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PERSPECTIVE OPEN ACCESS

Known and Unknown Biases: A Framework for Contextualising and Identifying Bias in Animal Behaviour Research

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ABSTRACT

Biases in animal behaviour research are inevitable consequences of our societal and cultural standpoint. To remove our biases, the first stage is to identify them. We call on individual researchers to adopt a more active approach to addressing bias within their research. We propose that biases exist within a matrix defined by the general acceptance of a bias's existence and the understanding of the impact this bias has on research outputs. Borrowing from a conceptual framework previously applied to the study of biodiversity, our matrix consists of four categories: “*known knowns*” are biases we are aware exist and are empirically tested; “*known unknowns*” are biases we know of but have limits to being mitigated against; “*unknown knowns*” are biases which we know exist but are overlooked; and “*unknown unknowns*” are biases we are unaware exist. Contextualising biases in this way, we believe, will lead to greater investment by individual researchers to locate and mitigate biases in their own research. To facilitate this process, we provide a set of self-reflective questions designed to help researchers critically evaluate the assumptions, limitations, and generalisability of their research. By acknowledging and addressing biases within this framework, we move toward a more robust and trustworthy scientific process.

1 | Introduction

Contrary to the rhetoric of scientific objectivity, scientists are not neutral, dispassionate observers. It has long been recognised that aspects of our social context—e.g., our upbringing, education, culture and beliefs—can strongly influence how we inquire about and interpret the world (Gould 1981; Keller and Scharff-Goldhaber 1987; Zuk 1993). Thus, as individuals, we construct our knowledge from a perspective that reflects our

own experiences and understandings (Sprague 2005). This perspective is influenced by those who created knowledge before us, who were themselves similarly influenced (Adams et al. 2015). It is therefore inevitable that scientific research will be prone to bias, and as scientists, it is our responsibility to address, mitigate, and remove biases from our research. This is particularly true for the study of animal behavior, which often requires a level of inference and subjective interpretation. Here, we aim to foster an environment where individual researchers take greater

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accountability for bias in their own work and actively seek out bias at every stage of the research process. Though biases will arise in all fields of science, here we illustrate our article with examples from animal behavior research and have a general focus toward quantitative science to illustrate the points we raise as this is our combined field of expertise. However, we believe the ideas can be applied to other fields.

There are a number of ways biases pervade in the research process. (i) When formulating our hypotheses, we may seek out processes which align with our expectations (Kamath and Wesner 2020; Urquiza-Haas and Kotrschal 2015; van Wilgenburg and Elgar 2013; Yanai and Lercher 2020). For example, many traits are studied in only one sex (Haines et al. 2020; Zucker and Beery 2010), possibly because we view certain traits as masculine or feminine (Green and Madjidian 2011). (ii) When planning our methodology, we may base our approach on what we assume is biologically plausible, setting the limits around our own perception of the environment and/or the subject's capabilities (Bennett et al. 1996; Hansell and Ruxton 2008). Sampling methods, particularly in animal behavioral research, are also particularly vulnerable to bias (Webster and Rutz 2020). For example, studies involving the trapping of animals may inadvertently select for 'bold' individuals (Carter et al. 2012). Animals used in both captive and wild behavioral studies may be non-random in many ways, from their genetic makeup to their previous experiences (Griffith et al. 2017; Webster and Rutz 2020), and sampling may be biased towards aesthetics, oddities and rarities, particularly in scientific collections (Pyke and Ehrlich 2010). (iii) Finally, even if we succeed in collecting unbiased data, our interpretations of that data may be influenced by our pre-existing ideas (Matlin and Stang 1978; van Wilgenburg and Elgar 2013). Acknowledging our inherent biases as individuals and maintaining awareness that our biases likely enter our research process facilitates improved detection and mitigation of bias.

Several frameworks exist which aid the mitigation of specific biases (see for example, Chadwick et al. 2024; Moher et al. 2015; von Elm et al. 2008; Webster and Rutz 2020). These frameworks have arisen by individual researchers locating a bias in their research field and taking steps to address this. We believe we need to create an environment where more researchers are taking an active approach such as these to locate bias in their own research. Zvereva and Kozlov (2021) found that researchers believed their own research was less prone to bias than that of other researchers, demonstrating a need for all individual researchers to acknowledge that their research is likely to be biased (Zvereva and Kozlov 2021).

How, as researchers, can we locate our bias? We suggest that by classifying biases, researchers will be better able to assess how their own work may be influenced by biases. Loxdale et al. (2016) applied a knowledge-data framework to the current understanding of biodiversity, given that both knowledge and data are not constant over time (Loxdale et al. 2016). They highlight that there are species known to science ('*known knowns*'); species we hypothesize to exist or have existed ('*known unknowns*'); species once recorded but likely to have now gone extinct, although this cannot be proven ('*unknown knowns*'); and finally, species yet to be discovered ('*unknown unknowns*'). Loxdale et al.'s framework

provided a tool to enable researchers to focus on areas of biodiversity research that lack knowledge and/or data. We believe adapting this approach can provide a key tool to address areas of animal behavior research which are impacted or prone to bias.

In this article, we apply the conceptual framework of '*knowns*' and '*unknowns*' to our current understanding of bias in a scientific framework as a tool for researchers to envision how biases may manifest in their research (Figure 1). For a bias to be fully mitigated against, it is crucial for all researchers to be aware of its presence and for the impact the bias has had to be fully investigated. We, therefore, modify Loxdale et al.'s matrix such that there are two linear factors describing bias in scientific contexts: (x) the level of acceptance of a bias' existence in the general scientific population (or field of research if the bias is specific to an area/field) and (y) how much current understanding there is of the impact the bias has on scientific research. Before a bias can be mitigated against, we need to be aware of its presence, and hence, the acceptance of a bias' existence (x-axis) is a crucial component to mitigating against bias. However, being aware of a bias cannot on its own lead to mitigation of the bias: we as researchers also need to take action to address the bias by fully understanding the impact the bias has on research.

Becoming aware of a bias's existence is innately difficult, as it requires us to challenge what we assume to be true (Zvereva and Kozlov 2021). How aware we are of a bias's existence is strongly intertwined with human culture and societal bias. There is a logical progression from being aware of how our culture and society views the world, to awareness of how our viewpoints are biased or influence our research (Figure 1, x-axis). It is innately challenging to be aware of a bias that has no obvious logical links to how we perceive the world. Therefore, this axis (x) is challenging to progress along, unlike the y-axis which is directly related to the amount of investment by researchers. Once a bias is located, it is up to us as individual researchers to invest in fully realising and mitigating against the bias. The understanding of a bias's impact on research (y-axis) does not necessarily mean the bias has been fully investigated and mitigated against but rather, it is receiving sustained attention from researchers, and the knowledge of how the bias has affected/is affecting research is increasing.

Our manuscript describes four categories of bias: '*known knowns*', '*known unknowns*', '*unknown knowns*' and '*unknown unknowns*' (summarised in Figure 1). '*Known knowns*' represent biases that we are readily aware of, and there has been sustained investment in understanding the impact of the bias. Researchers should strive for all biases to be '*known knowns*'. '*Known unknowns*' are biases of which we are aware, but there are barriers preventing empirical testing or mitigation. These biases risk becoming embedded in research practice without sustained investment in acknowledging they exist and efforts made to mitigate against them. '*Unknown knowns*' are biases of which we are aware but are overlooked. These biases often have fewer barriers preventing them from becoming '*known knowns*' but often the current research culture prevents motivation for researchers to invest in mitigating against them. '*Unknown unknowns*' are biases that remain outside of our awareness. By consciously making an effort to locate bias in our research, we are more likely to uncover '*unknown unknowns*'. We encourage researchers to

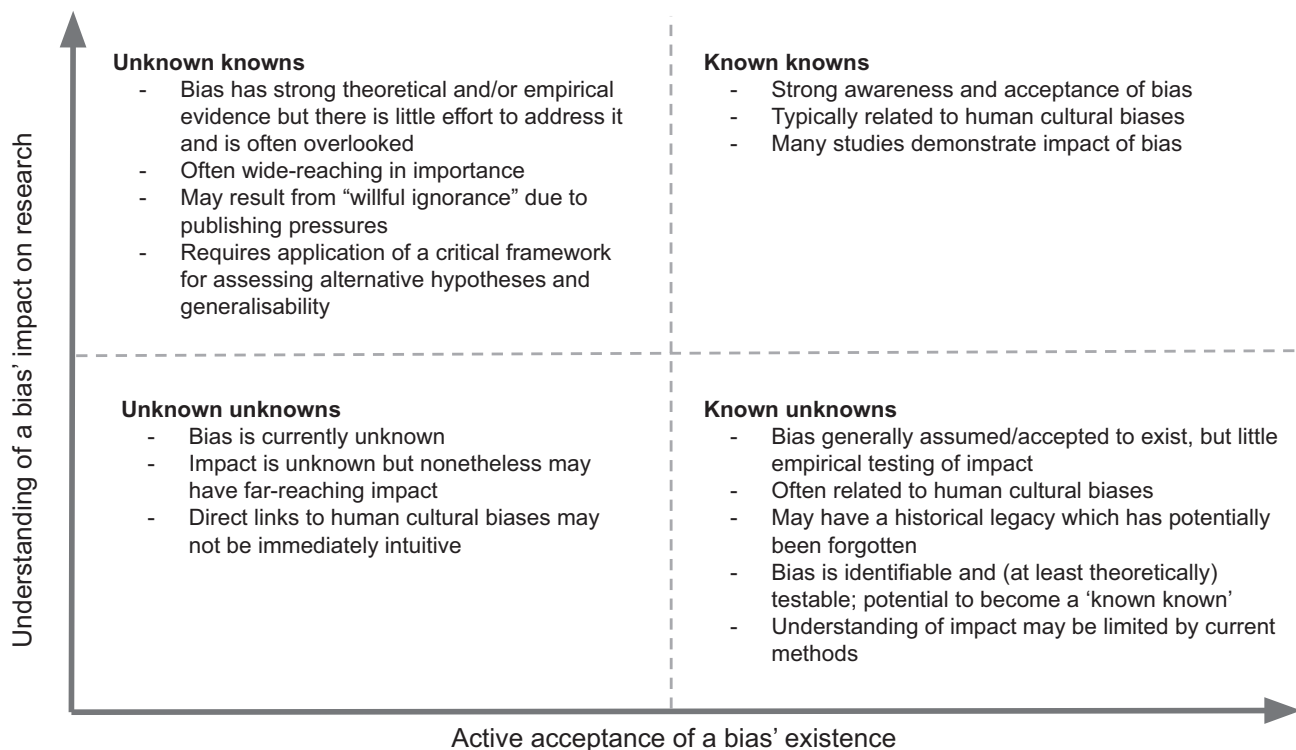


FIGURE 1 | A conceptual framework of 'known' and 'unknown' biases during the research process. The framework is based on a matrix where (x) represents the level of acceptance that a bias exists and (y) represents the current understanding of the impact a bias is having on scientific research, and each combination describes the features of a bias located along each axis. The four categories can be interpreted as coordinates (x, y).

assess every step of the research process for bias by considering that biases may present themselves in one of the four categories. Our framework provides a guide for researchers to explore and question their research process to locate biases, with the aim of full awareness and understanding of the impact of the bias.

Our framework provides an approach which, though aimed at the field of animal behaviour, can be applied across various fields of research and provides individual researchers with the tools to locate bias in their own research. It is important to note that uncovering unknown bias often involves an element of luck, such as a bias being revealed through new methodologies (e.g., Griffith et al. 2008). However, the likelihood of locating biases becomes significantly easier when we are actively searching. We believe our framework will encourage researchers to be more mindful of potential biases, thereby increasing the likelihood of identifying biases in their own work.

2 | Known Knowns: Biases We Know of That Are Empirically Tested

We propose 'known known' biases are those which are widely recognised, accepted, and empirically tested by researchers. These biases, therefore, feature highly in both the general acceptance (x) and there is a thorough understanding of the biases impact in research (y). It is notable that awareness of these biases is often, unsurprisingly, intertwined with cultural shifts in human society, such as in the case of the study of race, gender, and socio-economics (Gould 1981; Henrich et al. 2010; Saini 2019). In animal behaviour research, historically an anthropomorphised

view also meant behaviours were observed with preconceived stereotypes, such as sex roles. However, as researchers have become more aware of this bias, the field has made efforts to mitigate its influence, prompting shifts in research focus, changes in the language used to describe behaviour, and critical reflection of how the bias has shaped the field (Green and Madjidian 2011). Other 'known known' biases have less obvious societal links but are nevertheless impactful on scientific research. These biases are, more often than not, broad in terms of their impact across multiple fields. For example, observer bias is a well-known phenomenon that impacts multiple fields and disciplines. In the field of animal behaviour, observer bias occurs when a researcher's prior expectation influences how they score or interpret an animal's behaviour (Tuytens et al. 2014). Researchers have implemented standardised ways to mitigate this bias, such as observer blinding (Traniello and Bakker 2015). Biases in this category are not exclusively broad, but those which are area-specific tend to have clear definitions and have implemented practices in the research field to mitigate the effects. For example, citizen science projects can be prone to spatial bias, where there is variation in recorder activity such as 'hotspots' of spatial coverage close to high human population densities (Geldmann et al. 2016). This can be reduced through sampling design and accounted for during data analysis, for example, using spatial filtering (Robinson et al. 2018).

Not only are 'known known' biases widely accepted in the scientific community, but they also receive abundant attention in scientific literature, either through empirical testing. For example, studies on female sexually selected traits receive much more attention since it was highlighted that female sexual selection was

underrepresented in the literature (Amundsen 2000; Clutton-Brock 2009). It is now commonly assumed that laboratory and rearing conditions can lead to differences in observed behavior in laboratory studies (Griffith et al. 2017). ‘Known known’ biases have also seen the implementation of methods to mitigate against them, as is the case with publication bias (Møller and Jennions 2001) and observer bias (Traniello and Bakker 2015). There is also frequent discussion of the bias, for example, representation and diversity amongst researchers (Keller and Scharff-Goldhaber 1987; O’Brien et al. 2020), anthropomorphising animal behaviour (Pollo and Kasumovic 2022) and gender biased research (Zucker and Beery 2010). As such, ‘known known’ biases have the ability to gain traction once a bias is known to the scientific community, leading to an exponential increase in efforts to address and mitigate the bias. The discovery of ‘known knowns’ are of high value and researchers should aim to prioritize locating these biases and moving other biases into this category. Not only do they increase our knowledge of the given bias, making research more trustworthy, transparent, and reliable, but they also lead to various new avenues of research. This has been the case in the study of animal personalities/temperaments, where variation between individuals was once overlooked; there are now numerous studies and theories as to why individual variation in behavior is maintained, leading to deeper insights into how personality influences our understanding of evolution and ecology. For example, pace-of-life syndromes, bold/shy individuals, and dispersal syndromes (Laskowski et al. 2022; Réale et al. 2007; Wolf and Weissing 2012).

As researchers, we should aim to locate biases and fully explore them. That is, we should aim for all biases to become ‘known knowns’. However, it is important to highlight that when a bias becomes a ‘known known’, this is not the endpoint. The examples of specific biases above illustrate this, as we would not consider any of these examples to be completely understood and resolved. Researchers should strive to fully explore these biases, filling in the gaps in our knowledge, addressing and rectifying the bias’s influence to date, and expanding bias mitigation to other fields. Our framework provides a guide for researchers to locate biases and thereby increase awareness and understanding of their impact to move them within this category.

3 | Known Unknowns: Biases We Know of but Cannot Currently Be Mitigated Against

‘Known unknowns’ are biases which researchers are aware of (high in acceptance, (x)) but have barriers preventing mitigation or empirical testing (low in understanding of impact (y)). These biases are often specific to a research field and may be difficult to circumvent by experimental design. That is, biased methods may be unavoidable until new techniques or methodologies are developed. For example, certain demographics within a population may be missed due to sampling limitations. This is the case with the so-called ‘invisible fraction’ where a non-random subset of a population dies before it is sampled and leads to biased inference, such as on the strength of selection or the amount of genetic variance; and unless there is sufficient genealogical data, it may be impossible to account for this analytically (Grafen 1988; Weis 2018). Similarly, animal behaviour studies may also suffer from detection/sampling bias, where certain

individuals are more likely to be observed or captured, for example, bold individuals (Carter et al. 2012). There are also biases which require systematic changes to the academic process to resolve, as is the case with publication bias. Though methodologies have been developed to account for publication bias (as is the case in meta-analyses), there are still few, if any, areas of research where the published literature is truly representative of the natural world (Møller and Jennions 2001). As with the case of publication bias, ‘known unknowns’ are often a historical legacy. For example, the non-random collection of specimens that make up museum collections may result in biased conclusions (Meineke and Daru 2021).

‘Known unknown’ biases have the potential to become ‘known knowns’, and in some cases, relatively easily. However, progress is often hindered by a lack of resources available for dedicating effort towards finding resolutions to these biases, such as the call for investment in removing bias from aggregated datasets of biological collections (Meineke and Daru 2021). These should, therefore, be key targets for developing approaches for mitigating and removing the bias. However, they also run the danger of becoming stagnant areas that are never satisfactorily resolved, ultimately holding back progress in the field. It is often all too easy to justify a pre-existing method by simply citing its use elsewhere, without stating its limitations. This uncritical approach may lead researchers to increasingly ignore these biases and may risk the bias being forgotten about over time—see ‘unknown knowns’. By remaining aware of ‘known unknown’ biases throughout the research process and acknowledging them in our outputs, we improve our chance of developing new ways to address the bias or challenge the systems that hinder its mitigation.

4 | Unknown Knowns: Biases We Know of but Overlook

‘Unknown known’ biases are those that are easily located but nonetheless are overlooked or dismissed (low on acceptance (x), though there is general understanding of their impact (high in y)). These biases have limited empirical evidence despite there being no significant barriers to investigation. These biases remain underexplored, contributing to ‘research waste’ whereby studies are either not published due to biases such as publication bias or are published without sufficient detail to allow proper scrutiny or replication by other researchers (Purgar et al. 2022). ‘Unknown known’ biases may be particularly problematic since they often have wide-reaching importance across multiple fields, and as such, we generally understand the impact these biases have on research and the severe impact they have on research integrity and reliability but fail to act upon them. One of the clearest examples is confirmation bias and the failure to report alternative hypotheses to explain results (MacCoun 1998; Nickerson 1998; van Wilgenburg and Elgar 2013). In research of animal behaviour, for example, there is a tendency to interpret behaviours in a human-centric way rather than from the perspective of the animal (e.g., see, Amundsen 2000; Pollo and Kasumovic 2022; Tuytens et al. 2014; Wynne 2004). A failure to diversify the subjects of our research fully is a key contributor to the reproducibility crisis, as it limits the replicability and generalisability of our findings. Prominent examples in animal behaviour include the persistent sex bias in animal studies,

with males being more commonly used in animal experiments (Voelkl et al. 2020; Zucker and Beery 2010; Zuk 1993) and the over-reliance on model organisms, which are often highly unrepresentative of wild organisms, even those which are the closest free-living relatives of model organisms (Alfred and Baldwin 2015).

'Unknown known' biases have the potential to become 'known knowns' but require additional investment among researchers towards finding resolutions. For example, Marshall (2024) highlighted that inadequate experimental design is not uncommon in ecology and evolution but can be reduced significantly by dedicating time and effort to discussing experimental design (Marshall 2024). The progress of some biases from 'unknown knowns' to 'known knowns' is hampered by the pressure to publish in modern academia (Fanelli 2010). Biases such as publication bias and confirmation bias can be aided by journal initiatives, such as data-sharing. A recent study by Ivimey-Cook et al. (2025) showed that 61% of journals in ecology and evolution had either mandated or encouraged data-sharing in response to the reproducibility crisis, with a 96.5% compliance rate when mandated policies were enforced by editorial staff (Ivimey-Cook et al. 2025). This demonstrates that once the impact a bias is having on research is understood and awareness among the research community increases, initiatives such as journal mandates become increasingly adopted and are an effective strategy to mitigate the bias and increase researcher awareness. The current system encourages an environment where a cohesive, high-impact story is favored over reporting what are viewed as potential flaws such as viable alternative hypotheses or a lack of generalizability. Furthermore, more biases will be mitigated against if individual researchers take a more active role in locating and addressing these biases in their own research, which has been exemplified with frameworks such as STRANGE (Webster and Rutz 2020).

Mitigation of 'unknown known' biases can be aided by deliberate interventions to encourage researchers to identify and resolve biases during the research process. Within other fields, such as psychology and social science, the influence of social, political, and cultural factors has been acknowledged and confronted via critical theory (Kellner 1990). The application of critical theory aims to remove implicit and unconscious bias by identifying gaps, limitations, and assumptions of current understanding. We suggest this same approach can be applied to biological research and propose a formal critical approach, which will allow us to evaluate how bias influences our choice of research questions, development of theory, choice of methods, and interpretation of evidence (Green and Madjidian 2011; Haines et al. 2020) (Table 1).

In Table 1, we outline a novel formal critical approach to identify biases for animal behaviour research. The underlying theme is that critical theory can be implemented with explicit justification of every assumption, action and conclusion within the research process. This can be broken down into three reflective questions: 'What assumptions am I making?'; 'What limitations does my study have?'; and 'How generalisable is my research?'. We believe these three questions capture the core sources of bias in scientific research. For example, unstated assumptions can obscure theoretical and methodological biases, and is a

component leading to the reproducibility crisis (Ioannidis 2005). Limitations of studies are often under-reported so as to favour a 'high-impact' story or to increase the chance of publication (Price and Murnan 2004). The generalisability of findings has come under increased scrutiny as many studies rely on unrepresentative samples, such as STRANGE species in animal behaviour research (Webster and Rutz 2020). Identifying our biases in this way ensures each stage of our research process is rigorously examined, preventing biases from being overlooked. Though our questions are aimed at research within the field of animal behaviour, these self-reflective questions can be applied more broadly to other fields of biological research. For journals publishing animal behaviour research, we suggest that a statement on 'Assumptions, limitations, and generalisability' should be required as part of the submission process, and published alongside the results, to (a) ensure the authors engage with this process of critical reflection and (b) help the readers to objectively evaluate the findings. The guidelines provided in Table 1 provide the basis for writing such a statement.

5 | Unknown Unknowns: Biases We Do Not Know Exist

The final category, 'Unknown unknown' biases are arguably the most concerning since we are unaware of their existence (low in awareness (x)), causing an undetermined impact on our research (low in understanding of impact (y)). Routes to discovering these biases may not be immediately obvious. These, therefore, have the potential to mislead scientific thought and hamper our understanding of the natural world. An example of how the discovery of an 'unknown unknown' revolutionised scientific thinking is how molecular techniques revealed true genetic monogamy is rare in passerine birds (Griffith et al. 2008). Prior to this, our understanding of reproductive behaviour was biased by field observations suggesting most passerines are monogamous. This has revolutionised our understanding of both natural and sexual selection, revealing the importance of behaviours such as extra-pair paternity and female mate choice (Brouwer and Griffith 2019).

The challenge herein lies in finding ways to uncover these biases. Discovering an 'unknown unknown' often hinges significantly on luck and chance, such as unexpected results which may draw attention to previously unrecognised assumptions (Copeland 2019). However, we suggest two avenues that could lead to more and faster discovery of these biases. Firstly, researchers can take a more active approach in thinking about how unknown biases are impacting their own research. Specific to the field of animal behaviour, the STRANGE framework has been successful in enabling researchers to think critically about how generalisable their study species are to locate bias in their own research (Webster and Rutz 2020). By taking an epistemological approach and being critical at every stage of the research process, we are more likely to expose ways our research has been influenced by our standpoints (by adopting a critical perspective; Kellner 1990). Our self-reflective questions (Table 1) may provide a good starting point for researchers to investigate how bias may be introduced to research. Leading on from this is the limitation affecting our ability to be more self-critical; and so secondly, we suggest a call for funding that

TABLE 1 | Reflective questions to identify plausible biases in biological research. This framework can provide the basis for writing a statement on the assumptions, limitations, and generalisability of a study. Note that this is not an exhaustive list of questions but provides researchers with the foundation to assess bias in their own research.

	Ideas for example	Testing for example	Explaining for example
What assumptions am I making?	What is my justification for exploring this trait in the context I am studying it?	Am I exploring a trait in a context that is appropriate for the study organism/system?	Can my results be explained by an alternative hypothesis?
	Is my personal standpoint influencing my expectations?	Have I made assumptions that limit my observations?	Is my interpretation of my results subject to confirmation bias?
		Could my methods be inadvertently exploring a different trait to what I think I am exploring?	
What limitations does my study have?		Have I chosen appropriate methods/ subjects to test my hypothesis?	
	Are any of the assumptions I have made limiting the scope or validity of my research question?	Is my methodology designed to reduce or account for bias?	Are there limitations in my methods, data or results that could be misinterpreted or be misleading?
	Could I be missing alternative possibilities due to systematic biases (for example, publication bias)	Could my data be influenced by systematic biases?	Can I improve the accessibility of my research?
How generalisable is my research?		Is my data subject to an assumption or limitation that I cannot rectify retrospectively?	
	Does my hypothesis reflect the generalisability of my idea?	To what extent do my methods allow me to generalise beyond the trait or organism being tested?	Have I made clear whether/ how the trait is likely to vary in a different context/species?
	Is the generalisability of my research impacted by taxonomic or geographical bias?	Is my methodology repeatable for other systems or species?	
		Does my sampling approach allow me to generalise to the level I require?	

is dedicated specifically to identifying as yet unknown biases that could have fundamental importance to our understanding of biology. The UK Metascience Unit (UKRI 2024) and the Meta-Research Innovation Center at Stanford (METRICS; Stanford University n.d.) projects are leading examples of initiatives dedicated to identifying and reducing bias and increasing research integrity.

6 | Final Remarks

Biases are not static in the matrix framework proposed here. All biases are capable of becoming '*known knowns*' with varying degrees of ease. Biases can move between categories, and it is the responsibility of all of us to ensure bias in our work moves

towards becoming removed entirely, even if it is only able to currently be mitigated or acknowledged. Importantly, we must be cautious of biases becoming stagnant with no effort to remove them from the academic process.

Acknowledging the limitations of our own objectivity, and recognising that all existing knowledge within animal behaviour research may be influenced by known and/or unknown biases, should lead us to act with deliberate caution when designing and conducting our studies, and interpreting our findings. Furthermore, we should be transparent about the limitations of our research when reporting findings, being careful not to overstate results or the generalisability of the research. Our framework (Figure 1) and self-reflective questions (Table 1) we hope will enable researchers to be more mindful of potential biases

in their own research. Although this paper focuses on biases in animal behaviour research, many biases, such as sampling limitations, observer bias, and publication bias, are also common in other fields of research. Evaluating the assumptions, limitations, and generalisability of our work can be applied more broadly to other fields and all researchers. Regardless of discipline, researchers should consider how biases might impact their own research. Adopting an active approach to locating and mitigating bias will lead to a research culture of continuous improvement, creating a transparent and trustworthy scientific process.

Author Contributions

Lucy A. Winder: conceptualization, writing – original draft, visualization, writing – review and editing, project administration. **Emilie Brignall:** conceptualization, writing – original draft. **Francesca S. E. Dawson Pell:** conceptualization, writing – original draft. **Marion Germain:** conceptualization, writing – original draft. **Chay Halliwell:** conceptualization, writing – original draft. **James A. Hibberd:** conceptualization, writing – original draft. **Fay Morland:** conceptualization, writing – original draft. **Andreas Nord:** conceptualization, writing – original draft. **Mark Sutherland:** conceptualization, writing – original draft. **Jamie E. Thompson:** conceptualization, writing – original draft. **Nicola Hemmings:** conceptualization, writing – original draft, writing – review and editing, supervision.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

No new data were generated in this manuscript.

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