

Gum-feeder as environmental enrichment for zoo marmosets and tamarins

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Research highlights

- Wooden gum feeders can improve the welfare of zoo marmosets and tamarins by stimulating their natural species-specific behaviors and decreasing stress-related behavior. - and helping them to cope with stressful contexts.
- Zoo callitrichines, managed by adequate enrichment programs, may show positive welfare.

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Title: Gum-feeder as environmental enrichment for zoo marmosets and tamarins

Short running title: Gum-feeder as enrichment for marmosets and tamarins

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Abstract

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Tamarins and marmosets are small-bodied social callitrichines. Wild callitrichines feed on exudates, such as sap and gum; particularly, marmosets are mainly gummivores, while tamarins consume gums only occasionally and opportunistically. Zoo marmosets and tamarins are usually provided with gum Arabic as an alternative to the exudates normally found in the wild. The aim of this study was to evaluate the effects of a gum feeder on the behavior and well-being of four zoo-managed callitrichines. We studied four cotton-top tamarins (Saguinus oedipus), four red-handed tamarins (Saguinus midas), two pygmy marmosets (Cebuella pygmaea) and three Geoffroy's marmosets (Callithrix geoffroyi) housed at Parco Natura Viva (Italy). We conducted the study over two different periods, a baseline (control, without the gum feeder) and then a gumfeeder (when the gum feeder was provided) period. We used continuous focal animal sampling to collect behavioral data, including durations of social and individual behaviors. We collected 240 minutes of observations per period per study subject, with a total of 3,120 minutes for all the subjects in the same period and of 6,240 minutes in both periods. We analyzed data by using non-parametric statistical tests. First, we found that the gum feeder promoted species-specific behaviors, such as exploration, and diminished selfdirected behaviors, suggesting an enriching effect on tamarin and marmoset behavior. Moreover, in redhanded tamarins, the provision of the gum feeder reduced the performance of self-directed and abnormal behavior, specifically coprophagy. These results confirm that gum-feeders are effective foraging enrichment tools for zoo marmosets and tamarins.

Keywords: zoo primate welfare, callitrichines, gum Arabic

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Introduction

Tamarins and marmosets are small-bodied New World monkeys belonging to the subfamily of Callitrichinae (Groves, 2001). They live in geographical areas ranging from Costa Rica to South Brazil, Bolivia and Paraguay, with the greatest variety of species found in the Amazonian region (Emmons, 1990; Buckner *et al.*, 2015). They are cooperative breeders and may form familial groups made up of a breeding pair, several siblings and other members helping with parental care of the offspring. The breeding pair is dominant over other group members (Emmons, 1990; Smuts *et al.*, 1986).

The diet of wild tamarins and marmosets is affected by seasonality and availability of food resources; they mainly feed on fruit, insects, small vertebrates, leaves and nectar (Rosenberger, 1992; Garber, 1993; Bairrão Ruivo, 2010). In addition, their natural diet includes plant exudates, such as gum and sap, which are rich in carbohydrates and minerals (Bairrão Ruivo, 2010; Power, 2010). Many plant gum exudates consist of β-linked complex polysaccharides which often also contain glycoproteins and proteoglycans (Gashua et al., 2015) from which energy, water and minerals, particularly calcium, can be obtained after fermentation. Callitrichines require a high intake of calcium from their diet, as these species generally give birth to twins and plenty of milk is required during the nursing process (Garber, 1993; Kelly, 1993; Heymann and Smith, 1999; Pack et al., 1999; Passamani and Rylands, 2000; Taylor and Vinyard, 2004). The benefits of gum feeding have been widely investigated in the wild. Therefore, supplementing the diet of zoo non-human primate species with plant gum exudates, might improve the diet and general wellbeing of these primate species in captive environments (Garber, 1993; Kelly, 1993; Heymann and Smith, 1999; Pack et al., 1999; Passamani and Rylands, 2000; Taylor and Vinyard, 2004).

Plant gum exudates are an essential component of the marmoset diet, whilst it is less important for
other callitrichines (Power and Oftedal, 1996; Bairrão Ruivo, 2010; Power, 2010). For this reason,
marmosets show behavioral, morphological and metabolic adaptations to gum feeding. In particular, their
dental adaptations allow them to gouge trees and thereby stimulate gum exudate production as part of the
plants' response to wounding (Vinyard et al., 2003; Vinyard et al., 2004; Eng et al., 2009; Burrows & Nash,
2010); while modifications of the gastro-intestinal trait permit the digestion of gums and other plant exudates
(Coimbra-Filha and Mittermeier 1977; Heymann & Smith, 1999; Bairrão Ruivo, 2010). Tamarins tend to

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feed on gum and sap only opportunistically, when this food is available in trees wounded by other animals, with seasonal variation in terms of time investment for gum feeding (Garber, 1993; Power and Oftedal, 1996; Power, 2010).

Zoo tamarins and marmosets are generally provided with commercially available gum Arabic, as a replacement for the various plant gum exudates that they consume in the wild (Goodrum et al., 2000). Furthermore, these plant gum exudates may be crucial biochemical digestive challenges for the normal functioning of their digestive tract (Bairrão Ruivo, 2010). Wild tamarins show more frequent gum feeding during the afternoon, as gum digestion is time consuming and would therefore be easier during the night sleeping (Kelly, 1993; Heymann & Smith, 1999; Bairrão Ruivo, 2010). On the contrary, gummivore marmosets are known to eat gum frequently throughout the daylight hours as their gastrointestinal system is well-adapted to gum digestion (Kelly, 1993; Heymann & Smith, 1999; Bairrão Ruivo, 2010). Tamarins and marmosets are usually attracted to novel objects, and vigilant and aware of what happens in their surrounding environment (Menzel & Menzel, 1979). Promoting natural gum feeding behavior with specific devices might be important to enhance the feeding strategy and husbandry of zoo-managed callitrichines in order to improve their physical and mental well-being.

Giving zoo animals the opportunity to perform their species-specific behaviors represents one of the primary goals of modern zoological gardens. In addition, ethological parameters have been proven to be a valuable tool in assessing zoo animal welfare (Hill & Broom, 2009; Fontani et al., 2014). The occurrence of natural species-specific behaviors, such as exploratory behaviors, is considered an indicator of good welfare status and enriched environment (Mench, 1994; Mellor & Beausoleil, 2015), while abnormal behaviors, such as excessive inactivity, stereotypies and self-injurious behaviors, may indicate poor welfare or stressful scenarios (Renner et al., 2000; Manteca et al., 2016). Moreover, self-directed behaviors such as self-grooming and scratching, are usually benign activities that occur commonly in non-human primates (Maestripieri et al., 1992). However, in certain situations such as social tension and conflicting or frustrating contexts, self-directed behaviors can be associated with uncertainty and anxiety and have been considered as displacement activities (Troisi & Schino, 1987; Troisi, 2002; Lutz, 2014; Spiezio et al., 2017). Thus, a 59 54 decrease in self-directed behaviors might be considered as a positive welfare indicator, although these activities are normally included and well-represented in the species-specific behavioral repertoire of primate

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species (Spiezio *et al.*, 2017; Leeds & Lukas, 2018). Similarly, coprophagy occurs in both captive and wild non-human primates and may have an adaptive value in these species; however, in controlled environment this behavior has been related to factors such as nutritional deficiency and medical problems but also boredom and social stress (Krief *et al.*, 2004; Prates & Bicca-Marques 2005). Therefore, coprophagy has been classified as an abnormal behavior and has been identified as a possible indicator of poor well-being (*i.e.*, Prates & Bicca-Marques 2005; Lutz, 2018).

Environmental enrichment is a widespread practice among modern zoos and has been found to promote the performance of species-specific behavioral repertoire and to address as well as prevent abnormal behavior (Hosey et al., 2013). Though some callitrichines may not require gum to reach their nutritional needs in zoo settings, gum feeding may represent a behavioral necessity and could improve the diversity of the behavioral repertoire as well as the welfare status of zoo marmosets and tamarins (Bairrão Ruivo, 2010). In the current study, zoo tamarins and marmosets were provided with wooden drilled logs as gum-feeders. Previous research investigating the effects of a similar gum-feeder on zoo marmosets and tamarins suggested that this environmental enrichment could promote species-specific behaviors, reduce abnormal behaviors, such as stereotypies and coprophagy, and decrease inactivity (McGrew et al., 1986; Roberts et al., 1999; Taylor, 2002; Huber & Lewis, 2011). In particular, the provision of hanging feeder baskets and sticks smeared with Acacia gum promoted feeding and foraging while decreasing stereotypic behavior, specifically excessive coprophagy, in red-handed tamarins in zoo (Taylor, 2002). Similarly, common marmosets (Callithrix jacchus) in different social housing conditions have been found to benefit from foraging enrichments, specifically gum feeders, promoting natural behaviors and leading to a reduction in the time spent performing stereotypic behavior such as pacing (McGrew et al., 1986; Roberts et al., 1999). In general, providing zoo callitrichines with gum arabic in feeders that require specific feeding abilities has been found to promote naturalistic behaviors and feeding strategies described in the wild. In particular, artificial gum feeders are cheap and easy to build and can be helpful in enhancing the physical and mental well-being of these species, which are well-disposed to work for gum, enhancing also the educative value of zoo exhibit (McGrew et al., 1986; Huber & Lewis, 2011).

60 82 The aims of this study were to:

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Assess the effects of the gum-feeder on the behavior of two tamarin species (Saguinus oedipus and Saguinus midas) and two marmoset species (Callithrix geoffrovi and Cebuella *pygmaea*) which were scarcely investigated in the past. On the basis of prior work by other authors revealing that gum feeding represents a behavioral need and a digestive challenge for the digestive system of marmosets and, to a lesser extent, tamarins (Heymann & Smith, 1999; Bairrão Ruivo, 2010; Hosey et al., 2013; Roberts et al., 1999; Taylor, 2002), we predicted that the gum feeder would increase the performance of species-specific behaviors, such as explorative and feeding behaviors and reduce inactivity and abnormal behavior.

- Compare the duration of gum Arabic feeding between tamarins and marmosets, particularly the time spent feeding on gum between the morning and the afternoon within the two groups, to identify the optimal time for gum provision. Since gum digestion is time consuming and would therefore be easier during night sleeping, tamarins show gum feeding more during the afternoon (Heymann & Smith, 1999; Bairrão Ruivo, 2010). Thus, we expect that marmosets would perform gum feeding more than tamarins and during day time, while tamarins would eat gum during afternoon hours rather than in the morning. 1.e
- 38 99 **Materials and Methods**

Study subjects and area

⁴²101 We studied eight tamarins, specifically four cotton-top tamarins and four red-handed tamarins; and ⁴⁴₄₅102 five marmosets, three Geoffroy's marmosets and a pair of pygmy marmosets. The study subjects belonged to 47 103 two different age-groups: juveniles, including individuals aged less than two years (approximate age of 49</sub>104 sexual maturity, Tardif 1984; Ziegler et al. 1987; Abbott et al., 2003; Tardif et al., 2011) and adults, ⁵⁰ 51105 including individuals aged more than two years (Abbott et al., 2003; Tardiff et al., 2008; Tardiff et al., 2011) (Table 1).

All groups were housed at Parco Natura Viva-Garda Zoological Park (Bussolengo, Italy) in separated enclosures in the Tropical Green House. Although enclosures were not adjacent to each other, Geoffroy's marmosets, cotton-top tamarins and red-handed tamarins were in acoustic contact. Their enclosures were made of an outdoor and an indoor area and each area was approximately 30 m² and

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1 2 3 4 111 contained trees, branches and logs, ropes, wooden boxes, sheds and shelves; the indoor areas were heated 5 6 112 and provided with UV lamps. The pygmy marmoset enclosure was an 18 m² aviary. 7 8 ⁹ 113 During the study, the gum feeders were placed in the outdoor area of the enclosures. Tamarins and 10 $^{11}_{12}114$ marmosets were fed twice a day with fruits, multi-cereal pap, mealworms, gum Arabic, and occasionally 13₁₄115 meat and eggs. Fresh water was available ad libitum. Manipulative, sensory or food-related devices were 15 16</sub>116 provided daily as environmental enrichment. The study, which did not involve any invasive or stressful 17 18117 techniques, was conducted in accordance with the EU Directive 2010/63/EU and the Italian legislative 19 20118 decree 26/2014 for Animal Research. 21 22 23119 Procedure and data collection 24 25120 The study was made of two different periods, the baseline and the gum-feeder period. During the baseline, 26 27121 gum Arabic was provided in bowls at the time of the afternoon meal following the daily routine husbandry. 28 29122 In the gum-feeder period, gum Arabic was provided, using the new gum feeders, at the usual feeding times 30 31123 over the morning and the afternoon. The gum-feeder consisted of a wooden drilled disc, with 10 to 15 holes 32 ³³124 each, on both sides of the disc (Figure 1). The discs were hung with ropes on the enclosure trees and 34 ³⁵125 36 branches, approximately 1.5 meters above the ground. The daily amount of gum Arabic was put in the holes ³⁷₃₈126 of the feeder. In particular, the amount of gum Arabic per subject was prepared using approximately 8 grams ³⁹ 40¹²⁷ of powder and 5 grams of water. The entire study consisted of 6,240 minutes (104 hours) of observation 41 42128 divided in the baseline (52 hours) and in the gum-feeder period (52 hours) for all the subjects. Per period and 43 44129 per subject, a total of 240 minutes of behavioral observations were carried out and two sessions per day per 45 46130 monkey were run (one in the morning, one in the afternoon). The duration of the data collection sessions per 47 48131 monkey differed between species based on the sample size. In particular, per period, data on each cotton-top 49 50132 tamarin (N = 4) and on each red-handed tamarin (N = 4) were collected during eight 30-minute sessions. For 51 ⁵²133 53 the marmosets, per period and per monkey, data were collected during six 40-minute sessions for Geoffroy's ⁵⁴134 55 marmosets (N = 3) and four 60-minute sessions for pygmy marmosets (N = 2) for an overall of 240 minutes ⁵⁶ 57135 of observation for each subject within each period. Monkeys were observed in a prescribed sequence ⁵⁸ 59</sub>136 following a specific design to avoid time-of-day bias in data collection. Feeding and enrichment times were ⁶⁰137

the same over the study period, one in the morning and one in the afternoon.

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138 A continuous focal animal sampling method (Altmann, 1974) was used to collect durations of 139 normal and abnormal individual and social behaviors (Table 2). The time spent out-of-sight, here defined as 140 "not observed", was also recorded. The red-handed tamarins were the only subjects to perform abnormal $^{10}_{11}141$ behavior, specifically coprophagy, which was reported in three out of four monkeys, although for a very low 12 13142 percentage of the total observation time (ranging from 0.13% to 1.67%). In order to assess the effect of the 14 15143 feeder on the behavior of the red-handed tamarins, we created the category stress-related behaviors (SRB), 17144 including coprophagy and self-directed behaviors. Although these behaviors are found in wild animals and 19145 may be adaptive, they both have been related to stressful and conflict situations within controlled 20 21146 environments (Troisi & Schino, 1987; Troisi, 2002; Prates & Bicca-Marques 2005; Lutz, 2014; Spiezio et 22 23147 al., 2017; Lutz, 2018). The gum-eating behavior was included in the category "feeding/foraging" as in the 24 ²⁵148 26 first period gum Arabic was provided in bowls with other food; however, in order to compare the time spent ²⁷149 28 feeding on gum between marmosets and tamarins, the duration of gum feeding from the new feeder was also ²⁹ 30</sub>150 collected (Table 2).

³³ 34152 Data analysis

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35 36153 Data were analyzed using non-parametric statistic tests and significance level was set at p < 0.05 (Siegel & 37 38154 Castellan, 1992). In particular, the Wilcoxon test was used to compare the behavior of the study subjects 39 40155 between the two periods (baseline vs. gum-feeder), whereas the Mann-Whitney test was used to compare the 41 42156 time spent feeding on gum between the study groups (marmosets vs. tamarins) in the gum-feeder period. 43 ⁴⁴157 StatView version 5.0 (SAS Institute Inc.) was used for all the statistical analyses on behavioral data. In 45 46 47 158 addition, single-case analyses were used to test the effect of the gum-feeder on the behavior of each ⁴⁸159 49 individual, comparing the performance of SRBs between different periods within each individual of red-⁵⁰ 51</sub>160 handed tamarins (Fisch, 2001) performing stress-related behaviors, specifically abnormal behavior and self-⁵² 53161 directed behavior. For the single-case analyses, Wilcoxon-Mann-Whitney test (Marx et al., 2016) was used. ⁵⁴ 55162 For all behavioral categories, medians and interquartile range (IQR) are reported in the manuscript and 56 57163 tables. 58

- 59164 60
 - 165 **Results**

We investigated the effects of the gum-feeder on the behavior of the study subjects comparing the time spent

maintenance and self-directed were performed significantly more during the baseline than during the gum-

feeder period, whereas the opposite pattern was found for visual exploration. No other significant differences

were found (see Figure 2 and Table 3 for median, IQR and statistical values). On the other hand, within

social behaviors, interspecific behavior was performed more during the baseline than during the gum-feeder

period, whereas no other differences were found (see Table 3 for median, IQR and statistical values).

Finally, Wilcoxon test revealed that "not observed" was performed significantly more during the baseline

After evaluating the effects of the gum-feeder on all subjects, we focused on tamarins and marmosets

separately. Regarding tamarins, within individual behaviors, feeding/foraging and maintenance were

performed significantly more during the baseline than during the gum-feeder period, whereas no significant

differences were found in any other behavioral category (see Table 3 for median, IQR and statistical values).

Regarding marmosets, "not observed" was performed significantly more during baseline than during the

gum-feeder period (z = -2.023, p = 0.043), while no other significant differences were found between the two

Three red-handed tamarins (CS, OB and Normann) showed the abnormal behavior 'coprophagy'. In order to

test whether the gum-feeder positively affected the behavior as a measure of welfare of these individuals, a

than during the gum-feeder period (z = -2.481, p = 0.013) (Table 3).

When considering each individual behavior, Wilcoxon tests revealed that feeding/foraging,

in individual and social behaviors as well as "not observed" between the two periods (Figure 2, Table 3).

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Baseline vs. gum-feeder period

Effect of the gum-feeder on tamarins

Effects of the gum-feeder on marmosets

periods (see Table 3 for median, IQR and statistical values).

Effects of the gum-feeder on stress-related behavior in the red-handed tamarins

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1 2 3 4 166 5 6 167 7 8 168 9 ¹⁰ 11 12 13</sub>170 14 1₅171 16 17172 18 19173 20 21174 22 23175 24 ²⁵176 26 ²⁷28177 ²⁹30178 ³¹ 32</sub>179 33 ₃₄180 35 36181 37 38182 39 40183 41 42184 43 ⁴⁴185 45 46 47 186 ⁴⁸ 49</sub>187 ⁵⁰ 51</sub>188 ⁵² 53189 ⁵⁴ 55190 56 57191 58 59192 60

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single-case analysis was done to compare the performance of SRBs between different periods within each

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194 individual. For CS, the median duration of SRB was 113.5 (50) seconds in the baseline and 74.5 (51.25) 195 seconds in the gum-feeder period; for OB, the median duration of SRB was 90 (70.25) seconds in the 196 baseline and 52 (72.75) seconds in the gum-feeder period; for Normann, the median duration of SRB was ¹⁰₁₁197 101 (99.75) seconds in the baseline and 60 (70) seconds in the gum-feeder period. Wilcoxon-Mann-Whitney 12 13</sub>198 tests revealed that SRB were performed significantly more during the baseline than the gum-feeder period in 14 15199 CS (p = 0.037) but no significant differences were found for OB and Normann (p > 0.05).

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Gum-feeding in marmosets and tamarins

21202 Finally, we focused on the time spent feeding on gum Arabic- by the study subjects. The median time spent 23203 feeding on gum Arabic was 42.5 (90.8) seconds for tamarins and 235 (255.5) seconds for marmosets. 24 ²⁵204 26 Marmosets tended to spend more time eating gum Arabic than tamarins (U = 7, p = 0.056). When ²⁷205 28 considering gum feeding during the day within each group, the median time spent feeding on gum Arabic by ²⁹30206 tamarins was 31.5 (91.8) seconds in the morning and 0 (20) seconds in the afternoon. In the case of ³¹ 32²⁰⁷ marmosets, the median time spent feeding on gum Arabic was 116 (207) seconds in the morning and 114 ³³ 34208 (281) seconds in the afternoon. Wilcoxon tests revealed no significant differences between morning and 36209 afternoon in both tamarins (Z = -1.572, p = 0.116) and marmosets (Z = -0.365, p = 0.715) (Figure 3).

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40211 Discussion

42212 This study aimed to investigate the effects of a gum-feeder, a wooden disc drilled with holes, on the behavior 43 ⁴⁴213 45 of zoo tamarins and marmosets, to assess their welfare. Moreover, the study aimed to compare the time spent 46 47 214 feeding on gum Arabic between these species. First, each species interacted with the gum-feeders performing 48 49</sub>215 a new behavior to obtain gum Arabic, confirming the enriching role on the animal daily routine and feeding ⁵⁰ 51</sub>216 strategies of this device (McGrew et al., 1986; Roberts et al., 1999; Huber & Lewis, 2011). All the study ⁵² 53217 monkeys interacted with the gum-feeder and showed the behavior of eating Arabic gum from the holes. In 54 55218 particular, marmosets and tamarins had to cling on to the wooden disc holding with one or both hands and 56 57219 then retrieve gum directly with the mouth or grasping it with one hand. When retrieving the gum, marmosets 58 59220 gouged and scraped the disc moving the head and mouth similarly as in the tree-gouging behavior reported in 60 221 the wild: indeed, the jaws were widely open around the gum in the hole, the upper jaw anchored on the

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wooden disc whereas the lower jaws indented the area around the hole, favoring both gouging and scraping movements (Rylands, 1984; Vinyard et al., 2003; Vinyard et al., 2004; Burrows & Nash, 2010; Thompson et al., 2014). This movement was less pronounced in tamarins as they simply retrieved the superficial gum coming out the holes and consumed it.

First, we found that subjects were out-of-sight ("not observed") more during the baseline than during the gum-feeder period. This result seems to suggest that the gum-feeder could enhance the welfare of the study subjects, engaging them in the performance of species-specific behaviors and increasing the time they were visible to the public. This finding is consistent with previous research on gum-feeder provision in marmosets, reporting an increased animal visibility(Kelly, 1993).

Regarding individual behaviors, when data on tamarins and marmosets were pooled together, we found that feeding/foraging behaviors as well as maintenance behaviors were performed significantly less during the gum-feeder period than during the baseline. Within each group (tamarins and marmosets), we found the same patterns and significant differences for these categories in tamarins, whilst they did not differ significantly in marmosets. Gum Arabic is a high-energy food source, rich in carbohydrates and minerals, requiring longer time to be digested than other food items (Kelly, 1993; Power, 2010; Bairrão Ruivo, 2010). It is possible that the provision of this food item in the new gum-feeder elicited tamarin and marmoset interest, inducing them to eat gum Arabic before the rest of the food. Given the nutritional properties of the gum (Power & Oftedal, 1996; Power, 2010), we speculate that a small amount of this food could be sufficient for these small primates, leading to the reduction in the overall time spent feeding and foraging in the second period. Also, it is possible that the interest toward the gum feeder as a novel device might supply the callitrichines with the need of looking for food elsewhere in the enclosure (e.g. bowls, usual feeding points). In other words, the study monkeys may have spent less time feeding and foraging in the second period because they consumed gum Arabic first, and the same could extend to the decrease in maintenance behavior during the second period. However, the presence of the gum feeder increased visual exploration, which was performed significantly more during the gum-feeder period than during the baseline. This behavior is particularly relevant in callitrichines as they are curious, attracted to novel objects and aware of their surroundings (Menzel & Menzel, 1979). Such features of these small-bodied primates have been related to improved vigilance and anti-predator behaviors (Caine, 1984). Therefore, the gum-feeder seemed to

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250 encourage the performance of species-specific behaviors that are particularly important for the survival of 251 the species in the wild (Caine, 1984).

252 The presence of the gum-feeder also led to a significant decrease in self-directed behaviors, which $^{10}_{11}253$ have been described as potential behavioral indicators of stress and anxiety in non-human primates, at least 12 13254 in some situations (Maestripieri et al., 1992; Leeds & Lukas, 2018). As our results underlined a decrease in 14 15255 the performance of these behaviors, the presence of the gum-feeder seems to positively impact the behavior 17256 of the study monkeys (Spiezio et al., 2017; Leeds & Lukas, 2018). However, the study monkeys spent a 19257 relatively low amount of time performing self-directed behaviors in both the baseline and the gum-feeder 21258 periods (4% and 3% of the total observation time respectively) suggesting that this statistically significant 23259 change may not necessarily be biologically important. 24

²⁵260 26 Regarding social behaviors, during the second period, a significant decrease of interspecific ²⁷261 28 behaviors, including interacting with or directing attention to humans was also reported. As visitors may be ²⁹ 30²⁶² distressing for the study subjects, this finding seems to highlight that the gum feeder might help tamarins and ³¹ 32</sub>263 marmosets to better cope with humans, promoting the performance of species-specific social behaviors and ³³ 34264 discouraging possibly deleterious human-animal interactions. Together with the decrease in the time spent 36265 out-of-sight in presence of the feeder, this result highlights a possible positive effect of gum-feeding devices 38266 on callithricines' well-being in zoo environments.

40267 Except for the decrease in feeding/foraging and maintenance behavior reported in the gum-feeder 41 42268 period, no significant differences between periods were found in tamarins. The decrease in feeding/foraging 43 ⁴⁴269 behaviors within tamarins seems consistent with the same result obtained when data from both species were 45 46 47 270 pooled together. Indeed, tamarins do not have as many adaptations for gum feeding and digestion as ⁴⁸49271 marmosets (Heymann & Smith, 1999; Bairrão Ruivo, 2010; Hosey et al., 2013; Roberts et al., 1999; Taylor, ⁵⁰ 51272 2002); therefore, consuming the gum before other food items due to the new presentation modality might ⁵² 53</sub>273 have reduced the tamarin appetite and the performance of other food-related behavior, but had no effect on 54 55274 gummivore marmosets. However, the lack of significant differences in marmosets might be due to the small 56 57275 sample size and thus further research is needed to better investigate this aspect.

On the other hand, marmosets were out-of-sight ("not observed") significantly more during the 59276 60 277 baseline than the gum-feeder period, suggesting that for these species the presence of the drilled wooden

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discs might help to increase the visibility of these species to zoo visitors. Inactive behaviors, such as sleeping, were rather uncommon in the study subjects, as tamarins and marmosets tend to be very active during the day. However, the study subjects had rest in the wooden nest boxes in the highest part of the enclosure where the observer was not able to see them. Therefore, the behavioral category "not observed" also included resting, which was otherwise not recorded in the study periods. For this reason, it is possible, by decreasing the time spent out-of-sight, the presence of the gum-feeder also reduced inactive behavior of the study subjects, especially within marmosets. This is consistent with findings by other authors on common marmosets (Callithrix jacchus) (Roberts et al., 1999).

21286 When analyzing the behavior of each study subject during the two periods, we found that in three 23287 red-handed tamarins, the gum-feeder had a positive effect as reduced SRBs, such as self-directed behaviors 24 ²⁵288 26 and coprophagy. In addition, red-handed tamarins performed auto-coprophagy, which has been previously ²⁷289 28 related to physical or psychological stress in non-human primates (Anderson et al., 1991; Krief et al., 2004; ²⁹30 Prates & Bicca-Marques, 2005). One of the main effects of the gum-feeder was the reduction of SRBs, ³¹ 32</sub>291 including coprophagy. This was reported in other studies on gum-feeders in the red-handed tamarin, ³³ 34292 suggesting that gum-related enrichment might be particularly relevant to prevent coprophagy in this species 36293 (Taylor, 2002), and on laboratory common marmosets, where similar gum-feeding devices decreased 38294 stereotypies (Roberts et al., 1999).

40295 Based on data collected during the second period, this study aimed at verifying the presence of 41 42296 differences in gum-feeding between tamarins and marmosets and investigate whether the time spent feeding 43 ⁴⁴297 on gum Arabic differed between morning and afternoon within each group. First, although no statistically 45 ⁴⁶298 47 significant difference was found, marmosets tended to feed on gum more than tamarins. This finding agrees ⁴⁸299 with previous work on these species, as gummivore marmosets spend a large amount of time feeding on ⁵⁰ 51</sub>300 gum, based on the importance of this food in their diet, whereas tamarins rely more on fruit and invertebrates ⁵² 53³⁰¹ and cannot easily obtain and digest plant exudate (Kelly, 1993; Heymann & Smith, 1999; Bairrão Ruivo, ⁵⁴ 55302 2010). However, it could not be excluded that the greater interaction of the marmosets with the feeder was 56 57303 due to species differences in approaching the new enrichment devices, as previously suggested (Renner et 58 59304 al., 2000). 60

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305 Similarly, no differences in time spent feeding on gum Arabic were found between mornings and 306 afternoons, neither within marmosets nor within tamarins. In the wild, marmosets feed on exudates 307 frequently throughout the daylight hours, as their gastrointestinal system is well-adapted to gum digestion ¹⁰ 11</sub>308 (Kelly, 1993; Heymann & Smith, 1999; Bairrão Ruivo, 2010). Therefore, the gum-feeding behavior of the 12 13</sub>309 study Geoffroy's and pygmy marmosets resembles that reported in the wild, as the study subjects ate gum 14 15310 Arabic to the same extent in both the morning and the afternoon. In the case of tamarins, these species are 16 17311 known to eat exudates mainly during the afternoon, so that they can digest this food item overnight (Kelly, 18 19312 1993; Heymann & Smith, 1999; Bairrão Ruivo, 2010). However, we collected data on the time spent eating 20 21313 gum but not the amount of gum that was eaten. Again, providing gum in the new gum feeders might have 22 23314 incentivized the consumption of this food item regardless of the time of the day.

²⁵315 26 Our findings seem to highlight positive effects of the gum-feeder on the behavior of zoo ²⁷316 28 callithrichines, as previously reported in the species considered in the current study as well as in other ²⁹ 30³¹⁷ species of marmosets and tamarins (McGrew et al., 1986; Roberts et al., 1999; Taylor, 2002; Huber & ³¹ 32</sub>318 Lewis, 2011). However, the small sample size and the relatively small amount of data collected in our study ³³ 34319 hamper the ability to draw firm conclusions and future research is needed to investigate more deeply the 35 36320 gum-feeding behavior of zoo callitrichines, focusing on larger samples and longer periods of time.

40322 Conclusion

42323 In conclusion, our findings suggest that providing zoo tamarins and marmosets with gum Arabic in a 43 ⁴⁴324 naturalistic and innovative way, such as using a wooden gum feeder, might enhance the welfare of these 45 46 47 47 species by:

- ⁴⁸ 49</sub>326 1) promoting the performance of species-specific behaviors, such as visual exploration and decreasing ⁵⁰ 51</sub>327 self-directed behaviors, that can in some cases indicate a stressful situation of the animal;
- ⁵² 53³²⁸ 2) improving the visibility to zoo visitors by decreasing the time spent out-of-sight and in interspecific 54 55329 interactions;
- 57330 3) reducing the performance of abnormal behaviors, as reported in the study of red-handed tamarins;
- 59331 4) increasing the consumption of this crucial food item, at least in the first stages of the introduction of 60
 - 332 the feeder.

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Our results add to previous literature on gum-feeding devices in tamarins and marmosets, highlighting that the wooden gum feeders can improve the welfare of these species in controlled environments by stimulating natural behaviors and promoting naturalistic feeding strategies (McGrew et al., 1986; Roberts *et al.*, 1999; Taylor, 2002; Huber & Lewis, 2011).

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188x176mm (150 x 150 DPI)



Figure 1: The gum-feeder provided to marmosets and tamarins. In the gum-feeder period, tamarins and marmosets were provided with wooden drilled discs, hanged in the outdoor enclosure and filled with Arabic gum. The picture shows one of the study Geoffroy's marmoset interacting with the feeder.

188x176mm (150 x 150 DPI)







Figure 2: Behaviors of the study marmosets and tamarins. The bars report the % total duration in seconds of individual and social behaviors as well as "not observed" in the baseline and in the gum-feeder. Asterisks indicate categories that differed significantly between periods (Wilcoxon test: p < 0.05).

336x146mm (150 x 150 DPI)

Species	Name	Sex	Age
	Mum	F	Adult
Cotton-top tamarin	Franca	F	Juv
(Saguinus oedipus) N = 4	Rubik	F	Juv
	Dad	М	Adult
	CS	F	Adult
Midas tamarin	OB	F	Adult
(S. midas) $N = 4$	Norman	М	Adult
	CC	М	Adult
Cooffroy's marmosot	Mum	F	Adult
(Callithrix geoffroyi)	Dad	М	Adult
<i>N</i> = 3	Sbiru	М	Juv
Pygmy marmoset	Peace	F	Adult
(Cebuella pigmea) N = 2	Love	М	Adult

Table 1: Tamarins and marmosets involved in the study. For each species the table reports the subject name, sex (F = female; M = male) and age class (Adult: > 2 years of age; Juv: < 2 years of age).</td>

behavio u rs	
Abnormal behavio u r	Coprophagy.
Alert	Being watchful.
Exploration/Play	Exploring the environment, hunting, manipulating enrichment devices, leaves, twigs and other objects found in the enclosure by sniffing, biting, chewing, gouging, handling, pouncing on, grappling with.
Feeding/Foraging	Eating food found in the enclosure, either in bowls or foraging on trees, ground and other substrates.
Gum-feeder*	Eating gum from the gum-feeders (second period).
Locomotion	Moving around in the enclosure, walking, running or jumping along trees or walls of the enclosure
Maintenance	Peeing, defecating, drinking.
Scent-marking	Marking branches, shelves, ropes and other substrates with ano-genital, supra-pubic or sternal glands.
Self- groomingdirected b.	Cleaning or licking the own hair or other parts of the body, scratching with the hands or with the legs.
Visual exploration	Looking around quietly.
Social behaviour<u>behavior</u>	
Affiliative b.	Allo-grooming (using the hands and/or mouth to clean the partner fur or other parts of the body), being in contact with conspecifics, nuzzling (rubbing or pushing gently with the nose and mouth), anogenital inspection (orienting the face against or toward anogenital region of partner or use hands or mouth to investigate anogenital region of partner), stealing food/objects from the hands or from the mouth.
Agonistic b. Fighting (biting, clawing, and wrestling), attacking (lunging at or pouncing aggressively), cuffing, chasing, threatening (staring with lower eyebrows, fut tongue flicking), mounting between same-sex individuals.	
Interspecific b.	Interacting with or directing attention toward individuals of different species, such as humans (visitors, zoo-keepers <u>zookeepers</u>) or Azara agoutis (only in the Geoffroy's marmoset enclosure).
Not visible	
Not observed	The individual is not visible to the observer or it is not possible to identify the behaviourbehavior being performed.

*This behavioural category was recorded only in the second period, when <u>Arabic gum arabic</u> was put in the gum feeder and it was possible to detect clearly that the subjects were feeding on that food item.

Table 2: Study ethogram. Individual and social behaviours collected in the study periods.

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	All			Tamarins			Marmosets		
Individual behavior	Baseline	GF	Z and <i>p</i> -value	Baseline	GF	Z and p-value	Baseline	GF	Z and p-value
Abnormal	0 (0)	0 (9)	#	0 (60.8)	0 (79.5)	#	0 (0)	0 (0)	#
Alert	7 (17.5)	0 (4.5)	z = -1.868, p = 0.062	8 (15.8)	4 (9.5)	z = -1.016, p = 0.310	5 (18.5)	0 (0)	z = -1.826, p = 0.068
Expl/play	869 (1307.5)	663 (612)	z = -1.922, p = 0.055	974.5 (1569.5)	857.5 (1308.5)	z = -1.400, p = 0.161	321 (1509)	494 (723.5)	z = -1.214, p = 0.225
Feeding/Foraging	1219 (590.5)	826 (665.5)	z = -2.481, p = 0.031*	1312.5 (635.3)	1233.5 (864.5)	z = -2.100, p = 0.036*	1081 (653.5)	763 (485.5)	z = -1.483, p = 0.138
Locomotion	2920 (980.5)	2859 (1572.5)	z = -0.384, p = 0.701	2958.5 (890.3)	3340.5 (1155.8)	z = -2.260, p = 0.208	2920 (1199.5)	1622 (1432)	z = -1.483, p = 0.138
Maintenance	57 (70)	10 (32)	z = -3.111, p = 0.002*	83 (42.8)	13 (30.5)	z = -2.521, p = 0.012*	19 (11.5)	0 (22.5)	z = -1.761, p = 0.078
Scent-marking	38 (36.5)	22 (28.5)	z = -1.049, p = 0.294	27.5 (36)	28 (32.8)	z = -0.491, p = 0.624	49 (55.5)	19 (37)	z = -0.944, p = 0.345
Self-groomingdirected	420 (240.5)	392 (337)	z = -2.201, p = 0.028*	464 (257.3)	437.5 (183.3)	z = -1.260, p = 0.208	384 (250.5)	208 (354)	z = -1.753, p = 0.080
Visual expl	6891 (2946.5)	8097 (1760)	z = -2.201, p = 0.028*	6578 (3024.8)	7860.5 (2860.3)	z = -1.680, p = 0.093	7080 (2402.5)	8119 (2034)	z = -1.483, p = 0.138
Social behavior									
Affiliative	764 (632.5)	1331 (1180.5)	z = -1.712, p = 0.087	704.5 (753.5)	776.5 (798.8)	z = -0.560, p = 0.575	1110 (964)	1797 (1269.5)	z = -1.753, p = 0.080
Agonistic	0 (34.5)	8 (28.5)	z = -0.533, p = 0.594	9.5 (43.3)	7 (20.8)	z = -0.280, p = 0.779	0 (21.5)	8 (54.5)	z = -1.604, p = 0.110
Interspecific	0 (69)	0 (0)	z = -2.023, p = 0.043*	0 (59.3)	0 (0)	z = -1.342, p = 0.180	38 (94)	0 (0)	z = -1.604, p = 0.110
Not also much	05((001 5)	207 (552 5)	<i>z</i> = -2.481,	704 5 (94(5)	172 ((22)	z = -1.540,	1117 (740)	202 ((15)	z = -2.023,
<u>Not observed</u>	<u>950 (881.5)</u>	<u>207 (553.5)</u>	$p = 0.013^*$	<u>/24.3 (846.5)</u>	1/2 (633)	p = 0.124	<u>111/(/40)</u>	<u>303 (613)</u>	p = 0.043*

*Significant difference between the two periods (p < 0.05).

Table 3: Individual <u>behaviors</u>, and social behaviors and "not observed" in the baseline and in the gum-feeder period. For each behavioral category, the table reports the median (interquartile range) duration in seconds calculated in the baseline and in the gum-feeder period (GF), as well as the *z* and *p* values of the Wilcoxon test performed to compare the two periods. All: data collected on different species pulled together; Tamarins: analysis performed within the tamarin group (N = 8) (*Saguinus oedipus, Saguinus midas*); Marmosets: analysis performed within the marmoset group (N = 5) (*Callithrix geoffroyi, Cebuella pigmeapygmaea*).

For per Perieu