The role of antiparasite treatment experiments in assessing the impact of parasites on wildlife

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The role of antiparasite treatment experiments in assessing the impact of parasites on wildlife

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It has become increasingly clear that parasites can have significant impacts on the dynamics of wildlife populations. Recently, researchers have shifted from using observational approaches to infer the impact of parasites on the health and fitness of individuals to using antiparasite drug treatments to test directly the consequences of infection. However, it is not clear the extent to which these experiments work in wildlife systems, or whether the results of these individual-level treatment experiments can predict the population-level consequences of parasitism. Here, we assess the results of treatment experiments, laying out the benefits and limitations of this approach, and discuss how they can be used to improve our understanding of the role of parasites in wildlife populations.

The impact of parasites on individuals and wildlife populations

Parasites (defined broadly to include any disease-causing organism, from viruses and bacteria to parasitic helminths and ectoparasites) are ubiquitous in natural systems and can have significant effects on host survival and reproduction [1,2]. Conventional wisdom suggests that parasites negatively affect host fitness due to 'disease', the pathological state caused by parasite infection, growth, and replication within the host and by damage caused by the response of the host to infection. The potential impact that parasites can have on their wildlife hosts is most often apparent when a newly emerging disease sweeps through a population of conservation or economic importance, causing, in some cases, devastating losses [3,4]. Increasingly, we see examples in wildlife, such as chytridiomycosis decimating amphibian populations [5,6], ash dieback changing the distribution of ash trees [7], marine mammal morbillivirus causing population crashes [8], and a suite of pathogens associated with the collapse of honeybee colonies [9]. Typically, these occur through the introduction of a novel parasite into a naïve host population either directly, or indirectly through host species invasions, in which a host species moves into a new area, bringing its parasite community with it [10]. In these cases, there is a clear effect of the parasite on both the individual hosts in the new area (typically they die, or are otherwise severely and obviously affected) and their populations (the abundance is often dramatically reduced). These devastating epidemics rightly attract much attention and the seeming increase in these events has raised serious concern about the role of infectious disease for wildlife conservation [3,11,12].

However, less commonly considered is the 'everyday' impact that endemic parasites have on their wildlife host individuals, or the role they have in regulating or driving the dynamics of their native host populations. This may be either because parasites, by living inside their hosts, are literally overlooked or, by typically (although not always) being physically small, they are assumed to be inconsequential (but see [13]). Furthermore, unlike predator–prey relationships, where the interaction is clear (an individual gets eaten), the negative effects of endemic parasites on their hosts may be covert or sublethal, with hosts often being left alive and with no obvious adverse effects. Hence, quantifying (or even noticing) the impact of parasites on their host individuals is difficult. In addition, even if parasites are accurately quantified it can be hard to disentangle the cause-and-effect relationship between levels of parasitism and observed measures of host condition or fitness, for example, if weaker or sicker individuals tend to get higher levels of infection than healthy or stronger hosts [14]. These challenges are exacerbated when we move to assess the impact of parasites at the host population level. Historically, it has been argued that endemic diseases are unlikely to have a major role in regulating natural populations (e.g., [15]). However, the potential regulatory force of parasites on their host populations has been clearly demonstrated theoretically since the pioneering work of Anderson and May [16,17]. However, this body of theory also shows that detecting or demonstrating those impacts in natural host populations is far from straightforward ([18,19]; Box 1). In particular, this theory suggests that attempting to infer

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Box 1. Theoretical expectations of the effects of parasitism on host populations

Here, we present an overview of epidemiological theory that reveals the potentially complex relationships between parasite virulence (the detrimental effect that a parasite has on its individual host), prevalence of infection, and consequences for the population dynamics of the host [18,19].

The basic model considers a directly transmitted microparasite (e.g., a virus or bacteria) infecting a single host species (Equations I and II):

$$\frac{dU}{dt} = aH - H(b + BH) - \beta UI \quad [I]$$

$$\frac{dI}{dt} = \beta UI - I(b + BH) - I\alpha \quad [II]$$

where U is the density of uninfected hosts, I is the density of infected individuals, and H is the total population density ($H = U + I$). Hosts are born at per capita rate a and die at a density-dependent rate $b + BH$ (where B is the strength of density dependence); in the absence of the parasite, the host population stabilises at carrying capacity, $K = (a - b)/B$. Uninfected hosts become infected at density-dependent transmission rate β, and infected hosts suffer parasite-induced mortality at rate α. This model predicts a nonlinear relation between virulence at the individual host level (α) and host density at the population level (Figure IA, unbroken black line). In particular, the greatest population-level impact occurs at relatively low levels of individual-level virulence. Correspondingly, the predicted prevalence of infection rapidly declines with increasing virulence (Figure IA, broken red line). Together, these two results show that the greatest level of host population suppression is caused by a relatively benign parasite at the individual level, with a relatively low population-level prevalence. A qualitatively similar pattern is seen if we consider the parasite to be a macroparasite (e.g., parasitic helminth [16] and Box 2; Figure IB, where the broken red line shows mean parasite burden, rather than parasite prevalence).

This basic model can be modified to consider various alternative scenarios. For example, if the parasite affects host fecundity (e.g., by reducing body condition) rather than host survival, we see a rapid decline in host population size with increasing virulence, and no recovery of the host population at high virulence levels (Figure IC). Furthermore, the reduction in parasite prevalence with increasing virulence is less dramatic than seen under mortality-inducing parasites. Hence, parasites that greatly affect host fecundity rather than survival can have a substantial impact on the host population, with little impact on their own prevalence.

Finally, parasites may transmit in a frequency-dependent manner (e.g., sexually transmitted diseases; [60,61]). This can be modelled by replacing the transmission terms in both Equations I and II (βUI) with $\beta UI/H$. In this case, the parasite can greatly suppress the host population, potentially driving it locally extinct at low-to-intermediate virulence levels (Figure ID). However, high virulence levels can result in the parasite driving itself extinct. Notably, in this scenario, unlike that seen under density-dependent transmission, the parasite is able to maintain a relatively high prevalence while still suppressing the host population. In this case, reasonably highly prevalent parasites may be indicative of high potential for host population suppression.

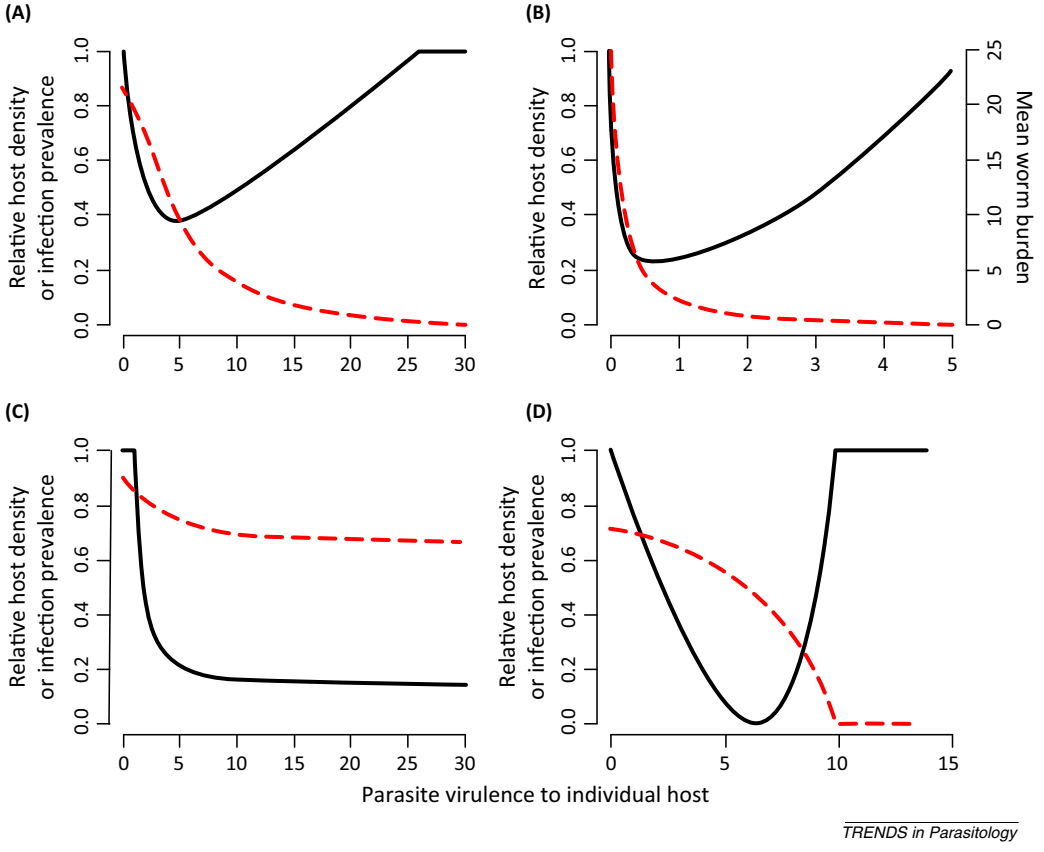

Figure I. Predicted scaling relations between parasite virulence at the individual level (additional host mortality rate or reduced host fecundity rate due to infection) and equilibrial host population abundance relative to an uninfected population (unbroken black lines) and parasite prevalence or mean burden (broken red lines). **(A)** A microparasite that affects host survival; **(B)** a macroparasite (helminth) that affects host survival (broken red line shows mean parasite burden); **(C)** a microparasite that affects host fecundity; and **(D)** a microparasite with frequency-dependent ('FD') transmission.

parasite impacts purely from observational data, as has historically been the case, can be challenging.

To overcome these challenges, an increasing number of studies have used antiparasite treatment experiments of wildlife hosts to perturb levels of parasitism and more directly quantify the impacts of infection. Given the increasing interest in adopting treatment approaches to understand host–parasite dynamics in their natural setting, it is timely to review the ways in which they are being used and their findings. Here, we collate examples of such studies from the literature to assess the reported effects of treatment on the target parasites, host health, and, where possible, host population dynamics. We highlight the power of these experimental approaches for understanding the impact of infection at the individual level, while acknowledging their limitations, and focus on how the combination of theory and large-scale experiments may improve our understanding of the consequences of parasitism for wildlife populations.

The use of treatment-based approaches for inferring the individual-level impacts of parasitism

We collated 51 studies reporting a total of 66 antiparasite treatment experiments on noncaptive wildlife populations (several papers reported experiments with more than one drug; Table 1). From the information provided in these papers we assessed: (i) the wildlife host–parasite systems and antiparasite drugs that have been used in these studies; (ii) how effective the drug treatment approaches were at reducing target parasites; (3) whether treatment improved individual host health and fitness; (4) whether treatment had any detectable effects on nontarget, co-infecting parasites; and, lastly, (5) whether treatment studies elucidated the population-level consequences of parasitism.

Which drug treatments have been used in wildlife studies and in which systems?

A range of antiparasitic drugs have been used to reduce parasitism in wild animal populations, with most drug-treatment experiments using anthelmintics to target nematode infections (41/66 experiments; Figure 1A). By far the most common drug used was the standard veterinary and human deworming drug ivermectin, which was used in 13 experiments to target nematode parasites (Table 1), but given its wide-target parasite spectrum, was also used in four experiments to target a range of ectoparasites (botflies, mites, and lice; Table 1). Since its discovery in 1973, ivermectin has been used to treat humans and domestic animals across the globe, because it is relatively inexpensive, effective across a broad range of parasites, and importantly, can be used to treat an individual successfully with a single oral or topical dose [20]. The other antinematode experiments tended to use common deworming drugs (e.g., levamisole, fenbendazole, and albendazole), while the remaining antiectoparasite experiments used common, topical pyrethrin-based insecticides (e.g., permethrin) (Table 1). There were also several less-common drug targets for other, non-nematode anthelmintic trials (e.g., targeting cestodes [21] or digenean trematodes [22]). Importantly, we found very few studies that used drugs to reduce or clear infection with microparasites (Figure 1A), including the use of antiprotozoals [23–25] and antibiotics [26,27].

While there was some taxonomic diversity in the hosts being treated, 38% of the host–parasite studies (25/66) investigated the impacts of parasitism in artiodactyl hosts, comprising deer species and wild or feral sheep (Table 1). The next most common host taxa were birds (16/66) and rodents (10/66), but there were also studies on lagomorphs (rabbits and hares; 7/66), and marsupials (4/66). Overall the majority of studies involved herbivore or omnivore hosts, although a small number of experiments (4/66) were carried out on carnivores (Figure 1A; Table 1).

Does drug treatment reduce parasite prevalence and/or intensity in wildlife?

Many studies provided information on the effect of treatment on the target parasite (55/66; 83%), assessed either in terms of the prevalence of infection (proportion of hosts infected), mean parasite abundance (mean number of parasites or parasite eggs shed per host), or mean parasite intensity (mean number of parasites or parasite eggs shed per infected host). The efficacy of antiparasite treatment was assessed by either comparing these metrics of parasite infection between treated animals and control animals (given water or a relevant placebo) or by measuring the reduction in these metrics before and after treatment. We summarise these results qualitatively, categorising them as either reporting a positive effect of treatment (a significant reduction in any of the above metrics post treatment) or no effect of treatment (no significant change in any of the above metrics); none of the studies reported an increase in the target parasite prevalence, abundance, or intensity post treatment. Most studies that provided information on efficacy (49/55; 90%; with the remaining studies not documenting the effects of treatment on the target parasites) reported a reduction in the target parasite following treatment, suggesting that antiparasite treatments often have a detectable, desired negative effect on the target parasite(s) (Figure 1B). However, for some drugs (particularly some of the less well-used antinematode drugs, such as cambendazole, thiabendazole, and dichlorvos), there was no reported effect of treatment (Figure 1B). By contrast, all insecticidal treatments were reported to cause some detectable reduction in the target parasite(s) (Figure 1B).

We also considered whether a reported effect was 'context dependent', that is, where the authors reported a reduction in parasite infection, burden, or intensity due to treatment, but only in a subset of the data (e.g., between host sex or age classes, or between target parasite species or years within the same study). Note that not all studies allowed assessment of context dependence in their design or analyses (i.e., they only had one cohort of individuals within one time period, etc.). Nevertheless, of the 55 studies that provided any information on efficiency, nearly half of the studies (27) reported context dependence in the effect of treatment such that, although a reduction in the target parasite was reported, this was only seen in a certain subset of the individuals (Figure 1B). In particular, significant reductions in the target parasite were often only detected in certain age or sex classes [28,29], or only by certain parasite species within mixed infections [29–31]. In addition, it was commonly found that the effect of the drugs

Table 1. Antiparasite drug treatment studies in wildlife

Drug type	Drug name	Target parasite(s)	Host species	Dose and/or administration	Refs
Anthelmintic	Ivermectin	Nematode, *Heligmosomoides polygyrus*	Yellow-necked mouse, *Apodemus flavicolis*	10 mg/kg, injection, repeated doses	[28]
			Wood mouse, *Apodemus sylvaticus*	10 mg/kg, oral, repeated doses	[32]
		Nematode, *Trichostrongylus retortaeformis*	Mountain hare, *Lepus timidus*	0.1 ml, injection, single dose	[62,63]
		Nematodes, *Protostrongylus* spp.	Mountain sheep, *Ovis canadensis*	0.5 mg/kg, injection, single dose	[64]
				Oral via feed, single and repeated doses	[34]
		Several nematode species	White-footed and deer mice, (*Peromyscus leucopus and Peromyscus maniculatus*)	200 mg/kg, oral, single dose	[39,45][a]
			Snowshoe hare, *Lepus americanus*	0.4 mg/kg, injection, single and repeated doses	[31]
				0.3 mg/kg, injection, repeated doses	[47,54]
			Svalbard reindeer, *Rangifer tarandus*	Oral, repeated doses	[37,50]
	Ivermectin, moxidectin and albendazole	Several strongyle nematode species	Kangaroo, *Macropus giganteus*	Single, injection, and oral	[65]
	Ivermectin, praziquantel and pyrantel pamoate	Several helminth species	Florida panther, *Puma concolor*	Mixed, injection, and oral; repeated	[66]
	Abamectin	Several nematode species	Svalbard reindeer, *R. tarandus*	23–45 μg/kg/day, slow-release capsule	[30]
	Moxidectin	Several nematode species	Svalbard reindeer, *R. tarandus*	0.2–0.4 mg/kg, injection, single dose	[37,50]
	Levamisole hydrochloride	Nematode, *Trichostrongylus tenuis*	Red grouse, *Lagopus lagopus scoticus*	2 mL, oral, single	[36,43,46][a]
	Levamisole hydrochloride	Several nematode species	White-footed mouse, *P. leucopus*	15 mg/kg, injection, single	[35]
	Tramisol (levamisole)	Several helminth species	Cottontail rabbit, *Sylvilagus floridanus*	8 mg/kg, oral, single	[67]
		Nematode, *Protostrongylus* spp.	Bighorn sheep, *Ovis canadensis*	17.8–24.4 mg/kg, oral, single	[68]
	Fenbendazole	Several nematode species	White-tailed deer, *Odocoileus virginianus*	30–60 g, oral (via feed)	[69]
		Lungworm, *Protostrongylus* spp.	Mountain sheep, *O. canadensis*	3 g, oral, repeated	[70]
		Several nematode species	Alpaca, *Vicunga pacos*	15 mg/kg, oral	[25]
		Several nematode species	Goshawk, *Accipiter gentilis* and white-tailed sea eagle, *Haliaeetus albicilla*	1–2 ml, sprayed nests, single dose	[71]
		Lungworm, *Protostrongylus* spp.	Bighorn sheep, *O. canadensis*	Oral via feed, repeated dose	[72]
		Mixed helminths	Brown pelican, *Pelecanus occidentalis*	22 mg/kg, oral, repeated dose	[73]
	Albendazole	Nematode, *Osteragia gruehneri*	Soay sheep, *Ovis aries*	Oral (via bolus), repeated dose	[53]
		Several nematode species	Svalbard reindeer, *R. tarandus*	Oral (via bolus), repeated dose	[37]
		Several nematode species	Kangaroo, *M. giganteus*	3.8 mg/kg, oral, repeated dose	[65]
		Mixed helminths	Brown pelican, *Pelecanus occidentalis*	10 mg/kg, oral, repeated dose	[73]
	Cambendazole	Lungworm, *Protostrongylus* spp.	Bighorn sheep, *O. canadensis*	30 mg/kg, oral, single	[68]
	Thiabendazole	Lungworm, *Protostrongylus* spp.	Bighorn sheep, *O. canadensis*	120 cc, oral, single	[68]
	Flubendazole	Several nematode species	Pheasant, *Phasianus colchicus*	Oral (via feed), repeated dose	[44][a]
	Pyrantel pamoate	Nematode, *Heligmosomoides polygyrus*	Yellow-necked mouse, *A. flavicolis*	100 mg/kg, oral, repeated dose	[38]

Table 1 (*Continued*)

Drug type	Drug name	Target parasite(s)	Host species	Dose and/or administration	Refs
	Pyrantel pamoate	Raccoon roundworm, *Baylisascaris procyonis*	Raccoon, *Procyon lotor*	90 mg, oral (bait), single/ repeated dose	[74]
	Dichlorvos	Lungworm, *Protostrongylus* spp.	Bighorn sheep, *O. canadensis*	120 cc, oral, single	[68]
	Netobimin	Several nematode species	Mouflon, *Ovis musimon*	7.5 mg/kg, oral, single	[29]
	Clorsulon	Mixed helminths	Brown pelican, *P. occidentalis*	10 mg/kg, oral, repeated dose	[73]
	Piperazine dihydrochloride	Mixed helminths	Brown pelican, *P. occidentalis*	109 mg/kg, oral, repeated dose	[73]
	Praziquantel	Mixed cestode species	Snow goose, *Chen cearulescens*	10 mg/kg, injection, single dose	[21]
		Alceolar echinococcosis, *Echinococcus multilocularis*	Red fox, *Vulpes vulpes*	50 mg, oral via bait, single/ repeated dose	[75]
Triclabendazole	Fascioloidiasis, *Fascioloides magna*	White-tailed deer, *O. virginianus*	10 mg/kg, oral, single dose	[22]	
Insecticide	Ivermectin	Botfly, *Cutebra* spp.	Townsend vole, *Microtus townsendii*	10 mg/mL, topical, single dose	[76]
		Louse, *Trichodectes canis*	Wolf, *Canis lupus*	200 μg/kg, injection, single dose	[77]
		Sarcoptic mange, *Sarcoptes scabiei*	Spanish ibex, *Capra pyrenaica*	0.2–0.4 mg/kg, injection	[78]
		Mite, *Psoroptes ovis*	Bighorn sheep, *O. canadensis*	500 μg/kg, injection, single dose	[79]
		Fly, *Philornis downsi*	Darwin's finches (*Geospiza* spp.)	1%, nests sprayed, single dose	[80]
	Fipronil	Fleas, to indirectly remove *Trypanosoma microti*	Field vole, *Microtus agresti*	10 mg/kg, oral, repeated dose	[81][a]
	Pyrethrum (pyrethrin based)	Lice, *Ischnocera* spp.	Rock dove, *Columba livia*	1%, fumigation repeated dose	[33]
	Permethrin (pyrethrin based)	Mixed ectoparasites	Red squirrel, *Tamiasciuris hudsonicus*	Topical, repeated dose	[82]
	Pyrethrin	Mixed ectoparasites	Goshawk, *A. gentilis* and white-tailed sea eagle, *H. albicilla*	Sprayed nests, single dose	[71]
	Dichlorvos (2-dichlorovinyl dimethyl phosphate)	Mixed ectoparasites	Cottontail rabbit, *Sylvilagus floridanus*	Topical (collar), single dose	[67]
Antiprotozoal	Toltrazuril	Coccidiosis, mixed coccidian species	Alpaca, *V. pacos*	15 mg/kg, oral	[25]
		Coccidiosis, *Isospora spp.*	Laughing thrush, *Dryonastes courtoisi*	12.5 mg/kg, oral, repeated dose	[24]
	Carnidazole	Trichomoniasis, *Trichomonas gallinae*	Pink pigeon, *Columba mayeri*	10 mg, oral, single/repeated dose	[23]
	Primaquine	Mixed protozoan blood parasites	Blue tit, *Cyanistes caeruleus*	0.1–0.05 mg, injection, single dose	[83]
Antibiotic	Oxytetracycline (tetracycline based)	Mixed bacterial species	Reindeer, *R. tarandus*	20 mg/kg, injection, single dose	[26]
	Doxycycline (tetracycline based)	Lyme disease, *Borrelia bugdorferei* and *Anaplasma phagocytophium*	Mixed rodent species	500 mg/kg, oral (via bait), repeated dose	[27]

[a]Indicates population-level drug treatments; all other references refer to individual-level treatment experiments.

varied with time, either on a short timescale, such that treated hosts were rapidly re-infected [24,25,32], or on longer timescales, such that the reported efficacy of treatment varied across the season or between years [30,33,34].

Does drug treatment benefit the health or fitness of treated individuals?

Across the range of studies, authors reported individual host fitness consequences of treatment in a variety of ways which, for simplicity, we group into effects on: (i) host survival; (ii) host fecundity (grouping various measures relating to the number of offspring produced or matured, or body condition of those offspring, depending on the information presented); and (iii) other metrics of host 'health', typically relating to measures of body condition (fat scores or weight relative to body length), or other physiological measures. For each of these categories, we scored the reported effects in terms of whether they were 'positive' (i.e., a significant improvement in any of the metrics post treatment, or compared with untreated control animals),

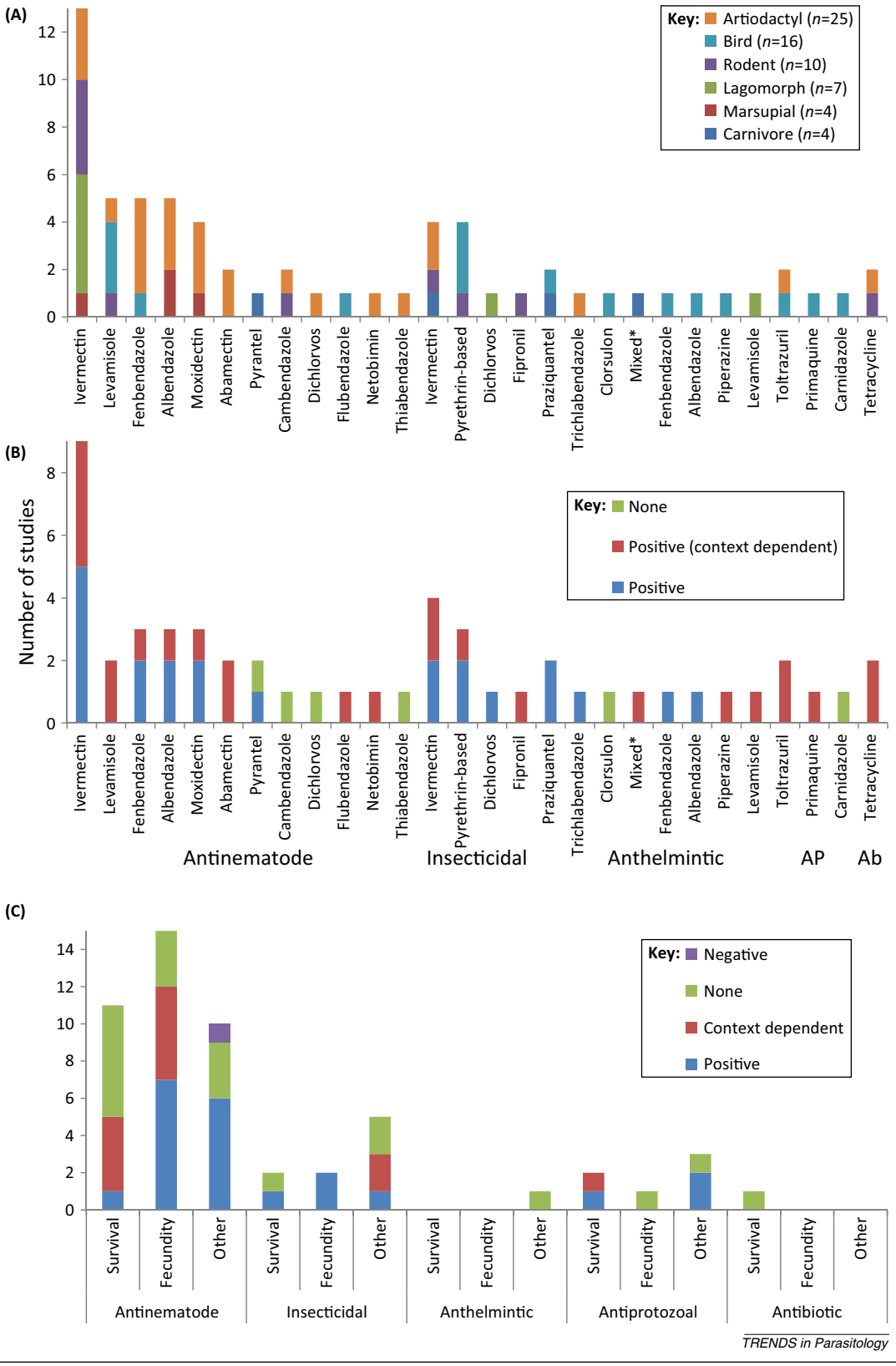

Figure 1. The number of antiparasite treatment studies in wildlife populations, showing **(A)** the drug used and host taxon studied, **(B)** reported measurements of efficacy of treatment against the target parasite, and **(C)** reported effects of treatment on host fitness (survival, fecundity or 'other' measure of host health; see main text for details). Numbers in each segment refer to the number of experiments reporting that effect. All drugs are broken down by whether the drug was primarily as an antinematode, insecticidal, anthelmintic (targeting helminth parasites other than nematodes), antiprotozoal (AP), or antibiotic (Ab). The treatment marked 'Mixed*' was a single study that used a combined treatment of ivermectin, praziquantel, and pyrantel pamoate to target mixed helminth infections.

'none' (no significant effect in any metrics), or 'negative' (a significant reduction in any metrics). Furthermore, as with our drug efficacy scores, we also considered whether a reported effect was 'context dependent' (i.e., a change in a host health metric that varied between subsets of individuals, such as ages or sexes). Again, many studies did not explicitly seek such context dependency in their analyses, so we were unable to assess its occurrence in all studies.

In total, 37/66 experiments (56%) provided information on the health effects of treatment, reporting a total of 53 measures of host effects (many studies sought more than one effect of treatment on the host). Of these, 33 (62%) reported a beneficial effect of treatment on the host by at least one of the above metrics (Figure 1C). However, as with the efficacy measures of treatment, many positive effects on host fitness that were detected were found to be context dependent, varying with host age or sex [21,35], or between different years (e.g., [36]) or with previous infection burdens [37]. In 19 out of the 53 reported measures of host fitness (36%), treatment was found to have no detectable effect on any metric of host fitness and, in one case (studying nematode and coccidia infections in alpacas), treatment was reported to reduce host health [25].

The consequences of treatment for the broader parasite community

Antiparasite treatment studies can not only highlight the consequences of the target parasite on host fitness, but also give insight into the occurrence of interactions between co-infecting parasites, although few studies explicitly monitor these indirect consequences of treatment for the nontarget parasite community. In the few examples in wildlife that have investigated these effects [32,38,39], the authors examined whether treatment to remove the target parasite species (these were nematodes in all cases) had consequences for the abundance or prevalence of another co-infecting parasite species (ticks [38]; coccidia and cestodes [39]; coccidia and blood-borne *Trypanosoma* spp. and *Bartonella* spp. [32]). In each of these cases, there was a corresponding increase in some of the co-infecting parasite species, suggesting that the target parasite was in some way suppressing infections (either directly, via the immune response of the host, or through competition for shared resources [40]) by the nontarget parasite species. Importantly, a recent analysis of two of these studies demonstrated that neither cross-sectional nor longitudinal observational data could accurately detect the within-host interactions demonstrated through antiparasite treatment studies [41]. Thus, these experimental studies are crucial for uncovering important within-host interactions among the parasites that may affect the efficacy of treatment and the net benefits of treatment for host health [42]. Clearly, however, more experimental studies are needed that look at a wider range of perturbations (treatment targets), monitor a wider range of nontarget parasite taxa, and follow the health and fitness of treated individuals.

Scaling the individual-level impact of parasites to the population-level consequences

From the above studies, it is clear that parasites can have important consequences for individual-level health and fitness, but what are the consequences for such impacts at the population level? Intuitively, we may expect highly virulent parasites and/or those at high prevalence to be having the greatest impact on their host population. If so, then comparing host densities across different populations with differing disease prevalences may be expected to be a viable means to infer disease impacts; if a given parasite is having a major effect, then populations with high infection levels may be expected to have lower mean host lifespans and/or smaller population sizes compared with those with low infection levels. However, such approaches are unable to decouple the dynamic relationship between host lifespan, host population size, infection risk, and the impact of infection. It may be, for example, that positive relationships between individual lifespan and infection state are observed if longer-lived hosts are more likely to accumulate parasites. Similarly, larger host populations may present more transmission opportunities, leading to positive relationships between population size and disease prevalence. Such effects are likely to obscure, or even overturn, any signal of the direct effect of infection on individual host health or host population size.

These concerns are supported by basic epidemiological theory ([18,19]; Box 1), which shows that the scaling relations between disease impacts at the individual and population levels are far from straightforward, often involving strong nonlinearities (Box 1, Figure I). In particular, highly virulent parasites at the individual level are likely to kill their hosts before they can transmit onwards, effectively burning themselves out, and so may be at low prevalence and are unlikely to have a significant effect at the host population level. The parasites that may have the greatest population-level impact are those of intermediate virulence, which have some (but not a substantial) effect at the individual level, and are able to transmit to many hosts, resulting in major effects at the population level. As such, parasites that are observed relatively rarely (or have relatively low mean infection burdens for parasitic helminths) and are relatively benign, may be the ones that are having the greatest population-level impact (Box 1).

Given these complexities, drug treatment experiments have the potential to reveal the true impact of parasites at the population level, for example, by comparing host abundance or dynamics between drug-treated and untreated populations. However, few studies have performed such population-level treatment experiments (but see [43–45]). This is probably because there are various logistical issues that mean the viability of this approach may be limited, for example, due to the lack of suitable replicate populations and appropriately matched controls, and constraints on the levels of drug efficacy and treatment coverage that can be attained, particularly for population-level assessments. For example, in Box 2 we show theoretically that high levels of both treatment coverage and drug efficacy may be needed to reveal the effect a macroparasite is having on its host population size. Furthermore, there may be additional problems associated with deciding the appropriate scale to define a coherent population in the wild, and determining the required duration of time needed to sustain treatment. However, these problems may not be insurmountable, and population-level drug treatment experiments can be

Box 2. Theoretical assessment of the utility of antiparasite treatment approaches to quantify the impact of parasites on host populations

Here, we modify the host–macroparasite used previously (Box 1; [18]) to incorporate the effect of anthelmintic treatment. Following the approach of [84], we assume sustained application of a treatment of efficacy h applied to a proportion g (drug coverage) of the host population. Treatment increases the parasite mortality at rate $c = -\log(1-gh)$ [84]. The full equations of this system are shown by Equations I and II:

$$\frac{dH}{dt} = aH - H(b + BH) - \alpha P \quad [I]$$

$$\frac{dP}{dt} = \frac{\beta\lambda PH}{\gamma + \beta H} - P(b + \mu + \alpha + BH + c) - \frac{(\alpha + \mu)P^2}{H}\frac{1 + k}{k} \quad [II]$$

where H is the host population density and P is the size of the parasite population. As before (Box 1), hosts are born at per capita rate a and die at a density-dependent rate $b + BH$, or due to parasite infection at per capita rate α. Parasites produce infective stages at rate λ, which die at rate γ and infect hosts at rate β. They die at rate μ or when hosts die (at rate $b + \alpha + BH$) or through treatment at rate c. Finally, parasites are distributed across the host population according to the parameter k, which is an inverse measure of the degree of aggregation [16].

This model shows that increasing treatment coverage increases the change in host population size relative to an untreated population (Figure I). However, this relationship is not linear, with little change in host population density over much of the range of treatment coverage; only at very high coverage do we see a dramatic increase in population size post treatment, because it is released from the regulatory effects of the parasite. Furthermore, if drug efficacy is <100%, there is a dramatic reduction in the treatment effect. Qualitatively similar results are seen if it is assumed the parasite affects host fecundity rather than host survival (not shown). Overall, although treatment experiments can reveal population-level impacts of disease, the levels of coverage required to detect this may be problematic for many wildlife systems.

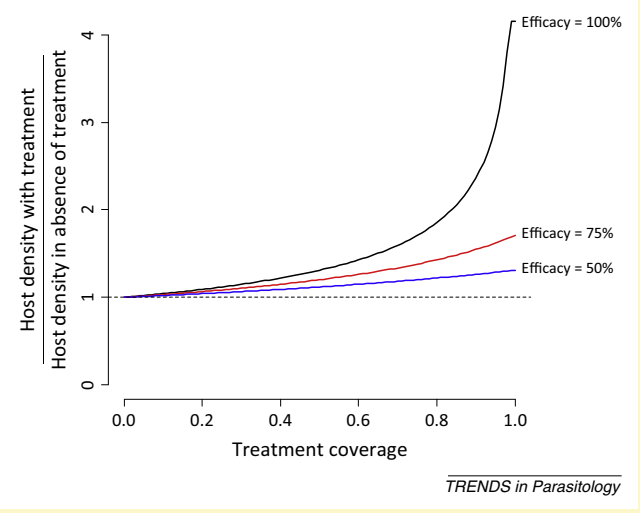

Figure I. The predicted relationship between anthelmintic treatment coverage (proportion of host population treated) and host population density relative to the untreated population, for different levels of drug efficacy.

informative for understanding the role of parasites in wildlife. The first, and arguably still the most complete, study of this kind documented both the individual- and population-level effects of the nematode *Trichostrongylus tenuis* in contributing to population crashes in red grouse populations in northern England ([36,43,46]; see Box 3 for details). Other studies investigated the role of parasites in wildlife populations, in combination with other important factors, such as nutritional status, predator–prey dynamics, and resource availability [35,45,47,48]. It is likely that the population-level effects of these sublethal endemic parasites may be best understood when measured in conjunction with key factors determining individual fitness and population survival. These population-level studies show the value of adopting integrated approaches that combine some or all of observational (ideally longitudinal) empirical studies, individual-level treatment experiments, mathematical modelling to predict population-level impacts, and ultimately population-level experiments, to test those predictions (Box 3).

Concluding remarks and future directions

It is well recognised that parasites can have devastating effects on their individual hosts and on their host populations [1,2]. However, detecting those effects, particularly for endemic parasites, is a major challenge in disease ecology and conservation. Theory shows that the scaling relationship between the individual-level impacts of disease and the population-level consequences of those impacts can be nonlinear and, indeed, non-monotonic ([18,19]; Box 1), meaning that it is difficult to understand the role of endemic parasites in shaping host population and community dynamics through observational studies alone [49,50]. For that reason, experimental perturbations are essential.

Conceptually, drug treatment experiments seem an ideal method of assessing the individual- and population-level impacts of parasitism. However, such approaches can be logistically challenging; in particular, for assessing population-level impacts, high levels of drug coverage (proportion of the population treated) are likely to be needed to be able to detect an effect of treatment (Box 2). Indeed, even at the individual level, there are challenges with conducting and interpreting the results of treatment experiments in wildlife. There is a variety of drugs that can be applied to wildlife systems, making it difficult to decide the appropriate drug, dose, and administration method to maximise the potential for a significant reduction in the target parasite ([51]; but see Table 1), while also minimising the potential for toxic effects on the host, or knock-on effects on the wider ecosystem as could happen for drugs that persist in the environment. Many available antiparasite drugs are known to have toxic effects on some animals, and their doses are closely regulated for veterinary use. Obviously it is essential that such guidelines are accurately followed in any wildlife treatment experiment, and all doses and frequencies of treatment are clearly described.

Our review of the literature showed that most of these drugs can reduce parasite infection, burden, or intensity in treated individuals and that this reduction is often, but not always, associated with a benefit for host condition,

Box 3. Experimental approach to link individual- and population-level effects of parasitism in the red grouse system

Although treatment experiments have been used to assess the impact of parasite infection in many systems, few have successfully linked individual and population levels as well as Hudson and colleagues [36,43,46,85]. By investigating *Trichostrongylus tenuis* infection in red grouse populations in Northern England, these authors demonstrated the strong role that this parasite can have in contributing to the famous multiannual grouse population cycles (4–8 years [86]).

Observational studies suggested that high levels of *T. tenuis* infection correlated with poor breeding success [87], and preliminary models proposed that this negative effect could underlie the multiannual fluctuations in red grouse abundance [86]. To move beyond the correlations, the authors directly assessed the impact of parasitism on bird fecundity by comparing (i) levels of parasitism and (ii) grouse 'breeding production' of control birds (which received water) with birds treated orally with an anthelmintic (levamisole hydrochloride) [36,46]. Treatment reduced both mean parasite eggs per gram (EPG; Figure IA) and the mean number of worms per bird (not shown), although there was variability in the extent of reduction between years; parasite EPG, for example, was reduced by ~95% in 1982 but only by ~70% in 1983. Treatment positively affected bird survival (Figure IB) and fecundity [clutch size (Figure IC), and also hatching success and number of chicks surviving (not shown)]. Again, there was substantial context dependency with between-year variation in effect size.

These data were used to parameterise a mathematical model to predict that *T. tenuis* drove the grouse population cycles [85]. Importantly, Hudson and colleagues then tested these predictions using a large-scale population-level treatment experiment across six sites followed over 9 years [43]. The authors treated grouse with replicated, timed population-level treatments, chosen to coincide with predicted crash years in abundance [$n = 2$; treated once, $n = 2$; treated twice (both coinciding with separate predicted crash years), and $n = 2$: control]. Even though estimated levels of treatment coverage ranged from 15% to 50%, these treatments were shown to dramatically reduce the tendency for the populations to cycle [43].

Overall, this body of work demonstrates the challenges inherent in understanding parasite impacts on wildlife: context dependency in effect sizes (e.g., varying treatment effects on parasites and host fitness across years, possibly due to varying parasite pressure) and logistical challenges (e.g., achieving and maintaining sufficient treatment coverage for population-level experiments). Nevertheless, through a combination of long-term observational data, individual-level treatment experiments, mathematical modelling, and population-level experiments, this work provided the first (and still one of few) demonstration of the impact of parasites on host individuals and populations.

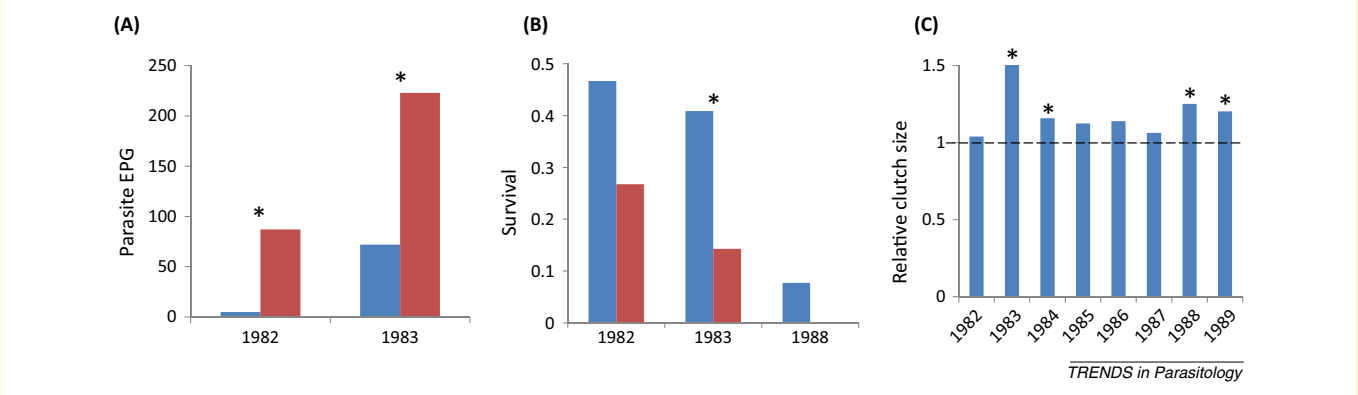

Figure I. The effect of levamisole treatment (blue bars) on **(A)** mean *T. tenuis* egg output per gram of faeces (EPG), **(B)** red grouse survival, and **(C)** mean red grouse clutch size, relative to those of untreated control birds (red bars) [46]. The asterisks denote statistically significant effects of treatment, as reported by the relevant papers.

survival, or reproduction. However, we also discovered that not all drugs were efficacious for treating wildlife parasites (i.e., there was not always a detectable effect of treatment on the target parasite) and that reducing parasite infection was not always good for the host; sometimes, there were no detectable benefits to the host of treatment, or those effects varied significantly across subsets of the population. One point to make here is that in 11 out of 66 treatment experiments there was no apparent attempt to assess the effect of treatment on the target parasite. Hence any subsequent change in host fitness or behaviour in those studies cannot be unambiguously attributed to parasite reduction (e.g., they may arise through the direct effect of the drug on the host). A second important finding was that drug efficacy can be variable, not only between different drugs and host–parasite systems, but also within a host–parasite system (Figure 1B); in many cases, there were notable context dependencies, such that the impact of treatment on the target parasite varied between years or months, or among subsets of the host population (i.e., with host age and/or sex) or between parasite species within mixed infections (similar parasite species specific responses to treatment have previously been noted in human drug treatment programmes [52]). Context dependencies were also observed in the impact of treatment on various metrics of host fitness (Figure 1C). Previous studies have shown that parasite effects on host fitness are often mediated through interactions with other forces (e.g., predation, competition, nutrition, or environmental stress [2,45,53]). Hence, it can be challenging to extrapolate results from one context (e.g., an experiment carried out in one year or one location) to another (e.g., the same experiment carried out in another year or another location). Ideally, what are needed are sufficiently extensive trials that span multiple contexts (e.g., years, locations, host groups, and parasite species) in concert with manipulation of other factors (e.g., predator presence and/or absence [54] or resource availability [45]), with appropriate randomized controls and levels of replication. Clearly, there are major logistical challenges associated with achieving such extensive studies, and it is possible that theory may be able to provide some guidance of the important contexts to consider, by exploring the extent to which different key parameters are affected by variation in different biological processes.

In addition, there are various considerations that need to be made when designing a treatment experiment in wildlife. One particular issue is that currently available drugs typically target broad taxonomic groups of parasites (such as 'nematodes' or 'ectoparasites') as opposed to specific parasite species, making it difficult to identify which parasites may be having the documented impact on host health or fitness [51]. Similar issues are well known in community-wide parasite control programmes in human populations, resulting in considerable variability in treatment success, both in terms of responses of target parasites and benefits to individuals [52,55–57]. With this in mind, it may be that using vaccination, as opposed to drug treatments, could provide the necessary finer-scale methods to target specific parasite species, providing greater precision than the coarse-scale approach adopted by current drug treatment experiments; however, there is only a limited set of vaccines currently available for wildlife parasites. A second issue that needs to be considered relates to the diversity of parasites typically infecting individual hosts in natural settings [40]. There may be unexpected increases in infections by potentially harmful, nontarget parasites following treatment [32,39], potentially masking or even adversely affecting host health responses to treatment [42]. Clearly, the choices of drug, dose, and frequency of administration then have to be carefully thought through, as do the levels of monitoring of target and nontarget parasites and host health metrics before and after treatment.

At the population level, the logistical challenges associated with carrying out the necessary large-scale experimental manipulations are magnified; it is over 15 years since this method was first used to demonstrate the effects of parasitism on host population dynamics [43], but that study remains one of the few that have been carried out at the population level (but see, for example, [45]). Although there are logistical issues with carrying out such large-scale perturbation experiments, those problems are not insurmountable, and need to be overcome. It is nearly 40 years since Anderson and May showed theoretically the potential for parasites to regulate their host population and alter host population dynamics [16], and there is currently great interest in the role of parasites in structuring larger ecological communities [58,59]; however, we still have few experimental tests of the theory or demonstrations of their impact at the population level, much less at the community level. Nevertheless, we would urge researchers and funders to appreciate the value of experimental manipulations, at both the individual and population levels, as perhaps the best tool for understanding the role of parasites in driving population and community dynamics.

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