ISABELA NORMANDO MASCARENHAS

HUSBANDRY PRACTICES ADOPTED IN *EX SITU* MANAGEMENT OF *Callithrix* spp. – A SYSTEMATIC REVIEW

Dissertation submitted to the Animal Biology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

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To wildlife, source of pure inspiration

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“Only if we understand, can we care.Only if we care, we will help. Only if we help, we shall be saved.”.

(Jane Goodall)

ABSTRACT

MASCARENHAS, Isabela Normando, M.Sc., Universidade Federal de Viçosa, March 2022. **Husbandry practices adopted in ex situ management of *Callithrix* spp.**. Adviser: Fabiana Cristina Silveira Alves de Melo. Co-advisers: Fabiano Rodrigues de Melo and Reggiani Vilela Gonçalves.

The *Callithrix* genus involves six species of marmosets widely distributed in *ex situ* management that aims to improve their use in biological research (mostly *C. jacchus*) as well as the success of endangered species in conservation programs (*C. aurita* and *C. flaviceps*). However, adaptation to captivity involves several conditions that cause stress to primates, which can cause physiological, behavioral, and clinical damages. Therefore, this systematic review aims to gather and highlight techniques that favor *Callithrix* spp. welfare and good performance through an structured search on Web of Science, Scopus and Pubmed data bases. This study was performed in accordance to the PRISMA guidelines. Twenty-seven studies were selected and allowed to correlate husbandry practices with behavior, physiological parameters and reproduction in *Callithrix* spp. in *ex situ* management. Studies that did not involve *Callithrix* sp. were excluded and publications of veterinary care, analysis of vocalization, associated treatments, and ecology were not considered. The *ARRIVE* Guidelines were used to assess the quality of the studies included, which demonstrated good scientific evidence. In general, reproduction potential in *Callithrix* spp. colonies were associated with group formation; social bond implied directly in cortisol levels and the expression of natural behaviors. Captive marmosets' welfare was enhanced by the adoption of specific husbandry practices, such as housing and feeding strategies, environmental enrichment and proper human-animal interaction. Together, these actions enables the expression of natural behaviors of the species. Marmosets were kept in *ex-situ* management for different purpose: in laboratories where they are used as an experimental model, in zoos or in conservation centers. The difference between the institution's context influences the applicability of different husbandry practices. However, animal welfare has been a concern regardless the purpose for which they are kept. Thus, the high scientific evidence of the studies that supported this systematic review, allows the main findings of this research to be used by decision makers in the field of *Callithrix* husbandries.

Keywords: *Callithrix.* Husbandry practices. *Ex situ* management.

RESUMO

MASCARENHAS, Isabela Normando, M.Sc., Universidade Federal de Viçosa, março de 2022. **Práticas adotadas no manejo *ex situ* de *Callithrix* spp.**. Orientadora: Fabiana Cristina Silveira Alves de Melo. Coorientadores: Fabiano Rodrigues de Melo e Reggiani Vilela Gonçalves.

O gênero *Callithrix* envolve seis espécies de saguis amplamente distribuídas em cativeiro que visam melhorar seu uso em pesquisas biológicas (principalmente C*. jacchus*) bem como o sucesso de espécies ameaçadas de extinção em programas de conservação (*C. aurita* e C. *flaviceps*). No entanto, a adaptação ao cativeiro envolve diversas condições que causam estresse aos primatas, podendo causar danos fisiológicos, comportamentais e clínicos. Dessa forma, esta revisão sistemática tem como objetivo reunir e destacar técnicas que favorecem o bem estar e o bom desempenho de *Callithrix* spp. em manejo *ex situ* através de uma busca estruturada nas bases de dados Web of Science, Scopus e Pubmed. Este estudo foi realizado de acordo com as diretrizes PRISMA. Vinte e sete estudos foram selecionados e permitiram correlacionar práticas de manejo em cativeiro com comportamento, parâmetros fisiológicos e reprodução em *Callithrix* spp. Estudos que não envolveram *Callithrix* sp. foram excluídos e não foram consideradas publicações de cuidados veterinários, análise de vocalização, tratamentos associados e ecologia. As diretrizes ARRIVE foram usadas para avaliar a qualidade dos estudos incluídos que demonstraram boa evidência científica. Em geral, o potencial de reprodução em populações de *Callithrix* spp. foram associadas à formação de grupos; o vínculo social implicado diretamente nos níveis de cortisol e na expressão de comportamentos naturais. O bem-estar dos saguis em cativeiro foi potencializado pela adoção de práticas específicas de manejo, como estratégias de alojamento e alimentação, enriquecimento ambiental e interação humana-animal adequada. Juntas, essas ações demonstraram favorecer a expressão de comportamentos naturais das espécies objetos deste estudo. Os saguis foram mantidos em manejo *ex-situ* para diferentes finalidades: em laboratórios onde são usados como modelo experimental, em zoológicos ou em centros de conservação. A diferença entre o contexto da instituição influencia a aplicabilidade das diferentes práticas de manejo. Porém, o bem-estar dos animais tem sido uma preocupação independentemente da finalidade para a qual eles

são mantidos. Portanto, a alta evidência científica dos estudos que embasaram essa revisão sistemática permite que os resultados principais dessa pesquisa sejam utilizados pelos tomadores de decisão da área do manejo em cativeiro de *Callithrix* spp.

Palavras-chave: *Callithrix*. Práticas de manejo. Manejo *ex situ*.

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LIST OF ACRONYMS AND ABBREVIATIONS

IUCN International Union for Conservation of Nature

ICMbio Instituto Chico Mendes de Conservação da Biodiversidade

LIST OF SYMBOLS

@ Arroba.

% Porcentagem.

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1 INTRODUCTION

The Callitrichidae family belongs to New World primates and is formed by the genera *Callithrix, Cebuella, Callibella, Mico, Saguinus, Leontocebus, Leontopithecus* and *Callimico* (RYLANDS; COIMBRA-FILHO; MITTERMEIER, 2009; RYLANDS et al., 2016). They represent the world's smallest primates and are widely distributed throughout neotropical habitats (RYLANDS et al., 1993).

The Atlantic Forest Marmosets, *Callithrix*, comprimeses six species: *C. jacchus, C. penicillata, C. geoffroy, C. kuhli, C. aurita* and *C. flaviceps* (MITTERMEIER et al., 1988). Of these, *C. jacchus* is widely bred and used marmoset in laboratories for scientific research. As the environment can be an additional source of suffering, improving housing and caring of these animals reduces the impact of experiments upon them, which might be considered for ethical, legal, and scientific reasons (PRESCOTT & BUCHANAN-SMITH, 2014).

Callithrix aurita and *Callithrix flaviceps* are listed by the International Union for Conservation of Nature (IUCN) as two of the twenty-five most endangered primate species on the planet (CARVALHO et al., 2019, SILVA et al., 2018). According to the Red Book of the Brazilian Threatened Fauna (ICMBio, 2018) these species was classified as “Endangered”, which is the second most serious state of conservation for species in nature. Among the main threats to their conservation, stand out habitat loss, competition and hybridization with invasive species of the genus *Callithrix* (CARVALHO et al., 2018; MELO et al., 2019). In view of the need to conserve *C. aurita*, the National Action Plan for the Conservation of Atlantic Forest Primates and Leashed Sloth highlights the need to create new areas of protection, the development of environmental education actions, the control of hybrids and the implementation of a conservation and management program in captivity (MMA, 2019). Establishing captive

breeding programs is also a strategy corroborated by the Mountain Marmoset Conservation Program – (MMCP) created in 2012 for the conservation of endemic endangered species from the Atlantic Forest of southeastern Brazil, namely: *Callithrix aurita* and *Callithrix flaviceps*.

However, adaptation to captivity involves several conditions that cause stress to primates, which can cause physiological, behavioral and clinical damage (PIZZUTTO et al., 2013). Thus, this systematic review aims to highlight the influence of techniques and practices in captivity on the cortisol levels, reproduction and behavior of *Callithrix* spp. In addition, our objective was to evaluate the methodological quality of each identified study, and the main sources of bias that may impair the quality of the evidence. We believed that the critical analysis of the current evidence can help the decision-makers choose better management practices for these animals and consequently improve the welfare of captive primates.

2 METHODS

2.1 Focus Question

The objective of this systematic review was to answer the following focus question: What is the influence of the practices adopted in captivity husbandry on the behavior and reproduction of *Callithrix* spp.? Second, is there a relationship between environmental enrichment, feeding, and reproduction in these species? What is the influence of human interaction and level of welfare in these species? Third, do the practices adopted in captivity husbandry exert influence on the physiology and behavior of these animals?

2.2 Search Strategy

This systematic review was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines, which were used as a guide for screening, selection, and eligibility of studies. After the question was established, data collection was carried out through an advanced search in the electronic databases Scopus (https://www.scopus.com/home.uri), Pubmed (https://pubmed.ncbi.nlm.nih.gov/), and Web of Science (www.webofknowledge.com) on September 11, 2020. At first, the search filters were developed for the PubMed database by combining the keywords: *Callithrix;* animal husbandry; animal breeding; animal housing. Both were combined with the Mesh Terms and the TIAB command to expand the search possibilities. The search strategy applied to PubMed was adapted for other databases. Only studies in English were considered.

2.3. Eligibility criteria

The PICOS (population, intervention, comparison, outcomes, and study design) strategy was employed to identify criteria for then the inclusion of studies in the systematic review (Methley et al., 2014). The studies found were exported and tabulated using Microsoft Excel®. Subsequently, the duplicates were eliminated. The selection of articles was based on an exhaustive reading of the titles and abstracts. Therefore, there was no date limitation in the search for articles. When in doubt, the entire publication was retrieved and reevaluated. The researchers independently analyzed the articles according to their perceived eligibility and all dissenting opinions were assuaged by consensus. The kappa test was performed to evaluate the agreement between two researchers (INM and MMS) which indicated a good level of agreement (kappa= 0,959).

Only original studies that involved at least one of the species of the genus *Callithrix* in *ex situ* management were included. Studies that were not primary studies were excluded, such as literature reviews, book chapters, notes, brief reports, and websites. Studies that did not involve *Callithrix* sp. were also excluded. Still, according to the exclusion criteria, studies of veterinary care, analysis of vocalization, associated treatments, and ecology were not considered. Finally, the selected studies were read in full.

2.4 Data extraction

Two independent reviewers (INM and MMS) extracted the essential data grouped into descriptive levels as follows:

(1) Publication characteristics: title, author, year, and country;

(2) Characteristics of the animal model: species, number, sex, age, and weight;

(3) Husbandry characteristics: food - diet, frequency, display, water intake, setting, dimensions of the enclosure, material of the enclosure, lighting, number of individuals per enclosure, restraint and transportation, behavior - parental, social, reproductive, conditioning, environmental enrichment, and reproduction.

When there was doubt, an arbitration was requested from other independent researchers (FCSAM and RVG) to decide whether any given study met the eligibility criteria previously defined, likewise to discard subjectivity in the data collection and selection process, the information was extracted independently and analyzed separately.

2.5 Bias Analysis

To ensure the scientific knowledge of this systematic review, we used the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments), which comprises a 20 items checklist to improve the reporting of research involving animals, aiming at high quality reports and reliability of scientific publications (Coelho et. al, 2016). The ARRIVE guidelines table enables a critical evaluation of the articles included in this study based on 5 domains and their subdomains, as described below: 1) Abstract; 2) Objectives; 3) Methods (ethical statement; study design; experimental procedures; experimental animals; husbandry and housing; experimental results and statistic methods); 4) Results (base data; numbers analyzed; results and estimate); 5) Discussion (scientific implications; generalization and financial) (**Table S1**).

3 RESULTS

3.1 Publications characteristics

The initial search in this study compromised 222 papers, with 69 from PubMed, 69 from Scopus, and 84 from Web of Science. Out of these, 76 were duplicates, remaining 146 studies. After reading the titles and abstract, inclusion criteria were met by 27 articles. The PRISMA diagram illustrates the process of studies selection **(Figure 1).**

Most studies originated from United States of America (22,2%, n= 6), Brazil (18,51%, n=5), followed by Italy and United Kingdom (14.81%, n= 4 each), Australia (11.11%, n=3), Scotland (7.40%, n=2), Japan, Scotland and Poland (3.70%, n=1 each).

3.2 Characteristics of experimental animals

All experimental animals included in this study belong to the *Callithrix* genus. Among them, most was *C. jacchus* (85,18%, n= 23), followed by *C. penicillata* (7.40%,

n= 2), *C. geoffroy* (3,70%, n=1) and *C. Kulli* (3,70%, n=1). The proportion of sex of animals was 29.62% (n=8) female, 3.70% (n=1) male, and 62.96% (n=17) both. This information was omitted in 3.70% (n=1) of the studies. Seventeen studies specified animals' age (62.96%) and ten (37.03%) did not report this information. Only 11.76% (n=2) of the studies mentioned marmosets' body weight, while 92.59% (n=25) did not describe it. Yet, 3 studies (11.11%) used up to 5 marmosets, 6 (22.22%) ranged from 6 to 15, 6 (22.22%) ranged from 16 to 25, and 9 (37.03%) experiments used up to 25 animals. 3 (11,11%) studies did not mention it.

3.3 Main findings

The present study highlighted techniques that are adopted in the captive management of *Callithrix* spp. Among them, it was possible to correlate husbandry practices with reproduction in 33.33% (n=9) of the studies. From these papers, 11.11% (n=3) were associated with reproductive failure (**Table 1**), while 22.22% (n=6) demonstrated to improve reproductive parameters (**Table 2**).

In this research, the *ex situ* management practices of marmosets were classified in four groups: social interaction and housing; feeding considerations; environmental enrichment; and others. Therefore, 55.55% (n=15) correlated it with marmosets' behavior (species-specific behaviors and abnormal behaviors). The implications of human interaction on the behavior of *Callithrix* spp. were considered in 7.40% (n=2). Physiological parameters, such as cortisol levels, reproductive hormones and cardiophysiological values were associated with those practices in 22.22% of the studies (n=6).

Considerations about feeding was identified in 18.5% (n=5) of the studies **(Figure 3.a)**; while 14.8% (n=4) discussed about environmental enrichment **(Figure**

3.b). Housing and group formation were approached in 33.33% (n=9) **(Figure 3.c)** and human animal interaction in 7.40% (n=2) **(Figure 3.d).**

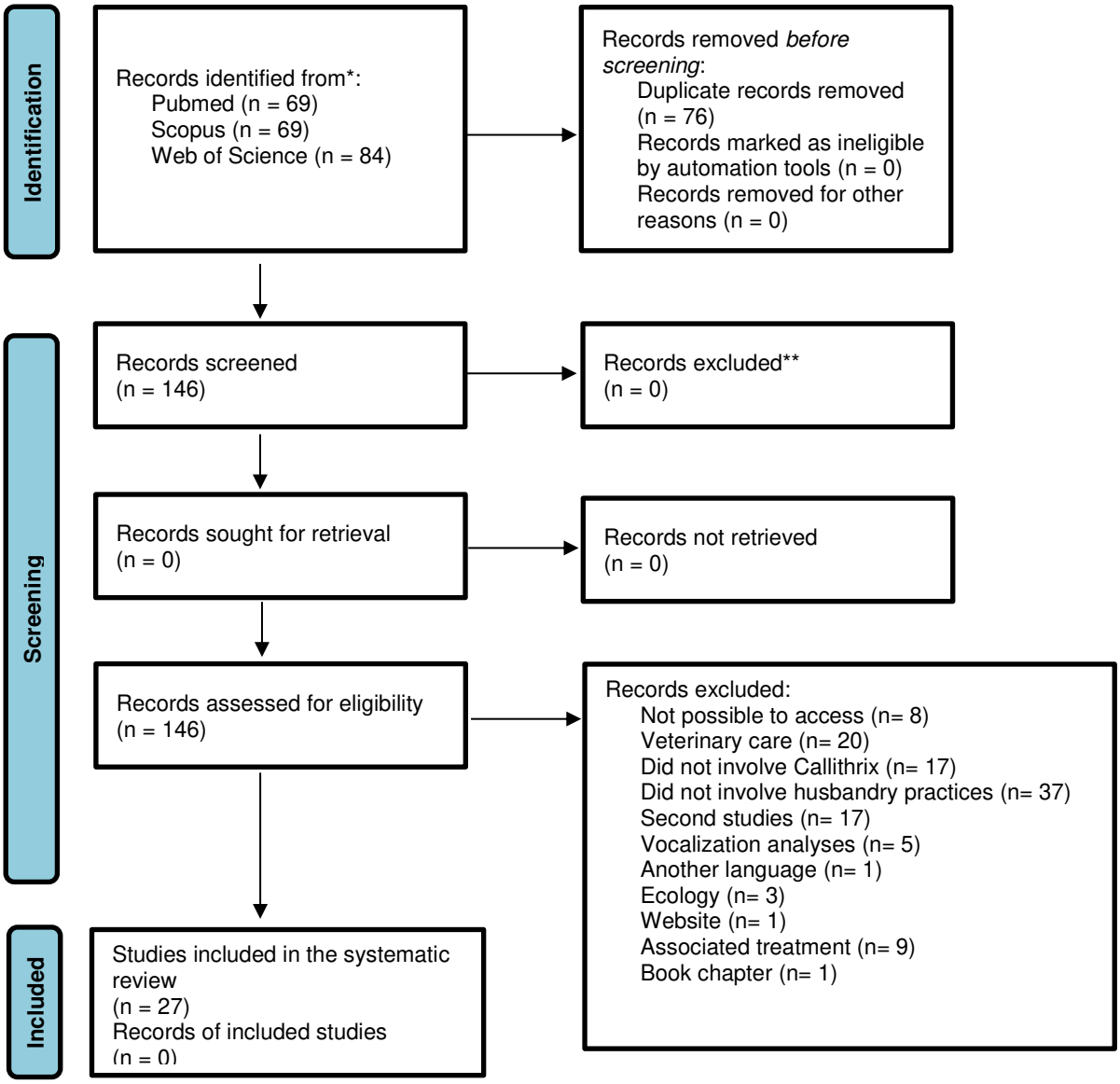

Figure 1. Flow diagram of the systematic review based on PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) . *From:* Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

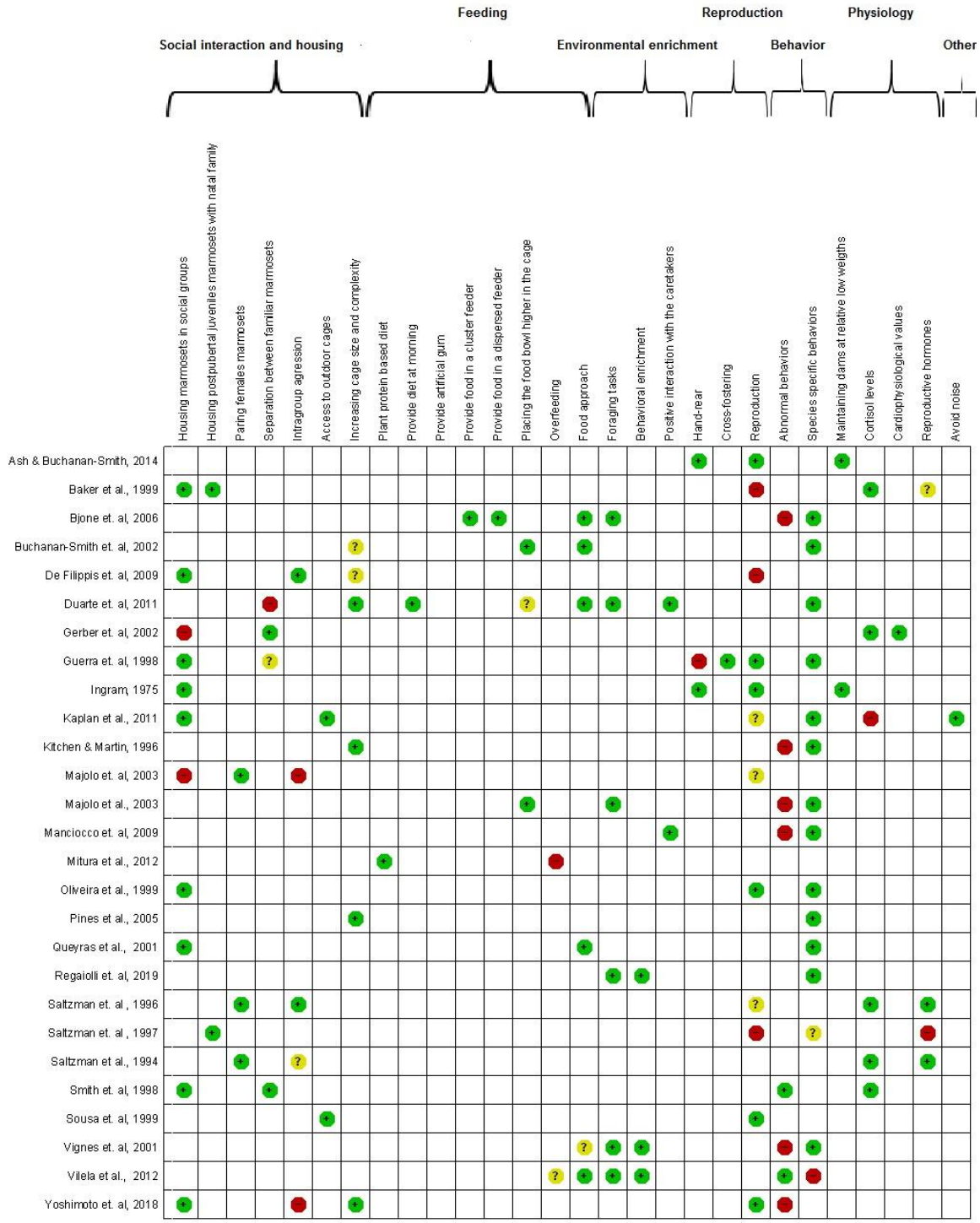

Figure 2. Main Techniques Adopted in the Captive Management of *Callithrix* sp. The strategies highlighted in this systematic review were grouped in three different domains: social interaction and housing; feeding; and environmental enrichment. To evaluate those strategies, the studies used parameters related to: reproduction; behavior and physiology.

Table 1. Reproductive failure in captive marmosets due to the group formation dynamic and its implication on individuals' physiology and behavior.

Reproduction	Husbandry practice	Physiology	Behavior
Reproductive failure	Housing daughters with the intact natal family	Social suppression of ovulation in acyclic daughters	Cyclic and acyclic daughters did not engage in sexual behavior with their father
	Housing postpubertal males common marmosets with their intact natal families	Sons did not have lower reproductive hormones levels (LH and testosterone) or higher cortisol levels than the father	Sons engaged in very low rates of sexual behavior
	Housing females in established social groups	Cortisol concentrations were strongly correlated with ovarian function	Dominant females tended to perform more aggression, less submission, and more display behavior to stimulus females
	Pairing younger females (<18 months) with males	Not included	Not included

Table 2. Strategies that improve the reproductive potential in captive marmosets due to the group formation dynamic and its implication on physiology and behavior.

Reproduction	**Husbandry practice**	**Physiology**	**Behavior**
Improve the reproductive potential	Housing daughters with the natal families in which the father had been replaced by an unrelated adult male	Not included	Cyclic daughters showed frequent courtship and sexual behaviors with the male, reduced affiliative interaction with the mother, and elevated frequencies of aggressive behavior
	Housing postpubertal common marmosets with their natal families in which the mother had been replaced by an unrelated adult female	Sons did not have lower reproductive hormones levels (LH and testosterone) or higher cortisol levels than the father	Sons engaged in significantly more sexual behavior
	Housing marmosets in large cages	Increased body weight	Stereotypical behavior was not observed as it was in small cages

Improve the reproductive potential	Induction of cross-fostering between two species of *Callithrix*	Bodyweight gain and locomotor activity were not impaired in the infant that was not very young.	Allogrooming, time spent in bodily interaction and resting together increased gradually as a function of time between orphans and step-parents
	Maintaining dams at relatively low weights	May help to reduce larger litters and dystocic birth	Not included
	In the case of triples, the 3rd can be hand-reared	Not included	Not included
	Use of outdoor cages allowed the animals to be aware of the seasonal variations	Not included	Not included

3.4 Bias Analysis

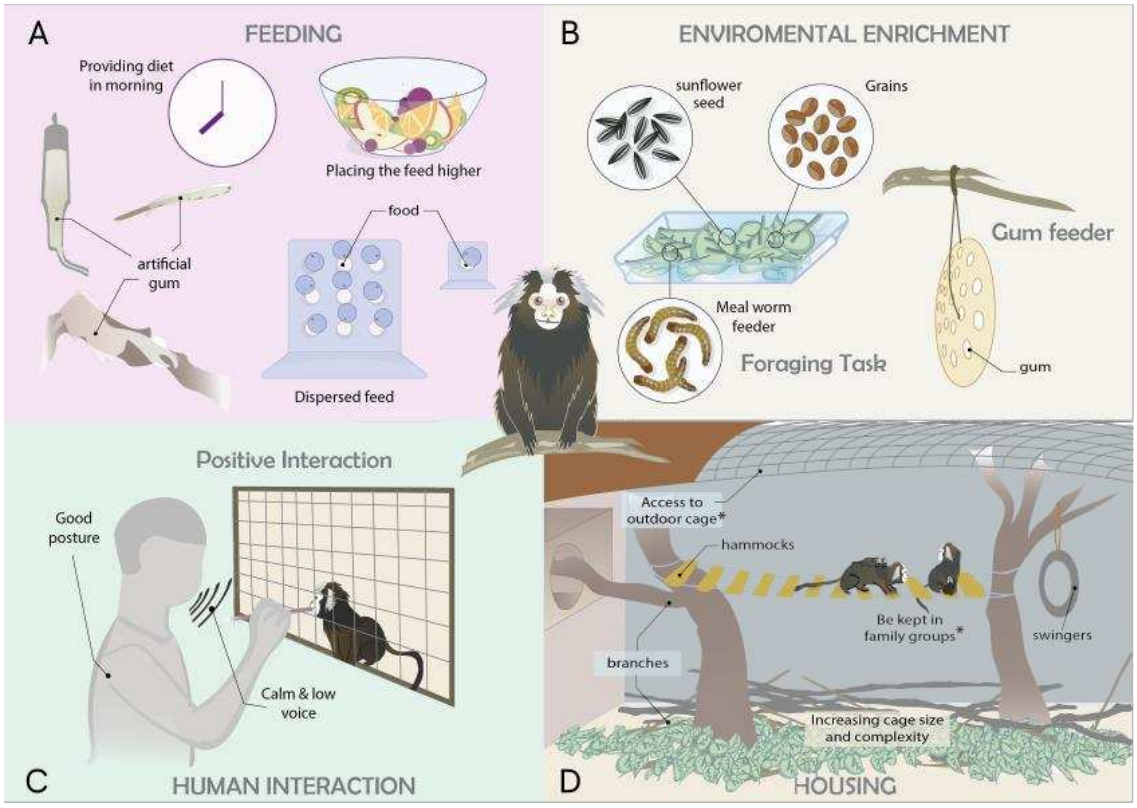

Figure 3. Techniques adopted in the captive management of *Callithrix* spp. that favors the expression of species-specific behavior. *Access to outdoor cages and housing marmosets in family groups minimizes stress in captive monkeys through reducing cortisol levels.

After critically analyzing the papers, it was possible to observe that 96.2% of the studies had precise and concise title descriptions of the article's content as well as accurate summary of the topic, research objectives, main methods, main results and conclusions. Also, 96.2% of the studies clearly described the primary and secondary objectives. 48% of the studies indicated the ethical permissions. Detailed husbandry practice were describe by 62.9% studies. All of them (100%) provided information regarding animal species, while 66.6% described animals' sex and age. It was observed that 96.2% of the studies reported husbandry conditions, while 88.8% described housing of experimental animals. Welfare related assesments were

observed in 55.5% of the experiments. Regarding the sample-size, 92.59% related the total number of animals used in each experiment, but only 77.7% explained how the number of animals was decided. Details on the statistical methods used for each analysis was provided by 77.7% of the studies. Only 44.4% reported relevant characteristics and health status of the animals. Among the evaluated discussion, 92.59% interpreted the results, taking into account the study objectives and hypotheses, current theory, and other relevant studies in the literature.

4 DISCUSSION

4.1 Considerations about reproduction

In general, reproduction potential in *Callithrix* spp. colonies were associated with group formation. Housing postpubertal common marmosets (*C. jacchus*) with their intact natal family showed that cyclic and acyclic daugthers did not engage in sexual behavior with their father (Saltzman et. al, 1997). Sons engaged in very low rates of sexual behavior but when the mother was replaced by an adult female, the rates of sexual behavior increased significantly (Baker et. al, 1999). Nevertheless, infant carrying and baby-sitting by other group members, other than parents, has been described in callitrhichids (Mittermeier et. al, 1988). This helpers benefits themselves by their gain in rearing experience (Rothe et. al, 1993). Furthermore, breeding cooperation in *C. jacchus* mitigate the mother's costs of infant care accrue from lactation, infant transport, reduced capacity to forage and increased suscebility to predation (Souza de Oliveira, 1999).

Reproductive parameters also was increased by the use of outdoor cages , that allows the animals to be aware of seasonal variations and times the reproductive activity (Sousa et. al, 1999). Also, maintaining dams at low weight disfavored the occurrence of dystocia due to macrosomia in *C. jacchus* and the need for surgical

intervention (Ash et. al, 2014). Dystocia is defined as an abnormality or difficulty, of maternal or fetal origin, at the time of delivery or expulsion of the fetus. Among the causes of dystocic birth in non-human primates, the occurrence of macrosomic fetuses and multiple fetuses has been documented in captive marmosets (Santana et al., 2021).

In case of triplets, human intervation becomes necessary since parents rarely rear more than two infants (Ash et. al, 2021). Hand-rearing them were pointed out as an efficient strategy to reduce infant mortality (Ash & Buchanan-Smith, 2014; Ingram, 1975). Supplementary feeding procedure, where infants remains in contact with each other when removed from the family, appears to minimize negative behavioural and physiological consequences of early family separation (Ash et. al, 2021); Cross fostering – the adoption of infants by non-specific adults – were also an useful technique for preserving the life of immature animals and occurred successfully between *C. jacchus* and *C. penicillata* individuals (Guerra et. al, 1998).

4.2 Animal welfare

Well-being in captive non-human primates is usually evaluated through behavior and hormonal analyses. Allowing captive animals to express their species-specific behaviors is a valuable tool in assessing animal welfare (Ragaiolli et al., 2020). In this systmatic review, the behaviors positively related to animal welfare were described as afiliattive behaviors (i.e.: allogrooming); exploratory behaviors; playful activities and feeding behavior (i.e.: foraging). On the other hand, undesirable behaviors, indicative of compromised well-being were correlated with the frequency of

stress-related behaviors (i.e.: sent marking, auto-grooming, self-scratching) and stereotypical behavior (i.e.: pacing).

Environmental enrichment is a technique widely adopted in many institutions around the world in attempt to promote the performance of species-specific behavior and to prevent abnormal behavior. It is also benefit for animal's physiology, primarily through stress reduction (Albanese et. al, 2021). The most desirable form of environmental enrichment for common marmosets (*C. jacchus*) is social housing. Captive marmosets should be kept in family groups - a breeding pair and a couple of offspring might be enough (Duarte et. al, 2011). Social attachment has an important role in welfare in captive marmosets. In the other hand, social isolation elevates cortisol levels markedly and should be avoid (Smith et. al 1998; Gerber et. al 2002; Kaplan et. al, 2011). However, crowding confined space can evoke several intragroup aggressions (Saltzman et. al 1994, 1996; De Filippis et. al 2009).

Besides that, foraging tasks alllow marmosets to express their intrinsic exploratory behavior. This strategies has the potential to increase exploration and decrease boredom and steryotipical behaviors. (Vignes et. al, 2001; Majolo et. al 2003; Bjone et. al, 2006; Duarte et. al, 2011; Vilela et. al, 2012; Ragaiolli et. al, 2019). Its effectiveness depends on the heigh of the device in the enclosure and the presence of higden food (Buchan-Smith et. al 2002; Majolo et. al 2003). Marmosets' foraging strategies include searching, processing, and consuming food that is spatially distributed, embedded, or hidden (Mittermeier et. al 1988). Provide gum feeder encourages specific-specific behaviors (Regaiolli et. al 2019), as marmosets are gummivore and spents a large amount of time eating gum (Mittermeier et. al 1988). Therefore, foraging tasks should contain food items from their daily diet. Even though, environmental enrichment might stimulates food consumption and monitoring

marmosets body weight should be associated with this practice, in order to avoid overfeeding and obesity (Vilela et. al, 2012).

Environmental enrichment has a particular importance for those animals that are kept alone. Marmosets has strong social needs and do not respond well to isolation (Scott, 1991). When reproduction and single-housing is to be avoid, marmosets can be housed in same sex-pairs. In female common marmosets (*C. jacchus*) this is a safe practice if one monkey is sexually immature (Majolo et. al, 2003). However, if individuals needs to be solitary, visual and auditory contact with conspecifics might be provided (Duarte et. al, 2011).

Under the right condition, human-animal interaction (HAI) is a valueble tool in improving animal welfare. After daily duties, such as cleaning the enclousures and feeding the monkeys, it was seen that the presence of the caretaker for over twenty minutes with each marmosets' family estimulated their affiliative behaviors, such as grooming and playful activities, as well as decreases stress-related behavior (i.e.: self-scratching) (Manciocco et. al, 2009). In this particulary study, the interaction consisted in grooming, playing and sweet-talking to the monkeys. If the staff have the right attitude and interact with marmosets in the right way, animal welfare will be improved (Duarte et. al, 2011). Captive primates tends to cope with routine laboratory procedures when they are early exposure to positive human interaction (Ash et. al, 2021). The relationship between humans and animals depends on the caregiver's attitude towards the animals and the knowledge and experience of the animals. Unique dyads were formed between keepers and zoo animals, which influenced animal behavior (Ward & Melfi, 2015). Recurrent capture and handling will build a negative relationship between keepers and the marmosets that they are dealing with, turning daily checks that are needed for these animals very difficult. The capture of a callitrichid

should be carried out when absolutely necessary (Ruivo, 2010). Of the twenty-seven articles selected for this systemic review, none of them elucidated the different methods used for the capture, handling and transport of marmosets and how their variations imply behavioral and physiologic parameters of these animals.

The concentration of glucocorticoid can be used as a parameter for monitoring adrenal activity and increases with stress (Altino et al., 2018). Chronic elevation of cortisol can compromises health and welfare of primates (Baker et. al, 1999; Gerber et. al, 2002). Therefore, non-invasive mensurement of cortisol has important implications for good husbandry practices in captive primates and it is a practice that must be stimulated by the different institutions that keep marmosets in captivity. Measuring behavior itself can be doubtful without a physiological measure of stress (Kaplan et. al, 2011). Unfortunatly, only 7,14% (n=2) in this systematic review correlated husbandry practices with cortisol levels in marmosets. Social bond implied directly in cortisol levels and the expression of natural behaviors (Smith et. al, 1998; Gerber et. al, 2002; Kaplan et. al, 2011). As social primates, marmosets in the wild usually lives in small family groups composed of a breeding pair and their offspring (Stevenson & Rylands, 1988). The separation between familiar marmosets to a familiar or to an unfamiliar environmental increases urinary and saliva samples of cortisol, and all of the caridiophysiological values and locomotor activity (Kaplan et. al, 2011). On the other hand, the presence of the heterosexual social partner can modulate the physiological consequences of exposure to a stressor, such as novel-cage housing (Gerber et. al, 2002). Still, according to Kaplan et. al, 2011, cortisol levels measured by saliva samples were significantly elevated in three situations: when removal a familiar marmoset from the home-room; in a roof repair situation and hailstorm; and human speech played on a FM radio. The same study showed that cortisol levels

measured by saliva samples decreased significantly when the animals had access to outdoor cages compared with access to indoor rooms. Isolation elevates cortisol levels markedly and should be avoid.

Although the measurement of cortisol levels, as well as other physiological parameters, are necessary to scientific research and to ensure animal health and well-being, it is important that the collections of biological material are not invasive or do not yet represent an additional source of stress to captive primates. Possible strategies involves voluntary cooperation and animal conditioning (Ferraz et. al, 2013). Methods which minimize any stress to the animal must be employed whenever possible, specially in callithrichids, since they are highly tuned to avoid predators in the wild (Ruivo, 2010). In this research, no specific studies on *Callithrix spp.* conditioning were identified.

5 CONCLUSION

This review aimed to gather information about the different *Callithrix* spp. husbandry practices in *ex-situ* management adopted in institutions widely distributed over the world and correlate them with reproduction potential, behavior and physiological implications in these species.

In this research, it was possible to classify these practices into four groups, including social interaction and housing; feeding practices; environmental enrichment and other (avoid noise). However, it was not possible to go deeper into one classification or another, but to make a general overview of what has been done, and identify the gaps of information, promoting necessary studies to the development and improvement of *Callithrix* colonies raised in captivity.

Finally, marmosets were kept in *ex-situ* management for different purpose: in laboratories where they are used as an experimental model or in zoos. However, the information obtained in this study must also be extrapolated to other classifications of *ex situ* management, such as conservation centers. Establishing captive breeding programs is a demand of the National Action Plan for the Conservation of Atlantic Forest Primates and Leashed Sloth for the protection of two endangered species: *Callithrix aurita* and *Callithrix flaviceps.* The difference between the institution's context influences the applicability of different husbandry practices. In all cases, animal welfare has been a concern regardless the purpose for which they are kept.

Table S1: Bias analyses (ARRIVE) of the studies

TITLE	Gerber et. al, 2002	Saltzman et. al , 1997	Saltzman et. al , 1996	Buchanan-Smith et. Al, 2002	Smith et. Al, 1998	Guerra et. al, 1998	Duarte et. al, 2011	Manciocco et. al, 2009	Majolo et. al, 2003	Bjone et. al, 2006	Regaiolli et. al, 2019	Ingram, 1975	Yoshimoto et. al, 2018	Ash & Buchanan-Smith, 2014	Vignes et. al, 2001	Sousa et. al, 1999	Majolo et. al, 2003	De Filippis et. al, 2009	Baker et. al, 1999	Queyras et. al, 2001	Kaplan et. Al, 2011	Kitchen & Martin, 1996	Vilela et. al, 2012	Oliveira et. al, 1999	Pines et. al, 2005	Saltzman et. al, 1994	Mitura et al., 2012		
Precise and concise description of the article's content	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	26	96.2%
ABSTRACT																													
Accurate summary of the topic, research objectives, main methods, main results and conclusions of the study.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	26	96.2%
INTRODUCTION																													
a. Includes sufficient scientific training	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	26	96.2%
b. Explanation of the experimental approach and rationale	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	26	96.2%
OBJECTIVES																													
Clearly describe the primary and secondary objectives of the study	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	26	96.2%
METHODS																													
Ethical statement																													
Indicates the nature of the review ethical permissions, relevant licenses	✓	✓	✓		✓				✓	✓	✓		✓	✓	✓				✓				✓			✓	✓	13	48.14%
Study design																													
a. Number of animals per group	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	26	96.2%

TITLE	Gerber et. al, 2002	Saltzman et. al , 1997	Saltzman et. al , 1996	Buchanan-Smith et. Al, 2002	Smith et. Al, 1998	Guerra et. al, 1998	Duarte et. al, 2011	Manciocco et. al, 2009	Majolo et. al, 2003	Bjone et. al, 2006	Regaiolli et. al, 2019	Ingram, 1975	Yoshimoto et. al, 2018	Ash & Buchanan-Smith, 2014	Vignes et. al, 2001	Sousa et. al, 1999	Majolo et. al, 2003	De Filippis et. al, 2009	Baker et. al, 1999	Queyras et. al, 2001	Kaplan et. Al, 2011	Kitchen & Martin, 1996	Vilela et. al, 2012	Oliveira et. al, 1999	Pines et. al, 2005	Saltzman et. al, 1994	Mitura et al., 2012		
Experimental procedures																													
Detailed describe the husbandry practice	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓										✓	17	62.9%
Duration of experimente	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓	24	88.8%
Time of day for data collection	✓	✓	✓		✓				✓	✓	✓				✓				✓		✓	✓		✓	✓	✓	✓	15	53.5%
Experimental animals																													
Information regarding animal species	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	27	100%
Animal strain	✓	✓	✓	✓	✓	✓		✓		✓				✓		✓			✓	✓		✓	✓	✓	✓	✓	✓	18	66.6%
Animal sex	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	18	66.6%
Animal weight range	✓													✓									✓		✓		✓	5	18.51%
Animal age	✓	✓	✓	✓				✓	✓	✓	✓		✓	✓	✓		✓		✓		✓			✓	✓	✓	✓	18	66.6%
Information related to a brief history of the animals	✓	✓	✓	✓	✓	✓		✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	24	88.8%
Husbandry and housing																													
Husbandry conditions	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	26	96.2%
Housing of experimental animals	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	24	88.8%
Welfare-related assesments and interventions that were carried out before, during or after the experiment	✓			✓	✓		✓	✓		✓	✓		✓		✓		✓				✓	✓	✓		✓	✓		15	55.5%

TITLE	Gerber et. al, 2002	Saltzman et. al , 1997	Saltzman et. al , 1996	Buchanan-Smith et. Al, 2002	Smith et. Al, 1998	Guerra et. al, 1998	Duarte et. al, 2011	Manciocco et. al, 2009	Majolo et. al, 2003	Bjone et. al, 2006	Regaiolli et. al, 2019	Ingram, 1975	Yoshimoto et. al, 2018	Ash & Buchanan-Smith, 2014	Vignes et. al, 2001	Sousa et. al, 1999	Majolo et. al, 2003	De Filippis et. al, 2009	Baker et. al, 1999	Queyras et. al, 2001	Kaplan et. Al, 2011	Kitchen & Martin, 1996	Vilela et. al, 2012	Oliveira et. al, 1999	Pines et. al, 2005	Saltzman et. al, 1994	Mitura et al., 2012		
Sample size																													
Total number of animals used in each experiment and the number of animals in each experimental group	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	25	92.59%
Explains how the number of animals was decided. Provides details of any sample size calculation	✓	✓	✓	✓	✓				✓	✓			✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	21	77.7%
Distribution of animals in experimental groups																													
Gives full details on how the animals were distributed into experimental groups		✓	✓	✓	✓			✓	✓	✓			✓	✓			✓		✓	✓	✓	✓	✓		✓	✓		18	66.6%
Describes the order in which animals in different experimental groups were treated and evaluated.						✓			✓	✓					✓		✓		✓			✓			✓	✓		9	33.3%
Experimental results																													
Clearly defines the evaluated primary and secondary experimental results	✓	✓	✓		✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	25	92,8%
Statistic methods																													
Provide details on the statistical methods used for each analysis		✓	✓		✓			✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	21	77.7%
Specifies the unit of analysis for each dataset (for example, single animal, group of animals, single neuron).		✓	✓	✓	✓			✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	22	81.4%
Describe any methods used to assess whether the data meet the assumptions of the statistical approach.	✓	✓	✓	✓	✓			✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	24	88.8%

TITLE	Gerber et. al, 2002	Saltzman et. al , 1997	Saltzman et. al , 1996	Buchanan-Smith et. Al, 2002	Smith et. Al, 1998	Guerra et. al, 1998	Duarte et. al, 2011	Manciocco et. al, 2009	Majolo et. al, 2003	Bjone et. al, 2006	Regaiolli et. al, 2019	Ingram, 1975	Yoshimoto et. al, 2018	Ash & Buchanan-Smith, 2014	Vignes et. al, 2001	Sousa et. al, 1999	Majolo et. al, 2003	De Filippis et. al, 2009	Baker et. al, 1999	Queyras et. al, 2001	Kaplan et. Al, 2011	Kitchen & Martin, 1996	Vilela et. al, 2012	Oliveira et. al, 1999	Pines et. al, 2005	Saltzman et. al, 1994	Mitura et al., 2012		
RESULTS																													
Base data																													
For each experimental group, it reports relevant characteristics and health status of the animals		✓			✓	✓					✓	✓		✓		✓	✓		✓					✓	✓		✓	12	44.4%
Numbers analyzed																													
Reports the number of animals in each group included in each analysis. Reports absolute numbers (eg 10/20, not 50% a).		✓	✓			✓		✓	✓		✓			✓	✓	✓		✓	✓	✓	✓	✓			✓	✓	✓	17	62.9%
If any animals or data were not included in the analysis, explain why?		✓	✓	✓			✓	✓	✓					✓		✓			✓	✓					✓	✓		12	44.4%
Results and Estimate																													
																													85.1%
Report the results for each analysis performed	✓	✓	✓	✓	✓			✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		23	

TITLE	Gerber et. al, 2002	Saltzman et. al , 1997	Saltzman et. al , 1996	Buchanan-Smith et. Al, 2002	Smith et. Al, 1998	Guerra et. al, 1998	Duarte et. al, 2011	Manciocco et. al, 2009	Majolo et. al, 2003	Bjone et. al, 2006	Regaiolli et. al, 2019	Ingram, 1975	Yoshimoto et. al, 2018	Ash & Buchanan-Smith, 2014	Vignes et. al, 2001	Sousa et. al, 1999	Majolo et. al, 2003	De Filippis et. al, 2009	Baker et. al, 1999	Queyras et. al, 2001	Kaplan et. Al, 2011	Kitchen & Martin, 1996	Vilela et. al, 2012	Oliveira et. al, 1999	Pines et. al, 2005	Saltzman et. al, 1994	Mitura et al., 2012		
DISCUSSION																													
Scientific interpretation/implications																													
a. Interpret the results, taking into account the study objectives and hypotheses, current theory, and other relevant studies in the literature.	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	25	92.59%
b. Comments on the limitations of the study, including any potential sources of bias and imprecision associated with the results		✓	✓	✓			✓	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓		20	74%
Generalization / translation																													
It comments on whether, and how, the results of this study are likely to translate to other species or systems, including any relevance to human biology.						✓			✓	✓	✓					✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	15	55.5%
Financing																													
Lists all funding sources (including grant number) and the role of funders) in the study	✓	✓	✓		✓	✓	✓					✓		✓					✓				✓	✓	✓	✓	✓	14	51.8%
Results	26	32	30	25	28	17	13	27	30	30	27	9	25	30	27	26	28	22	32	25	25	28	27	25	33	32	29		

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