PHILOSOPHICAL TRANSACTIONS B

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Cite this article: Trillo PA, Bernal XE, Hall RJ. 2023 Mixed-species assemblages and disease: the importance of differential vector and parasite attraction in transmission dynamics. *Phil. Trans. R. Soc. B* **378**: 20220109. https://doi.org/10.1098/rstb.2022.0109

Received: 16 October 2022
Accepted: 13 January 2023

One contribution of 21 to a theme issue 'Mixed-species groups and aggregations: shaping ecological and behavioural patterns and processes'.

Subject Areas:
behaviour, ecology, evolution

Keywords:
mixed-species chorus, mixed-species groups, cues, vectors, parasites, infectious disease

Author for correspondence:
Paula A. Trillo
e-mail: ptrillo@gettysburg.edu

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6461005.

THE ROYAL SOCIETY PUBLISHING

Mixed-species assemblages and disease: the importance of differential vector and parasite attraction in transmission dynamics

Paula A. Trillo[1], Ximena E. Bernal[2,3] and Richard J. Hall[4,5,6]

[1]Department of Biology, Gettysburg College, Gettysburg, PA 17325, USA
[2]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA
[3]Smithsonian Tropical Research Institute, Apartado 0843-03092, Panama, República de Panama
[4]Odum School of Ecology, [5]Department of Infectious Diseases, College of Veterinary Medicine, and [6]Center for the Ecology of Infectious Diseases, University of Georgia, Athens, GA 30602, USA

PAT, 0000-0001-5994-5189; XEB, 0000-0001-6155-5980; RJH, 0000-0002-6102-4183

Individuals from multiple species often aggregate at resources, group to facilitate defense and foraging, or are brought together by human activity. While it is well-documented that host-seeking disease vectors and parasites show biases in their responses to cues from different hosts, the influence of mixed-species assemblages on disease dynamics has received limited attention. Here, we synthesize relevant research in host-specific vector and parasite bias. To better understand how vector and parasite biases influence infection, we provide a conceptual framework describing cue-oriented vector and parasite host-seeking behaviour as a two-stage process that encompasses attraction of these enemies to the assemblage and their choice of hosts once at the assemblage. We illustrate this framework, developing a case study of mixed-species frog assemblages, where frog-biting midges transmit trypanosomes. Finally, we present a mathematical model that investigates how host species composition and asymmetries in vector attraction modulate transmission dynamics in mixed-species assemblages. We argue that differential attraction of vectors by hosts can have important consequences for disease transmission within mixed-species assemblages, with implications for wildlife conservation and zoonotic disease.

This article is part of the theme issue 'Mixed-species groups and aggregations: shaping ecological and behavioural patterns and processes'.

1. Introduction

Infectious diseases are often spread in ecological communities where species vary in their ability to acquire and transmit pathogens. Since ecological interactions can influence pathogen exposure [1,2], understanding the community context of transmission can be crucial for shaping conservation and public health interventions. Investigations of disease dynamics in communities usually focus on the effects of the density or relative frequency of hosts that vary in their susceptibility to, or tolerance of, infection [2,3]. Often ignored, however, are host-specific biases in vector and parasite foraging behaviour that could influence disease transmission. Cue-oriented vectors and parasites are organisms that harm hosts indirectly through the transmission of infectious agents or directly through parasitism, and that exploit host-emitted cues to locate or identify hosts. This diverse group, including blood-feeding mosquitoes, ticks, bats and sap-feeding aphids [4], relies on host cues from a variety of sensory modalities, including olfaction [5], vision [6] and audition [7]. Such cues mediate choice between host species [8], and result in differential host attraction that can alter infection dynamics. While it has long been recognized that sensory cues influence vector and parasite attraction to focal host individuals or species

[8,9], it is less well-understood how cue-oriented foraging behaviour affects disease dynamics in a community context.

One widespread and ecologically important grouping of species that could influence pathogen dynamics are mixed-species assemblages, which we define as a gathering of individuals of at least two species, from the same trophic level, that are within close spatial proximity. This definition includes both assemblages where individuals actively choose to join a group (mixed-species groups) and those where individuals aggregate based on the distribution of shared resources (mixed-species aggregations) [10,11]. Mixed-species assemblages are found in diverse clades including mammals, birds, fish, amphibians and arthropods, in contexts such as feeding guilds, mating arenas or communal nesting or roosting sites. Assemblages can help individuals find food, detect and avoid predators, or exploit suitable breeding or nesting sites [11]. Compared to multi-trophic communities, where hosts have less spatially and temporally restrictive associations and vary widely in abundance, foraging style and traits influencing pathogen transmission, mixed-species assemblages often contain fewer, more closely related species with similar life histories.

In this study, we propose that the composition of mixed-species assemblages can modulate the attraction of cue-oriented vectors and parasites to the assemblage as a whole, as well as influence vector and parasite attraction to host species within the assemblage, ultimately impacting infection levels and feeding back to influence assemblage composition. We first highlight empirical studies of cue-oriented vectors and parasites attacking mixed-species assemblages. We then look beyond mixed-species assemblages at examples of vector and parasite biases within and between host species. We discuss how biases can influence the number and search efficiency of cue-oriented vectors and parasites recruited to mixed-species assemblages, and how this could interact with differential attraction to hosts within the assemblage to affect disease dynamics. We extend this conceptual framework by integrating insights from a natural system with those provided by a mathematical model. We develop a case study based on mating assemblages where different frog species are attacked by an eavesdropping vector and present a two-host one-vector model that investigates how cue-mediated changes in vector attraction to the assemblage, search efficiency, and bite attraction to hosts modulate transmission dynamics. Ultimately, we provide a broad perspective about how disease transmission in mixed-species assemblages can shape, and be shaped by, host composition, and identify future directions to further examine this phenomenon.

2. Cue-oriented vectors and parasites in mixed-species assemblages

The diverse and recurrent interactions that occur in mixed-species assemblages have important consequences for the ecology of communities that likely extend to host–pathogen dynamics. Mixed-species assemblages may decrease transmission risk for individuals by diluting the number of conspecific hosts with high competence for infection (ability to obtain and transmit pathogens) [12,13]. Alternatively, elevated transmission (amplification) might result from increased exposure of cue-oriented vectors or parasites to competent hosts of multiple species [11,14]. Pathogen dilution and amplification are widely documented for vectors and parasites infecting diverse host communities [2,15]. Ultimately, processes contributing to dilution and amplification at the scale of mixed assemblages may either reinforce or counteract those at the larger scale of the community.

Although some studies discuss disease transmission in mixed-species assemblages based on direct or environmental transmission [16–20], fewer have focused on cue-oriented vectors and parasites. Moreover, those studies provide mixed evidence regarding the effect of host diversity on disease outcomes. Studies investigating vector-borne avian haemoparasites suggest mixed-species groups can result in disease amplification. For example, birds in mixed flocks experienced increased prevalence of *Haemoproteus and Leucocytozoon* blood parasites [21] and ticks [22] compared to species not found in flocks. Similarly, a study comparing mixed-species and single-species groups showed that bird species in mixed flocks had higher rates of mosquito-borne *Plasmodium* infection than those in single-species flocks [23]. While these results suggest disease amplification, other findings provide contrasting evidence, in some cases for the same parasites but in different communities, complicating identification of overarching patterns [21,23,24].

Patterns seen at the community level might not be equivalent to those experienced by vectors and hosts at the level of mixed-species assemblages, and individual-level effects of participating in mixed assemblages can be species-dependent. For example, in mixed-nesting colonies of cliff swallows (*Petrochelidon pyrrhonota*) and invasive house sparrows (*Passer domesticus*), sparrow nests had lower prevalence of Buggy Creek virus (BCRV), transmitted by swallow bugs (*Oeciacus vicarius*), compared to nests in single-species colonies [25]. This benefit was not, however, symmetrical. Cliff swallows experienced similar BCRV prevalence in nests from mixed-species and single-species colonies. Overall infection prevalence across colonies was high, presumably driven by the presence of sparrows. Thus, participation in mixed-breeding colonies decreased infection rates for individual house sparrows while contributing to disease amplification at the community level.

Mixed-species communal roosts are common for bird species associated with west Nile virus (WNV), including the American robin, *Turdus migratorius* [26]. Dilution of transmission in mixed-species contexts has been invoked as a potential cause of reduced WNV infection rates among mosquitoes from communal roosts [26,27]. To our knowledge, however, the effects of mixed-species roosting on vector-borne disease dynamics have not been directly assessed, nor compared to those found in single-species roosts. House sparrows placed in communal roosts of American robins experienced lower WNV risk and encounter rates with mosquitoes than sparrows in non-roost locations [28]. While this study did not interpret the results from a mixed-species perspective, it suggests that heterospecific hosts can influence transmission rates in mixed-species roosting sites.

Studies of disease prevalence in mixed-species assemblages reveal that while they can modulate disease dynamics associated with cue-oriented vectors and parasites, the direction of these effects is difficult to predict. In addition, host vulnerability in mixed assemblages may be affected by the species identities of group members. For example, mixed flocks often include nuclear species, which influence decisions of heterospecifics to join or remain in the flock, encouraging group formation and cohesion [11]. These nuclear species can be

more frequently infected with haemoparasites in mixed flocks [21]. Thus, species composition, and in particular, the role and host competence of certain species may influence disease prevalence in mixed-species assemblages.

Evaluating interspecific variation in disease prevalence within mixed-species assemblages provides a general perspective of the influence of cue-oriented vectors and parasites on disease dynamics of these groups. This approach, however, ignores the mechanisms underlying differential disease outcomes for members of those assemblages. Including the effect that different species composition, and thus different cue combinations, have on vector and parasite attraction to the assemblage, as well as attraction to individuals once at the assemblage, may increase predictive power for disease dynamics in mixed-species assemblages.

3. Cue-oriented vector and parasite biases outside of mixed-species assemblages

While few studies have investigated the dynamics of cue-oriented vector and parasite infections in mixed-species assemblages, it is well-known that not all members of a community are attacked at the same rate [9,29,30]. In this section, we draw from literature that examines how host type influences foraging patterns in cue-oriented vectors and parasites, including studies examining how host type influences attack rates at the intraspecific (single-species) and interspecific (multi-species) levels. We interpret patterns from this literature, assuming that factors producing biases in vector and parasite attacks in non-assemblage contexts act similarly within mixed-species assemblages.

(a) Intraspecific variation in host-emitted cues and vector and parasite biases

Within species, cues emitted by individuals can differ greatly, potentially modulating their attractiveness to vectors and parasites. In humans, for example, variation in volatile skin chemicals is partially responsible for inter-individual variation in relative attractiveness to haematophagous vectors [31]. Several factors, including reproductive status or diet, can result in differential attack rates. Pregnant women, for example, are more likely to be bitten by *Anopheles* and *Mansonia* mosquitoes than non-pregnant women [32,33], and hosts that recently consumed bananas or beer receive more mosquito visits [34,35]. Similarly, host size can also influence cue-oriented vector and parasite attack rates. Haematophagous flies (*Carnus hemapterus*) prefer larger nestling European bee-eaters (*Merops apiaster*) [36], and *Culex* mosquitoes are more attracted to odours of adults than nestling house sparrows [37]. In some cases, vector bias toward larger individuals is driven by quantitative differences in host metabolites. *Macrocheles* mites, for example, preferentially feed upon *Drosophila* fruit flies that produce higher quantities of CO_2 [38]. Mixed-species assemblages with individuals from species varying in size or metabolic rates could create similar biases.

Acoustically oriented vectors and parasites home in on host calls and are often attracted to particular call variants. Ormiini parasitoid flies, for example, prefer specific cicada and cricket song variants. *Therobia leonidei* are biased toward male bush-crickets (*Poecilimon thessalicus)* producing songs that are also more attractive to females [39]. Similarly, *Ormia ochracea* preferentially orient to male variable field cricket (*Gryllus lineaticeps*) songs with faster and longer chirps [40] and to field crickets (*Teleogryllus oceanicus)* producing longer chirps and shorter interpulse intervals [41]. Finally, tachinid fly parasitoids of the bush-cricket *Mecopoda elongata* are more likely to attack crickets producing one of three alternative song types [42]. Attraction biases are also apparent in eavesdropping vectors. Frog-biting midges (*Corethrella* spp.), which transmit trypanosomes [43,44], preferentially attack túngara frogs (*Engystomops pustulosus*) producing more complex calls [45].

(b) Interspecific variation in host-emitted cues and vector and parasite biases

Cue-oriented vectors and parasites can exhibit strong species-specific host biases. To study patterns in host use, molecular analysis is often used to identify the source of recent blood meals [46]. While this method is vital in documenting the outcome of vector foraging behaviour in the wild, it is influenced by the relative abundance of host species within a community. Here, we focus on field and laboratory choice tests as more direct measures of biases in host-seeking behaviour. We include bloodmeal identification studies when the relative abundances of local hosts are also quantified.

Biases of mosquitoes either towards or against human hosts have been widely studied [9]. In choice trials, where human or calf odour was paired with CO_2, *Anopheles* and *Aedes* mosquitoes were more likely to approach human over calf hosts, while *Culex* mosquitoes showed higher visitation to calf odours [47]. Studies with wild mosquito and host communities also show clear feeding biases. Bloodmeal analyses reveal that some hosts are attacked more often than expected given their relative abundances. *Culex* mosquitoes, considered principal vectors of WNV, overuse American robins relative to their local abundances and abundances of other avian hosts [48].

Several other cue-oriented dipteran vectors feed on a narrower subset of species within a broader range of available hosts. Tsetse flies (*Glossina* spp.) are more attracted to chemical cues of non-human mammals than those of humans [49]; and sand flies (*Phlebotomus* spp.) are differentially attracted to humans and various domestic and wild hosts [50]. Similarly, ixodid ticks exhibit strong biases for the odours of certain host species over other viable hosts both in the laboratory and field [51,52].

Acoustically oriented vectors also discriminate between multiple host species based on their calls. Attack biases of frog-biting midges for some call types extend beyond within-species variation [53,54], and while flesh flies (*Emblemasoma erro*) parasitize a wide range of cicadas, they prefer hosts that call with certain amplitude and spectral characteristics [55]. In populations where *Ormia ochracea* exploits multiple cricket species, these flies express interspecific host song preferences, but preference strength is lower compared to flies from single-host populations [56]. *Therobia leonidei* flies, by contrast, are more attracted to *Poecilimon* bush-crickets with longer songs, regardless of whether they are native to the region, perhaps because these songs are easier to detect and localize [57].

The pattern of interspecific preferences in cue-oriented vectors and parasites is not confined to terrestrial environments.

In choice trials, trematode parasites (*Ribeiroia ondatrae*, *Alaria marcinae* and *Cephalogonimus americanus*) had strong preferences for particular frog and newt tadpole host species [58]. Similarly, in separate choice trials, trematodes (*Euparyphium albuferensis* and *Echinostoma friedi*) differentially infected four species of freshwater snails [59]. Ultimately, disparities in host species' attractiveness provide the raw material for host-use biases in mixed-species assemblages.

(c) Effects of vector and parasite host-use biases on disease dynamics

Mathematical models demonstrate that disparities among host types in attack rates, such as those described above, can strongly affect community-level disease dynamics. In cases where hosts vary in competence, amplification generally results from attack biases toward more competent hosts, and dilution from biases toward less competent hosts [60–62]. While models often assume that vector and parasite biases are static, these biases and their effects on disease dynamics can change. Some lineages of dog ticks (*Rhipicephalus sanguineus*), for example, shift to more strongly prefer human versus canine hosts as ambient temperatures rise, increasing disease transmission risks to humans [63]. Some pathogens are hypothesized to manipulate infected vectors to preferentially target higher-competence hosts [64]; this is supported by experiments examining the foraging behaviour of malaria-infected mosquitoes and by modelling that predicts small infection-induced behavioural changes can elicit substantial effects on transmission [65].

4. Differential attraction and disease dynamics in mixed-species assemblages

While many studies show that cue-oriented vectors and parasites can differentially assort among hosts species, less is known about how local mixed-species assemblages might influence vector and parasite recruitment to those communities. Because host species are often unevenly distributed across the landscape as they aggregate around resources or actively associate with other individuals, mixed assemblages emitting particularly attractive cues or combinations of cues could experience higher attack rates from cue-oriented vectors and parasites through mechanisms operating at the assemblage and individual-host levels. At the assemblage level, species composition and relative abundance of different host cues could alter the number of vectors and parasites drawn to the group [66,67]; we call this '*assemblage attraction*'. The combination of cues in an assemblage could also draw vectors and parasites more quickly, influencing their search efficiency. As vectors spend less time searching, cues can ultimately affect the rate at which they bite hosts (*bite rate*). These factors are rarely addressed in mixed-species assemblages.

Once within the assemblage, vectors and parasites use cues to choose between the available host species, a process we call '*bite attraction*'. This term is similar to 'preference' used in Trillo *et al*. [66] to describe the proportion of eavesdroppers attacking one prey species over another within mixed-species assemblages. Although 'preference' is commonly used in vector-borne disease studies, it has been used to describe both the relative number of bites experienced by different host types in the wild, where encounter rates with vectors vary, as well as the result of choice trials, where host availability to vectors is carefully controlled. Here, we use 'bite attraction' rather than 'preference' to focus exclusively on contexts where vectors and parasites have opportunities to assess multiple host types. Both 'assemblage attraction' and 'bite attraction' are broadly defined to include perceptual, cognitive and motor processes underlying choice. Vector and parasite decisions at these two levels may combine to influence overall infection rates [66].

The range over which cues are detected by vectors and parasites may vary between host species. Vectors and parasites attracted from a distance by cues with large ranges may, once at the assemblage, either continue to target these cues or switch to cues with more restricted ranges. Thus, cues of one species that attract greater numbers of vectors or parasites to an assemblage may also increase bite attraction to this same species within the assemblage, increasing its disease risk relative to other species. The contrasting situation, in which one host species' cue is more attractive to enemies at a distance, while another hosts' cue attracts more bites within the assemblage, is also plausible and may create nuanced patterns of infection. Mismatches in 'assemblage attraction' and 'bite attraction' may occur when vectors and parasites sequentially assess different cues as they approach a host. Flesh flies in the genus *Emblemasoma*, for example, are attracted to acoustic cues when locating cicada hosts but rely on visual cues in close proximity [55]. Likewise, *Aedes* and other mosquitoes are drawn to hosts from a distance via CO_2 cues and volatile skin odorants but use visual cues, temperature and humidity surrounding the host's body at closer distances [30,68,69].

Considering the influence of assemblage attraction on multi-host disease dynamics requires new focus on the long-range assessment and localization behaviours of cue-oriented vectors and parasites. Vector and parasite biases are often tested over short distances, but host-seeking behaviour often involves distant targets. For example, tsetse flies (*Glossina* spp.) respond to hosts' odour cues from 30–90 m away and to visual cues at distances over 100 m [70,71], whereas tabanid flies (horse flies) travel toward odours emanating from hosts up to 80 m away [72]. Mosquitoes vary in the range at which activation and orientation behaviour towards the host are elicited, but some can navigate toward non-host visual cues up to 35–40 m away [68]. Below we discuss a case study where there is differential attraction to host species' mating signals used as cues by eavesdropping midges. This case study informs our mathematical model, which investigates how differential vector attraction alters disease dynamics.

5. Case study: mixed-species frog choruses and eavesdropping vectors

Many anurans aggregate in choruses to advertise to mates. Because these aggregations often form in wetlands and water bodies used as breeding habitat, it is common for multiple species to chorus in the same location [73]. Such mixed-species assemblages can attract frog-biting midges (Corethrellidae) and mosquitoes (Culicidae) that cue in on calls of male frogs and ultimately bite them. Here we focus on a community of frogs in Panama that often form mixed-species assemblages, and the eavesdropping frog-biting midges associated with them. In this community, two

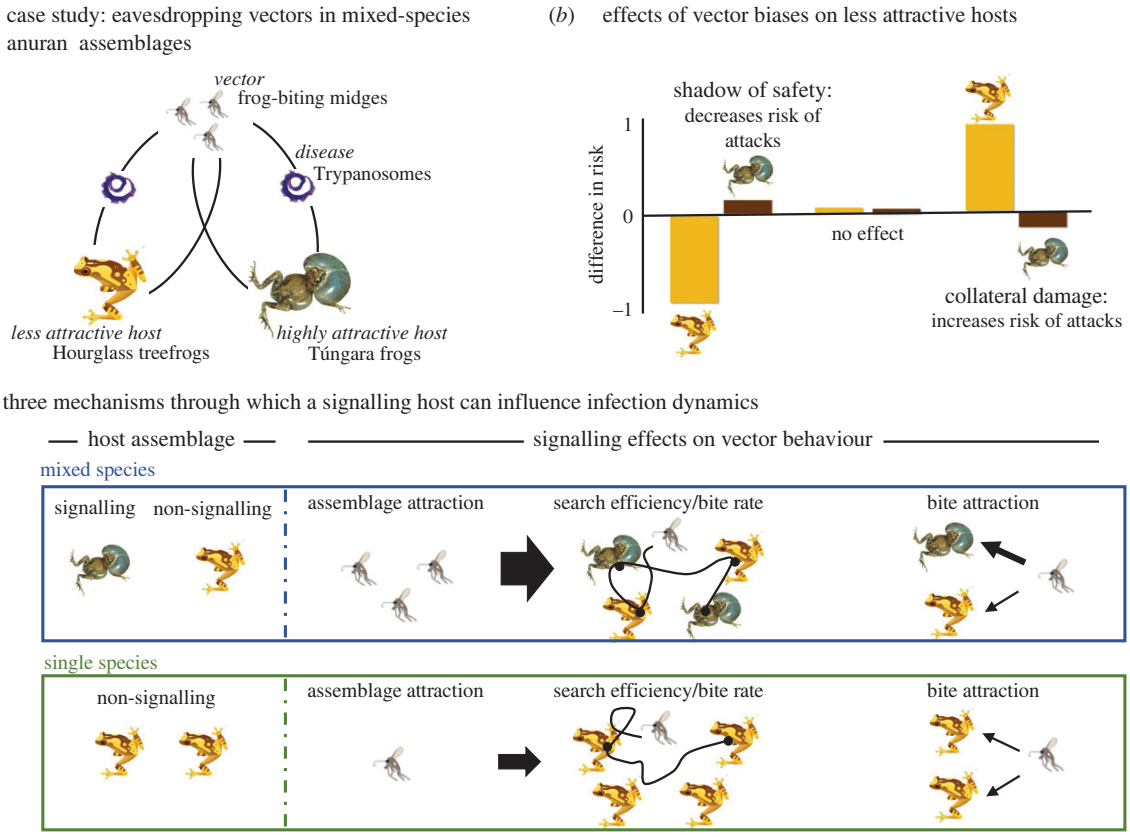

Figure 1. Schematic of a two-host, one vector case study, potential outcomes of vector attraction bias and mechanisms underlying the model developed in this study. (*a*) Case study examining hourglass treefrogs and túngara frogs, whose mating calls differentially attract frog-biting midges, vectors of trypanosomes. Ellipses depict the life cycle of the trypanosomes, which use frogs as their only host. (*b*) Effects of vector bias on attack risk experienced by species producing less attractive cues. The *y*-axis represents the difference in attack risk when the less attractive species signals close to a more attractive heterospecific versus close to a conspecific. While collateral damage has been shown in this system, both processes could occur in mixed-species assemblages. (*c*) Mechanisms included in the model by which attractive signalling hosts affect disease dynamics. Top and bottom panels contrast mono- and mixed-species assemblages, depicting more vectors attracted to the assemblage, higher search efficiency and higher bite attraction to signalling hosts in mixed-species assemblages. Arrow thickness represents the strength of attraction.

anuran species, túngara frogs (*Engystomops pustulosus*) and hourglass treefrogs (*Dendropsophus ebraccatus*), often aggregate to call at the same puddles and ponds [74], where cue-oriented midges (*Corethrella* spp.) are attracted in large numbers to frog calls [45] rather than CO_2, or other cues commonly used by haematophagous vectors [75].

Frog-biting midges attack multiple frog species in a given community [53,54,76]. Calls produced by túngara frogs, however, are more attractive to the midges than those of treefrogs, a bias that affects attack rates suffered by these species when calling from the same assemblage (figure 1*a*). Hourglass treefrogs calling nearby túngara frogs attract more frog-biting midges than if calling near conspecific males [77]. The effect of incurring higher vector attack risk by calling next to a more attractive signaller was termed 'collateral damage' (figure 1*b*). The level of collateral damage for hourglass treefrogs depends on the relative abundance of túngara frogs in the assemblage [78]. Thus, the relative abundance of species highly attractive to cue-oriented vectors and parasites in mixed-species assemblages may influence risk for less attractive species.

The opposite pattern, where calling nearby a more attractive signaller is beneficial to less attractive species, may arise if vectors attracted to the assemblage preferentially attack signallers of the more attractive species, overlooking less attractive individuals calling nearby. This mechanism was termed 'shadow of safety' (figure 1*b*). The 'collateral damage' and 'shadow of safety' scenarios present contrasting consequences resulting from vector and parasite biases that could contribute to asymmetries in assemblage and bite attraction. Therefore, at a given mixed-species assemblage, differential effects among pairs of species could result in diverse landscapes, with collateral damage and shadow of safety effects scattered across the network of species interactions. Ultimately, this framework can guide investigations of how cue-oriented vector attack rates vary due to assemblage composition.

Frog-biting midges are vectors of trypanosome parasites [43,44] and other pathogens. Trypanosome transmission can result from fecal deposits touching wounds, consumption of infected vectors by hosts or, in the case of frog-biting midges, during blood feeding via saliva [77]. Therefore, differential attack rates at conspecific versus mixed-species choruses likely shape trypanosome infection rates for hosts. Anuran trypanosomes are often found in a single family of hosts [79,80], suggesting limited ability of trypanosomes to survive across the many hosts bitten by frog-biting midges. In the case of choruses of túngara frogs and hourglass treefrogs, increased vector bite rates for dead-end hosts could result in dilution. Further studies, however, are needed

to better understand host competency and trypanosome transmission dynamics within this species pair.

Phenomena illustrated in this case study may be more common than previously anticipated. Cue-oriented haematophagous flies are common vectors at anuran choruses. Eavesdropping on frog calls to obtain a bloodmeal is a widespread strategy among Diptera, recognized in over 191 species across 23 genera [76]. Several of these species can transmit pathogens. This includes *Culex peccator*, a carrier of Eastern Equine Encephalitis virus [81], and *Uranotaenia unguiculata*, a vector of WNV [82]. Frog-biting midges are also suspected vectors of *Batrachochytrium dendrobatidis* [83], a chytrid fungus implicated in anuran population declines worldwide [84]. Though examinations of vector-borne disease dynamics in anurans are still limited, they offer promising opportunities to broaden our understanding of disease transmission in mixed-species assemblages.

Overall, hosts across taxonomic groups that participate in mixed-species assemblages are susceptible to suffer from collateral damage or could benefit from shadow of safety effects, and these changes in vector attack rate could influence transmission dynamics in previously unanticipated ways. Lessons from the case study presented here likely extend to mixed-species assemblages in a variety of taxa. While some vectors and parasites in those systems use cues in other sensory modalities, biases in the sensory filters that mediate detection and responses to host-emitted cues are likely universal.

6. Model

To explore the consequences of cue exploitation for disease dynamics in mixed-species assemblages, we develop a simple mathematical model based on the Ross-Macdonald model for vector-borne disease dynamics [85] for an assemblage consisting of two host species: a 'signalling' host that emits cues that vectors detect (with population size H_1); a second non-signalling host (H_2); and a biting vector (V). While we refer to the first host species as 'signalling', the model is applicable to any host-emitted cue that the second host does not produce. Hosts and vectors are tracked by their infection status: susceptible (S_j) or infected (I_j) ($j = 1, 2, V$). The equations describing population and infection dynamics are:

$$\frac{dS_1}{dt} = m_1(K_1 - S_1) - a_{1V}b\left(\frac{pH_1}{pH_1 + H_2}\right)\frac{S_1}{H_1}I_V, \tag{6.1}$$

$$\frac{dI_1}{dt} = a_{1V}b\left(\frac{pH_1}{pH_1 + H_2}\right)\frac{S_1}{H_1}I_V - (m_1 + v_1)I_1, \tag{6.2}$$

$$\frac{dS_2}{dt} = m_2(K_2 - S_2) - a_{2V}b\left(\frac{H_2}{pH_1 + H_2}\right)\frac{S_2}{H_2}I_V, \tag{6.3}$$

$$\frac{dI_2}{dt} = a_{2V}b\left(\frac{H_2}{pH_1 + H_2}\right)\frac{S_2}{H_2}I_V - (m_2 + v_2)I_2, \tag{6.4}$$

$$\frac{dS_V}{dt} = \lambda - m_V S_V - bS_V\left(a_{V1}\left(\frac{pH_1}{pH_1 + H_2}\right)\frac{I_1}{H_1} + a_{V2}\left(\frac{H_2}{pH_1 + H_2}\right)\frac{I_2}{H_2}\right) \tag{6.5}$$

and

$$\frac{dI_V}{dt} = bS_V\left(a_{V1}\left(\frac{pH_1}{pH_1 + H_2}\right)\frac{I_1}{H_1} + a_{V2}\left(\frac{H_2}{pH_1 + H_2}\right)\frac{I_2}{H_2}\right) - m_V I_V, \tag{6.6}$$

where for host j, K_j is the species' maximum disease-free population size or carrying capacity and m_j and v_j are respective *per capita* rates of natural and disease-induced mortality. Vectors are attracted to the assemblage at rate λ and bite hosts at a rate b; p describes attraction to the signalling host within the assemblage that results in biting (i.e. how much more likely the signalling host is to be bitten than the non-signalling host if they were equally abundant); and a bite causes infection to species j from species k with probability a_{jk}. Cues from the signalling host can alter infection dynamics through three mechanisms (figure 1*c*): (i) increasing the vector numbers attracted to the mixed-species assemblage, λ (i.e. *assemblage attraction*); (ii) increasing vector search efficiency, and thus *bite rate*, b; and (iii) increasing *bite attraction* to the signalling host within the assemblage (p). We assume that assemblage attraction and bite rate scale with the number of signalling hosts. Defining x_0 as the value of parameter x ($x = \lambda, b$) in the absence of cues, and θ_x as the fold change in x when the signalling host is at carrying capacity (figure 2), these can be expressed as:

$$x = x_0\left(1 + (\theta_x - 1)\frac{H_1}{K_1}\right). \tag{6.7}$$

Locally, we assume that host-emitted cues alter bite attraction to signalling hosts, so that

$$p = \theta_p p_0, \tag{6.8}$$

where p_0 is the bite attraction to signalling hosts within the assemblage in the absence of cues, and θ_p is the fold change in bite attraction due to cue detection.

To focus on how vector responses to host cues influence dynamics, we analyze a simplified model where in the absence of signalling, the two hosts are ecologically and epidemiologically identical (i.e. they have the same carrying capacity and mortality rates; hosts and vectors have an equal probability of infection from a bite, and hosts experience the same bite rates). Under these assumptions, we determine the effect of host cues on outbreak potential by calculating the pathogen basic reproduction number (electronic supplementary material S1–S3), the number of new infections arising from an index case in an infection-naive assemblage:

$$R_0 = R_0(0)\theta_b\sqrt{\frac{2\theta_\lambda(1 + \theta_p^2)}{(1 + \theta_p)^2}}, \tag{6.9}$$

where $R_0(0)$ is R_0 in the absence of signalling effects, θ_b and θ_λ describe how bite rate and assemblage attraction increase with signalling host density, and θ_p describes how bite attraction increases with individual host signals. This expression shows that outbreak potential increases most rapidly (linearly) with signalling effects on bite rate, sub-linearly with assemblage attraction, and increases but saturates with bite attraction (figure 3*a*). Combined effects of signalling on two or more of these processes act to further increase R_0, with bite attraction to the signalling host having the weakest positive effect.

We solved the model numerically to determine the species-level effects (electronic supplementary material, S4, figure S1) and assemblage-level effects of signalling on equilibrium infection prevalence in the assemblage (fraction of hosts in the assemblage that are infected), assemblage size (total number of hosts in the assemblage) and assemblage

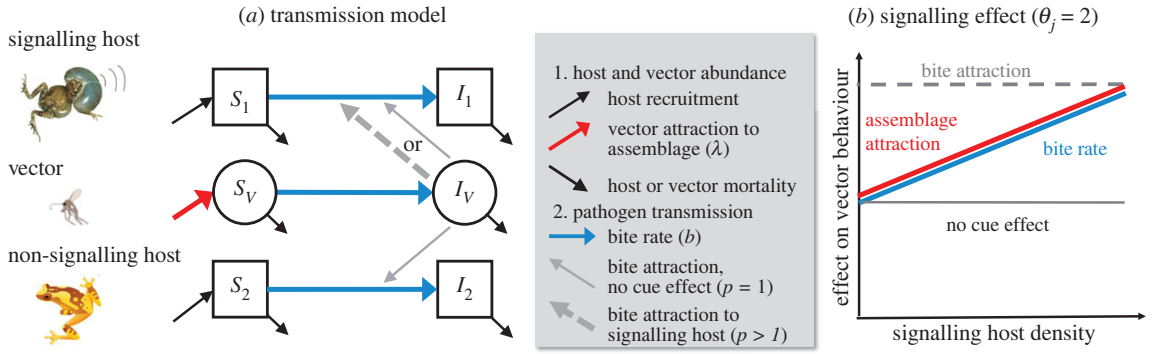

Figure 2. Schematic showing the model structure. (*a*) Transmission model: boxes represent host abundance in the assemblage by infection status (susceptible or infected): signalling hosts (S_1, I_1) and non-signalling hosts (S_2, I_2); circles denote vector abundance (S_V, I_V). Arrows represent transitions between classes due to recruitment, mortality or transmission. Solid, coloured arrows represent processes whereby signalling alters vector attack on the assemblage, through increased vector attraction (red) or search efficiency (i.e. bite rate; blue); dashed arrows indicate effects of signalling on bite attraction to the signalling host. (*b*) Signalling effect: functional relationships between signalling and vector foraging behaviours. Vector attraction rate to the assemblage and bite rate are assumed to increase linearly with host density. Bite attraction to signalling hosts is density independent. Relationships illustrated for a signalling effect of 2, corresponding to a doubling of vector attraction and biting rates when signalling hosts are at carrying capacity, and a doubling of bite attraction to signalling hosts.

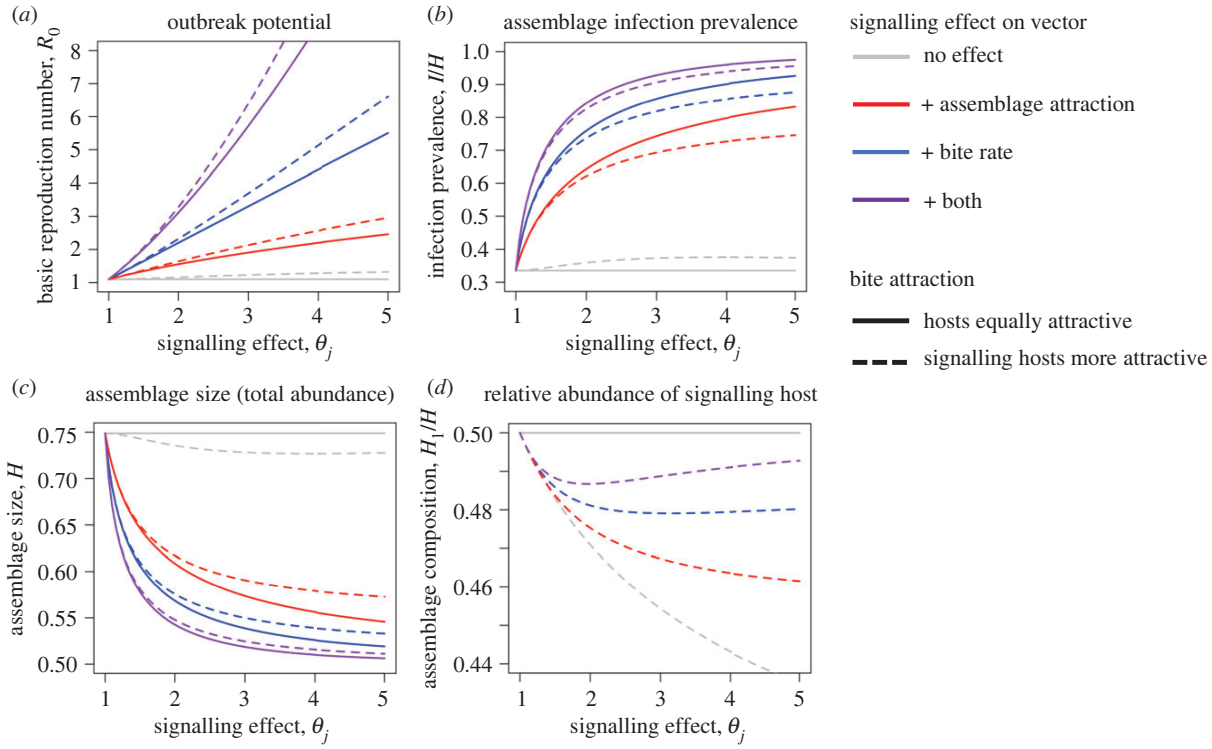

Figure 3. Effect of the strength of signalling host cues (i.e. the fold change in effect size on vector behaviours (θ_j, $j = b,\lambda,p$) on (*a*) pathogen outbreak potential in a disease-free assemblage (R_0), and assemblage-level outcomes; (*b*) infection prevalence (fraction of assemblage infected, I/H); (*c*) assemblage size (total number of hosts, H); and (*d*) assemblage composition (fraction of signalling hosts in the assemblage, H_1/H). Each line corresponds to a scenario where different vector behaviours are altered by host cues, independently or together. Colours represent assemblage-level effects on vector attraction (λ, red), bite rate (*b*, blue) or both (purple). Solid lines indicate no differential attraction to signalling hosts (p), and dashed lines indicate that cues increase bite attraction to signalling hosts within the assemblage. The parameter values used are detailed in electronic supplementary material S2.

composition (relative abundance of signalling hosts in the assemblage; figure 3*b–d*). Infection prevalence has an increasing, saturating relationship with the strength of each signalling effect (figure 3*b*) and assemblage size has a decreasing, saturating relationship (figure 3*c*). Signalling effects on bite rate cause the most pronounced increase in prevalence (and thus the strongest regulation of assemblage population size), followed by effects on assemblage attraction, and their combined effects have the strongest positive effect overall. Cues that increase bite attraction to signalling hosts within the assemblage cause a small increase in assemblage prevalence and decrease in assemblage size. However, in combination with other signalling effects, bite attraction slightly decreases assemblage prevalence and reduces the negative effects on assemblage size. This is because, while bite attraction within the assemblage always

increases prevalence in the signalling host (electronic supplementary material, figure S1a), the concurrent reduction in prevalence in the non-signalling host through bite redistribution (electronic supplementary material, figure S1b) causes a net reduction in assemblage prevalence.

The effects of signalling on bite attraction are most strongly observed on assemblage composition. Signalling effects on bite rate or assemblage attraction do not change species composition without bite attraction to the signalling host within the assemblage, because vectors distribute bites equally among hosts once at the assemblage. However, bite attraction to signalling hosts decreases the relative abundance of the signalling host (figure 3*d*). This decline is most pronounced when cues affect bite attraction only (figure 3*d*). When cues also influence bite rate and assemblage attraction, the effects on host composition are weaker and can become nonlinear (i.e. the relative abundance of the signalling host is lowest at intermediate signalling effects). This is because signalling-induced increases in bite rate and assemblage attraction cause increases in vector attacks to both host types, and thus infection prevalence (electronic supplementary material, figures S1a,b). This, in turn, decreases both hosts' abundance (electronic supplementary material, figures S1c,d) and counteracts the effect of bite attraction to the signalling hosts.

Altogether, results from this simplified model suggest that host signalling effects on vector host-seeking behaviour can have profound impacts on pathogen outbreak potential, assemblage size and composition. These effects could be further exacerbated in host assemblages where host species differ in life history and competence for infection. Consideration of biases in cue-oriented vectors and how they are expressed at different scales may contribute to understanding disease-diversity relationships in multihost-vectored pathogen systems.

7. Discussion

(a) Implications for transmission dynamics in assemblages

Mirroring previous studies, our model indicates that the relative attractiveness of host species can strongly influence disease dynamics. We focus here on contexts where hosts are spatially concentrated in mixed-species assemblages and conceptualize foraging behaviour of cue-oriented vectors and parasites as having two stages: (i) attraction to the assemblage, where the relative abundance of different host cues can mediate the number of vectors or parasites drawn to the group (assemblage attraction) and/or their foraging efficiency (bite rate), and (ii) attraction to a host within the assemblage, where vectors or parasites use cues to choose and ultimately bite hosts (bite attraction). Our model highlights that an interplay between these cue effects on vector abundance and foraging can affect disease prevalence as well as the differential risks faced by each host species within the assemblage. Further, when disease induces host mortality, vector biases in attack rates could ultimately feed back to shape assemblage structure.

The findings and concepts presented in this study are broadly applicable. Mixed-species assemblages span a broad range of taxa and contexts and act as arenas for vector-borne and zoonotic diseases [21,23,25,27]. Natural contexts for such assemblages are diverse and include mixed flocks or communal roosts of birds and bats, herds of grazing ungulates, fish shoals or aggregations surrounding submerged structures such as reefs, vertebrate or invertebrate communities within vernal pools, scavengers and detritivores at carcasses and breeding aggregations of amphibians. Mixed-species assemblages also result from human activity and include communally housed livestock, cohabitation of domestic animals and humans, markets selling live animals, mixed herds of grazing livestock and resource subsidies for wildlife and zoos. In any of these contexts, the mixture of cues propagating to foraging enemies outside of an assemblage, and the cues used to assess hosts once at the assemblage, could influence overall infection rates and infection prevalence in each host species.

One context where assessing the consequences of assemblage and bite attraction processes for transmission may be particularly fruitful is mating aggregations of closely related species, where assemblage members may share vectors and have similar competence for some pathogens [86]. As hosts are producing mating signals, however, they are likely to differ in their attractiveness to eavesdropping vectors and parasites. Frog species in the *Engystomops petersi* complex in the Amazonian Ecuador call from the same ponds [87], and produce mating calls that vary in complexity [88,89]. Given that similar variation in call complexity results in differential attraction of frog-biting midges in congeneric túngara frogs [45], mixed-species assemblages in this complex may experience differential pathogen exposure modulated by asymmetries in assemblage and bite attraction. Similarly, in temperate regions, eavesdropping *Culex territans* mosquitoes bite frogs from closely related species (*Lithobates clamitans*, *L. catesbiana* and *L. virgatipes*) that often call at the same choruses, and share the same trypanosome species [90].

(b) Future research directions

Our model and conceptual framework for understanding disease dynamics in mixed-species assemblages suggests several directions for further research. One intriguing result from the model is that cue-induced vector biting biases can alter assemblage size and composition, with potentially important ecological and evolutionary consequences. Given conservation and public health threats from emerging and zoonotic vector-borne pathogens, this warrants future exploration. Additionally, because host competence, tolerance and avoidance behaviours can greatly influence disease dynamics, especially when paired with vector or parasite bias for certain host-types [62], incorporation of interspecific variation in host ecological and epidemiological features into our model could be important for predicting and managing zoonotic risk. As disease control strategies often focus on a single or few hosts that play comparatively large roles in amplifying pathogen transmission [91], these could include highly competent hosts that are attractive to vectors within an assemblage. Our results suggest that host types whose cues disproportionately attract vectors to an assemblage can have a strong amplifying effect even if they are bitten less, and could, thus, be apt targets for control.

Our understanding of how biases in attraction change as cue-oriented vectors or parasites approach a host is still limited. The 'activation' and 'orientation' phases of foraging have been well studied in some mosquitoes [69], and early experiments with tsetse flies isolated different cues used for long- versus

short-range attraction [71]. Extending this approach to a broader range of vectors and parasites attacking hosts in both mono- and mixed-species assemblages, and a more thorough quantification of the active spaces of host-emitted cues, are necessary to deepen our understanding of vector and parasite attraction.

Empirical data are needed from explicit comparisons of infection prevalence for given species in mono-specific versus mixed-species assemblages in both wild and domestic contexts. Differences in prevalence between these contexts provide opportunities to investigate the relative importance to transmission dynamics of cue-modulated attraction to the assemblage and attraction within the assemblage. Moreover, understanding how these mechanisms alter disease dynamics when housing particular combinations of domestic or wild animals together will inform development of policies to minimize potential disease outbreaks. Consideration of changes in ecological interactions and assemblage composition due to resource supplementation by humans, potentially connecting wild and domestic host species [92], will also be critical to understanding how vector and parasite biases modulate disease dynamics in anthropogenic environments.

Finally, although we focused on disease dynamics in mixed-species assemblages, our model and conceptual framework integrating assemblage attraction, search efficiency/bite rate and within-assemblage bite attraction are applicable to any gathering of multiple host types. This includes conspecific groupings where vectors or parasites show differential attraction to multiple host types, such as infected and healthy, male and female or larger and smaller individuals. The relevance of our model will be determined by the degree of variation in host-emitted cues among assemblage members, whether those cues propagate beyond the assemblage, and whether cue variation results in attraction biases and changes in search efficiency by vectors and parasites. In such contexts, our model brings new perspectives aimed at contributing to a more complete understanding of transmission dynamics.

Data accessibility. No data are included in this paper, but R code for the model is included as electronic supplementary material [93].

Authors' contributions. P.A.T.: conceptualization, investigation, project administration, visualization, writing—original draft, writing—review and editing; X.E.B.: conceptualization, investigation, visualization, writing—original draft, writing—review and editing; R.J.H.: conceptualization, formal analysis, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. The authors acknowledge funding from the National Science Foundation (grant no. RJH DEB-2022897; XEB IOS-2054636). P.A.T. acknowledges funding from the Smithsonian Institution (Scholarly Studies Award).

Acknowledgements. We are grateful to E. Goodale for the invitation to contribute to this Special Issue on 'Mixed-species groups and aggregations: shaping ecological and behavioural patterns and processes'. We are also thankful to M.S. Caldwell for constructive comments on the manuscript and support that improved the quality of this work. Finally, we thank our families for their patience while we worked on final revisions for this manuscript during the holidays.

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