JAGIELLONIAN UNIVERSITY · FACULTY OF BIOLOGY

Methodological Workshop in Evolutionary Biology

Practical Part

Spring 2025

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About the Course

Active training based on the own ideas of the doctoral students in the field of evolutionary biology, ecology and environmental biology. The course is devoted to practical aspects of conducting systematic search of literature on the selected topic:

- Formulating aims
- Planning of systematic search
- Performing the search and analyzing results
- Writing the report in accordance with the standards of scientific publication
- Writing a review in accordance with standards of peer-review of the manuscript
- Presenting results

In the Spring 2025 edition, the following topics were proposed and considered during the course. Eventually, the students worked on one common topic which appeared to be of interest to all of them.

Proposed Research Topics

1. Digital Media Exposure and Brain Development in Children
2. VR in Schizophrenia / Autism Treatment
3. Ways to Assess (Self) Consciousness in Invertebrates
4. Paradox of Long-term Asexual Reproduction on Fitness
5. Impact of Caffeine/Energy Drinks (Taurine) on Children/Young Adults (or Immature Animals)
6. Born to Kill? The Developmental Pathway from Childhood Trauma to Psychopathic Traits in Adulthood

Participants have chosen topic number 3.

Chasing a Rabbit in the Mirror: A Systematic Review of Self-Recognition Tests in Animals

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Abstract
Self-recognition in non-human animals is a topic of active scientific interest, but studies vary widely in species examined and methods employed. We conducted a systematic review to synthesize which research approaches are used to assess self-recognition in non-human animals. A comprehensive literature search was performed in Scopus and Web of Science, yielding 4736 records. After removing duplicates and non-empirical sources, 2719 studies were screened, and 118 studies met our inclusion criteria. These studies span over 60 years and include a diverse range of taxa, from primates and birds to invertebrates. Our analysis identified the mirror self-recognition test as the predominant method, often applied to visually oriented species, though olfactory, auditory, and interoceptive modalities were also represented. Most studies focused on adult individuals and rarely incorporated experimental manipulations or neurological assessments. Birds, particularly corvids, emerged as the most frequently tested group, surpassing even primates. While research has broadened taxonomically and methodologically in recent decades, significant biases remain toward certain taxa, sensory modalities, and test types. We highlight the need for more methodologically diverse approaches, especially in underrepresented species and life stages, to advance our understanding of animal self-recognition.

Introduction

Research of signs of self-awareness has spread throughout all taxa, challenging scientists to find out methodologies to assess such complex features in animals, which present much diverse physiology, some being even distant relatives of humans in the phylogenetic tree. To undertake this challenge, a definition of self-awareness is needed. Self-awareness involves understanding one's internal state to navigate the world. Lage et al. (2022) define it in three evolutionary levels of complexity, based on the idea that evolution is a gradual phenomenon and the appearance of complex cognitive abilities is a result of a continuum over the course of evolution. So, the first level is basic bodily self-recognition. The second involves metacognition—awareness of emotions and memories. The third, Theory of Mind, includes understanding beliefs, desires, and others' mental states. We focused on the first level described by Lage et al.: self-recognition as self-awareness. Research on self-recognition in non-human animals requires vast variation in methodologies that reflect the sensory systems of the animals being tested. For example, the mirror test is a gold standard for assessing visual animals, but it is not suitable for animals which rely on auditory or chemical stimuli. Therefore, some animals may not show signs of self-recognition in response to tests which do not consider specific types of stimuli. Furthermore, the detection of self-recognition may also be affected by the life stages of animals, making the interpretation difficult. In this systematic review, we present a comprehensive catalog of self-recognition studies in animals and discuss how the diversity of research approaches may contribute to the variation observed in self-recognition assessment outcomes. We base our systematic review on the following criteria: the study addresses self-recognition (as the definition previously given) in non-human animals (human studies are excluded); the study must be an empirical research, and must be written in English. Furthermore, we addressed different variables within the chosen studies, namely: (i) animal taxa used, (ii) life stages included (juvenile or adult), (iii) modalities of stimuli used (e.g., visual, auditory, chemical), (iv) experimental manipulations prior to testing, and (v) whether the study addresses the neurological basis of self-recognition. We aim to identify knowledge gaps that need to be addressed to enhance our understanding in the evolution of self-perception, thus providing opportunities for future research—for instance, through the application of consistent methodologies across evolutionarily distant taxa to enhance cross-species comparability and strengthen investigations in this field.

Methods

We conducted a systematic literature search to identify peer-reviewed empirical studies investigating self-awareness/self-recognition in non-human animals. The search was performed on 6 May 2025 using two databases: Scopus and Web of Science, without any restrictions on publication year. The Scopus search string was:

TITLE-ABS (self W/1 aware OR *cognit* OR conscious* OR perce* OR ident*) AND TITLE-ABS (animal* OR *vertebrate* OR reptile* OR bird* OR fish* OR mammal* OR arthropod* OR insec*),*

while the Web of Science string was:

TS=((self NEAR/1 (aware OR *cognit* OR conscious* OR perce* OR ident*)) AND (animal* OR *vertebrate* OR reptile* OR bird* OR fish* OR mammal* OR arthropod* OR insect*)).*

The combined search yielded 4736 records (2352 from Web of Science and 2384 from Scopus). All references were imported into Zotero reference manager (https://www.zotero.org/), where duplicates (n = 1673) were removed. Additionally, book chapters and book sections were excluded (n = 344), resulting in 2719 articles for initial screening.

Screening was performed using Rayyan (https://www.rayyan.ai/). Three pairs of researchers independently reviewed titles, abstracts, and keywords. Disagreements were resolved through discussion within each pair. Studies were excluded during this phase based on the criteria listed in Figure 1. To be included, studies had to be written in English, be empirical in nature, involve non-human animals, and contain a test or assessment related to self-awareness/self- recognition or closely related constructs.

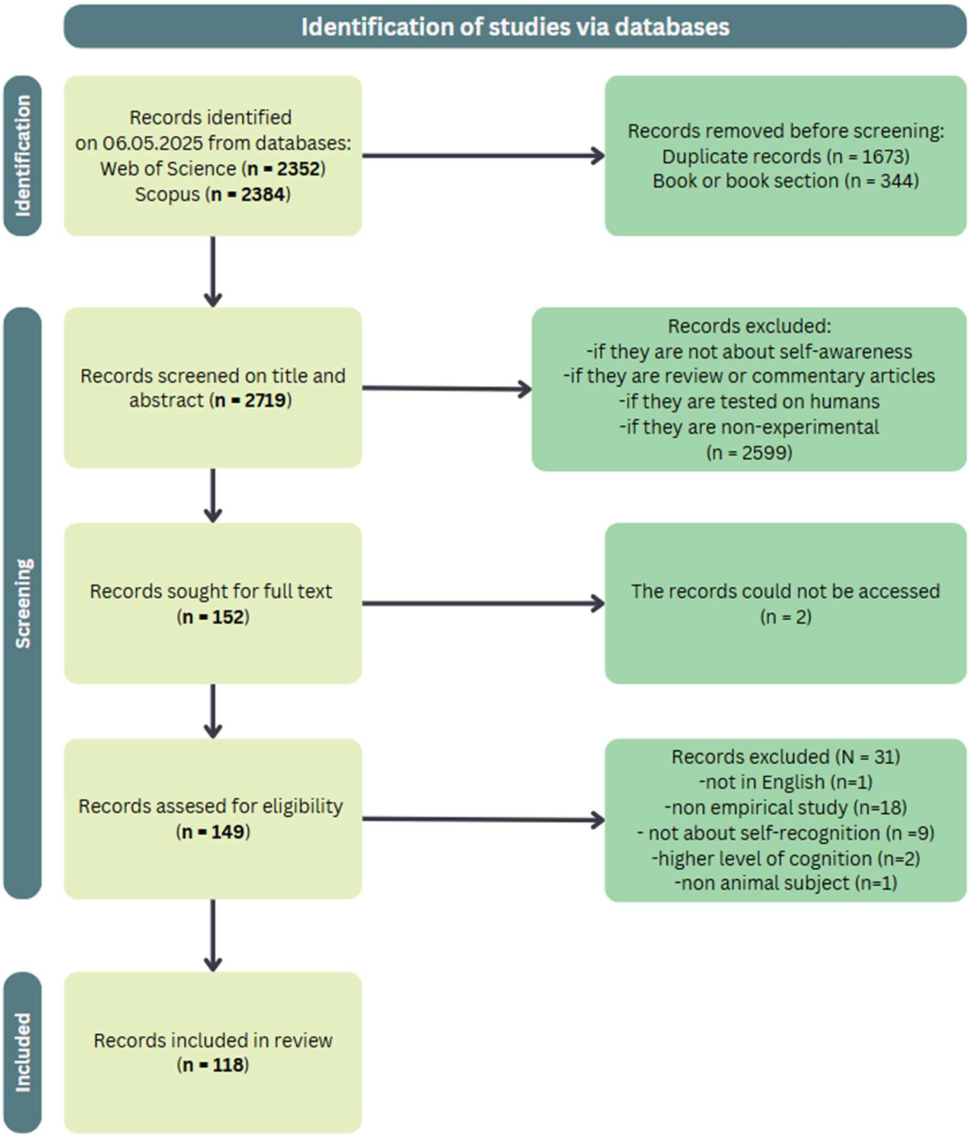


Figure 1. PRISMA flow diagram of study selection process for a systematic review on animals self-awareness.

This resulted in 152 studies selected for full-text screening. Seven researchers were assigned approximately 20–25 papers each. Two articles could not be accessed and were excluded. The remaining 149 full texts were assessed for eligibility based on predefined inclusion criteria.

Studies focusing solely on metacognition or other higher-order cognitive processes without addressing self-recognition were excluded (n = 31).

The final set of 118 studies was systematically reviewed and coded based on several key variables: (1) the species involved, including the English name; (2) the life stage of the individuals tested—categorized as juvenile, adult, both, or not specified, with "adult" referring to organisms that are independent and no longer reliant on parental care; (3) the type of stimulus used in the self-recognition tests, classified as auditory, chemical, visual, not specified, or other; (4) the specific type of self-recognition test employed; (5) whether there was any experimental manipulation conducted prior to testing, coded as "yes" if it altered the animal's condition in a way not central to the test itself, and "no" if it was a direct part of the self-awareness/self-recognition assessment; and (6) whether the study included an investigation into the neurological basis of self-recognition, such as through EEG.

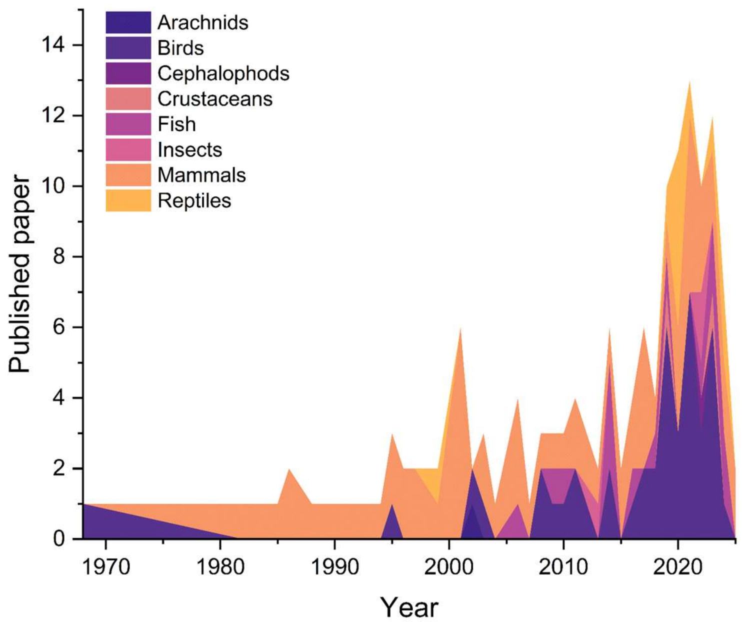
To capture the range of approaches across the literature, self-recognition tests were grouped into the following categories: (1) standard mirror mark tests involving a visible mark applied to the subject; (2) contingency behavior only, where subjects match their movements to a mirror image without a mark; (3) delayed mirror tests using time-delayed reflections; (4) auditory self-recognition, such as recognizing one's own vocalizations; (5) olfactory self-recognition based on scent cues; (6) self body-size perception, involving awareness of one's physical dimensions; (7) self-motion perception, recognizing movement as self-generated; (8) perception of physiological processes like heartbeat detection; (9) video playback recognition using live or recorded visual feedback; (10) other visual alternatives, including touchscreen-based tasks; and (11) other, for tests that did not fit any predefined category and were classified during coding.

The data were extracted using structured Google Forms, which enabled consistent and systematic coding across all included studies. Once collected, the data were organized and categorized in Microsoft Excel. Further data visualizations were carried out using Origin Pro 2024.

Results and Discussion

We based our review on 118 publications included at the final screening step. To allow detailed analysis, we further separated studies by animal taxa and test types, yielding 135 individual cases in total. The field of animal self-recognition study can be dated back to at least six decades ago and spans through diverse vertebrate and invertebrate taxa (Fig. 2A). There were only 16 studies before 2000, which were mostly conducted on primates and birds. The field gained more interest in the last three decades, where research expanded to other animal groups, including non-primate mammals, reptiles, fishes, arthropods and certain cephalopods (octopuses). Unexpectedly, self-recognition studies in birds (N=43) exceed those in primates (N=32) (Fig. 2B). Certain bird taxa, especially corvids, evolve large brains relative to body size and high intelligence comparable to primates (Seed et al. 2009), thus undoubtedly the most studied group of birds. The trend is similar within primates, where most studies addressed great apes, i.e., chimpanzees, gorillas, bonobos, and orangutans. When primates are grouped with other mammalian taxa, the combined number of studies (N=60) exceeds that of birds. Artiodactyls are the second most studied mammalian groups (N=10). This includes cows, pigs and sheep, as well as dolphins and killer whales, which are well-known for their high cognitive ability. These animals are reasonably the focus of research, given the rising concern for the welfare of livestock and captive animals. Rodents (N=9) are also well-represented among mammals, likely due to their widespread use in laboratory research. Studies on reptiles (N=11), fishes (N=14), and invertebrates (N=6; including insects, crustaceans, arachnids, and octopuses) together account for a quarter of the reviewed publications, adding notable taxonomic diversity to the field. The rapid increasing number of publications and the growing diversity of tested animal taxa highlight self-recognition as an actively expanding field, especially after 2020 (Fig. 2A).

A)



B)

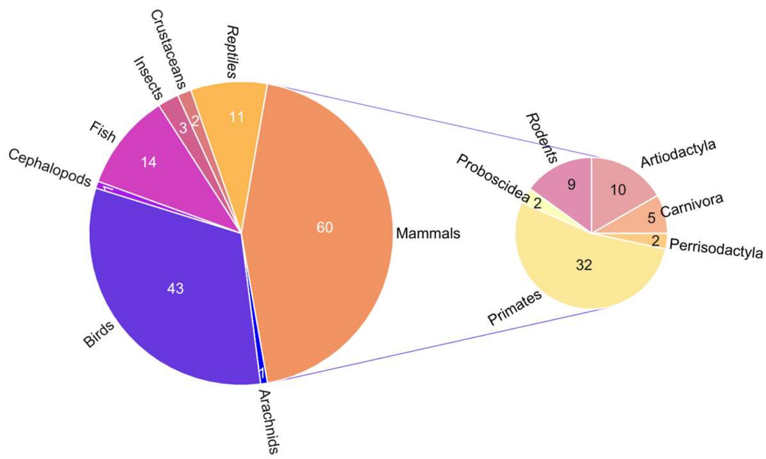


Figure 2. A) Trends in published studies on self-recognition across Animal Taxa (1968–2025), the colors represent different groups, including mammals, birds, reptiles, fish, insects, cephalopods, crustaceans, and arachnids; B) main pie chart (the big one) shows distribution of animal classes and a secondary pie chart further breaks down the Mammal category

The life stage of animals used in experiments can influence whether they demonstrate self-recognition. Including individuals from different life stages allows researchers to track the development of self-recognition abilities over time, which may parallel the maturation of the nervous system. For instance, studies in humans have shown that children do not recognize themselves in front of the mirror before the age of 15–18 months (Lewis & Brooks-Gunn, 1979; Amsterdam, 1972). In our review, the majority of studies focus on adults (N=91), Fig. 3). Although some studies included both adult and juvenile stages (N=20), limited sample sizes often hinder statistical comparisons across life stages, a common challenge in animal research. A few studies examined only juvenile animals (N=13), raising questions that the tested animals may have been too developmentally immature to possess and/or exhibit self-recognition. Some studies even did not specify the life stage of the animals (N=9). These cases may partly reflect the limitation in the research area due the difficulty in age determination, particularly in non-captive animals.

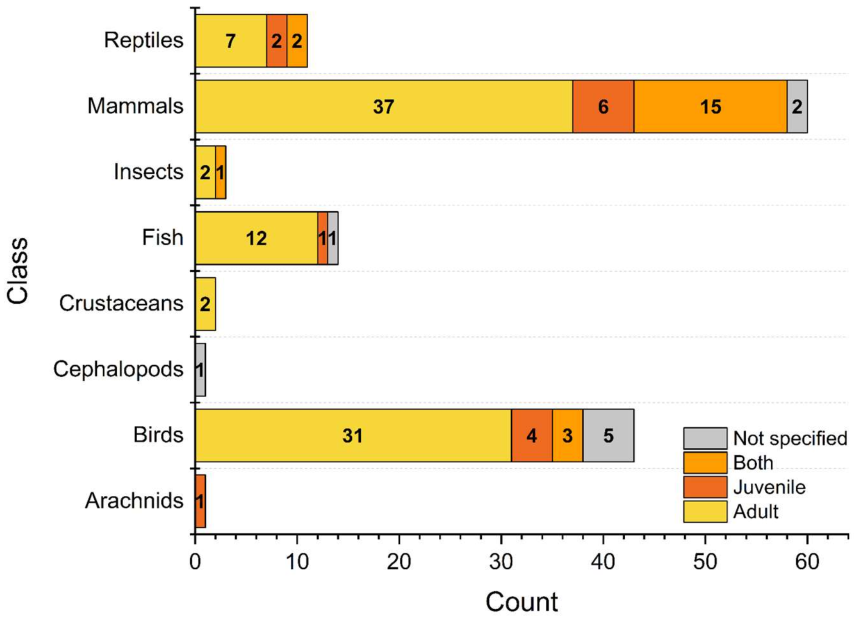


Figure 3. Distribution of animal age stages across different classes, the count of various animal classes (Reptiles, Mammals, Insects, Fish, Crustaceans, Cephalopods, Birds, and Arachnids) categorized by the age stage (Adult, Juvenile, Both, or Not specified) primarily studied in research

The stimulus modality used in the experiment is one of the most important factors in testing animal self-recognition. A mismatch between an animal's dominant sensory modality and the modality employed in the test can result in false-negative outcomes. As shown in Fig. 4, the mirror test is by far the most commonly used method (N=94), particularly the standard version involving a visible mark applied on the animals (N=55). The popularity of the mirror test, along with other vision-based methods (N=7, e.g., touchscreen tasks, screen-based feedback or video playback recognition), reflects an anthropocentric bias toward visual stimuli. Despite this bias, most reviewed studies appropriately applied these methods to taxa that are highly visual, such as birds and mammals. To our surprise, the mirror test is not restricted to use in vertebrates but has also been successfully applied to invertebrates, such as wasps (Riojas-Schnier & Toth, 2022), crabs (Robinson, 2023), and octopuses (Amodilo & Fiorito 2022), emphasizing its general application as a gold standard and the importance of visual perception in these animals. Besides mirror self-recognition, some studies paid more attention to olfactory or chemically mediated self-recognition (N=14), especially in fishes and reptiles. Certain species of snakes, for example, pass the so-called ‘chemical mirror test’ by discriminating their own odour from others (Freiburger et al., 2024; Burghardt et al., 2021). Auditory stimuli have also been used in testing animal self-recognition, although we found only one study in our review, which demonstrated zebra finches’ recognition on their own vocalizations (Geberzahn et al., 2021). The rest (N=19) have explored self-recognition through internal signals (e.g., heartbeat perception), bodily awareness (e.g., body size or position perception) and other specific aspects (e.g. self body-color perception), which have been considered alternative forms of self-recognition (Lage et al., 2022).

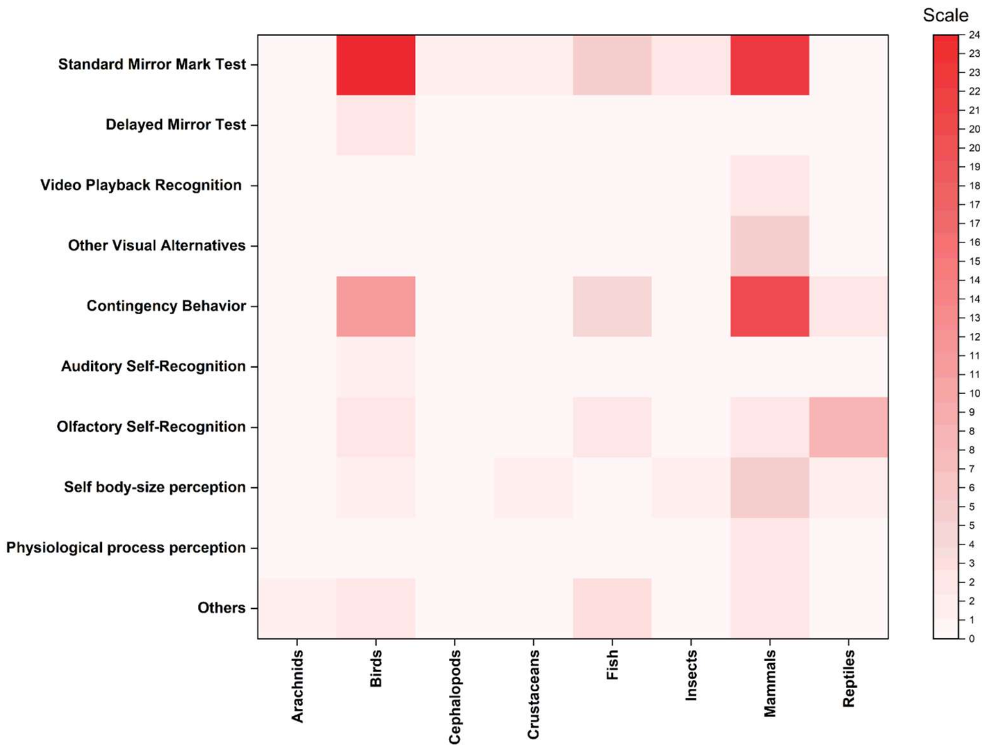


Figure 4. Heatmap of test types applied across different animal classes. The color scale indicates the number of papers that uses a specific test, with darker red indicating the higher amount.

Some studies we review suggested that simply matching the sensory modality is not sufficient; further adjustment may be required. For instance, elephants did not demonstrate self-recognition unless they were tested with a sufficiently large and close mirror (Plotnik et al., 2006 compared to Povinelli, 1989). Moreover, more than one sensory modality can be tested within the same study to make an insightful comparison, such as research on visual and chemical self-recognition in *Liolaemus* lizards (Ruiz-Monachesi et al. 2020), or visual and own body self-recognition in chimpanzees and gorillas (Vanhooland et al., 2025). These examples indicate that investigating self-recognition in diverse species often requires carefully tailored experimental designs, informed by a deep understanding of the species’ natural behavior and a willingness to explore unconventional or innovative approaches. In the latter case, experimental manipulations and neuroscience-based approaches may provide valuable tools for expanding our understanding of this behavior. Of the 135 cases reviewed, nine employed experimental manipulations prior to testing for self-recognition, mostly in vertebrate taxa and one in spiders (Fig. 5). A variety of approaches were used, including dietary manipulations (e.g., type or amount of food; Burghardt et al., 2021; Bilde et al., 2002), social isolation (Salzen & Cornell, 1968), and neuronal or hormonal interventions (e.g., Zhou et al., 2022; Ferkin et al., 2008). Only five studies directly addressed the neurological basis underlying self-recognition. Among these, molecular neuroscience techniques were commonly employed and show high potential for future investigations (e.g., Yokose et al., 2024; Desjardins & Fernald, 2010).

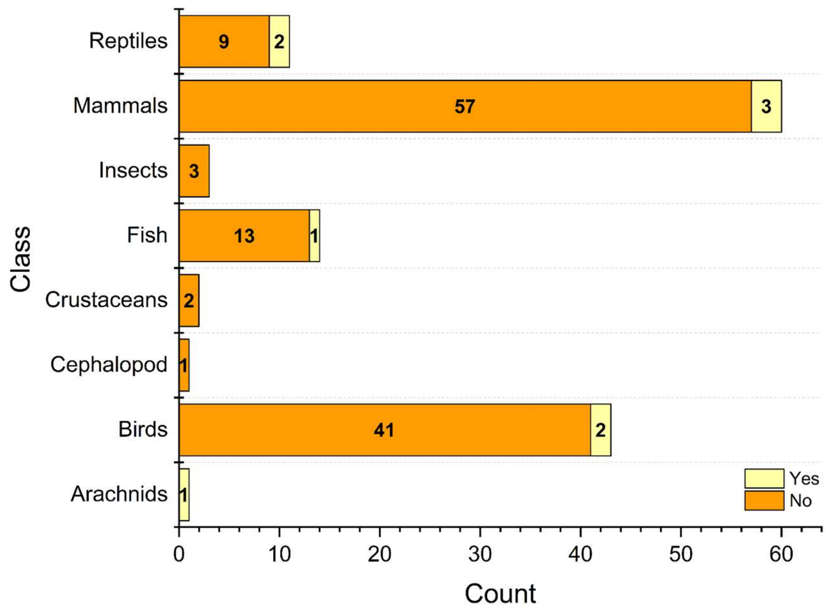


Figure 5. Experimental manipulation conducted prior to experimental design in the study.

Conclusion

This systematic review offers a comprehensive overview of current advancements in self-recognition research in non-human animals. We analyzed 118 studies, encompassing 135 individual experiments across a wide taxonomic range, from primates and birds to invertebrates, using various methodological approaches.

Our analysis reveals that while interest in animal self-recognition has expanded over the past six decades, research efforts remain unevenly distributed in terms of species examined and types of stimuli used. Visual-based paradigms, particularly mirror tests, still dominate the field, often at the expense of methods more suited to species that rely on other sensory modalities. Most studies also focus on adult individuals, with limited attention to developmental trajectories.

To move the field forward, it is crucial to diversify experimental methodologies and integrate tools from neuroscience, such as neuroimaging, electrophysiology, or molecular markers, to uncover the underlying mechanisms of self-recognition. The central question is not merely whether an animal shows signs of self-recognition, but how, when, and why such capacities emerge. These efforts can help distinguish genuine self-recognition from behavior driven by simpler mechanisms, clarify how self-related cognition develops, and shed light on its possible evolutionary origins.

Author Contributions

The topic was initiated by A.B. and conceptualized by A.D., F.N., K.P., N.E., S.D., and J.R. All authors contributed equally to the formulation of the search string, abstract screening, and full-text screening. K.P. and S.D. designed the Google Form survey. F.N. and J.R. collected and organized articles from the database. S.D. curated and finalized the datasheet. A.D. summarized excluded articles. F.N. constructed the PRISMA diagram, and both F.N. and S.D. contributed to data visualization. A.B., A.D., K.P., and N.E. wrote the original manuscript. J.R. supervised the project.

Declaration of generative AI and AI-assisted Technologies

During the preparation of this work the author(s) used ChatGPT in order to check for English grammar and spelling, smooth and refine the writing, and have suggestions for the title. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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