmentation on nematode prevalence in skinks in the first and third decades after fragmentation. We used a generalized linear model and specified a binomial distribution with logit link function. Fragmentation was a fixed effect with three levels: fragments, matrix, and continuous forest. For nematode prevalence in skinks (presence/absence) and amphipod presence in skinks, we used bias reduced models using the *brglm2* R package, version 0.5.1, because the absence of nematodes and amphipods in skinks from the matrix caused complete separation of the response variable between treatments (Kosmidis et al., 2017). No random effects were included because they cannot be included in a bias-reduced model.

Fourth, we examined nematode presence/absence in skinks in pitfall traps from amphipod abundance in the same pitfall trap using a generalized linear model with binomial distribution and logit link function in the first and third decades after fragmentation. Because nematodes found in skinks may reflect infections from previous years, for the first decade of the experiment, we fit models using amphipods collected from the same pitfall trap for the same year that the skink was collected and the previous 1, 2, and 3 years. For the third decade after fragmentation, we fit models for the same year and previous 1 and 2 years of amphipod data. We used Akaike's Information Criterion corrected for small sample size (AIC$_c$) to select the best time lag (1, 2, or 3 years). The significance of fixed effects was assessed using likelihood ratio tests.

Finally, we used a structural equation model (SEM) to compare the relative effect of different factors on nematode presence in skinks. We used the *piecewiseSEM* R package (Lefcheck, 2016) to fit linear mixed models to log-transformed skink and amphipod abundance and nematode presence (empirical logit). Additionally, for the third decade, we included linear mixed models for mean

daily temperature (skinks) and percent canopy cover (amphipods) at each site because these variables are the main determinants of the abiotic niche of skinks (Tuff, 2016) and amphipods (Bitters, Hicks, et al., 2022). Abiotic data were not collected in the first decade after fragmentation. Because the model for the third decade included abiotic data collected at the site scale, the analysis was at the site scale, whereas the model for the first decade was at the skinks-within-sites scale and contained an extra random effect of site. Otherwise both models include the random effects of replicate and patch. All analyses were run in R (R Core Team, 2018).

RESULTS

The proportion of skinks infected with at least one individual *H. wogwogensis* more than doubled from 18% (34/186) to 37% (53/142) between the first and third decades of the experiment. This overall increase in landscape-wide prevalence was associated with an increase in the proportion of skinks infected in the fragments (Figure 2a; Appendix S1: Table S2). Nematode prevalence in skinks was lower in both fragments and the matrix compared to continuous forest in both time periods (Figure 2a; first decade: $\chi^2 = 49.0$, df = 2, $p < 0.001$; third decade: $\chi^2 = 14.6$, df = 2, $p < 0.001$). Among skinks collected in the third decade after fragmentation, prevalence was 52% (27/52) in the continuous forest, 33% (26/79) in the fragments, and 0% (0/11) in the matrix. Notably, while nematode prevalence in the fragments increased, though not significantly, from 11% to 33% from the first to the third decade of the experiment, it still does not equal the prevalence found in the continuous forest.
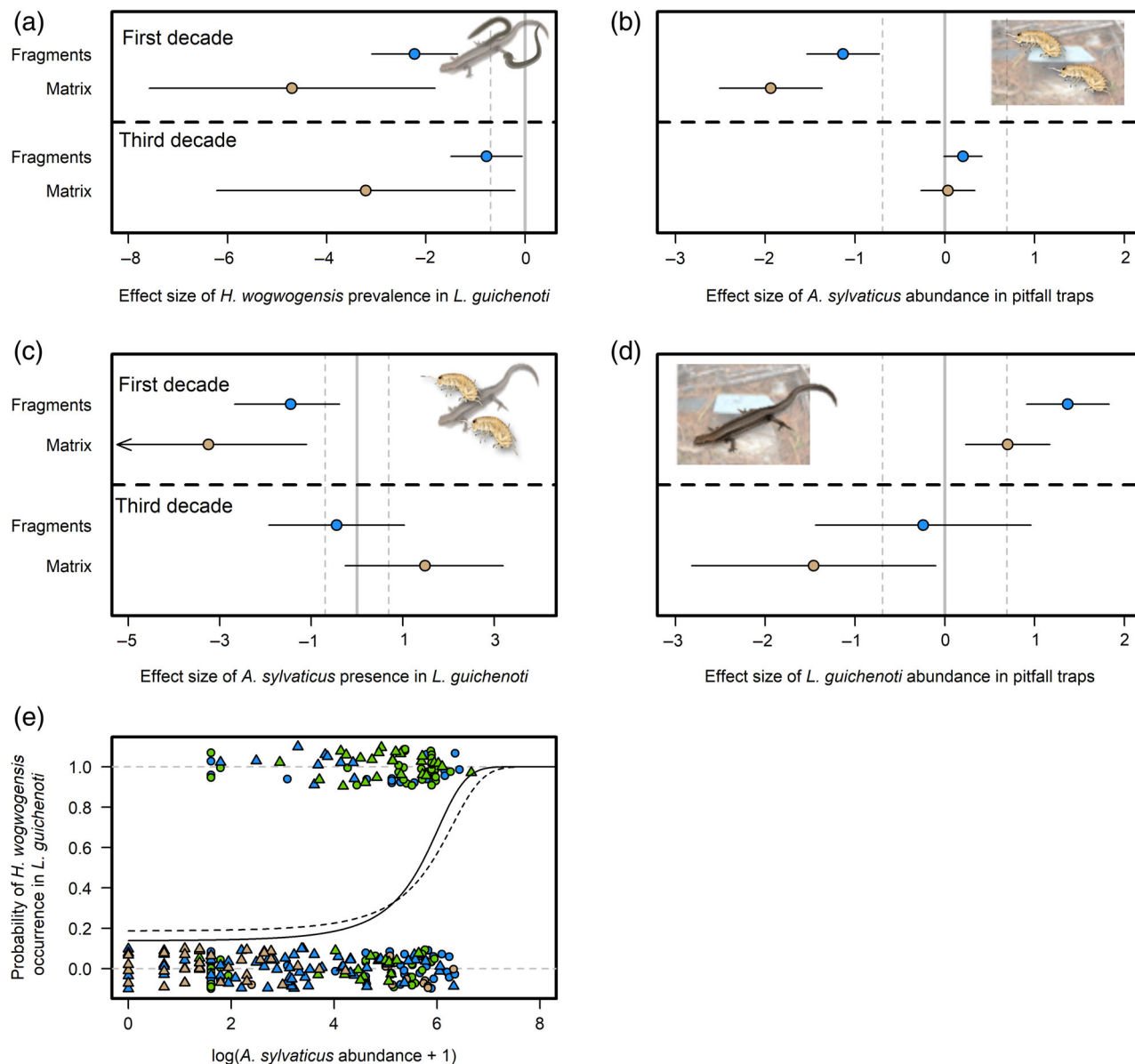
In the first decade after fragmentation, amphipod abundance in pitfall traps was significantly lower in fragments and the matrix compared to the continuous forest (Figure 2b; Appendix S1: Table S2; $\chi^2 = 16.6$, df = 2, $p < 0.001$). In the third decade after fragmentation, amphipod abundance was higher in fragments than in the continuous forest while abundance in the matrix was not different from the continuous forest (Figure 2b; $\chi^2 = 4.0$, df = 2, $p = 0.03$). Showing a similar pattern, in the first decade after fragmentation, amphipod presence within skink guts in fragments and the matrix was significantly lower than in the continuous forest (Figure 2c; $\chi^2 = 14.6$, df = 2, $p < 0.001$). In the third decade after fragmentation, amphipod presence within skink guts was not significantly different in fragments or the matrix compared to continuous forest (Figure 2c; $\chi^2 = 4.6$, df = 2, $p = 0.10$). For skinks with identifiable prey items in their gut, 8% (4/52) from the continuous forest contained at least one amphipod, 5% (4/79) from the fragments

contained at least one amphipod, and 27% (3/11) from the matrix contained amphipods. In the first decade after fragmentation, skinks were more abundant in both fragments and the matrix than in continuous forest (Figure 2d; $\chi^2 = 22.27$, df = 2, $p < 0.001$). In the third decade after fragmentation, skinks were less abundant in the matrix than in continuous forest, and there was no difference in abundance between fragments and continuous forest (Figure 2d; $\chi^2 = 7.54$, df = 2, $p = 0.02$).

We used logistic regression to predict the presence of nematodes in skinks at a given pitfall trap site based on the number of amphipods at that site. The best model included the previous 4 years of amphipod data from the first decade after fragmentation and the previous 2 years of amphipod data from the third decade after fragmentation. In both decades, amphipod counts from pitfall traps were positively related to nematode prevalence in skinks (Figure 2e; first decade: coefficient = 0.007, log odds ratio = 1.007, z value = 4.2, $p < 0.001$; third decade: coefficient = 0.004, log odds ratio = 1.004, z value = 3.7, $p < 0.001$). This relationship was not different between sampling periods ($\chi^2 = 0.01$, df = 1, $p = 0.90$).

The piecewise SEM analysis shows the relative effect of different factors on nematode presence in skinks (Appendix S1: Figure S2). Note that we cannot compare models including abiotic variables in both decades given that abiotic data were not available for the first decade. In the first decade, fragmentation had a large negative effect on amphipods, which in turn led to a large effect on nematode presence even though the direct effect of amphipod abundance on nematode presence was comparatively small. Fragmentation also had a positive effect on skink abundance, but skink abundance had no effect on nematode presence. Finally, fragmentation had a large direct negative effect on nematode presence separate from its effects on skink and amphipod abundance, suggesting that there were additional unmeasured variables influencing nematode presence. In the third decade after fragmentation, the direct effect of fragmentation on nematode, amphipod, and skink abundances was no longer significant. Fragmentation had a positive effect on canopy cover in fragments and the matrix, compared to continuous forest, as the pine plantation matured. Canopy cover positively predicted amphipod abundance. While the negative effect of fragmentation on temperature was not detected here (but see Tuff, 2016), temperature positively predicted skink abundance. Amphipod and skink abundance predicted the presence of nematodes in skinks.

From the 12,110 amphipods collected and dissected from pitfall traps from 2010 to 2013, 19 *H. wogwogensis* individuals were found at various life stages from nine individual amphipods (0.002% prevalence). Additionally,

FIGURE 2 The top half of panels (a)–(d) represent data from the first decade after fragmentation (years 1987–1999; Resasco et al., 2019) and the bottom half represent data from the third decade after fragmentation (years 2010–2013). Blue circles indicate effect sizes (log odds ratio) ± 95% confidence intervals of fragments and tan circles indicate effect sizes of matrix. Effect sizes are differences in abundance, presence, and prevalence in fragments compared to continuous forest and the matrix compared to continuous forest (solid gray line). An effect size with confidence intervals (CIs) that cross the solid gray line at zero indicates that we cannot reject the null hypothesis that there is no difference between fragments and the continuous forest and the matrix and continuous forest. Dashed gray lines show either a doubling or halving in the log odds of occurrence and abundance of each species. (a) Effect sizes and 95% CIs of *Hedruris wogwogensis* prevalence in the guts of *Lampropholis guichenoti*. (b) Effect sizes and 95% CIs of *Arcitalitrus sylvaticus* abundance in pitfall traps. (c) Effect sizes and 95% CIs of *A. sylvaticus* presence in the guts of *L. guichenoti*. (b) Effect sizes and 95% CIs of *L. guichenoti* abundance in pitfall traps. (e) Probability of occurrence of *H. wogwogensis* in *L. guichenoti* as a function of *A. sylvaticus* counts in pitfall traps. Triangle points represent data from the first decade after fragmentation (Resasco et al., 2019) and circle points represent data from the third decade after fragmentation. Green points represent a skink from continuous forest, blue points represent a skink from fragments, and tan points represent a skink from the matrix. Points are jittered to reduce overlap. Amphipod abundance is log-transformed on the *x*-axis. The solid black line is the probability curve from the first decade and the dashed black line is the probability curve from the third decade after fragmentation.

from the 3994 amphipods collected and dissected from additional pitfall trapping and litter sifting from 2015 to 2018, 171 *H. wogwogensis* individuals were found from 72 individual amphipods (0.018% prevalence). Infected amphipods were found in all three treatments of the experiment. We note that it is possible that prevalence is

higher and that we were unable to detect eggs and the tiniest larvae.

DISCUSSION

In the first decade after fragmentation, nematodes completely disappeared from the matrix and all but disappeared from their definitive skink host in fragments (Resasco et al., 2019), and by the third decade after fragmentation had not recovered in the matrix and only partially recovered in fragments (Figure 2a). Low prevalence of the nematode was associated with the low abundance of one or the other host in different decades: low abundance of the intermediate amphipod host in the first decade and low abundance of the definitive skink host in the third decade. Low host abundances were associated with changes to the abiotic environment. After the land surrounding fragments was cleared and planted with pine seedlings, the matrix, and to a lesser extent fragments, became sunny and dry compared to the continuous forest (Tuff et al., 2019). Amphipods require shady, moist habitat and so disappeared from the now sunny and dry matrix habitat and steeply declined in abundance in fragments (Figure 2b; Bitters, Hicks, et al., 2022; Margules et al., 1994). In contrast to amphipods, skinks favor sunny conditions, and their abundance was positively associated with the temperature of sample sites (Tuff, 2016). Even though skink abundance increased in the matrix and fragments, which may have been in response to the sunny conditions (Figure 2d), nematodes were absent from skinks in the matrix, and nematode prevalence was very low in skinks in fragments (Figure 2a). Further, skink guts contained zero amphipods in the matrix and few amphipods in fragments (Figure 2c). It is thus likely that the absence of amphipods in the matrix caused the absence of nematodes there by breaking this connection in the life cycle. By the third decade of the experiment, the pine plantation matrix was mature, and amphipod abundance completely recovered in the matrix and fragments (Figure 2b). However, in response to the now shady conditions in the matrix and fragments, skinks became extremely rare in the matrix (only 11 individuals caught in 4 years of sampling), and skink abundance declined in fragments compared to the continuous forest (Figure 2d; Tuff, 2016; Tuff et al., 2019). It is very likely that, as a consequence of the rarity of skinks in the matrix, nematodes continued to be absent from skinks in the matrix and nematode prevalence in skinks only partially recovered in fragments, from 11% to 33%, still less than the continuous forest (Figure 2a).

Our study experimentally illustrates how disturbance can cause local extinction in parasites with complex life cycles by reducing host populations resulting in a bottleneck at one or more critical life stages of the parasite (Lafferty, 2012). In the first decade after fragmentation, nematodes went extinct in matrix habitat when the intermediate host amphipod disappeared. When the intermediate host population then recovered in the third decade, skink populations were now too small to facilitate nematode reinfection. Thus, out-of-sync impacts on matrix intermediate and definitive host populations drove the local extinction of the nematode there. This result is predicted by food web models where parasites with complex life cycles are at greater risk of extinction than generalists because they rely on more than one obligate host to survive (Lafferty, 2012). Empirical evidence supports this prediction. In a meta-analysis assessing the impacts of fishing on parasites, complex life cycle parasites tended to decline in abundance in response to fishing; in contrast, directly transmitted parasites tended to increase in abundance (Wood & Lafferty, 2015). In a meta-analysis assessing differences in endoparasitism between mammals in urban and non-urban habitats, in carnivore and primate hosts that can persist in urban settings, parasites with complex life cycles were less prevalent in urban than in non-urban populations (Werner & Nunn, 2020). In forest island fragments in the Panama Canal, the abundance and species richness of ticks were positively related to the abundance and richness of wildlife; critically, specialist tick species were only present in fragments where their definitive hosts were also found (Esser et al., 2019).

Further, our study may provide evidence of a threshold effect in which nematode infection depends on the abundances of the intermediate and definitive hosts; when hosts are rare, the nematode is not present (Figure 2; Appendix S1: Figure S2). The persistence threshold hypothesis predicts that a host-density threshold must be exceeded to sustain a pathogen or parasite population (Bolker & Grenfell, 1996; Lloyd-Smith et al., 2005). Evidence from natural systems is rare, but examples include a persistence threshold for cowpox virus determined by the abundance of host bank vole and wood mice on islands off northwest England (Begon et al., 2003), and a persistence threshold for plague determined by the abundance of its gerbil host in Kazakhstan (Davis et al., 2004).

An alternative hypothesis for the decline in nematode infections in the first decade after fragmentation is that in the matrix and fragments, increased solar radiation and temperature and decreased humidity could have reduced nematode egg survival through desiccation. This could lead to fewer viable nematode eggs in the matrix and fragments regardless of the number of foraging amphipods and skinks. However, canopy cover was greater in both the matrix and fragments than in

continuous forest by the third decade of the experiment (positively impacted by fragmentation; Appendix S1: Figure S2). If abiotic impacts on egg survival were the main driver of nematode declines, then nematode abundance should have recovered in the matrix and fragments, compared to continuous forest, by the third decade of the experiment but did not. A second alternative hypothesis is that matrix pine needle litter could have been inhospitable to nematode eggs compared to *Eucalyptus* forest litter. However, if pine needle litter was the cause, as trees matured enough to accumulate needles in the litter layer, we would expect both a delay followed by a gradual decline in nematode abundance, which also did not happen. Thus, absence of one or the other host appears the most parsimonious interpretation of the data.

Our result highlights the vulnerability of parasites to habitat fragmentation, especially in species with life cycles dependent on more than one host species, because fragmentation impacts each member species of fragmented communities differently. Some species (potential hosts) increase in abundance, while others decline (Davies et al., 2000; Haddad et al., 2015). Further, when the matrix is dynamic, as is common (e.g., agriculture, production forestry), species abundances change in time in response to the changing matrix (e.g., Evans et al., 2017). Therefore, parasites may be especially sensitive to the impact of fragmentation because mismatches in the presence or abundance of multiple obligate hosts over time are more likely to occur. As discussed above, in our study, through impacts of the matrix, the abundance of the intermediate host was initially severely negatively affected and then recovered, whereas the definitive host was initially unaffected but was later severely negatively affected.

The underappreciated sensitivity of parasites to habitat fragmentation has significant implications for the integrity of food webs, communities, and ecosystems in fragmented landscapes. Parasites structure communities by modifying competitive and trophic interactions (Hudson et al., 2006) and increase the connectivity of food webs (Lafferty et al., 2008). Parasites can significantly increase nutrient cycling rates in communities (Mischler et al., 2016) and contribute substantial biomass to communities (Kuris et al., 2008). The loss of parasite species from communities can impact food web stability (Dobson et al., 2008). The loss, and change in prevalence, of parasite species, like we illustrate here, could have far-reaching impacts for fragmented ecosystems.

In January 2020, the entire Wog Wog experiment burned in the "Black Summer" fires, which were severe and widespread in southeastern Australia. Future research should determine whether the synergistic impacts of fire and forest fragmentation ultimately drive the nematode locally extinct, given the nematode's already decreased prevalence in fragmented habitat. The Wog Wog experiment is currently the only known location of the nematode, *Hedruris wogwogensis* (Jones & Resasco, 2016). Early evidence shows that the fire burned more intensely in the fragmented landscape than continuous forest (A. Spiers, B. A. Melbourne, and K. F. Davies, *unpublished data*). Given the dependence of the nematode on the abundance of two different host species, the nematode's persistence in the fragmented landscape may be tenuous, especially if the abundance of both hosts was severely reduced in fragments. The sensitivity of parasite species with complex life cycles to habitat fragmentation could have dire implications for fragmented ecosystems with increased fire intensity and frequency under climate change (Carlson et al., 2017). Finally, future studies should also examine the impacts of fragmentation and fire on within-host parasite community dynamics (Hassell et al., 2021).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
Data (Bitters, Meyers, et al., 2022) are available in Dryad at https://doi.org/10.5061/dryad.98sf7m0kz.

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