Integrative and Comparative Biology

Integrative and Comparative Biology, volume 0, pp. 1–17
https://doi.org/10.1093/icb/icad127

Society for Integrative and Comparative Biology

INVITED REVIEW

The "Seven Deadly Sins" of Neophobia Experimental Design

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Abstract Neophobia, an aversive response to novelty, is a behavior with critical ecological and evolutionary relevance for wild populations because it directly influences animals' ability to adapt to new environments and exploit novel resources. Neophobia has been described in a wide variety of different animal species from arachnids to zebra finches. Because of this widespread prevalence and ecological importance, the number of neophobia studies has continued to increase over time. However, many neophobia studies (as well as many animal behavior studies more generally) suffer from one or more of what we have deemed the "seven deadly sins" of neophobia experimental design. These "sins" include: (1) animals that are not habituated to the testing environment, (2) problems with novel stimulus selection, (3) non-standardized motivation, (4) pseudoreplication, (5) lack of sufficient controls, (6) fixed treatment order, and (7) using arbitrary thresholds for data analysis. We discuss each of these potential issues in turn and make recommendations for how to avoid them in future behavior research. More consistency in how neophobia studies are designed would facilitate comparisons across different populations and species and allow researchers to better understand whether neophobia can help explain animals' responses to human-altered landscapes and the ability to survive in the Anthropocene.

Introduction: What is neophobia, who studies it, and why?

Neophobia, an aversive response to novelty, is an often highly repeatable behavior expressed in a wide variety of animal taxa (Dardenne et al. 2013; Cohen et al. 2015; Pintor and Byers 2015; Joyce et al. 2016; Mitchell et al. 2016; Mazza et al. 2021). In both natural and human-altered environments, animals may encounter a variety of novel objects, foods, environments, scents, sounds, and creatures. Some of these new things, such as novel food sources, present opportunities to wild species. Others, like novel predators, present dangers. Therefore, a willingness to explore novelty may positively affect an individual's fitness by increasing opportunities for food and nesting sites, but it may also decrease fitness through increased predation and disease risk (Greenberg 1983, 2003; Sih et al. 2004; Réale et al. 2007). Neophobia has critical ecological and evolutionary relevance for wild populations because it directly influences a species' ability to adapt to new environments and exploit novel resources (Greenberg and Mettke-Hofmann 2001; Greggor et al. 2016; Magory Cohen et al. 2020). There is some evidence neophobia is genetic (Mettke-Hofmann 2017) and can be driven by eco-evolutionary history when novel stimuli are encountered by previous generations (Crane et al. 2020). Neophobia may be a type of fear generalization (Asok et al. 2019), where individuals are overgeneralizing novel stimuli as dangerous. Neophobia may also help explain why some individuals, populations, and species are invasive, or capable of persisting in human-altered landscapes, whereas others are not (Candler and Bernal 2015; Greggor et al. 2016; Magory Cohen et al. 2020).

We should note that exactly where neophobia fits into existing theoretical frameworks of animal personality traits is a matter of some debate. As per Carter et al. (2013), a personality trait can be defined as a specific quantifiable part of an animal's behavioral repertoire showing between-individual variation and within-individual consistency. By this definition, neophobia is a personality trait in many species. However, some researchers classify neophobia as a type of exploratory behavior (Réale et al. 2007), others as a type of boldness (Dougherty and Guillette 2018), others as an approach-avoidance conflict (Cowan 1977), while others consider it as a distinct category of behavior (Greenberg and

Advance Access publication November 23, 2023

Mettke-Hofmann 2001). Defining exactly what type of personality trait neophobia is, or attempting to classify different types of neophobia tests by the personality traits they reveal, is beyond the scope of this review (though see the section "Beyond the 7 deadly sins: other considerations" for a brief discussion of how the results of novel environment tests often differ from the results of other types of neophobia tests). In any case, neophobia is a behavior that is interesting and worth studying for its own sake, for the reasons discussed above.

Early studies of neophobia focused on quantifying anxiety, exploratory, and aversive behaviors (Berlyne 1950; Barnett 1958; Rozin 1968), identifying brain regions associated with these behaviors (Nachman and Ashe 1974; Kesner et al. 1975; Krane et al. 1976), and determining drugs that attenuate these behaviors in laboratory models (Mitchell et al. 1977; Archer et al. 1981). One of the first researchers to explore the ecological and evolutionary relevance of neophobia in wild model systems was Russell Greenberg (but see Cowan 1977 and Barnett 1958 for even earlier investigations of the ecological aspects of neophobia). Through an elegant series of laboratory and field experiments, Greenberg identified a role for neophobia in foraging preference and ecological plasticity in generalist vs specialist wild bird species (Greenberg 1983, 1984, 1987, 1989, 1990, 1992). Since then, studies have investigated how neophobia varies with respect to age (Greggor et al. 2020), sex (Ensminger and Westneat 2012; Bednarz and Zwolak 2022), social environment (Kelly et al. 2020; Rasolofoniaina et al. 2021; St. Lawrence et al. 2021), prior exposure to risk (Brown et al. 2014), diet (Middelkoop et al. 2020; Ventricelli et al. 2022), invasion success (Liebl and Martin 2014; Magory Cohen et al. 2020), urbanization (Bókony et al. 2012; Mazza et al. 2021), domestication (Moretti et al. 2015; Suzuki et al. 2021), fitness (Ferrariet al. 2015), and physiology (Baugh et al. 2017; Kelly et al. 2022). Depending on the species, neophobia has been correlated with other behavioral traits such as risk-taking (Bókony et al. 2012), innovation (Miller et al. 2021), exploration (Verbeek et al. 1994), aggression (Kozlovsky et al. 2014), boldness (Pârvulescu et al. 2021; De Meester et al. 2022), and proactive/reactive tendencies (Drent et al. 2003; Van Oers et al. 2004). Additionally, neophobia is still used to study the neural circuits of aversion and is used as a proxy for anxiety-related behavior in applied neuroscience (Emmerson et al. 2020; Shinohara and Yasoshima 2021; Ramos et al. 2022).

Neophobia is a behavior of interest in a broad range of published research, including several recent meta-analyses (Crane and Ferrari 2017; Takola et al. 2021) and a growing literature in comparative organismal biology. For example, neophobia is the focal behavior for the first multi-site collaborative ManyBirds project (Lambert et al. 2022), which is modeled after similar large-scale comparative studies in cognition and behavior in primates, human infants, and dogs (Altschul et al. 2019; Byers-Heinlein et al. 2020; Alberghina et al. 2023). However, in our view, many neophobia studies do not adequately address important factors that may affect results. The goal of this review was to survey the literature for broad trends in neophobia research and encourage optimization of neophobia paradigms through specific suggestions falling under seven broad categories ("sins") that many studies "commit." Greggor et al. (2015) and Crane and Ferrari (2017) have previously described the lack of consensus across studies and disciplines in measuring and interpreting neophobia behavior and presented several important considerations for designing neophobia tests that we include in our recommendations. For example, Greggor et al. (2015) outline the importance of novelty selection, and Crane and Ferrari (2017) discuss the importance of including control trials. Other recommendations are based on broad principles for sound experimental design in animal behavior research (Shettleworth 2009; Webster and Rutz 2020; Batesson and Martin 2021). In particular, the STRANGE framework proposed by Webster and Rutz (2020) shares similar considerations (e.g., acclimation or exposure to new testing situations).

Our intention for this review is not to present these recommendations as the be-all and end-all of neophobia experimental design. Our framework of "the seven deadly sins" is meant to be tongue-in-cheek, and we think our paper may be most useful for researchers who are new to neophobia research (or other areas of animal behavior research), as we ourselves were recently. In fact, we have committed some of the "seven deadly sins" in our own past work, and we became aware of some of these methodological pitfalls through conversations with colleagues at conferences and through the peer review process. We understand that there may be valid reasons for doing things differently than our recommendations—for example, to maintain consistency with past research—and we acknowledge that there may be crucial experimental design issues that we are missing here or that may be less important in specific circumstances. However, we believe the issues we raise here are critical to consider, even if neophobia is only a small component of the intended research project.

Literature search and analysis

There is currently no broad consensus on how to assess neophobia (Greggor et al. 2015); therefore, many different approaches are used across studies and disciplines. We performed a literature search to

Table 1 Numbers of papers that have committed the "seven deadly sins of neophobia experimental design." Poor novelty selection is defined as having treatment with biologically relevant features (eyes, mouths, likeness to food, or nesting material) or having multiple treatments with similar features (color, odor, texture, etc.). Papers "somewhat committed" pseudoreplication by having one replicate for one type of neophobia test and multiple replicates for another (e.g., 1 novel object, 3 novel foods). Papers "somewhat committed" having no controls by only having one control. Fixed treatment order (6a) (i.e., the order of control vs novelty treatments was the same for all individuals) and fixed order of novelty presentation (6b) (i.e., the order of objects was the same for all individuals) were considered two different types of one sin (not randomizing treatments, which does not allow researchers to look for the independent effect of treatment order or stimulus type)

Sin	Committed	Did not commit	Somewhat committed	Not applicable	Total
(1) Novel testing environment	36 (9%)	99 (26%)	0	248 (65%)	383
(2) Poor novelty selection	87 (23%)	181 (47%)	0	115 (30%)	383
(3) Not standardizing subjects' motivation	147 (38%)	236 (62%)	0	0	383
(4) Pseudoreplication	195 (51%)	159 (41%)	29 (8%)	0	383
(5) Lack of sufficient controls	203 (53%)	85 (22%)	95 (25%)	0	383
(6a) Fixed treatment order	212 (55%)	48 (13%)	0	123 (32%)	383
(6b) Fixed order of novelty presentation	98 (26%)	85 (22%)	0	200 (52%)	383
(7) Arbitrary thresholds	231 (60%)	41 (11%)	0	111 (29%)	383

understand the breadth of approaches and to identify potential weaknesses in experimental design. The following criteria were applied to select relevant articles:

1. Because we were interested in the ecological implications of neophobia, and biomedical neophobia research has specific criteria and motivations related to human disease, we focused on studies on non-model organisms and excluded human studies and studies on laboratory rats and mice. We did include studies of wild rodents, domesticated animals (chickens, pigs, etc.) and primate studies. Animals could be housed in captivity or studied in the field.

2. The study had to include an *a priori* reason to quantify neophobia; this excluded research that discussed neophobia as a potential *post-hoc* explanation for unexpected results.

3. Articles were deemed relevant if authors set out to measure "neophobia," even if they might have actually been measuring exploration or neophilia (see the section "Not standardizing subjects' motivation to approach novelty" for more details on this).

To find articles, the following search query was entered in Web of Science on March 24, 2023, accessed through Louisiana State University: *TOPIC "neophobia" NOT human NOT patient∗ NOT child NOT tourism NOT citizen NOT people NOT participant NOT nutrition*. The database found 1346 papers with the term "neophobia" in the title, abstract, or keywords. Filtering for primary literature brought this initial search down to 1211 results. We further refined our results to 1001 papers by excluding the following topics: *food science technology, appetite, food quality, foods, food research international, and British food journals* (to help filter out the many studies on human food neophobia). These 989 articles were then manually screened for relevance. Of these, 383 articles were deemed relevant (see Supplementary Material for a complete list).

The following information related to experimental design was extracted from each article (Table 1): taxon, journal, year, number of novelty treatments (e.g., the number of different novel objects tested), description of novelty treatments (e.g., red ribbon, purple plastic ball, etc.), number of control treatments, treatment order with respect to control vs novelty treatments (fixed or randomized), novelty presentation order (fixed or randomized), whether researchers standardized subjects' motivation to approach novelty (e.g., through fasting and object placement near food), whether subjects were tested in a new or familiar environment, how researchers measured neophobia (e.g., latency to approach, latency to feed), and how researchers analyzed neophobia (e.g., linear models, survival curves). A complete list of extracted information can be found in the Supplementary Material.

An overview of neophobia testing paradigms

Novelty recognition requires the coordination of both perception and memory (Hughes 2007), and any kind of stimulus can be novel. Novel stimuli span a broad range of categories, including, but not limited to, objects, foods, environments, smells, and sounds. Generally, the novel stimulus is paired with a positive stimulus (e.g., food or a nest box), which helps to standardize the subject's motivation to approach (see the

section "Not standardizing subjects' motivation to approach novelty" for more details). The subject is then observed until the desired behavior (e.g., time to approach or feed) is completed or until a predetermined time threshold is reached. Data may be collected by observing subjects in real-time using a hide, blind, or one-way observation glass; by recording videos of subjects interacting with novel stimuli and watching and annotating them later; or by using an automated data collection system, e.g., a radio frequency identification system that reads individual tags, and which is attached to a computer that records visits to a specific location (Fox et al. 2009; Grunst et al. 2019; Pârvulescu et al. 2021). Individuals are typically classified as neophobic if they are slow to approach or never interact with the novelty, non-neophobic if they appear indifferent to the novel stimulus, and neophilic if they approach and interact with the novel stimulus either faster than with a familiar stimulus or in the absence of a positive stimulus. What varies most among studies are the many factors of experimental design, such as testing environment, novelty choice, replication, controls, and data analysis methods. There can also be separate criteria for field vs laboratory tests, some of which are outlined below.

Novel objects are the most common type of stimulus used to measure neophobia behavior. In a field setting, novel objects are often presented on or near a positive stimulus like food (Greenberg 1989; Rasolofoniaina et al. 2021b) or a nest box (Vrublevska et al. 2015; Greggor et al. 2017). Note that a novel object presented in the absence of a positive stimulus may actually be measuring neophilia (a preference for or attraction to novelty; see the section "Not standardizing subjects' motivation to approach novelty"). One limitation of field studies is that researchers typically have little control over whether animals have recently eaten or interacted with their nests, and therefore over subjects' level of motivation. In a laboratory setting, novel objects are usually paired with a food reward, and researchers are able to fast subjects prior to testing (St. Lawrence et al. 2021; Szabo and Ringler 2022).

Food neophobia is often measured as an aversion to approach a new food (similar to object neophobia), the latency to try a new food, or an animal's wariness to incorporate a new food into its diet (known as dietary conservatism) (Marples and Kelly 1999). There is no current consensus on whether dietary conservatism and food neophobia are synonymous (Marples and Kelly 1999; Prasher et al. 2019; Szabo and Ringler 2022). Dietary conservatism paradigms often use a familiar food or prey item that has been altered to induce wariness (e.g., an unfamiliar color: Marples et al. 2007; Eccles et al. 2021; Szabo and Ringler 2022). Many applied behavior studies are interested in mitigating dietary conservatism in farm animals by testing animals' habituation to novel feeds (Burritt and Provenza 1997; Meagher et al. 2017; Sadeghi et al. 2019). Food neophobia is primarily tested in a laboratory setting with unfamiliar food items most likely not encountered in the wild (e.g., a colorful fruit-flavored breakfast cereal: Seok An et al. 2011; O'Hara et al. 2017; Kimball et al. 2022) with a similar setup as is used in object tests. Field studies assessing food neophobia are not as common, but a few have tested the incorporation of novel prey items (Beissinger et al. 1994; Marples et al. 1998; Ward-Fear et al. 2018), while others have measured latency to approach or eat novel foods (Visalberghi et al. 2003; Modlinska and Stryjek 2016), though these studies interpreted this response as exploration or boldness rather than neophobia.

Exploration of a novel environment is the most common paradigm used for spatial neophobia. Whether in a laboratory setting or in the field, researchers will introduce the subjects to an unfamiliar environment (e.g., a tent erected in the field [Liebl and Martin 2012], a novel testing arena [Michelangeli et al. 2016], or a new room [Verbeek et al. 1994]) and measure an animal's latency to enter the new space, its movement around the space, and the amount of space explored. In some studies, a different type of neophobia is measured in a novel environment; for example, novel object responses are sometimes tested in a novel testing environment, which may confound the two measurements (see the section "Novel testing environments").

While novel objects, foods, and environments are the most popular stimuli used to assess neophobia, there are other paradigms that target different sensory systems. For example, in species that use olfaction to communicate, novel scent cues are commonly used in both laboratory (Ferrariet al. 2015; Crane and Ferrari 2016) and field settings (Brown et al. 2013; McCormick et al. 2017). Researchers have targeted mechanoreception in fish by investigating responses to novel mechanical stimuli (Meuthen et al. 2016, 2019). The use of novel sounds has been used to explore neophobia in birds (Fulmer et al. 2016) and lizards (Walsh et al. 2018). And although novelty in an animal's environment can include novel heterospecifics and predators, it is much less common to use "novel heterospecific" or "novel predator" stimuli in neophobia tests than other types of stimuli (although see Jones et al. 2016; Crane and Ferrari 2017; Jolly et al. 2021; Feyten et al. 2022).

Overall patterns in comparative neophobia research

Using search criteria that excluded studies in humans and laboratory rodents, most of the papers we reviewed

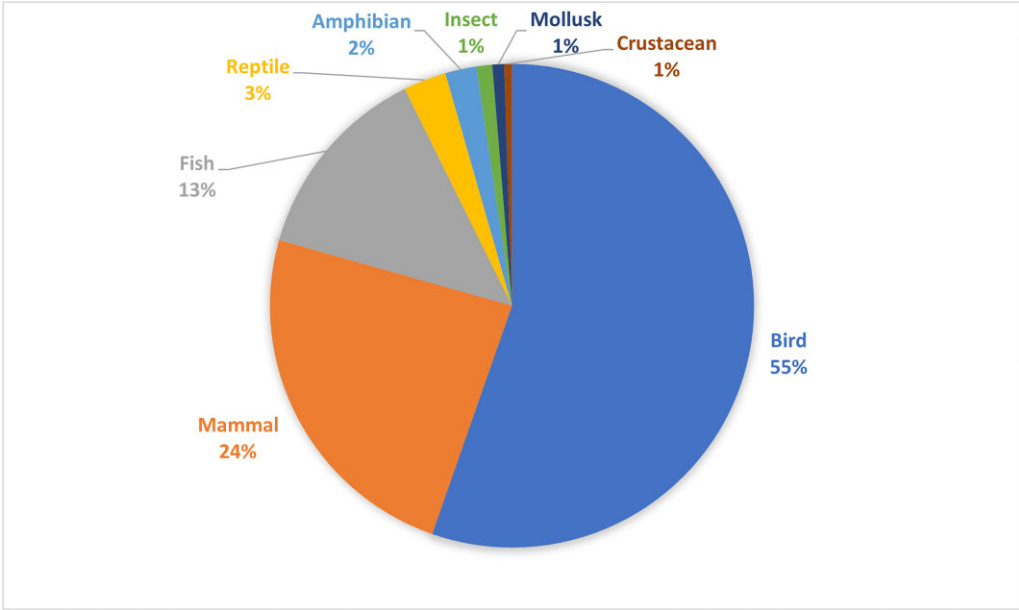

Fig. 1 Percentage of comparative neophobia studies by taxon ($n = 383$ publications, see text for details of the literature search). Human and laboratory rodent studies were excluded. Birds were the most represented taxon in neophobia research.

assessed neophobia in birds (55%). The next most common taxa were mammals (24%) and fish (13%). Reptiles, amphibians, insects, mollusks, and crustaceans were much less represented (<3%; Fig. 1), which highlights the need for more neophobia testing in these groups. Comparative neophobia research has been published in 91 different journals (Fig. 2), though it is possible our search may have missed some studies that assessed neophobia but did not include it in the title, abstract, or as a keyword. Unsurprisingly, most neophobia literature has been published in behavior journals: Animal Behavior (15%), Behavioral Ecology (7%), Applied Animal Behavior Science (6%), Animal Cognition (5%), and Behavioral Processes (5%). However, journals that target a broader audience have also published comparative neophobia research, including PLoS ONE (4%), Proceedings of the Royal Society B (3%), and Scientific Reports (2%). Lastly, neophobia publications have been rising over the years, particularly in the last two decades (Fig. 3), showing increased interest across disciplines. It is likely that this large rise in interest in neophobia in the 2000s was at least partly spurred by the pioneering work that Russell Greenberg and his lab members published in the 1980s and 1990s.

The "seven deadly sins" of neophobia research

While reading through the experimental design choices of the relevant articles, we noticed seven major repeated weaknesses ("sins"). We describe below why we consider these major weaknesses sins of experimental design, explain how these sins might influence neophobia results, and propose several suggestions for optimization. Again, it should be noted that we make these recommendations after years of careful optimization of our own experimental design and after committing some of these sins ourselves. Sins are ordered from least committed to most committed.

Novel testing environments

Many novel object, food, smell, taste, or sound studies (36%) tested subjects' neophobia in a new environment rather than a familiar environment like the home cage (note that this excludes spatial neophobia studies). Of those 138 studies, 36 did not acclimate test subjects to the new environment before testing for object, food, smell, taste, or sound neophobia (9% of total papers, Table 1). Note that this number includes studies that did not mention an acclimatization period, and therefore it was assumed that there was no acclimatization period. Using a novel testing environment without an acclimation period is not recommended, because it confounds different types of neophobia (e.g., object neophobia with spatial neophobia).

Recommendation

Either test individuals in a familiar environment (e.g., their home cage) or allow for an appropriate acclimation period prior to testing. Using control trials (see

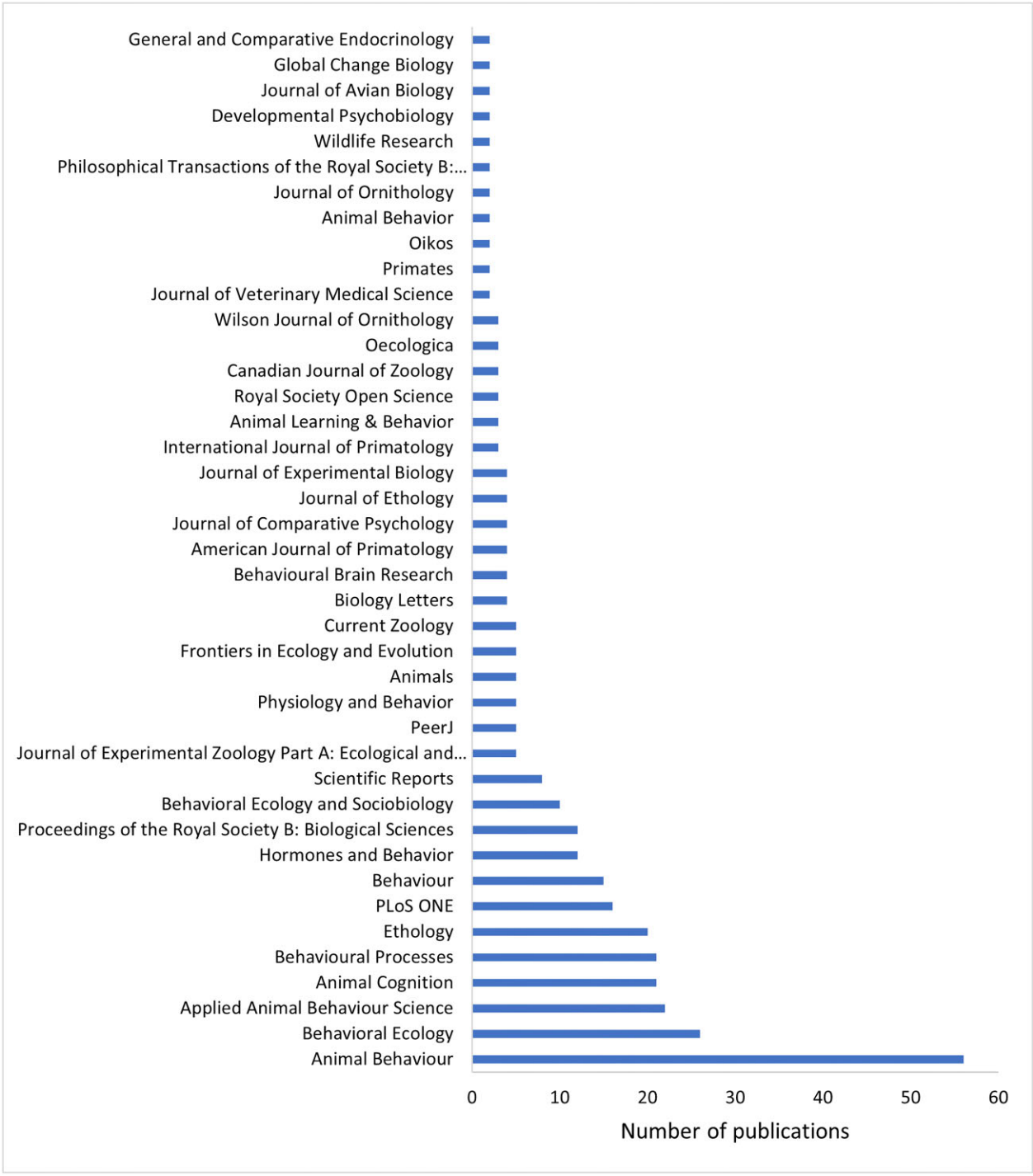

Fig. 2 Number of comparative neophobia studies by journal ($n = 383$, see text for details of the literature search and exclusion criteria). Behavior journals have published the most neophobia research. Only journals with two or more neophobia publications are shown above. For the entire list of journals, please refer to the Supplementary Materials.

the section "Lack of sufficient controls") can help determine whether animals have acclimated; acclimated animals should respond quickly to a positive stimulus in a control trial, whereas non-acclimated animals may hang back if experiencing spatial neophobia.

Poor novelty selection

To measure a subject's neophobia, it is important that the novel stimulus be truly novel, and not bear too close a resemblance to familiar objects. Many studies included novel objects with animal-like features such as

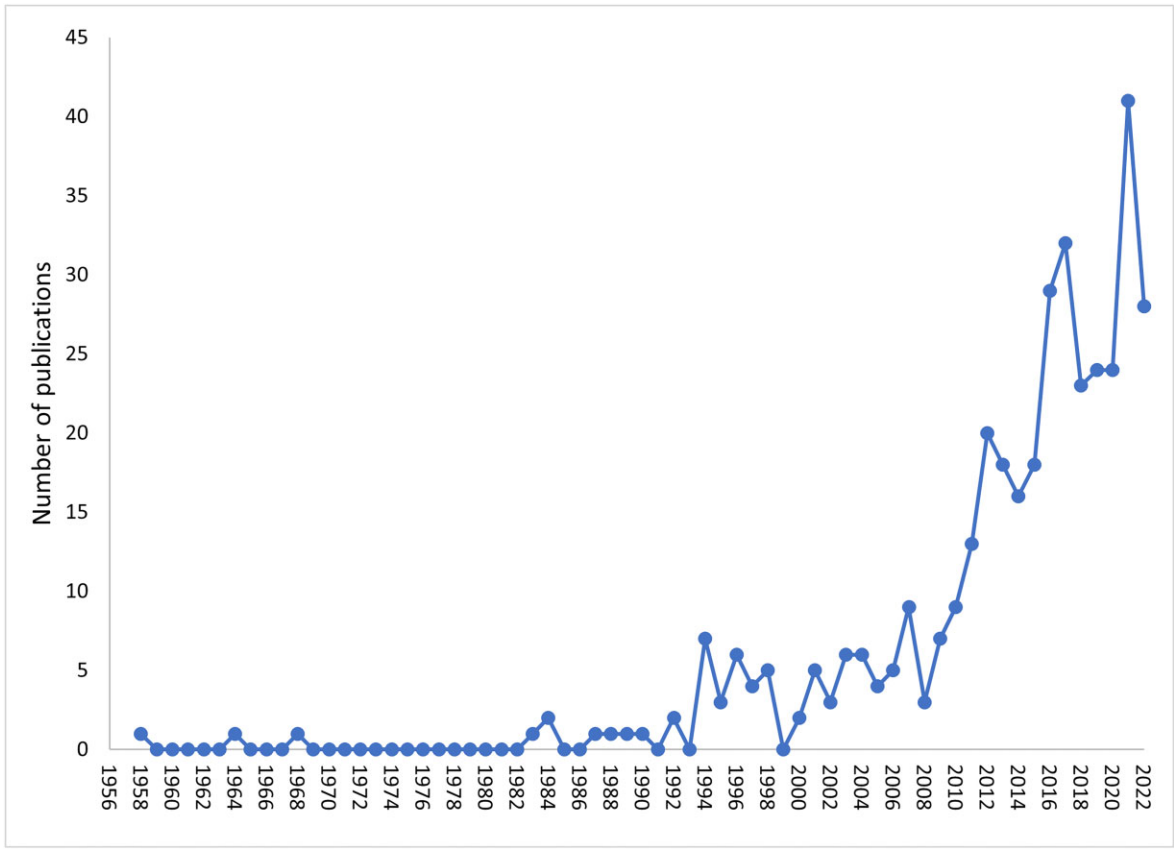

Fig. 3 Distribution of comparative neophobia publications over time ($n = 383$, see text for details of the literature search and exclusion criteria). There was a sharp increase in publications in the past two decades (2002–2022).

eyes and mouths (e.g., a toy animal). Similarly, many novel object and novel environment tests used items with plant-like features (e.g., a fake tree) that strongly resemble familiar food and nesting materials. Also, if the study organism is caught from the wild, it is important to consider other items that the individual may have previously encountered and therefore might not be perceived as novel. For example, in our lab, we avoid using Mardi Gras beads as a novel object because house sparrows may have encountered this object in the many urban environments they inhabit in south Louisiana. As discussed in Greggor et al. (2015), inclusion of features that trigger ecologically relevant cognitive biases can trigger innate fear responses that may also be distinct from the response to novelty. For example, the colors red and yellow are often associated with aposematic prey (Rowe and Guilford 1999). Certain colors or patterns may also have species-specific associations with sexual signals, such as the colors red and iridescent blue in guppies (Kodric-Brown 1985). Another repeated issue seen with novelty selection is studies that used multiple novel stimuli with very similar features (e.g., multiple objects made from brown cardboard). If different stimuli are too similar to each other, there is a chance the test subjects will not perceive them as distinct and novel, resulting in habituation. Overall, 23% of studies committed the sin of poor novelty selection in at least one of these aspects.

Recommendation

Researchers should select novel objects that do not have obvious "eyes" or "mouths," and which are not too similar to materials that subjects are likely to have previously encountered. Note that if your goal is to measure responses to a novel biological stimulus (e.g., a novel predator), then features such as "eyes" or "mouths" are appropriate and ecologically relevant. Ideally, researchers should use objects and foods that maximize the diversity of different colors and textures in novel stimuli, with special care not to use only red, yellow, or sexually selected colors: perhaps one object can be red, but not all objects should be red. For novel spatial tests, researchers should ideally use familiar objects in new locations so that only the space is novel, and not the objects within it, which would conflate object and spatial neophobia. For example, our spatial neophobia paradigm involves opening access to a new cage that has the same features and objects as the home cage, but the objects are in different locations (Kimball and Lattin 2023). Similarly, for studies using novel scents

or sounds, the scent should ideally not be applied to a novel object, and the sound should ideally not be emitted from a visible novel object like a speaker. We also strongly recommend that novel stimuli be validated with a small pilot group of individuals before use in a full study, because it is impossible to predict how selected stimuli will be perceived. We have found that some objects or foods that we selected for novelty tests did not increase subjects' average latency to feed compared to control conditions (e.g., a colorful fruit-flavored breakfast cereal compared to mixed seed and Purina lab diet, Kimball et al. 2022), indicating that these objects or foods may be too similar to items previously encountered, or not sufficiently novel to induce neophobia. Similarly, if a novel stimulus induces too strong a response (i.e., where no individuals approach it during the testing period), that suggests that it may not be suitable for studying inter-individual differences in neophobia (it represents a "strong situation" in psychology research [Carter et al. 2013]). For example, in our pilot trials, we found that a white tasseled keychain clipped above the food dish was unexpectedly aversive, to the point that no house sparrows approached or fed near it. We had no *a priori* reason to expect this tasseled keychain to induce such a strong response, so pilot trials were essential in determining that it was too aversive to use in our neophobia research. Note that the animal's sensory system should also be considered when choosing novel stimuli. For example, birds are able to see a broad spectrum of colors and have strong visual acuity (Blackwell et al. 2009; Shimizu and Watanabe 2012); therefore, varying the color of visual stimuli is appropriate. For a monochromatic species, it may be more appropriate to vary the size and texture of an object.

Not standardizing subjects' motivation to approach novelty

As mentioned previously, many studies measure neophobia by presenting something novel near a familiar food source, typically after a period of fasting that is usually several hours or overnight for homeotherms (e.g., Campbell et al. 2017; Kimball et al. 2022), and potentially a day or more for ectotherms (e.g., Candler and Bernal 2015; Szabo and Ringler 2022). Other studies present a novel stimulus near another type of positive stimulus, such as access to a nest box for breeding songbirds (Bókony et al. 2017; Morinay et al. 2020). In these types of studies, if the animal does not approach something it normally would, it is neophobic. If it does approach, it is not neophobic. However, several of the studies we reviewed did not present food or another type of positive stimulus during trials as a motivation to approach the novel stimulus (38% Table 1) and instead simply offered the novel stimulus as an option for the animal to approach, or not. Several more studies that did use food as a positive stimulus did not standardize subjects' motivation to eat by fasting individuals before trials (27% of total papers).

If novelty is presented on its own, and not in the context of food or another positive stimulus, then what is truly being measured is *neophilia* (an interest in or even preference for novelty) rather than neophobia (Mettke-Hofmann et al. 2002; Miranda et al. 2013). Interestingly, the baseline for many laboratory rodents is to show an active preference for novelty over control stimuli (Hughes 2007), which is rarely seen in wild species, with a few notable exceptions (e.g., some primates [Bergman and Kitchen 2009] and birds [Greenberg 2003]). If novelty is presented near food without standardizing motivation across subjects, then it is possible that some subjects that appear "neophobic" may have just eaten and therefore are not motivated to approach. Control treatments can allow researchers to determine whether their period of fasting is appropriate to standardize motivation (i.e., determine a time period where all animals approach and feed in the control condition).

Recommendation

Make sure what you are studying is neophobia and not neophilia. It is impossible to completely control for motivation across different subjects, because some individuals are just more reward-motivated than others, perhaps due to ecological, physiological, neurobiological, or genetic differences (Nader et al. 1997; Barron et al. 2010). Therefore, standardizing subjects' motivation to approach novel stimuli by associating them with ecologically relevant positive stimuli will help ensure that individual differences in approach times are due to neophobia, not neophilia. When food is used as a positive stimulus, a taxon-appropriate fasting period will also help standardize motivation across subjects. Note that if the fasting period is too long, this may also homogenize the response to novel stimuli across individuals. Many avian studies have successfully used overnight fasts (Marples and Roper 1996; de Bruijn and Romero 2020). This is a time period when birds normally do not feed but is long enough to create high motivation across subjects, which can be seen as a rapid approach during control conditions and a mix of neophobic and non-neophobic responses during novel stimulus trials.

Pseudoreplication

Many studies (51%) used only one novel stimulus in all subjects—e.g., one novel object or one novel environment—and suggested that the response to this one stimulus was indicative of animals' response to

"novel objects," "novel environments," or even "novelty" more generally (Table 1). This is pseudoreplication, which can occur when treatments are not replicated or when replicates are not statistically independent (Hurlbert 1984). Pseudoreplication was initially conceptualized in the field of ecology, but behavioral ecologists also became concerned with pseudoreplicaton in birdsong research (Kroodsma et al. 2001), where one song recording from one male was used to measure female responses (essentially measuring female responses to this one specific male's song rather than to male song more generally). This experimental design flaw was fixed by using multiple song recordings from multiple males. This example can be applied broadly to behavioral studies, and it is important to consider at what level replication is needed (Hurlbert 1984). In birdsong studies, replicates are needed at the level of male song recordings, while in neophobia studies, replicates are needed at the level of novelty treatments. If replicates are not added, a single novel object test may not test a subject's general response to novelty, but instead their specific response to one specific stimulus—for example, the response to a red plastic ball. This matters partly because research from our own lab (Kimball et al. 2022) and others (Camín et al. 2016) shows that not all novel objects and foods are equally aversive to all subjects. Further, using additional trials with different novel stimuli increases the likelihood of capturing an individual's "true" neophobic response and decreases the likelihood that an individual subject's response will be affected by stochastic unmeasured variables, e.g., an aggressive interaction with a conspecific immediately before testing a group-housed animal. Use of only a single novel stimulus is particularly a problem in studies testing object neophobia (48% of reviewed papers).

Recommendation

Researchers should include at least two different novel stimuli in their paradigm to be able to test for a general novelty response instead of the specific response to one object, food, etc. This will also allow for measures of reliability (also called consistency or repeatability) of neophobia within individuals (Réale et al. 2007; Bell et al. 2009). If responses are less repeatable across different objects, it may be necessary to add more novel stimuli to capture an animal's "true" response to novelty.

Lack of sufficient controls

Many studies did not include any control treatments (53%, Table 1) or only included one control treatment (22%, Table 1). Without a control treatment (e.g., in a novel object study, including trials assessing the subject's latency to approach the regular food dish with no novel objects present), it is impossible to know if an animal's response is specific to the novel stimulus (Greggor et al. 2015) or, perhaps, represents an aversion to the entire testing procedure (in which case, you may wish to exclude this individual from the study, because their response is not neophobia as much as it is general fearfulness). Further, when manipulations are used, controls are essential to be able to isolate effects of experimental manipulations on the response to novelty specifically. For example, we demonstrated that reducing circulating corticosterone in house sparrows (*Passer domesticus*) using a single injection of the drug mitotane reduced the latency to feed in novel object trials but not in control trials (Kelly et al. 2022). Without this necessary control, we would not have been able to rule out an alternative explanation for our data: that sparrows' reduced latency to feed in the presence of novel objects was because mitotane-injected birds were hungrier and faster to feed overall. As mentioned above for object tests, multiple control treatments are better than single tests to better capture an animal's "true" control response.

Recommendation

Whenever possible, researchers should include control trials that include as many of the test elements as possible (but which exclude the novelty). While one control is better than none, using multiple control trials reduces possible stochastic effects of testing day or other uncontrolled effects. If individuals are excluded from a study, we recommend using a strong threshold that only excludes subjects who fail to approach during all control trials.

Fixed treatment order

For studies that included controls, most of the studies we reviewed used a fixed treatment order (i.e., the order of control vs novelty treatments was the same for all individuals) (55%, Table 1), and some also used a fixed order of novelty presentation (i.e., the order of objects was the same for all individuals) (26%, Table 1). Using a fixed order of novel vs control trials makes it hard to disentangle possible order effects from treatment effects or to test for the effect of habituation to "novelty trials" generally. It is generally important when designing your experiment to consider variables that may affect neophobia but are not of interest. These variables that may be an undesired source of variation are often called "nuisance variables" (Kirk 2014). In our research, we are often interested in the effects of factors like circulating hormone levels or social context on neophobia; therefore, our nuisance variables include the total number of trials, the order of treatments vs controls, and individual object effects, and we randomize each of these aspects

Fig. 4 Example five day-novel object testing timeline for three test subjects (001, 002, and 003) using (A) a randomized design, (B) a balanced design, (C) randomized novelty presentation and fixed treatment order, and (D) a fixed order of novelty presentation and treatment order. Design (A) has both treatment order and novelty presentation randomized, which we believe is the ideal study design if you want to prevent order from effecting neophobia. Design (B) is ideal if you are interested assessing the effect of order on neophobia responses. Design (C) has novelty presentation randomized and treatment order fixed; however, some studies we screened had fixed novelty presentation and randomized treatment order.

in our study design. However, if these variables are of primary interest to a researcher studying neophobia, a different study design (such as a balanced design) would be more appropriate. Additionally, a few studies we reviewed did not expose all individuals to all the different novel stimuli used in the study. Ensuring that all individuals experience all novel stimuli allows researchers to test for specific stimulus effects (e.g., one object eliciting a much more neophobic response than the other objects) and to control for these effects statistically. Some novel stimuli may be perceived as "scarier" than others by study subjects, and it is important to be able to identify those effects.

Recommendation

We recommend that researchers consider all potential variables that could influence neophobia in their experimental design and either standardize or randomize any nuisance variables to prevent unwanted variation in neophobia. For example, randomizing the order of control and novelty treatments and exposing all subjects to all novel stimuli will prevent order from effecting neophobia, and allow you to assess if some objects are "scarier" than others. If multiple novel stimuli are used (as they ideally should be, see the section "Pseudoreplication"), the testing order of different novel stimuli should also be randomized, or else it is impossible to disentangle order effects from object effects. However, if you have a small sample size, randomization may not allow for equal representation of orders across individuals. Additionally, if you are interested in assessing the effects of treatment order on neophobia (e.g., determining whether exposure to multiple novel object tests several days in a row affects an animal's latency to feed on control days), you would want to use a balanced design. Balancing would still allow you to assess effects of different objects and also allow order effects to occur in sufficient numbers to assess this as an outcome. For examples of randomized, balanced, and fixed designs see Fig. 4. In general, using a randomized treatment and novelty order or a balanced order are more ideal designs (Fig. 4A and B).

Arbitrary thresholds

Just as there are many different ways that neophobia is measured (see the section "An overview of neophobia testing paradigms"), there are also a variety of ways to analyze neophobia data (e.g., linear models, Cox proportional hazard models). The problem with many linear model approaches—and any other analysis that assigns arbitrary maximum threshold values—is that they treat individuals that do and do not perform the behavior as the same. For example, an individual who feeds during the last minute of a 30-min trial would be treated the same as an individual who does not feed at all, when in fact these are very different responses. Generally, it is preferable to use Cox proportional hazard models, which do not require subjects to perform the behavior to be included in the analysis, because they do not assign somewhat arbitrary threshold values to individuals (aka data censoring). We found that 60% of neophobia studies used an arbitrary threshold for their analysis (Table 1). The extent of this "sin" may depend on the distribution of non-approachers in a study. For example, if few individuals fail to approach, then an arbitrary threshold is less of an issue.

Recommendation

If your data involve latency to perform a behavior (time to approach, time to feed, etc.), we recommend using Cox proportional hazard models using the "coxme" package (Therneau 2020) in R (R Core Team 2020) so that there is a distinction between subjects that complete the behavior and those that do not (see Fig. 5). Completion of behavior should be indicated as a binary variable (i.e., 0 or 1) (see Table 2 for an example data sheet). Note that data from Cox proportional hazard models are typically visualized using survival curves (Kassambara et al. 2021). Also, pilot studies are important to make sure that the length of neophobia trials is appropriate for your species and testing paradigm. If you are using 5-min trials and no individuals have approached the novel stimulus by the end of the testing period, this is clearly not a long enough time period to begin to distinguish individual variation in neophobia.

Beyond the 7 deadly sins: other considerations

As mentioned previously, there are many different ways to measure behavior during neophobia trials, e.g., as latency to approach a food dish, latency to first feed near novelty, number of visits to a novel feeder, total amount of time spent near novelty, or closest approach to novelty. Sometimes multiple measures may be of interest to researchers. For example, some studies have treated different responses toward novel foods as separate traits; Camin et al. (2016) denoted the time to approach a novel food as "food neophobia" and time to actually consume the food as "dietary conservativism" (although in this study these two traits were statistically indistinguishable). However, it often makes sense to examine whether different measures are correlated and choose the one that is most repeatable (both within and across different observers) (Kimball et al. 2022) or which is most ecologically relevant for the question at hand. If you are interested in how neophobia affects animals' access to beneficial resources, time to first eat a novel food might be a more relevant measure than time to first approach. Another way to manage correlated behavior data is to combine different measures using a principal components approach (Damas-Moreira et al. 2019).

Another major consideration in neophobia research is that not all types of "neophobia tests" may be measuring the same underlying behavioral trait, which can cause jingle-jangle fallacies (Bell 2007; Carter et al. 2013). For example, in wild-caught house sparrows tested in the lab, we found that individual responses to novel object, novel food, and novel object habituation tests were correlated within individuals (Kimball et al. 2022), suggesting that these tests are measuring the same trait (Carter et al. 2013). However, in other studies, individual responses to novel objects, foods, and spatial stimuli were either correlated or uncorrelated depending on the experimental setup and species (Fox et al. 2009; Damas-Moreira et al. 2019; Szabo and Ringler 2022). These different responses may be controlled by different neural circuits, especially since evolutionary constraints may act on object, food, and spatial neophobia differently for different species, which could explain why we may see uncorrelated neophobia responses across different contexts (Fox et al. 2009; Greggor et al. 2015). For example, the risk of trying a novel food vs the possible reward from doing so may be different than the risk of exploring a novel environment vs the possible reward from doing so.

Conclusions

Overall, based on numbers of published papers, we found that interest in neophobia has increased significantly over the past two decades, although most studies have focused on birds, mammals, and fish, with many fewer assessing neophobia in reptiles, amphibians, and invertebrates. Unfortunately, we found that most papers (97%) contained at least one of what we are calling the "seven deadly sins of neophobia research": aspects of the experimental design that may limit researchers' ability to be sure that they are assessing an animal's true neophobia response. In some cases, it may be impossible to avoid committing one or more of these "sins" due to practical limitations. In other cases, a study may have

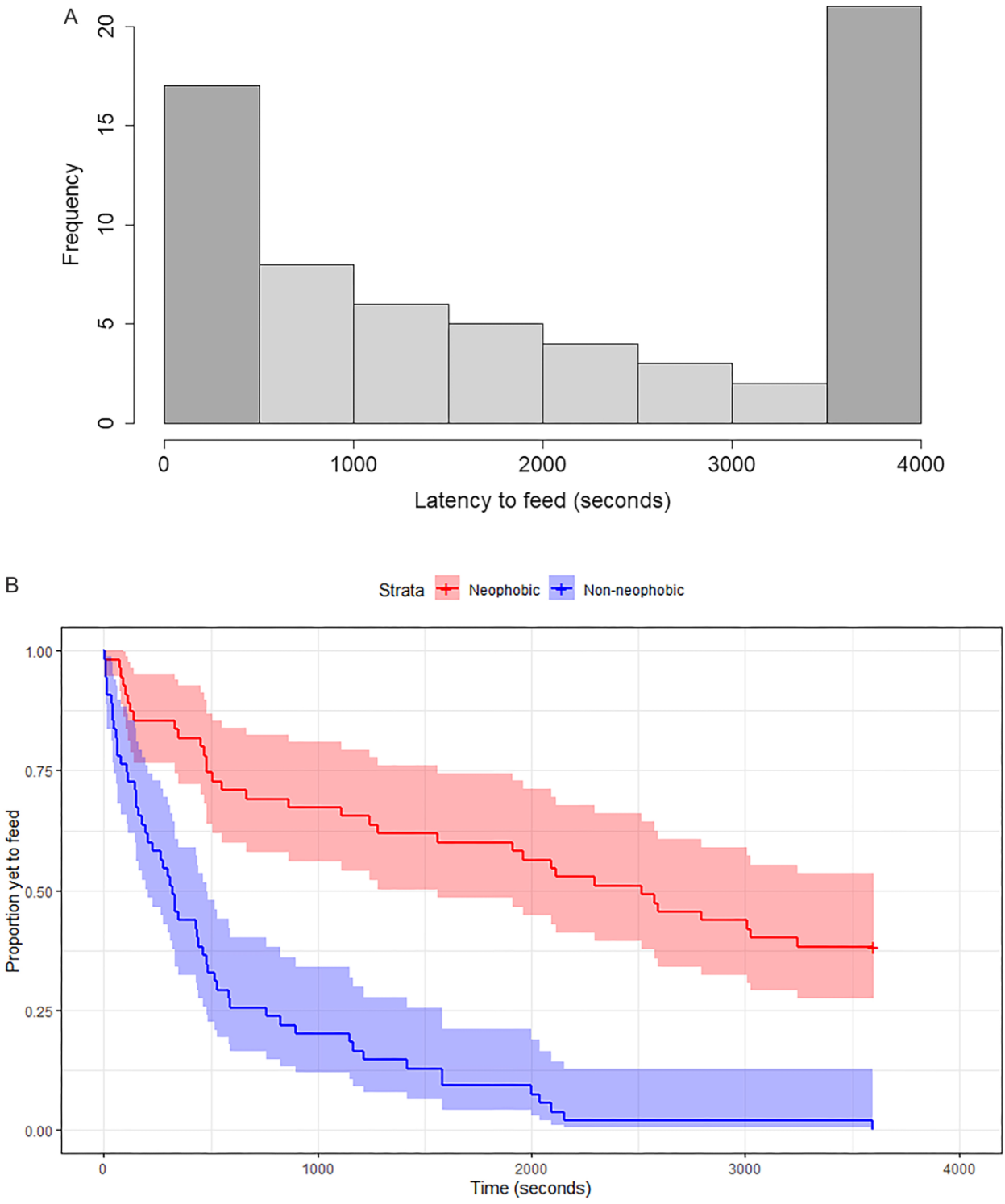

Fig. 5 Object neophobia data from house sparrows ($n = 22$) from Kimball et al. (2022) displayed in two different ways: (A) as a histogram where individuals that failed to approach were assigned an arbitrary threshold of 1 h, when the test ended, and (B) as a Cox proportional hazard model displayed using a survival curve, where individuals that failed to approach were assigned a value of 0, or failure to approach during the 1 h test period. Panel (B) was published in Behavioral Brain Research. 428. M.G. Kimball et al. Novel objects alter immediate early gene expression globally for ZENK and regionally for C-Fos in neophobic and non-neophobic house sparrows. 113863. Copyright Elsevier (2022).

Table 2 Example data for object neophobia trials showing approach and feed times for one individual over three consecutive trials. Note that if an individual has a "0" value for feeding status or approach status, the time to approach or feed is not used in a Cox regression

ID	Sex	Approach time (s)	Feeding time (s)	Object	Trial number	Trial date	Feeding status	Approach status
503	M	3600	3600	Cover	1	December 6, 2022	0	0
503	M	1100.73	1112.35	Red dish	2	December 7, 2022	1	1
503	M	30.34	31.29	Control	3	December 8, 2022	1	1

different goals than optimally characterizing an animal's neophobia phenotype, e.g., using neophobia trials that also serve to habituate an animal to an experimental apparatus (Slevin et al. 2020). However, currently, neophobia studies are so heterogeneous in design that it can be difficult to compare results across different projects. Standardized behavioral paradigms like the open-field test and elevated plus maze that are used in laboratory rodents have led to great progress in biomedical research (Walf and Frye 2007; Gould 2009; Võikar and Stanford 2023). A more standard experimental design would allow neophobia researchers to compare across studies more easily and better delineate any patterns in neophobia that may exist across different populations, species, or taxa.

Although there is considerable debate around the influences of urbanization on behaviors like neophilia and neophobia (Griffin et al. 2017), as urbanization increases across the globe, non-neophobic animals may be more successful than neophobic animals because of their increased ability to access to novel resources in human-altered environments (Miranda et al. 2013; Greggor et al. 2016). However, although being less neophobic may give animals more opportunities to find food and nesting sites, it may also increase exposure to predation and disease (Sih et al. 2004; Réale et al. 2007). Therefore, accurately and reproducibly characterizing neophobia is critical to understand when neophobia is beneficial and when it is a liability, as well as to understand the potential plasticity of this behavior (Kelly et al. 2020, 2022) and determine what physiological and environmental factors can change it. Future neophobia research should aim to incorporate the considerations outlined in this review to optimize experimental design and contribute more comparable data to the field. Again, we want to emphasize that this is not a one-size-fits-all approach. There are important and valid reasons to not follow every recommendation, but we encourage researchers to consider these recommendations, include information about them when publishing neophobia research, and justify their experimental design choices, both for transparency of study design and to facilitate comparisons across studies. Many of the recommendations in this review are relevant to not only neophobia research, but also animal behavior and cognition research more generally (Shettleworth 2009; Batesson and Martin 2021). Further, we know that other improvements to experimental design are possible beyond these "seven deadly sins," and we hope that this review spurs further conversation and consideration of how to best optimize neophobia research and animal behavior research more broadly.

Funding

C.R.L. was funded by the National Science Foundation (IOS 2237423).

Supplementary data

Supplementary Data available at *ICB* online.

Conflict of interest

The authors declare no competing interests.

Data availability

Supplementary material associated with this article is available in the online version.

References

Alberghina D, Bray EE, Buchsbaum D, Byosiere E, Espinosa J, Gnanadesikan GE, Hare E, Horschler DJ, Huber L, Kuhlmeier VA et al. 2023. ManyDogs Project: a big team science approach to investigating canine behavior and cognition. Comp Cogn Behav Rev 18:1–49.

Altschul DM, Beran MJ, Bohn M, Call J, DeTroy S, Duguid SJ, Egelkamp CL, Fichtel C, Fischer J, Flessert M et al. 2019. Establishing an infrastructure for collaboration in primate cognition research. PLoS One 14:1–19.

Archer T, Ogren S-O, Ross SB. 1981. N-2-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP4), a new selective noradrenaline neurotoxin, and taste neophobia in the rat. Psychobiology 9:197–202.

Asok A, Kandel ER, Rayman JB. 2019. The neurobiology of fear generalization. Front Behav Neurosci 12:1–15.

Barnett SA. 1958. Experiments on 'neophobia' in wild and laboratory rats. Br J Psychol 49:195–201.
Barron AB, Søvik E, Cornish JL. 2010. The roles of dopamine and related compounds in reward-seeking behavior across animal phyla. Front Behav Neurosci 4:1–9.
Bateson M, Martin P. 2021. Measuring Behaviour. An introductory Guide. 4th ed. Cambridge, UK: Cambridge University Press.
Baugh AT, Witonsky KR, Davidson SC, Hyder L, Hau M, Van Oers K. 2017. Novelty induces behavioural and glucocorticoid responses in a songbird artificially selected for divergent personalities. Anim Behav 130:221–31.
Bednarz PA, Zwolak R. 2022. Body mass and sex, but not breeding condition and season, influence open-field exploration in the yellow-necked mouse. Ecoly Evol 12:1–10.
Beissinger SR, Donnay TJ, Walton R. 1994. Experimental analysis of diet specialization in the snail kite: the role of behavioral conservatism. Oecologia 100-100:54–65.
Bell AM. 2007. Future directions in behavioural syndromes research. Proc Biol Sci 274:755–61.
Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behaviour: a meta-analysis. Anim Behav 77:771–83.
Bergman TJ, Kitchen DM. 2009. Comparing responses to novel objects in wild baboons (*Papio ursinus*) and geladas (*Theropithecus gelada*). Anim Cogn 12:63–73.
Berlyne DE. 1950. Novelty and curiosity as determinants. Br J Psychol 41:68–80.
Blackwell BF, Fernández-Juricic E, Seamans TW, Dolan T. 2009. Avian visual system configuration and behavioural response to object approach. Anim Behav 77:673–84.
Bókony V, Kulcsár A, Tóth Z, Liker A. 2012. Personality traits and behavioral syndromes in differently urbanized populations of house sparrows (*Passer domesticus*). PLoS One 7:e36639.
Bókony V, Pipoly I, Szabó K, Preiszner B, Vincze E, Papp S, Seress G, Hammer T, Liker A. 2017. Innovative females are more promiscuous in great tits (*Parus major*). Behav Ecol 28:579–88.
Brown GE, Chivers DP, Elvidge CK, Jackson CD, Ferrari MCO. 2014. Background level of risk determines the intensity of predator neophobia in juvenile convict cichlids. Behav Ecol Sociobiol 68:127–33.
Brown GE, Ferrari MCO, Elvidge CK, Ramnarine I, Chivers DP. 2013. Phenotypically plastic neophobia: a response to variable predation risk. Proc Biol Sci 280:20122712.
Burritt EA, Provenza FD. 1997. Effect of an unfamiliar location on the consumption of novel and familiar foods by sheep. Appl Anim Behav Sci 54:317–25.
Byers-Heinlein K, Bergmann C, Davies C, Frank MC, Hamlin JK, Kline M, Kominsky JF, Kosie JE, Lew-Williams C, Liu L et al. 2020. Building a collaborative psychological science: lessons learned from ManyBabies 1. Can Psychol 61:349–63.
Camín SR, Martín-Albarracín V, Jefferies M, Marone L. 2016. Do neophobia and dietary wariness explain ecological flexibility? An analysis with two seed-eating birds of contrasting habits. J Avian Biol 47:245–51.
Campbell SA, Beck ML, Sewall KB. 2017. Hatching asynchrony impacts cognition in male zebra finches. J Exp Zool 327:89–97.
Candler S, Bernal XE. 2015. Differences in neophobia between cane toads from introduced and native populations. Behav Ecol 26:97–104.
Carter AJ, Feeney WE, Marshall HH, Cowlishaw G, Heinsohn R. 2013. Animal personality: what are behavioural ecologists measuring? Biol Rev 88:465–75.
Cohen S, Benjamini Y, Golani I. 2015. Coping with space neophobia in *Drosophila melanogaster*: the asymmetric dynamics of crossing a doorway to the untrodden. PLoS One 10:1–14.
Cowan PE. 1977. Neophobia and neophilia: new-object and new-place reactions of three *Rattus* species. J Comp Physiol Psychol 91:63–71.
Crane AL, Brown GE, Chivers DP, Ferrari MCO. 2020. An ecological framework of neophobia: from cells to organisms to populations. Biol Rev 95:218–31.
Crane AL, Ferrari MCO. 2016. Uncertainty in risky environments: a high-risk phenotype interferes with social learning about risk and safety. Anim Behav 119:49–57.
Crane AL, Ferrari MCO. 2017. Patterns of predator neophobia: a meta-analytic review. Proc R Soc B Biol Sci 284:20170583.
Damas-Moreira I, Riley JL, Harris DJ, Whiting MJ. 2019. Can behaviour explain invasion success? A comparison between sympatric invasive and native lizards. Anim Behav 151:195–202.
Dardenne S, Ducatez S, Cote J, Poncin P, Stevens VM. 2013. Neophobia and social tolerance are related to breeding group size in a semi-colonial bird. Behav Ecol Sociobiol 67:1317–27.
De Bruijn R, Romero LM. 2020. Prior restraint stress inhibits habituation to novel objects in the European starlings (*Sturnus vulgaris*). J Exp Zool A Ecol Integr Physiol 333:88–95.
De Meester G, Pafilis P, Van Damme R. 2022. Bold and bright: shy and supple? The effect of habitat type on personality–cognition covariance in the Aegean wall lizard (*Podarcis erhardii*). Anim Cogn 25:745–67.
Dougherty LR, Guillette LM. 2018. Linking personality and cognition: a meta-analysis. Phil Trans R Soc B 373:20170282.
Drent PJ, Oers KV, Noordwijk AJV. 2003. Realized heritability of personalities in the great tit (*Parus major*). Proc R Soc Lond B 270:45–51.
Eccles GR, Bethell EJ, Greggor AL, Mettke-Hofmann C. 2021. Individual variation in dietary wariness is predicted by head color in a specialist feeder, the Gouldian finch. Front Ecol Evol 9:1–19.
Emmerson MG, Spencer KA, Brown GR. 2020. Social experience during adolescence in female rats increases 50 kHz ultrasonic vocalizations in adulthood, without affecting anxiety-like behavior. Dev Psychobiol 62:212–23.
Ensminger AL, Westneat DF. 2012. Individual and sex differences in habituation and neophobia in house sparrows (*Passer domesticus*). ethology 118:1085–95.
Ferrari MCO, Brown GE, Chivers DP. 2015. Time-sensitive neophobic responses to risk. Behaviour 152:1623–35.
Ferrari MCO, McCormick MI, Meekan MG, Chivers DP. 2015. Background level of risk and the survival of predator-naive prey: can neophobia compensate for predator naivety in juvenile coral reef fishes? Proc Biol Sci 282:20142197.
Feyten LEA, Demers EEM, Ramnarine IW, Brown GE. 2022. Assessing effects of predator density and diversity on neophobia in Trinidadian guppies. Behav Processes 201:104717.
Fox RA, Ladage LD, Roth TC, Pravosudov VV. 2009. Behavioural profile predicts dominance status in mountain chickadees, Poecile gambeli. Anim Behav 77:1441–8.
Fulmer AG, Santema P, Hauber ME. 2016. Intrabrood rank, age and adult presence predict novelty seeking in individual Arabian babblers, *Turdoides squamiceps*. Anim Behav 114:93–9.

Gould T. 2009. Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests, Neuromethods. NJ: Humana Totowa.
Greenberg R. 1983. The role of neophobia in determining the degree of foraging specialization in some migrant Warblers. Am Soc Nat 122:444–53.
Greenberg R. 1984. Neophobia in the foraging-site selection of a neotropical migrant bird: an experimental study. Proc Natl Acad Sci USA 81:3778–80.
Greenberg R. 1987. Social facilitation does not reduce neophobia in Chestnut-Sided Warblers (Parulinae: *Dendroica pensylvanica*). J Ethol 5:7–10.
Greenberg R. 1989. Neophobia, aversion to open space, and ecological plasticity in song and swamp sparrows. Can J Zool 67:1194–9.
Greenberg R. 1990. Feeding neophobia and ecological plasticity: a test of the hypothesis with captive sparrows. Anim Behav 39:375–9.
Greenberg R. 1992. Differences in neophobia between Naive Song and Swamp Sparrows. ethology 91:17–24.
Greenberg R. 2003. The role of neophobia and neophilia in the development of innovative behaviour of birds: why study ecological innovation?In: SN, KN, editors. Animal Innovation. Oxford, UK: Oxford University Press. p. 175–96.
Greenberg R, Mettke-Hofmann C. 2001. Ecological aspects of neophobia and neophilia in birds. In: V, CF, editors.Current Ornithology, Vol. 16. Boston, MA: Springer. p. 119–78.
Greggor AL, Clayton NS, Fulford AJC, Thornton A. 2016. Street smart: faster approach towards litter in urban areas by highly neophobic corvids and less fearful birds. Anim Behav 117:123–33.
Greggor AL, Masuda B, Flanagan AM, Swaisgood RR. 2020. Age-related patterns of neophobia in an endangered island crow: implications for conservation and natural history. Anim Behav 160:61–8.
Greggor AL, Spencer KA, Clayton NS, Thornton A. 2017. Wild jackdaws' reproductive success and their offspring's stress hormones are connected to provisioning rate and brood size, not to parental neophobia. Gen Comp Endocrinol 243:70–7.
Greggor AL, Thornton A, Clayton NS. 2015. Neophobia is not only avoidance: improving neophobia tests by combining cognition and ecology. Curr Opin Behav Sci 6:82–9.
Griffin AS, Netto K, Peneaux C. 2017. Neophilia, innovation and learning in an urbanized world: a critical evaluation of mixed findings. Curr Opin Behav Sci 16:15–22.
Grunst AS, Grunst ML, Pinxten R, Eens M. 2019. Personality and plasticity in neophobia levels vary with anthropogenic disturbance but not toxic metal exposure in urban great tits: urban disturbance, metal pollution and neophobia. Sci Total Environ 656:997–1009.
Hughes RN. 2007. Neotic preferences in laboratory rodents: issues, assessment and substrates. Neurosci Biobehav Rev 31:441–64.
Hurlbert SH. 1984. Pseudoreplication and the design of ecological field experiments. Ecol Monogr 54:187–211.
Jolly CJ, Smart AS, Moreen J, Webb JK, Gillespie GR, Phillips BL. 2021. Trophic cascade driven by behavioral fine-tuning as naïve prey rapidly adjust to a novel predator. Ecology 102:e03363.
Jones BC, Bebus SE, Ferguson SM, Bateman PW, Schoech SJ. 2016. The glucocorticoid response in a free-living bird predicts whether long-lasting memories fade or strengthen with time. Anim Behav 122:157–68.
Joyce BJ, Demers EEM, Chivers DP, Ferrari MCO, Brown GE. 2016. Risk-induced neophobia is constrained by ontogeny in Juvenile convict cichlids. Anim Behav 114:37–43.
Kassambara A, Kosinski M, Biecek P, Scheipl F. 2021. survminer: Drawing Survival Curves using "ggplot2". R package version 0.4.9. R Packag version 047.
Kelly TR, Kimball MG, Stansberry KR, Lattin CR. 2020. No, you go first: phenotype and social context affect house sparrow neophobia. Biol Lett 16:20200286.
Kelly TR, Lynch KI, Couvillion KE, Gallagher JN, Stansberry KR, Kimball MG, Lattin CR. 2022. A transient reduction in circulating corticosterone reduces object neophobia in male house sparrows. Horm Behav 137:105094.
Kesner RP, Berman RF, Burton B, Hankins WG. 1975. Effects of electrical stimulation of amygdala upon neophobia and taste aversion. Behav Biol 13:349–58.
Kimball MG, Gautreaux EB, Couvillion KE, Kelly TR, Stansberry KR, Lattin CR. 2022. Novel objects alter immediate early gene expression globally for ZENK and regionally for c-Fos in neophobic and non-neophobic house sparrows. Behav Brain Res 428:113863.
Kimball MG, Lattin CR. 2023. Exploration of a novel environment is not correlated with object neophobia in wild-caught house sparrows (*Passer domesticus*). Behav Processes 210:104913.
Kirk R. 2014. Experimental design: procedures for the behavioral sciences, experimental design: procedures for the behavioral sciences. In: L, G, editors. Thousand Oaks, CA, USA: SAGE Publications.
Kodric-Brown A. 1985. Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). Behav Ecol Sociobiol 17:199–205.
Kozlovsky D, Branch C, Freas CA, Pravosudov VV. 2014. Elevation-related differences in novel environment exploration and social dominance in food-caching mountain chickadees. Behav Ecol Sociobiol 68:1871–81.
Krane RV, Sinnamon HM, Thomas GJ. 1976. Conditioned taste aversions and neophobia in rats with hippocampal lesions. J Comp Physiol Psychol 90:680–93.
Kroodsma DE, Byers BE, Goodale E, Johnson S, Liu W-C. 2001. Pseudoreplication in playback experiments, revisited a decade later. Anim Behav 61:1029–33.
Lambert M, Farrar B, Garcia-Pelegrin E, Reber S, Miller R. 2022. ManyBirds: a multi-site collaborative open science approach to avian cognition and behavior research. Anim Behav Cogn 9:133–52.
Liebl AL, Martin LB. 2012. Exploratory behaviour and stressor hyper responsiveness facilitate range expansion of an introduced songbird. Proc Biol Sci 279:4375–81.
Liebl AL, Martin LB. 2014. Living on the edge: range edge birds consume novel foods sooner than established ones. Behav Ecol 25:1089–96.
Magory Cohen T, Kumar RS, Nair M, Hauber ME, Dor R. 2020. Innovation and decreased neophobia drive invasion success in a widespread avian invader. Anim Behav 163:61–72.
Marples NM, Kelly DJ. 1999. Neophobia and dietary conservatism: two distinct processes? Evol Ecol 13:641–53.

Marples NM, Quinlan M, Thomas RJ, Kelly DJ. 2007. Deactivation of dietary wariness through experience of novel food. Behav Ecol 18:803–10.
Marples NM, Roper TJ. 1996. Effects of novel colour and smell on the response of naive chicks towards food and water. Anim Behav 51:1417–24.
Marples NM, Roper TJ, Harper DGC. 1998. Responses of wild birds to novel prey: evidence of dietary conservatism. Oikos 83:161–5.
Mazza V, Czyperreck I, Eccard JA, Dammhahn M. 2021. Cross-context responses to novelty in rural and urban small mammals. Front Ecol Evol 9:1–16.
Mccormick MI, Chivers DP, Allan BJM, Ferrari MCO. 2017. Habitat degradation disrupts neophobia in Juvenile coral reef fish. Glob Change Biol 23:719–27.
Meagher RK, Weary DM, Von Keyserlingk MAG. 2017. Some like it varied: individual differences in preference for feed variety in dairy heifers. Appl Anim Behav Sci 195:8–14.
Mettke-Hofmann C. 2017. Neophobia. In: J, T, editors. Encyclopedia of Animal Cognition and Behavior. Cham: Springer. p. 1–8.
Mettke-Hofmann C, Winkler H, Leisler B. 2002. The significance of ecological factors for exploration and neophobia in parrots. ethology 108:249–72.
Meuthen D, Baldauf SA, Bakker TCM, Thünken T. 2016. Predator-induced neophobia in juvenile cichlids. Oecologia 181:947–58.
Meuthen D, Ferrari MCO, Lane T, Chivers DP. 2019. High background risk induces risk allocation rather than generalized neophobia in the fathead minnow. Behav Ecol 30:1416–24.
Michelangeli M, Wong BBM, Chapple DG. 2016. It's a trap: sampling bias due to animal personality is not always inevitable. Behav Ecol 27:62–7.
Middelkoop A, Kemp B, Bolhuis JE. 2020. Early feeding experiences of piglets and their impact on novel environment behaviour and food neophobia. Appl Anim Behav Sci 232:105142.
Miller R, Garcia-Pelegrin E, Danby E. 2021. Neophobia and innovation in critically endangered Bali myna. R Soc open sci 9: 211781.
Miranda AC, Schielzeth H, Sonntag T, Partecke J. 2013. Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity? Glob Change Biol 19:2634–44.
Mitchell D, Fairbanks M, Laycock JD. 1977. Suppression of neophobia by chlorpromazine in wild rats. Behav Biol 19:309–23.
Mitchell MD, Chivers DP, Brown GE, Ferrari MCO. 2016. Living on the edge: how does environmental risk affect the behavioural and cognitive ecology of prey? Anim Behav 115:185–92.
Modlinska K, Stryjek R. 2016. Food neophobia in wild rats (*Rattus norvegicus*) inhabiting a changeable environment-a field study. PLoS One 11:2–13.
Moretti L, Hentrup M, Kotrschal K, Range F. 2015. The influence of relationships on neophobia and exploration in wolves and dogs. Anim Behav 107:159–73.
Morinay J, Forsman JT, Germain M, Doligez B. 2020. Behavioural traits modulate the use of heterospecific social information for nest site selection: experimental evidence from a wild bird population. Proc Biol Sci 287:20200265.
Nachman M, Ashe JH. 1974. Effects of basolateral amygdala lesions on neophobia, learned taste aversions, and sodium appetite in rats. J Comp Physiol Psychol 87:622–43.
Nader K, Bechara A, Van Der Kooy D. 1997. Neurobiological constraints on behavioral models of motivation. Annu Rev Psychol 48:85–114.
O'hara M, Mioduszewska B, Von Bayern A, Auersperg A, Bugnyar T, Wilkinson A, Huber L, Gajdon GK. 2017. The temporal dependence of exploration on neotic style in birds. Sci Rep 7:1–9.
Pârvulescu L, Stoia DI, Miok K, Ion MC, Puha AE, Sterie M, Vereş M, Marcu I, Muntean MD, Aburel OM. 2021. Force and boldness: cumulative assets of a successful crayfish invader. Front Ecol Evol 9:1–12.
Pintor LM, Byers JE. 2015. Individual variation in predator behavior and demographics affects consumption of non-native prey. Behav Ecol 26:797–804.
Prasher S, Thompson MJ, Evans JC, El-Nachef M, Bonier F, Morand-Ferron J. 2019. Innovative consumers: ecological, behavioral, and physiological predictors of responses to novel food. Behav Ecol 30:1216–25.
Ramos R, Wu C-H, Turrigiano GG. 2022. Strong aversive conditioning triggers a long-lasting generalized aversion. Front Cell Neurosci 16:854315.
Rasolofoniaina B, Kappeler PM, Fichtel C. 2021. Wild narrow-striped mongooses use social information to enhance behavioural flexibility. ethology 127:253–66.
Rasolofoniaina BN, Kappeler PM, Fichtel C. 2021. Neophobia and social facilitation in narrow-striped mongooses. Anim Cogn 24:165–75.
R Core Team. 2020. R: a language and environment for statistical computing, R A Lang Environ Stat Comput R Found Stat Comput Vienna, Austria.
Réale D, Reader SM, Sol D, Mcdougall PT, Dingemanse NJ. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82:291–318.
Rowe C, Guilford T. 1999. The evolution of multimodal warning displays. Evol Ecol 13:655–71.
Rozin P. 1968. Specific aversions and neophobia resulting from vitamin deficiency or poisoning in half-wild and domestic rats. J Comp Physiol Psychol 66:82–8.
Sadeghi M-H, Sari M, Abadi TM, Rezai M. 2019. Effect of a short-term training on intake of *Halocnemum strobilaceum* forage by sheep. Appl Anim Behav Sci 213:74–80.
Seok An Y, Kriengwatana B, Newman AE, MacDougall-Shackleton EA, MacDougall-Shackleton SA. 2011. Social rank, neophobia and observational learning in black-capped chickadees. Behaviour 148:55–69.
Shettleworth SJ. 2009. Cognition, Evolution, and Behavior. 2nd ed., Oxford, UK: Oxford University Press.
Shimizu T, Watanabe S. 2012. The avian visual system. In: OF, T, EA, editors. How Animals See the World: Comparative Behavior, Biology, and Evolution of Vision. Oxford, UK: Oxford University Press. p. 473–82.
Shinohara K, Yasoshima Y. 2021. Inactivation of the ventral hippocampus facilitates the attenuation of odor neophobia in rats. Behav Brain Res 401:113077.
Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 19:372–8.

Slevin MC, Houtz JL, Bradshaw DJ, Anderson RC. 2020. Evidence supporting the microbiota–gut–brain axis in a songbird. Biol Lett 16:20200430.
St. Lawrence S, Rojas-Ferrer I, Morand-Ferron J. 2021. Does the presence of a conspecific increase or decrease fear? Neophobia and habituation in zebra finches. ethology 127:1033–41.
Suzuki K, Ikebuchi M, Kagawa H, Koike T, Okanoya K. 2021. Effects of domestication on neophobia: a comparison between the domesticated Bengalese finch (*Lonchura striata* var. *domestica*) and its wild ancestor, the white-rumped munia (*Lonchura striata*). Behav Processes 193:104502.
Szabo B, Ringler E. 2022. Fear of the new? Geckos hesitate to attack novel prey, feed near objects and enter a novel space. Anim Cogn 26:537–49.
Takola E, Krause ET, Müller C, Schielzeth H. 2021. Novelty at second glance: a critical appraisal of the novel object paradigm based on meta-analysis. Anim Behav 180:123–42.
Therneau T. 2020. coxme: mixed effect cox models.
Van Oers K, Drent PJ, De Goede P, Van Noordwijk AJ. 2004. Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities. Proc R Soc Lond B 271:65–73.
Ventricelli M, Gratton P, Sabbatini G, Addessi E, Sgaraglia G, Rufo F, Sirianni G. 2022. Individual variation in response to novel food in captive Capuchin Monkeys (*Sapajus* spp.). Front Ecol Evol 10:820323.
Verbeek MEM, Drent PJ, Wiepkema PR. 1994. Consistent individual differences in early exploratory behaviour of male great tits. Anim Behav 48:1113–21.
Visalberghi E. 2003. Response toward novel foods and novel objects in wild *Cebus apella*. Int J Primatol 24:653–75.
Võikar V, Stanford SC. 2023. The Open Field Test. In: J, editor. Psychiatric Vulnerability, Mood, and Anxiety Disorders: Tests and Models in Mice and Rats. New York, New York, USA: Springer. p. 9–29.
Vrublevska J, Krama T, Rantala MJ, Mierauskas P, Freeberg TM, Krams IA. 2015. Personality and density affect nest defence and nest survival in the great tit. Acta Ethol 18: 111–20.
Walf AA, Frye CA. 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2:322–8.
Walsh S, Goulet CT, Wong BBM, Chapple DG. 2018. Inherent behavioural traits enable a widespread lizard to cope with urban life. J Zool 306:189–96.
Ward-Fear G, Brown GP, Pearson DJ, West A, Rollins LA, Shine R. 2018. The ecological and life history correlates of boldness in free-ranging lizards. Ecosphere 9:1–13.
Webster MM, Rutz C. 2020. How STRANGE are your study animals? Nature 582:337–40.