Few studies of wild animal performance account for parasite infections: a systematic review

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Abstract

1. Wild animals have parasites. This inconvenient truth has far-reaching implications for biologists measuring animal performance traits: infection with parasites can alter host behaviour and physiology in profound and sometimes counterintuitive ways. Yet, to what extent do studies on wild animals take individual infection status into account?
2. We performed a systematic review across eight scientific journals primarily publishing studies in animal behaviour and physiology over a 5-year period to assess the proportion of studies which acknowledge, treat or control for parasite infection in their study design and/or analyses.
3. We explored whether parasite inclusion differed between studies that are experimental versus observational, conducted in the field vs the laboratory and measured behavioural vs physiological traits. We also investigated the importance of other factors such as the journal, the trait category (e.g. locomotion, reproduction) measured, the vertebrate taxonomic group investigated, and the host climatic zone of origin.
4. Our results show that parasite inclusion was generally lacking across recent studies on wild vertebrates. In over 680 filtered papers, we found that only 21.9% acknowledged the potential effects of infections on animal performance in the text, and only 5.1% of studies treated animals for infection (i.e. parasite control) or considered infection status in the statistical analyses (i.e. parasite analysis). Parasite inclusion, control and analysis were higher in laboratory compared to field studies and higher for physiological studies compared to behavioural studies but did not differ among journals, performance trait categories and taxonomic groups. Among climatic zones, parasite inclusion, control and analysis were higher in tropical, subtropical, and temperate zones than in boreal and polar zones.

5. Overall, our literature review suggests that parasites are sorely under-acknowledged by researchers in recent years despite growing evidence that infections can modify animal performance. Given the ubiquity of parasites in the environment, we encourage scientists to consider individual infection status when assessing performance of wild animals. We also suggest ways for researchers to implement such practices in both experimental and observational studies.

Key-words : performance, physiology, behaviour, pathogens, whole organism, PRISMA

Introduction

All living organisms, from bacteria to humans, have evolved in some way to deal with infections by parasites and pathogens (hereafter parasites). Here, we define parasites as organisms that live on or in another species (i.e. the host) and derive nutrients or other resources from their host causing them harm (Poulin & Morand, 2000). Parasites are ubiquitous in the environment and parasitism is considered the most common feeding strategy for nutrient acquisition (Lafferty et al., 2008; Marcogliese, 2004): some estimates suggest that roughly half of all living organisms are parasitic at some stage in their life cycle (Weinstein & Kuris, 2016). They are phylogenetically diverse, ranging from microscopic protozoa, bacteria and fungi to multicellular plants, worms, arthropods, fishes and even birds and mammals (Greenhall, 2018; Poulin & Morand, 2000; Twyford, 2018; Weinstein & Kuris, 2016). Although they are typically small in size compared to their hosts, parasite infection can disrupt host homeostasis; they can incite or suppress immune responses, alter normal behaviours and physiological processes, influence population dynamics and ultimately drive trait evolution (Poulin, 2011). Yet, despite their ubiquity, taxonomic diversity, and significant impact on their hosts, parasites are often overlooked or ignored by researchers studying wildlife, unless they have a significant economic impact (i.e. sea lice in salmonids, brucellosis in ungulates), are associated with zoonosis (e.g. rabies, bird flu, Lyme disease) or have clear conservation implications (e.g. white-nose syndrome in bats, Chytridiomycosis infection in amphibians). This oversight may be partly explained by the inherent difficulty in detecting and identifying parasites on wild hosts: many parasites are microscopic in size and found inside their hosts (i.e. endoparasites) making them difficult to study without sacrificing the host and specialized equipment/training for proper identification (Marcogliese, 2004; Poulin & Morand, 2000).

Parasites can impose an energetic burden on hosts either through using host resources for their own growth, causing an energetically costly immune reaction in hosts and/or negatively affecting host performance capacity. Performance capacity is a measure of how well an individual can accomplish a fitness-enhancing task such as foraging, moving (i.e. flying, swimming, running), mating and avoiding predators, through the use of morphological, behavioural and/or physiological traits (Arnold, 1983; Binning et al., 2017; McElroy & de Buron, 2014). Overlooking the impact of parasites on hosts can be problematic for researchers measuring animal performance. Indeed, numerous studies suggest that host performance capacity is a typical target of manipulation by parasites (Barber & Wright, 2005; Binning et al., 2017; McElroy & de Buron, 2014). For example, parasite infection tends to increase movement costs, leading to shorter migration distances, slower swimming, flying and running speeds in a range of animals from arthropods to mammals (Binning et al., 2013; Bradley & Altizer, 2005; Debeffe et al., 2014; Oppliger et al., 1996; Palstra et al., 2007; Wagner et al., 2003). Metabolic rates, commonly measured physiological traits underlying aerobic performance (Careau et al., 2008; Claireaux & Lefrançois, 2007), can also be affected by parasite infection. However, the direction of this relationship appears to be system-specific: studies report both increases and decreases in host aerobic metabolic performance with infection (Behrens et al., 2014; Booth et al., 1993; Caballero et al., 2015; Careau et al., 2012; Guitard et al., 2022; Ryberg et al., 2020) making their general effects on host metabolic performance difficult to predict and, thus, account for. The effects of parasites on host behaviour are similarly difficult to generalize. Parasite infection is related to both increased and decreased grouping behaviours (Arnal et al., 2015; Barber & Huntingford, 1995; Krause & Godin, 1994; Spagnoli et al., 2015), foraging (Barber et al., 2000; Meadows & Meadows, 2003), movement (reviewed in Binning et al., 2017), and activity (Arnal et al., 2015; Binning et al., 2017). As a result, ignoring parasite infection in studies of host performance can lead to biases, misinterpretations and erroneous conclusions that can be impossible to account for post-hoc (Timi & Poulin, 2020).

There are several ways that researchers may take parasites into account in their studies. First, researchers may acknowledge the existence of known parasites in their systems, but take no further steps to account for them in their design (i.e. parasite inclusion). For example, while assessing whether escape performance predicts survival in the northern quoll (*Dasyurus hallucatus*), Rew-Duffy et al. (2020) acknowledged that parasites could also affect survival, without specifically assessing the parasite load of the tested specimens. Acknowledgement with no further action may be the case when the host species, measured traits and/or experimental designs make it such that quantifying parasite infection is technically too challenging, costly or unethical to feasibly manage or simply when researchers think that infection is not a major factor. Second, researchers may control for individual infection in their study by removing infected individuals from the sample or treating individuals prophylactically or therapeutically prior to testing (i.e. parasite control). For example, Rangel & Johnson (2018) specified that wild-caught bluebanded goby (*Lythrypnus dalli*) were quarantined for 24 hours prior to their transfer in their holding tanks to minimize parasite transmission between individuals and to identify and remove infected fish pre-emptively. Furthermore, researchers may also specify when ectoparasites are forcibly removed and animals have been exposed to an anti-parasite treatment or spray prior to experiments. For example, Brusch IV et al. (2020) used an anti-parasite spray (Frontline, Merial Inc., Duluth, GA, USA) on European lizards (*Zootoca vivipara)* and removed visible parasites using forceps. A treatment upon arrival in the laboratory is another efficient option. For example, Christensen et al. (2021) treated wild-caught round gobies (*Neogobius melanostomus*) in a 1:5000 formalin bath for 30 minutes to kill ectoparasites prior to placing them in their laboratory aquarium facilities. Parasite control/removal may be more likely when infected individuals are easily identifiable by researchers, parasites are clearly visible (i.e. some ectoparasites), animals are tested in a laboratory setting, treatment is easy to administer and/or treatments are unlikely to influence the performance trait to be measured. Indeed, many research labs require that wild animals are quarantined and treated for common parasites/diseases prior to joining the laboratory population. Alternatively, researchers may deliberately choose not to remove parasites, as the stress caused by the manipulation could lead to potential changes in performance and reduce the ecological relevance gained from keeping natural levels of parasites. Parasite load influences the intensity of the immune response built by the host to respond to infection, which may result in different levels of physiological or behavioural performance capacity impairments (Ayres & Schneider, 2012). Researchers may thus explicitly quantify infection status (infected or not) and/or parasite load (number of parasites on a host) and include this variable in their statistical analysis (i.e. parasite analysis). The likelihood of parasites being included in the analyses may be higher when wild animals are handled or when studying traits that are already known to be heavily influenced by infection (see reviews by Barber et al., 2000; McElroy & de Buron, 2014). The degree to which parasites are considered in studies of animal performance may also depend on where study animals are collected. Although tropical climates are home to a greater biodiversity of hosts, and thus parasites (Kamiya et al., 2014; Martins et al., 2021), surveys and knowledge of vertebrate parasite fauna is greater in temperate zones (Poulin, 2010) suggesting that studies on hosts from temperate biomes may be more likely to take infection into account than tropical biomes. Despite the many documented effects of parasites on hosts, the extent to which studies on performance capacity in wild vertebrates take parasite infection into account is unknown, making it difficult to assess potential biases in the literature.

To address this knowledge gap, we conducted a systematic review of eight journals primarily publishing research on animal behavioural and physiological performance over a 5-year period and quantified if and how parasites were considered. We first identified articles which mentioned parasites at any point in the text (hereafter "parasite inclusion"), and then qualified the degree of parasite consideration in the study design and analyses. We then explored whether parasite inclusion depended on factors such as the journal, the study type (i.e. experimental or observational), the setting (i.e. field or laboratory), the research topic (i.e. behaviour or physiology), the vertebrate taxonomic group investigated, and the host climatic zone of origin. We predicted that the majority of studies would ignore parasite infection. Because parasites can have strong effects on both behaviour and physiology, we predicted that parasite inclusion would not differ between study topics. We also predicted that parasite inclusion should be higher in more controlled environments such as experimental and laboratory studies, and thus, especially in taxa often transported and studied in the laboratory such as fish and birds (Kamiya et al., 2014; Martins et al., 2021). We finally predicted that parasite inclusion may be higher in studies on temperate species because of the greater taxonomic knowledge of vertebrate parasite fauna in temperate zones (Poulin, 2010). Among studies that did have parasite inclusion, we assessed to what extent researchers considered parasites in their study design and analyses (hereafter "parasite control" and "parasite analysis"). We expected that a small proportion of studies would consider parasite infection in their design and analyses, with a higher predominance in experimental vs observational designs and for studies conducted under laboratory vs field conditions.

Methods

Search strategy

We conducted a systematic literature search (January 31st 2021), following PRISMA guidelines (Page et al., 2021), with pre-specified inclusion/exclusion criteria. In Web of Science, we used the advanced search tool and the following search key: TS=(performance OR trait AND behavio* OR physiolo* NOT cellul* NOT molecul*) AND SO=(JOURNAL NAME), where TS and SO refer to topic and publication name, respectively. This process was repeated for eight peer-reviewed journals (*Animal Behaviour;* ANBE, *Behavioral Ecology;* BEEC*, Canadian Journal of Zoology;* CJZ*, Functional Ecology;* FUNE*, Journal of Animal Ecology;* JAE*, Journal of Experimental Biology;* JEB*, Journal of Experimental Marine Biology and Ecology;* JEMB*, Proceedings of the Royal Society B: Biological Sciences;* PRSB) publishing most of the behavioural and physiological papers in ecology, by replacing the publication name parameter (SO). The aim of this selection was to have a diversity of journals publishing original research focused in large part on studies of wild animal performance. We thus intentionally avoided journals that are taxa-specific (e.g. *Journal of Fish Biology*), have an explicit focus on disease (i.e. *Journal of Parasitology*, *Journal of Wildlife Diseases*), are focused broadly on ecology including non-animal taxa (i.e. *Ecology*, *Ecology Letters*) or largely publish on topics outside of the biological sciences (e.g. *PNAS*, *Nature*). This first search yielded a total of 3039 references (Fig 1). We thus built and shared a document with all criteria and definitions and independently screened all references according to the eligibility criteria using a series of filters to discard non-relevant studies. We first filtered the studies by their title by removing them from the list if it contained any keyword that did not correspond to our inclusion criteria. If there was ambiguity as to whether the article met our inclusion criteria based on the title alone, it was kept in the reference list. We excluded 40% (1216 papers) of the studies at this step. We performed a second filter of studies based on reading their abstracts following the same criteria as above. We removed 29.3% (535 papers) of the remaining studies at this stage. We screened the remaining papers (1409 papers) in their entirety, from which we retained a total of 680 relevant publications (22.38% of papers remaining after full paper screening).

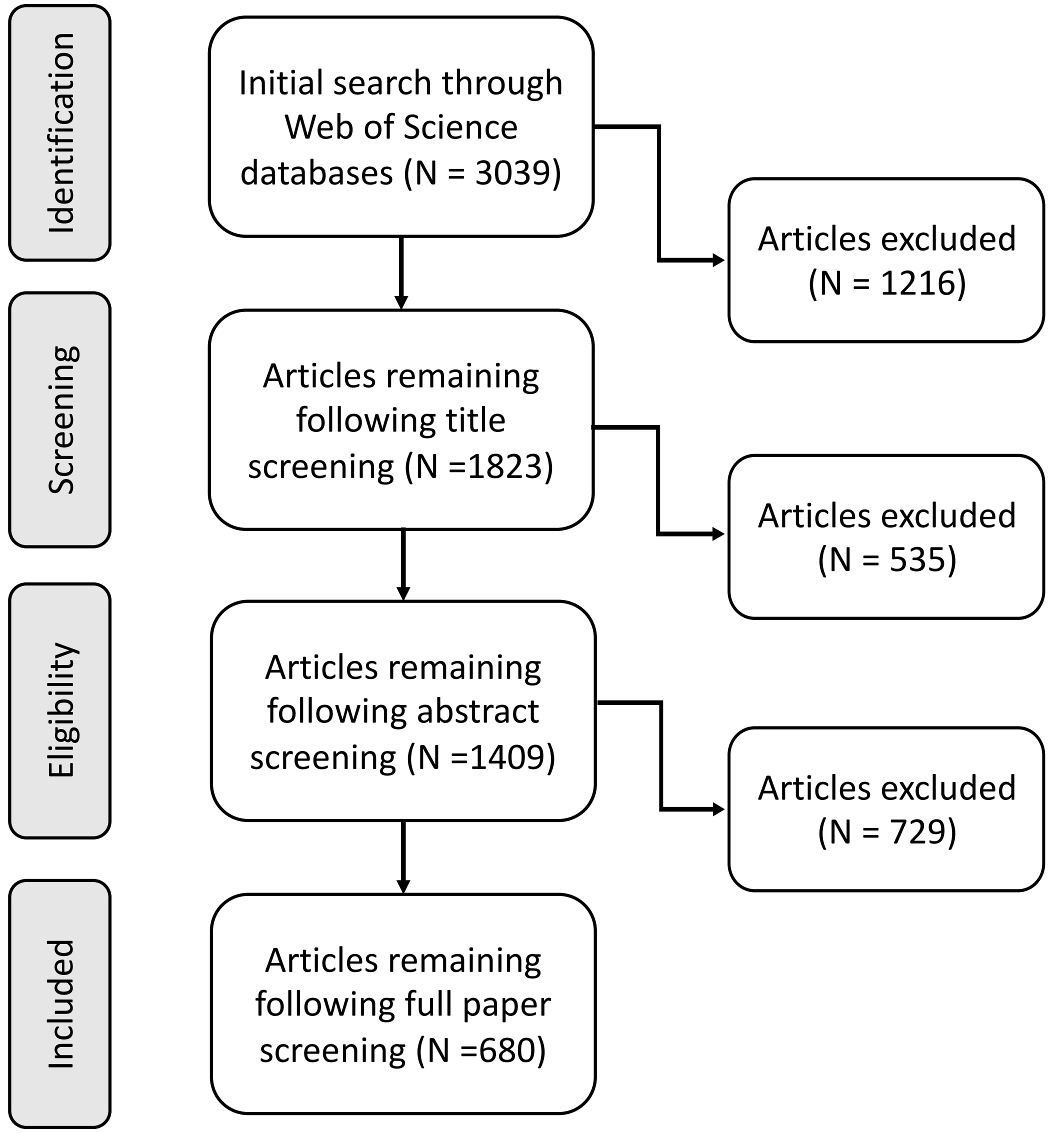

Figure 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart.

Eligibility criteria

To build a recent portrait of parasite inclusion, searches were limited to peer-reviewed empirical research articles published in eight English language journals between January 2016 and December 2020. Thus, studies using databases collected by other researchers, meta-analyses, reviews, methodological or theoretical studies, as well as studies with a medical purpose (human and nonhuman animal health) were excluded. We also excluded studies that did not measure at least one behavioral or physiological performance trait in an animal. We defined “performance” as actions that enable organisms to "regulate water, ions and temperature; [...] feed, digest, move, and grow that are crucial for [...] survival and reproduction"; Kingsolver & Huey, 2003). We focused our search on studies assessing performance traits that were measured on whole, live organisms. We thus excluded studies exclusively using carcasses or one-time samples of organs, body parts, body fluids (e.g. blood, milk) as well as studies focused at the nest/territory (if no trait was measured on one or both parents/individuals), population, community, or species level. We excluded all studies on humans and only considered studies on vertebrates originating from wild populations (including urban populations). We excluded studies measuring the performance of individuals born in laboratories, farms, or breeding centers (i.e. eggs collected in the wild but incubated and/or hatched in a laboratory setting, offspring reared in lab even if produced by parents collected from the wild, animals raised in captivity, domesticated animals and livestock).

Data collection process

After the selection process, we defined and used 14 variables for the data collection process (Table 1). Specifically, for each paper, we created a unique paper ID and recorded the journal name and the year of publication. We defined the study type as experimental (i.e. collection of data on individuals that have been purposely exposed or manipulated to a treatment/condition/factor of interest) and/or observational (i.e. collection of data on individuals that have not been purposely manipulated). We also recorded the study setting as those conducted in laboratory (i.e. person-made controlled settings), or field (i.e. in natural habitat) environments. We noted the study topic (i.e. behaviour or physiology) and characterized 12 categories of the main physiological and behavioral traits measured. Each unique article could include several study types, settings and/or topics collected as different observations within the same paper ID. To categorize the performance traits measured, we considered all response variables (y) presented in statistical analyses and figures of each study. As each trait was only assigned to one category, the decision was made based on the general topic of the paper and discussed when the trait was difficult to categorize. We also noted the taxa (fish, reptile, amphibian, bird, mammal), species names and the climatic zone (tropical, subtropical, warm temperate, cold temperate, boreal, polar). Specifically, we used the map (figure 1) published in Sayre et al. (2020) and determined in which among the six defined climatic zones the study subjects were captured.

For each selected paper, we qualified how parasite infection was accounted for using three different levels: inclusion (i.e. does the study mention parasites in the text), control (i.e. does the study treat or control parasite infection in subjects prior to or during data collection), analysis (i.e. is infection status considered in the data analysis; Table 1). Here, we considered parasites to include all live micro (i.e. bacteria, fungi, viruses) and macroparasites (i.e. helminths, arthropods, protozoa). Hosts included all non-human vertebrates regardless of their lifestyle (including brood parasites or kleptoparasites that could be potentially infected by a non-vertebrate parasite). We did not consider published articles using a non-infectious part of a virus/bacteria injected into subjects (e.g. vaccines / inactivated viral or bacterial proteins) nor published articles focusing on brood parasites or kleptoparasitism. We defined parasite inclusion as a binary consideration (i.e. yes or no) of parasite infection by screening all papers for key words associated with infection (i.e. infect-; parasit-; disease; pathogen-; virus; bacteria; quarantine) as well as reading the methods section. In published articles that scored a yes for parasite inclusion, we then considered as parasite control whether subjects were either excluded for parasite infection or received any treatment against it. Finally, we characterized parasite analysis as whether any type of quantification of parasitic load and inclusion in the study (e.g. as a predictor variable in models) was included or not during statistical analyses or whether the effect of parasite infection was one of the main goals of the study.

Patterns of parasite inclusion, control, and analysis across studies were explored in R 4.1.2 (R Core Team, 2021) using *plyr* (Wickham, 2011) and visualized using *ggplot2* (Wickham, 2016). Published articles sometimes included multiple studies conducted in different settings (i.e. both laboratory and field), types (i.e. both observational and experimental), topics (i.e. measuring physiological and behavioral traits), and/or quantified different types of performance traits (e.g. locomotion, reproduction, etc.). As a result, our dataset comprised 986 measures of host performance extracted from 680 unique articles. To assess whether our measure of parasite inclusion was biased by the unbalanced number of observations among journals, performance trait categories, vertebrate group, or climatic zone, we computed spearman correlations between the number of articles with and without parasite inclusion for each level of these categorical variables using cor.test(). The number of observations and relative frequency of observations were provided with each figure, to easily disentangle unbalanced absolute counts and proportions, either directly on the figure or in the caption.

Table 1. Variables used during the data collection process with their definition.

Variable	**Definition**
paper ID	journal code_year_article number
journal	ANBE / BEEC / CJZ / FUNE / JAE / JEB / JEMB / PRSB
year of publication	2016 : 2020
study type	experimental / observational
study setting	field / laboratory

study topic	behaviour / physiology
performance trait category	aerobic or anaerobic performance / cognition / communication / energy reserve and allocation / foraging / locomotion / morphology / parental care / personality / regulation and stress response / reproduction / sociality
taxonomic group	amphibian / bird / fish / mammal / reptile
species name	*sp*.
climatic zone	polar / boreal / cold temperate / warm temperate / subtropical / tropical (figure 1 in Sayre et al., 2020)
parasite inclusion	0 (no) / 1 (yes)
parasite control	no / exclusion of infected individuals / treatment to control or prevent infection by parasites
parasite analysis	no consideration of parasites / confounding effect / main effect

Results

General description

From the eight journals and five years screened, we identified 680 unique articles across 706 different species that studied at least one behavioural or physiological performance trait in wild vertebrates (986 performance measures in total). We found no difference in the number of articles per journal that met our search criteria across years (136 ± 13 articles, mean ± standard error; $F=0.003$, $p=0.95$). However, the number of articles found varied among journals, trait categories, vertebrate groups and climatic zones (Fig. 2). Articles from JEB, ANBE and BEEC represented more than half of those selected (i.e. respectively 26.0%, 22.7% and 15.4%; Fig. 2A). Articles quantified between 1 and 5 different performance trait categories, with the majority (65%) assessing only one. Reproduction and locomotion were the two most common performance traits studied, followed by aerobic/anaerobic performance, regulation and stress response, and personality (Fig. 2B). Birds were studied in 34.8% of the articles while articles on fish represented 22%, mammals 19.1%, reptiles 15.2% and amphibians 8.9% (Fig. 2C). The number of articles selected differed among climatic zones, with cool and warm temperate climatic zones representing 64.4% of the studies, and tropical/subtropical zones and boreal/polar zones representing 29.1% and 6.5% of the articles, respectively (Fig. 2D). The proportion of articles selected across study types was almost identical (i.e. experimental: 50.1%, observational: 49.9%), and balanced for study topics and settings. Specifically, articles on behavioural topics were slightly more common than those on physiology (56.4% and 43.6 % respectively) and there were slightly more field than laboratory studies (52.7% and 47.3% respectively). In addition, the sample size bias was reinforced when these three categories were combined, such that there was an overrepresentation of laboratory experimental studies focusing on physiology (21.8%) as well as field and observational studies focusing on behaviour (27.4 %, Fig. 3A).

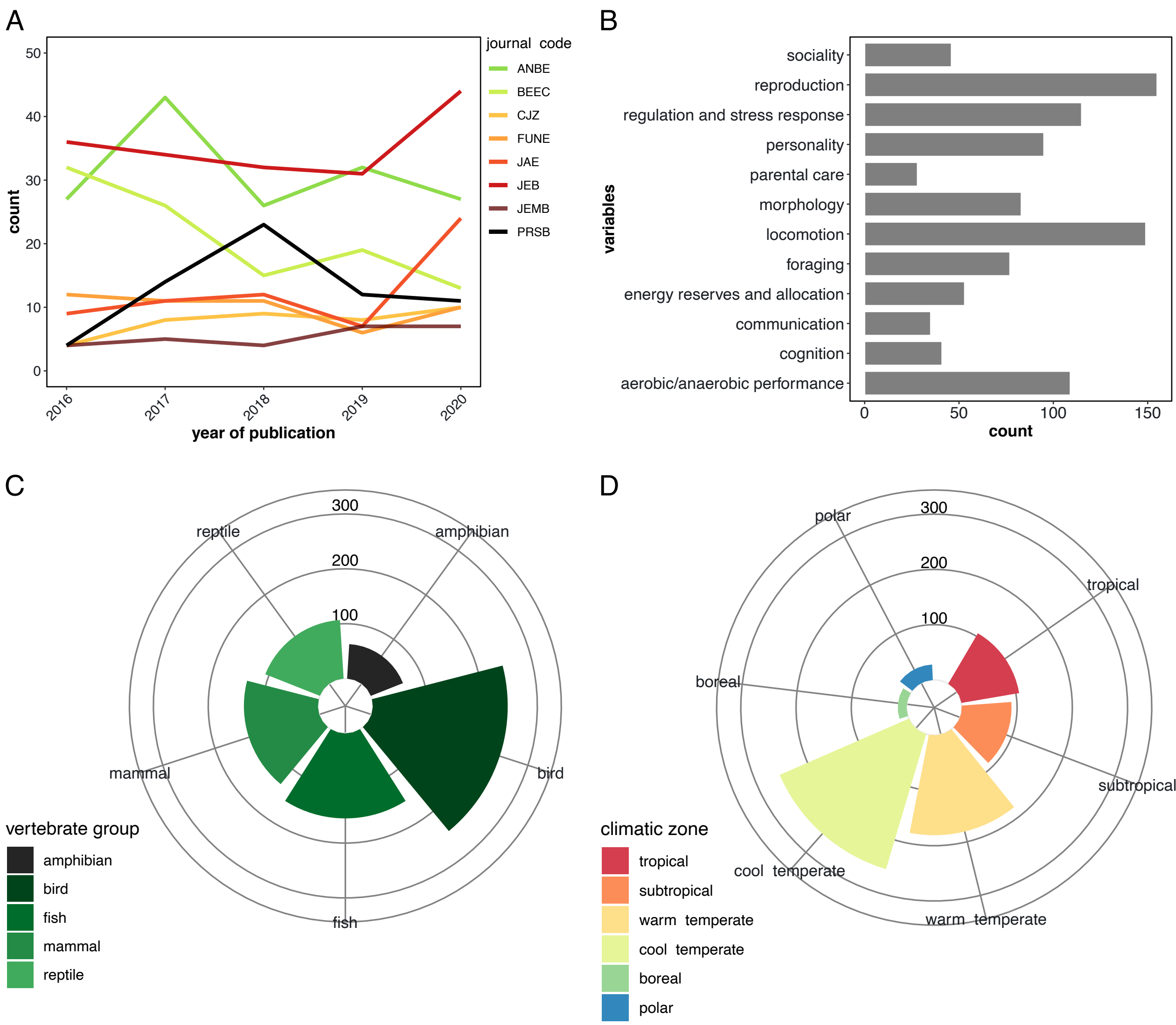

Figure 2. A) Number of articles assessed per journal across years, B) the number of articles that assessed each performance trait category, C) the number of taxa included in articles per vertebrate group, and D) the number of articles assessed per climatic zone.

Parasite inclusion, control and analysis

The majority of articles did not acknowledge parasite infection (78%). Of 680 unique articles, 149 mentioned (at least once) the possible effects of parasite infection in the manuscript (i.e. parasite inclusion; 21.9%). Among these 149 articles, no further parasite control was taken in 115 articles, 29 treated individuals for parasite infection (19.5%, representing 4.4% over all articles assessed), and 5 excluded infected individuals (3.4%, representing 0.7% over all articles assessed). Parasites were not included in the analyses in 114 of these 149 articles, while 10 included parasite infections as confounding factors (6.7%, representing 1.5% over all articles assessed), and 25 directly studied the effect of parasite infections on individual performance (i.e. main factor; 16.8%, representing 3.7% over all articles assessed). In most cases, studies included either parasite control or parasite analysis, resulting in a total of 58 articles with parasite control and/or analysis (8.5% over all articles assessed). Both treatment for parasite infection and inclusion in analyses as main factor occurred in 11 articles (1.6% over all articles assessed).

Although parasite inclusion did not vary with study type, setting or topic when considered separately, differences emerged when the effects of these factors were observed in combination. For observational studies, parasite inclusion was overall higher in laboratory compared to field studies, and higher for physiological studies compared to behavioural studies (Fig 3A). As a result, in observational physiological studies, parasite inclusion was higher for studies conducted in the laboratory (34.6%) than for studies conducted in the field (22.1%). In contrast, for experimental studies, we found no general trend in parasite inclusion, as 25.9 ± 1.0% articles (average ± standard deviation) acknowledged parasite infection, regardless of the study type and the topic (Fig. 3A). Among articles with parasite inclusion, parasite control (i.e. treatment and exclusion) was higher in studies conducted in laboratory rather than in a field setting (i.e. 27.9 ± 12.0% and 17.1 ± 11.9% respectively; Fig. 3B). In contrast, parasite analysis (including parasites as a main or confounding factor) was two times higher in studies conducted in the field rather than in a laboratory setting (i.e. 38.4 ± 12.4% and 16.2 ± 3.7% respectively; Fig. 3C).

Parasite inclusion among journals, vertebrate groups, performance traits and climatic zones was highly dependent on the number of articles assessed that fell under each category (Fig. S1). For instance, the number of articles with or without parasite inclusion were highly correlated for journals (rho= 0.929, p=0.002), performance trait categories (Spearman's rho= 0.788, p=0.002) and climatic zones (rho= 0.943, p=0.017) but marginally correlated for each taxonomic group (rho=0.900, p=0.083). This bias prevented us from highlighting the effect of these variables. Moreover, differences in parasite inclusion and control among journals, taxonomic groups, or performance trait categories (Fig. S2) reflected the differences observed among study setting or type as well (Fig. S3-4). For instance, the highest proportion of articles in which individuals were treated for parasite infection were conducted on fish, which were also the most common vertebrate group used in experimental studies in a laboratory setting (Fig. S3C). Finally, even if studies on temperate climatic zones were overrepresented in our sample, levels of parasite control and analysis were similar for cool temperate, warm temperate, tropical and subtropical studies (Fig. 4B-C). Of the five articles describing studies that excluded infected subjects, four of them occurred in subtropical zones (Fig. 4B). In addition, the number of articles including parasite infection as a main factor was higher for subtropical studies compared to the other climatic zones (Fig 4C).

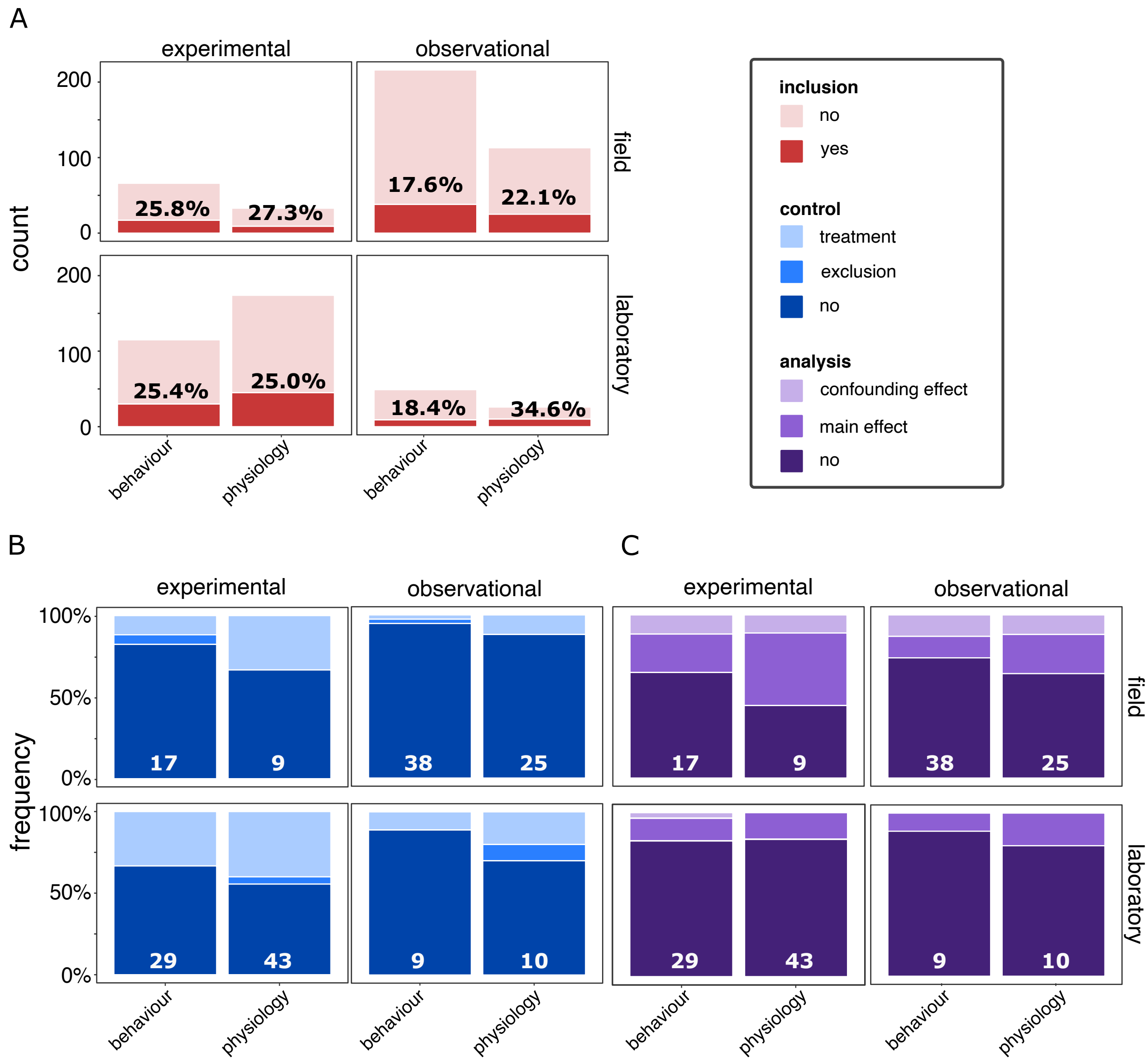

Figure 3. Parasite A) inclusion, B) control and C) analysis depending on the study type, setting, and topic (N=789). A) Number of articles with (dark red) and without (light red) parasite inclusion. B) Proportion of articles controlling (light blue), treating (blue) or not considering parasite infection (dark blue) in the study design. C) Proportion of articles considering parasite infection as a confounding factor (light purple), as a main effect (purple) or not considering it in the study design (dark purple). The percentages on panel A represent the proportion of studies with parasite

inclusion while the counts on panels B and C represent the total number of studies with parasite inclusion for each level of study type, setting, and topic.

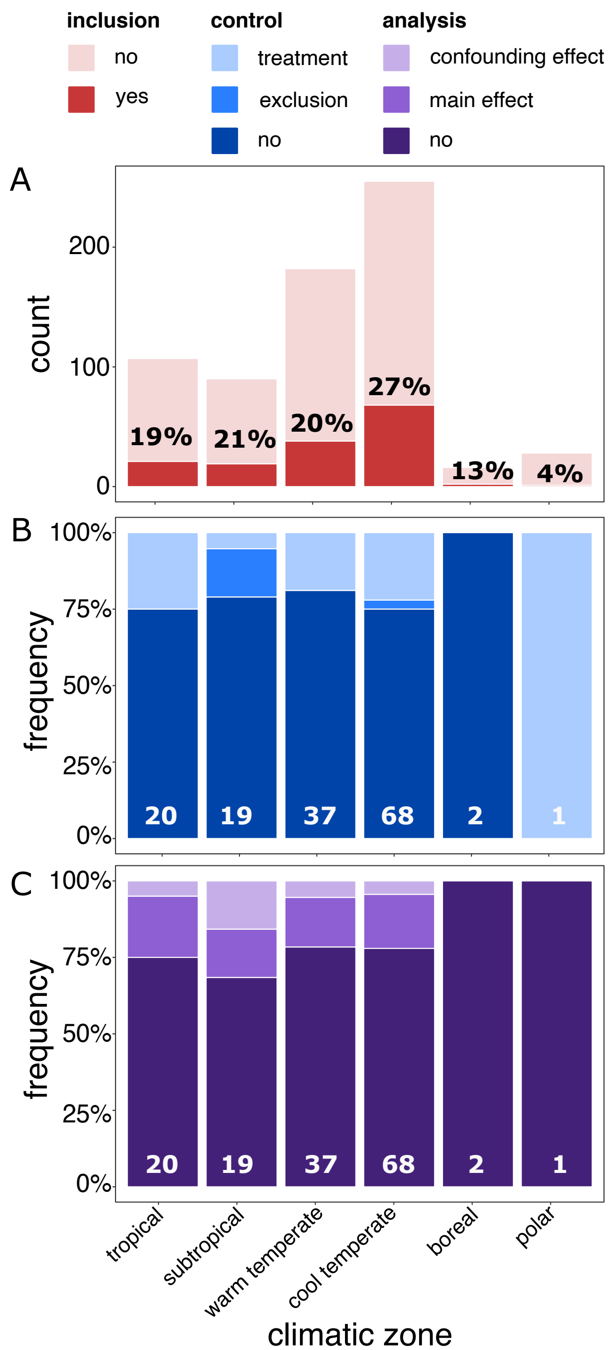

Figure 4: Parasite inclusion (A), control (B) and analysis (C) depending on the climatic zone (N=676). A) Number of articles with (dark red) and without (light red) parasite inclusion. B)

Proportion of articles excluding (light blue) infected individuals, treating (blue) or not considering parasite infection (dark blue) in the study design. C) Proportion of articles considering parasite infection as a confounding factor (light purple), as a main effect (purple) or not considering it in the analyses (dark purple). The percentages on panel A represent the proportion of studies with parasite inclusion while the counts on panels B and C represent the total number of studies with parasite inclusion for each level of climatic zone.

Discussion

Parasites are prevalent in all types of ecosystems (Caballero et al., 2015; Kuris et al., 2008; Poulin & Morand, 2000) and can influence their host's performance (e.g. Barber et al., 2000; Binning et al., 2017; Guitard et al., 2022; Poulin, 1994). Despite the large body of literature on parasite ecology, our results show that parasite infection is often overlooked or ignored by researchers (Timi & Poulin, 2020). Although the potential effects of infection on animal performance were acknowledged in 21.9% of articles assessed, we found that only 8.5% of these articles explicitly mentioned that they controlled for and/or included measures of infection in their analyses. This lack of consideration is potentially problematic as parasites can be a confounding factor that can lead to false conclusions if not accounted for, especially in studies on wild animals (Timi & Poulin, 2020). In the following paragraphs, we explore potential explanations for this gap between inclusion, control and analysis of parasites, and propose methodological and/or statistical tools that can help researchers tackle this problem in the future.

Parasite inclusion, control and analysis

We expected to find differences in parasite inclusion, control and analysis among study types, settings, and topics. In contrast, we found that parasite inclusion did not vary according to any of these factors when taken separately, but was overall lower for observational studies focusing on behavioural performances, regardless of the study setting (Fig. 3A). The highest proportion of control for parasite infection or exclusion of infected individuals was observed in experimental studies on physiological performance conducted in laboratory settings (Fig. 3B). Trends for parasite analysis were similar for the study type and topic, however, parasite consideration in analyses was higher for studies conducted in the field rather than in a laboratory setting (Fig. 3C). These results are partly in line with our predictions, as we expected that both parasite control and analysis would be more common in controlled environments, such as a laboratory. This could suggest that if parasite infections are controlled for, there is no need to consider parasites in analyses. Conversely, inclusion of parasite measures in analyses may be more relevant when no parasite control is performed on study animals, either because such action was impossible or because researchers wanted to account for individuals' parasite load in order to maintain ecological relevance. This could explain why both parasite control and parasite analysis were observed in only 11 articles included in this review. Hence, both research objectives and the level of manipulation of animals during research could influence how researchers account for parasites in their studies. Interestingly, most parasite control reported in the selected articles involved treatment of parasite infection rather than exclusion of infected subjects, while most of the parasite analyses involved including parasite measures as a main factor rather than as a confounding factor (Fig. 3B-C). This could indicate that metrics of parasite infection are tedious to quantify when they are not an a priori focus of the study. The fact that wild animals may be released at the end of a study could prevent the collection of parasite measures, and explain this lack of consideration in some analyses. In such cases, visible external parasites (ectoparasites, abrasions or lesions caused by infection), may be easier to consider as an estimation of an animal's infection status, and included as a confounding variable in data analyses. This strategy should be used with care and its limits acknowledged, as visible infection can be an imperfect indicator of the overall infection status of an animal (Guitard et al., 2022), but it has been demonstrated to be a good proxy of performance impairment in some taxa (e.g. willow ptarmigan, Holmstad et al., 2008).

The difficulty of collecting metrics of parasite infection may also explain why there were no differences in parasite inclusion, control and analysis among taxonomic groups, journals, performance trait categories, and climatic zones. We found, in contrast, strong biases in sample sizes and correlations among variables (Fig. 2). For instance, we found that treatment of parasites was often conducted on fish (Fig. S2), which was also the most represented group in experimental studies in a laboratory setting, and mostly published in *Journal of Experimental Biology* (Fig. S3). Among performance trait categories, more than 40% of studies with parasite inclusion investigating aerobic/anaerobic performance included parasite control (Fig. S2), but this trait was most frequently studied in a laboratory setting (86.2%; Fig. S4). The unbalanced sample sizes in certain study types and settings limited our ability to draw definitive conclusions on parasite inclusion for taxonomic groups, journals, and performance trait categories. Despite the unequal sample sizes among climatic zones, our results show similar parasite inclusion, control and accounting in statistical analysis in tropical, subtropical, and both temperate zones (Fig. 4 B-C). In contrast, we predicted that parasite inclusion would be highest in studies on temperate species because of the greater taxonomic knowledge of vertebrate parasite fauna in these zones. Despite a more limited knowledge of species taxonomy in tropical climates, parasites are at least as diverse and prevalent in these regions as in temperate zones. Indeed, tropical climatic zones may in fact harbour more parasites than other climatic zones. While there is no evidence of a relationship between latitudinal gradients and prevalence and diversity of parasites in vertebrates (Poulin, 2010), positive relationships between host diversity and parasite diversity have been observed across taxa (Kamiya et al., 2014). For instance, positive correlations between ectoparasite diversity and temperature have been reported for marine fishes (Poulin & Rohde, 1997; Rohde & Heap, 1998), yet the pattern between endoparasite diversity and temperature in marine (Rohde & Heap, 1998) or freshwater fish hosts (Choudhury & Dick, 2000; Poulin, 2001) is the opposite. Taken together, these trends suggest a higher parasite diversity is likely in the tropics, where temperature variations are small and host diversity is high, making it unsurprising that researchers working on tropical species would be as likely to consider parasites in their studies as those working on temperate host species.

Parasite consideration is likely higher than reported

We scored parasite inclusion based on explicit mention of parasite presence, control, treatment or quantification in the text of articles assessed. However, it is possible that some articles did consider parasites in their treatment and statistics without explicitly reporting it in their paper. Quarantine periods or treatment to control for parasite infections may not be reported because such practices are common in university animal care facilities or for specific study animals. Such practices may even be mandatory in some cases to obtain an animal care certificate or a research permit (e.g. California State University, article ID JEMB_18_18, Table S1). Since reporting of compliance to animal care protocols is required by scientific journals, one might suggest that in such cases researchers did not deem it relevant to add details of parasite control in their manuscript. In addition, infected individuals could also have been discarded during capture events, and their quantity not included in the sample size reported in studies. Similarly, mortality during transport or experimentation may not be explicitly reported but could very well be due to parasite infections. In statistics, researchers may not report results of exploratory analyses conducted to rule out confounding variables, such as estimates of infection or sickness noted in the field. Several criteria we used in this systematic review might also have discarded published articles that account for parasite infections. For instance, we removed animals bred in outdoor captivity (e.g. fish farms) because breeders usually control for most infections through the administration of antibiotics or other therapeutic treatments. Given these and other potential oversights, the proportion of studies which do consider parasites is likely higher than we have reported here. Whether researchers decide to actively treat or remove infected individuals or not, we nonetheless argue that they should always report practices in place to account for effects of parasite infection to ensure repeatability of studies and raise awareness of the importance of infection in measured traits.

Recommendations for parasite consideration

Our systematic review allowed us to summarize a range of techniques currently employed by researchers in the field (Table S1). First, non-invasive techniques can be used to assess individual health status for studies on animals that are not captured or are captured and held temporarily (i.e. seconds to minutes) prior to release. For instance, photographing individuals can help identify lesions, discolouration, malformation or some ectoparasites. Infrared thermography is also an increasingly used technique that can be used to identify febrile individuals in endotherms (Mota-Rojas et al., 2021). When individuals can be handled for a short period of time, a quick assessment of ectoparasite load can be performed. These can be left on or removed depending on the type of infection and the study objectives. In some cases, a small amount of blood can be sampled and assessed in the laboratory for bacterial-killing ability and/or leukocyte count (indicators of immune activity). Blood/tissue samples can also be screened for known pathogens through molecular techniques (Lind et al., 2020). Second, for animals that are captured and held over a period of days-weeks, a quarantine period prior to testing should be performed and explicitly mentioned. Anti-infection treatments should also be carried out and detailed (e.g. doses and timing of drugs administrated, Table S1). Alternatively, if anti-parasite treatments are deemed invasive or likely to affect performance measures, records of infection status should be taken. Third, when individuals can be captured and euthanized following trait measurement, it is valuable to screen individuals for endoparasite infection. Although a full assessment of parasite richness and abundance may not be logistically feasible without a dedicated team of parasitologists, we suggest that key organs be examined quickly under a stereoscope for noticeable infections or pathologies that may impact performance. For instance, metabolically active organs such as the liver should be examined as lesions to the liver caused by infection can cause depletion of energy reserves and thus, affect aerobic performance (Ryberg et al., 2020). Similarly, energy allocation shifts during an immune response ultimately affect aerobic performance and can be easily assessed by measuring differences in spleen morphology (e.g., enlargement) which is a widely used proxy of immune status in fish (Lefebvre et al., 2004). Other organs could be screened depending on the performance trait measured, such as gonads for reproduction (Albery et al., 2020), muscles for movement and activity (Umberger et al., 2019), and brain for cognitive performance (Townsend et al., 2022). This targeted approach may help reduce the burden of conducting a full analysis of parasite fauna while maximizing the chances that potentially relevant infections can be quantified. Finally, we encourage more researchers to explicitly include parasite infection as a main factor in their study design. Parasites are important features of wild animal populations and individuals show a gradient in parasite load. If researchers wish to interpret the results of their studies in an ecological context, it is imperative that research is conducted on subjects experiencing biotic stressors such as infections. Conclusions drawn solely on individuals that have been treated for infection and/or are healthy may not be representative of how animals perform in nature, where infections and immune costs can severely compromise optimal performance. We hope that our study encourages more researchers to embrace parasite infection as a feature, not a bug, of their study systems and bring more awareness to these overlooked, yet critically important pieces in the eco-evolutionary puzzle.

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Conflict of Interest

The authors declare no conflict of interests.

Contribution statement

J. de Bonville and S.A. Binning conceived the idea. E. Chrétien, M. Barou-Dagues, J. de Bonville and J. Guitard designed methodology; E. Chrétien, M. Barou-Dagues, J. de Bonville, J. Guitard, A. Kack, É. Melis, S.A. Binning, V. Thelamon, M. Levet conducted the search process; E. Chrétien, M. Barou-Dagues, J. de Bonville, J. Guitard, A. Kack, É. Melis, V. Thelamon, A. Côté, M. Gradito, A. Papillon. collected the data; E. Chrétien and M. Barou-Dagues analysed the data and produced the figures; E. Chrétien, M. Barou-Dagues, J. de Bonville and S.A. Binning wrote the manuscript. All authors contributed to the drafts and gave final approval for publication.

Data availability statement

The data underlying this article are available in the Zenodo repository and can be accessed here: doi: 10.5281/zenodo.7296510.

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