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4 1 **Positive reinforcement training: a tool for care and management of captive vervet**
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6 2 **monkeys (*Chlorocebus aethiops*)**
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26 12 **Running title:** Reinforcement training in vervet monkeys
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4 17 **Abstract**
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6 18 In modern zoos, training should be an integral component of the animal care and management.
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8 19 The benefits of training include the opportunity for positive interactions with caretakers. This
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10 20 study was carried out with a group of vervet monkeys (*Chlorocebus aethiops*) housed at the
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12 21 Garda Zoological Park. Using focal animal sampling, we observed the behaviour performed by
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14 22 all group members from December 2007 to August 2008. The group took part in a training
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16 23 programme to be isolated in a familiar area before the subjects were included in a cognitive
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18 24 study. We collected behavioural data during a pre-training period to assess the social behaviour
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20 25 of the colony and during the training period to investigate the effects of the training programme
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22 26 on the behaviour of individuals. Additionally, a second phase of the study was conducted and
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24 27 training sessions with individual monkeys were video-recorded to determine the behaviour of
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26 28 animals during each training session and thus to confirm that they were suitable for participating
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28 29 in the procedure. Our results suggest that the training programme enriched the daily routine of
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30 30 these captive primates by increasing affiliative behaviours while decreasing agonistic
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32 31 behaviours. Furthermore, there was behavioural response variability among the individuals
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34 32 under training procedure. However all the individuals were trained to calmly enter in a familiar
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36 33 area and to be isolated from other members of the group. In conclusion, our findings highlight
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38 34 the importance of using the positive reinforcement training to reduce the tension directly
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40 35 associated with potentially stressful procedures by allowing primates to voluntarily participate
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42 36 in these procedures. In addition, the training was found to be an enrichment tool for vervet
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44 37 monkeys.
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48 39 **Keywords:** animal welfare; captivity; enrichment tool; husbandry refinement; operational
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50 40 conditioning; stress
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44 Introduction

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46 In the early 1900s, Skinner suggested that the best way to understand animal behaviour is to
47 look at the causes of an action and its consequences. This approach is called “operant
48 conditioning” (Skinner 1981), and it entails the changing of behaviour by use of reinforcement,
49 which is provided after the desired response. The Skinner theory was based on the “Law of
50 Effect” (Thorndike 1911) with the addition of a new term, “reinforcement,” emphasizing that
51 behaviour that is reinforced tends to be repeated (i.e., strengthened).

52 Operant conditioning techniques can be applied successfully to improve the behavioural
53 management of nonhuman primates in research settings (Owen & Amory 2011). It is essential
54 that zoo curators review the literature to assess objectively whether specific positive
55 reinforcement training methods may enhance captive management and research procedures with
56 the animals (Schapiro et al 2003).

57 Operant conditioning with positive reinforcement has been shown to be the optimal tool for
58 training captive primates to calmly enter an experimental/training area while remaining isolated
59 from the rest of the group, thus achieving the voluntary cooperation of individuals in cognitive
60 research (Desmond & Laule 2005; Prescott & Buchanan-Smith 2003). These types of training
61 programmes are used because individual primates appear to be more relaxed when they are in
62 groups rather than isolated (Prescott & Buchanan-Smith 2003). Separating animals from their
63 groups can be stressful, both for the individuals removed from the group and for those who
64 remain behind. However, carrying out training sessions with primates within their social context
65 may limit their performance (Shapiro et al 2003).

66 Various aspects of captive environments can increase stress levels and jeopardize the well-being
67 of captive animals. The use of positive reinforcement training techniques enables researchers
68 and caretakers to reduce the tension associated with potentially stressful procedures and
69 situations (Carlstead 2009). The role of training in the management of captive populations has
70 changed significantly over time, and it has evolved into a series of techniques that allow for

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4 71 medical treatment and behavioural research and improve animal welfare (Laule 1993). As a
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6 72 method of training captive animals, operant conditioning with positive reinforcement is a
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8 73 practice that is increasingly recognized by zoos as a valuable addition to standard husbandry
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10 74 and behavioural management methods (Crowell-Davis 2008; Fuller et al 2012; Laule 2003).
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12 75 Animal training is effective not least because the animals themselves contribute to an
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14 76 improvement of their own handling free of stress (Colahan & Breder 2003).

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16 77 Positive reinforcement training improves care and reduces stress by enlisting a primate's
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18 78 voluntary cooperation with targeted activities, including husbandry and cognitive research
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20 79 activities (Laule & Whittaker 2007; Pomerantz & Terkel 2009). Although training should not be
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22 80 the only form of enrichment, it can be an integral part of any enrichment programme (Mellen &
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24 81 Mac Phee 2001). Recent studies (Mattison 2012; Owen & Amory 2011) indicated that the use
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26 82 of positive reinforcement training considerably reduced the potential for stress and improved
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28 83 welfare during the capture and containment of New World monkeys.

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30 84 Husbandry training is widespread in zoos and often considered helpful as environmental
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32 85 enrichment technique (Melfi 2013). However, although several studies assessed the effect of
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34 86 environmental enrichment programmes on animal behaviours, few studies empirically evaluated
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36 87 the impact of training on animal welfare - especially outside the training sessions (reviewed in
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38 88 Melfi 2013).

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40 89 The purpose of the present study was to evaluate whether a training programme for vervet
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42 90 monkeys (*Chlorocebus aethiops*) could be used to induce them to cooperate in behavioural
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44 91 management (i.e., to elicit voluntary participation in routine husbandry, animal transport, and
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46 92 health-care procedures). Specifically, the study aimed to detect if the monkeys could be trained
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48 93 to be isolated in a familiar area. In addition, we tried to assess whether this training programme
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50 94 is a multifunctional tool that can be used to create a variety of enrichment opportunities for
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52 95 captive animals.

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56 57 97 **Materials and Methods**

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6 99 A group of ten (four males and six females) vervet monkeys (*Chlorocebus aethiops*) housed at
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8 100 Garda Zoological Park (Italy) was involved in the training programme (**Table 1**). The vervet
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10 101 monkeys were trained to be isolated in the training area, while the other members of the colony
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12 102 remained in the indoor enclosure without visual or olfactory contact with the isolated
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14 103 individuals. A positive reinforcement technique (Schapiro et al 2003), using guillotine doors,
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16 104 was employed to train the subjects to calmly enter the experimental/training area in order to
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18 105 achieve their voluntary cooperation in a problem-solving study.
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21
22 107 -----Table 1-----
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26 109 The training area consisted of a 10-m² tunnel linking the 29-m² indoor with the 419-m² outdoor
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28 110 enclosure, whereas the training apparatus was an open rectangular wooden box hanging in the
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30 111 tunnel.

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32 112 Before the training session, the individuals were habituated to stay in the tunnel by providing
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34 113 them with food in the apparatus, then the individuals were separated by the group by closing the
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36 114 guillotine doors dividing the tunnel from indoor and outdoor enclosures. During the training
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38 115 session, if entered successfully the tunnel each subject could take the reward from the apparatus.

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40 116 This reward was a cube (1.5x1.5 cm) of jelly for primates consisting of a mixture of vegetables,
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42 117 fruits and nuts (“Delicacy Gelée” supplied by Viten®, Udine, Italy). ~~The training sessions lasted~~
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44 118 ~~differently (but never more than three minutes each), on the basis of the emotional state of each~~
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46 119 ~~individual. The length of each training session varied (but never exceeded 3 minutes) depending~~
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48 120 ~~on the emotional state of each individual.~~

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50 121 In the first phase of the study we used focal animal continuous sampling to assess the behaviour
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52 122 of the subjects within their social context (Altmann 1974). Each animal was observed during
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54 123 15-minute sessions in three different periods (each period made by ten sessions per subject for a
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56 124 total of 100 sessions) for a total of 75 hours: the ‘baseline’ before the training period, the ‘first
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4 125 period' once training had begun, and the 'second period' during the training. In the so-called
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6 126 'first period' and 'second period' we observed the animals during 15-minute focal animal
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8 127 sessions immediately after each training session when they were all housed together in the
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10 128 outdoor enclosure; this was to investigate the effects of the training programme on their group
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12 129 behaviour. Each study period lasted two weeks; within each period the training sessions were
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14 130 always conducted at the same time of day. All individuals were tested in each session and
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16 131 trained spontaneously in a random order.

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18 132 We collected data for social and individual behaviours. A comprehensive ethogram (Adeyemo
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20 133 1997; Cheney & Seyfarth 1990; Fedigan 1972; Fedigan & Fedigan 1988) was adapted to cover
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22 134 a range of social and individual behaviours (**Table 2**). Social behaviours were further grouped
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24 135 according to agonistic and affiliative behaviours. Agonistic behaviours included dominant and
25
26 136 submissive behaviours. Affiliative behaviours include measures of grooming, body contact,
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28 137 social play, sexual behaviour, social resting and all "other affiliative" behaviours. A category
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30 138 designed to quantify time spent engaged in individual behaviours included self-grooming,
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32 139 exploration, alert, locomotion, individual play, foraging, maintenance and resting.

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36 141 -----Table 2-----
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41 143 | The second phase of the study, consisting of additional 14 training sessions per individual,
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43 144 started a week after the first phase and was conducted exactly as the first one (i.e., the ten
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45 145 individuals were separated from their group and had to take a reward). This phase aimed at
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47 146 completing the isolation training process and fully prepare the animals for cognitive studies.
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49 147 These last sessions were video-recorded to monitor the behaviour of each individual during the
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51 148 | isolation training session. We ~~collect~~collected data about the displacement (set of anxiety-
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53 149 related behaviours including actions directed toward themselves; Maestriperi 1991) of the
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55 150 subjects and whether or not they took the reward.

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4 151 The first phase of the study, the previous three study periods, focussed on training effects on the
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6 152 whole colony (i.e., positive or negative effects as a result of the isolation training), whilst in the
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8 153 second phase the psychological well-being of the subjects during isolation and the feasibility of
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10 154 starting the problem solving trials (i.e., cognitive studies on individual and social learning which
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12 155 we plan to conduct) were assessed.

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14 156 Data analysis of the whole study was based on duration of behaviours. We used nonparametric
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16 157 statistical tests (Siegel & Castellan 1992). In particular, we used the Friedman test with a series
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18 158 of post-hoc Wilcoxon tests with Bonferroni correction on each combination of periods (to
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20 159 compare the three different periods - the 'baseline' before the training period, the 'first period'
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22 160 once training had begun, and the 'second period' during the training). In addition, in the second
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24 161 phase of the study we used the Wilcoxon test to compare behaviours between the first and the
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26 162 last sessions. All tests were two-sided, and the significance level was set to $p < 0.05$. Analyses
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28 163 were performed with StatView for Windows and Macintosh (version 5.0).

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31 165 **Results**

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36 167 Observing the group behaviour, during the first phase of the study, locomotion was displayed
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38 168 significantly more during the “first period” than the “baseline” and the “second period”
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40 169 (Friedman test: $\chi^2 = 9.80$; $p = 0.0074$); in particular, locomotion was significantly less displayed
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42 170 in the 'second period' compared to the 'first period' (Wilcoxon test with Bonferroni correction:
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44 171 $z = -2.80$; $p = 0.0051$) (**Figure 1**) along with the progress of the training programme.

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49 173 -----Figure 1-----

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53 175 In order to investigate whether the training programme could be considered an enrichment tool
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55 176 for captive animals, we focused on social behaviour: agonistic and affiliative behaviours. In
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4 177 particular, dominant behaviours were carried out most frequently during the baseline whilst
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6 178 gone significantly down during the “first period” and “second period” (Friedman test: $\chi^2 =$
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8 179 7.09; $p = 0.0289$) (**Figure 2**). On the contrary, social resting was shown more during the “first”
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10 180 and “second” period than during the baseline (Friedman test: $\chi^2 = 6.73$; $p = 0.0346$) (**Figure 3**).
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12 181 In addition, we found no significant ~~variation~~different in other affiliative behaviours (such as
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14 182 grooming, body contact, social play and sexual behaviours) (Friedman tests: N.S.) when
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16 183 comparing the different study periods.
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185 -----Figure 2-----

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187 -----Figure 3-----

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189 Furthermore, in order to assess whether a training programme for vervet monkeys could be used
190 to induce them to cooperate in behavioural management and cognitive research, the fourteen
191 video-recorded sessions of the second phase of the study were analysed. Comparing the first
192 two sessions with the last two sessions of the 14 sessions, vervet monkeys showed significantly
193 less displacement (running back and forth) during the final training sessions compared to the
194 first sessions (Wilcoxon test: $z = -2.03$; $p = 0.0425$) (**Figures 4.a and 4.b**).

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196 -----Figures 4.a & 4.b-----

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198 Moreover, over the last two sessions of the training programme, all the monkeys took their own
199 reward whereas in the first two sessions of the training programme only 40% of subjects ~~were~~
200 ~~calm enough to take~~took their own reward (Wilcoxon test: $W = 0$; $p < 0.05$) (**Figure 5**).
201 However, the number of training sessions needed to achieve the final stage (i.e., being calm and
202 taking the reward) varied depending on each individual.

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6 204 -----Figure 5-----7
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10 206 **Discussion and Conclusion**

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14 208 Results of the first phase of the study, focussing on training effects on the whole colony,
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16 209 revealed a significant decrease in locomotion across the training sessions suggesting an
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18 210 improvement in the well-being of the vervet monkey, as increased locomotion have been
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20 211 reported as non-invasive indicator of stress in other captive primate species (Box & Rohrhuber
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22 212 1993; Chamove et al 1988; Hosey & Druck 1987; Mitchell et al 1992; Schmidt 2010;
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24 213 Schoenfeld 1989). However, we were unable to address significant changes between the
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26 214 “second period” and the “baseline”. As a consequence, our results for locomotion should be
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28 215 regarded as preliminary and more trials would be necessary to state that there is definitely an
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30 216 improvement of well-being. Since other behaviours (such as social behaviours, and particularly
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32 217 dominant behaviours and proximity between individuals) are considered to be indicators of
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34 218 animal welfare (Melfi & Thomas 2012), the decrease of dominant behaviours together with the
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36 219 increase of social resting highlighted that the training programme reduced aggressiveness and
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38 220 improved socialization - appearing to be an important part of environmental enrichment
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40 221 programmes that improve the daily routine of captive animals, as described previously by other
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42 222 authors (Laule et al 2003; Laule & Desmond 1998; Laule et al 2003). Since no significant
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44 223 differences in other affiliative behaviours were found when comparing the baseline and the
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46 224 “second period”, no negative impact on welfare as a result of the training procedure was
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48 225 reported (Whitehouse et al 2013). Results of this study suggest that the training programme
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50 226 seems to help vervet monkeys to be isolated in a familiar area and voluntarily participate in the
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52 227 procedure, as described previously for other primate species (Fuller et al 2012; Owen & Amory
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54 228 2011; Prescott & Buchanan-Smith 2003). This can be useful for health purposes (i.e., to allow
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56 229 and facilitate the monitoring of vervet monkeys, by capturing and keeping without providing
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4 230 stress to these animals). Thus, our results provide support for previously published findings
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6 231 (Carlstead 2009; Laule 2003; Owen & Amory 2011; Pomerantz & Terkel 2009) that positive
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8 232 reinforcement training contributes to the behavioural management and well-being of captive
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10 233 nonhuman primates.

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12 234 Observations from the first video-recorded training sessions underlined that the individuals in
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14 235 the study group showed undesirable behaviours, such as displacement (Barros et al 2004;
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16 236 Bassett et al 2003; Kessel and Brent 2001), and did not take the reward most likely because they
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18 237 were not calm enough.

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20 238 These findings confirmed that the isolation of a vervet monkey from his group could be
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22 239 stressful, especially for the individual removed from the group (Shapiro et al 2003) - this was
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24 240 probably due to social features of vervet monkeys (Cheney & Seyfarth 1990). However, at the
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26 241 end of the training procedure ~~the vervet monkeys were~~each individual was calm enough to
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28 242 remain isolated in a familiar area while eating a reward.

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30 243 In conclusion, this study empirically evaluates the impact of training zoo animals within and
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32 244 outside of the training session, to fill a gap in the literature (Melfi 2013). Our results highlight
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34 245 that establishing a training programme might be a valuable tool that can be used to accustom
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36 246 captive vervets to isolation in a familiar area through positive reinforcement. In addition, the
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38 247 positive reinforcement training could also be used as valuable tool for an enrichment
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40 248 programme addressing elements of well-being for captive primates. Future research work
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42 249 should examine the effect of husbandry training techniques on abnormal behaviours, activity
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44 250 budget and proximity between individuals. In addition, we focused on the behavioural approach
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46 251 but we neglected the endocrine component. However, the ability to collect and analyse both
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48 252 physiological and behavioural data is crucial for evaluating the stress responses and welfare of
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50 253 animals in captivity (e.g., Peel et al 2005); in particular, one useful indicator of stress is the
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52 254 measurement of cortisol levels, whereas little is known about how testosterone and progesterone
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54 255 vary in stressful situations - such as cases of isolation (Fontani et al 2014). Actually, to assess
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56 256 the impact of stress comprehensively, multiple components of the stress response (i.e.,
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4 257 behavioural, hormonal, and immunological factors) should be monitored, and the links among
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6 258 these components should be considered as well (Peel et al 2005).
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10 260 *Animal welfare implications*
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14 262 Results show reduction in aggressive behaviours and increase of positive social interactions,
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16 263 suggesting that the training can also be used as husbandry refinement. Indeed, the positive
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18 264 reinforcement training provides animals with wider choice and control over their lives.
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20 265 Furthermore, to train animals in order to voluntarily enter into a familiar area and be isolated
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22 266 from other group members might support these animals to voluntarily cooperate in husbandry
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24 267 and veterinary procedures. Therefore, it might contribute to decrease both the use of anesthesia
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26 268 and the stress for trained animals and the entire group as well (Laule et al 1992; Luttrell et al
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28 269 1994, Veeder et al 2009).
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Table 1 - Group size (N=10) and composition (sex and dominance rank) housed at Parco Natura Viva-Garda Zoological Park at the beginning of the study (June 2008).

Name	Sex	Age (years)
Alf *	Male	15
Fauna	Female	12
Orni	Female	12
Fiamma	Female	10
Ghisma	Female	9
Freccia	Female	7
Nicola **	Male	3
Florio	Male	2
Chicca	Female	2
Pansa	Male	1

* alpha

** beta

Table 2 - Ethogram, based on previous comprehensive ethogram (see Adeyemo 1997), modified to cover a range of social and individual behaviours.

Behavioural class	Behavioural subclass	Behavioural category	Description	
Social behaviours	Agonistic behaviours	Dominance with conflict	Aggression toward an individual (hit, fight, bite, etc.)	
		Dominance without conflict	Hugging an individual's back without copula, others actions that express dominance toward an individual, different from the aggression	
		Dominance without submission by the receiver	The individual receiving threats or aggression does not display submissive behaviour	
		Redirected aggression	An individual who received aggression from a second individual is aggressive toward a third individual not involved in the conflict	
	Submissive behaviours	Submission with conflict	An individual shows submissive behaviour (crouching, sexual presentations, showing the back, fear expressions, escaping from an individual) after a physical aggression	
		Submission without conflict	An individual shows submissive behaviour (crouching, sexual presentations, showing the back, fear expressions, escaping from an individual) but no physical aggression was present	
	Affiliative behaviours	All other affiliative behaviours		All other affiliative behaviours not included in the ethogram
		Body contact		Being in contact with other individuals with attention to the surrounding environment
		Grooming		Cleaning another individual's fur with hands or mouth
		Sexual behaviours		Receiving or doing ano-genital inspection
Social play		Non-agonistic interaction: fight-play, somersaults, chase		
Social Resting		Resting in contact with other individuals		

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11	Individual behaviours	Alert	Looking around carefully to detect potentially dangerous situations
12		Exploration	Investigating and examining different areas of the enclosure and environmental enrichments
13		Foraging	Search and ingestion of food available in the enclosure
14		Individual play	Playing with objects or interacting with the environment
15		Locomotion	Walking, running, climbing
16		Maintenance	Eating, drinking, urinating, defecating
17		Resting	Resting alone
18		Self-grooming	Cleaning one's self fur with hands and mouth, sexual self-inspection, scratching
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3 **Figure 1 - Locomotion (N=10).** Comparison of the locomotion across the three study periods
4 (baseline, first period, second period); error bars stand for the standard deviation; locomotion, first
5 period vs. second period (Wilcoxon test with Bonferroni correction: $z = -2.803$; $p = 0.0051$).
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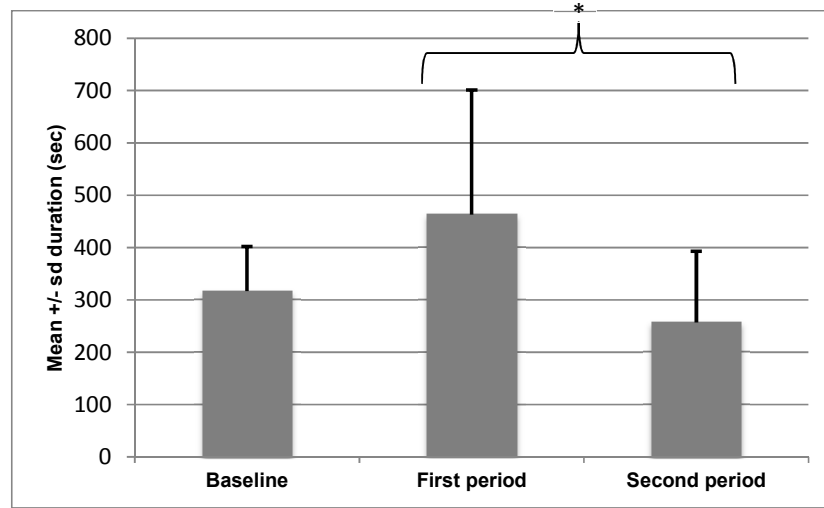


Figure 2 - Dominance and submission (N=10). Comparison of dominant and submissive behaviors across the three study periods (baseline, first period, second period); error bars stand for the standard deviation; dominant behaviors, baseline vs. second period (Friedman test: $\chi^2 = 7.091$; $p = 0.0289$).

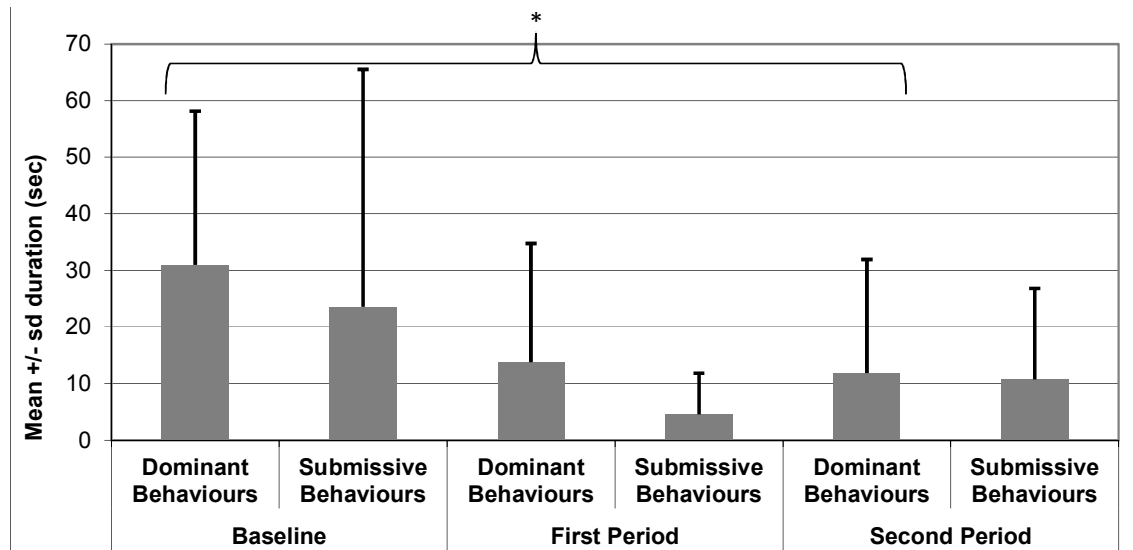
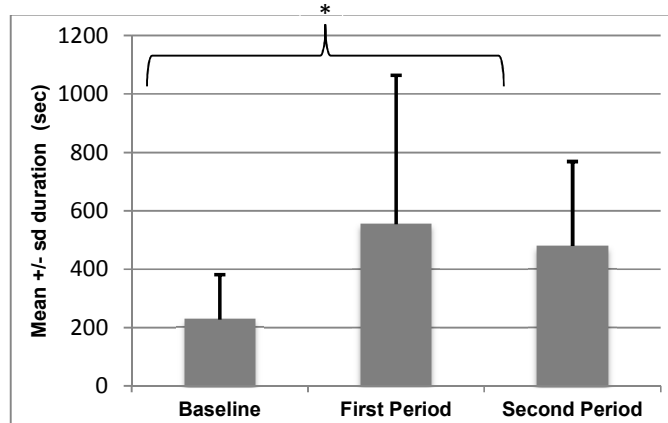


Figure 3 - Social resting (N=10). Comparison of social resting across the three study periods (baseline, first period, second period); error bars stand for the standard deviation; social resting, baseline vs. first and second period (Friedman test: $\chi^2 = 6.727$; $p = 0.0346$).



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3 **Figure 4.a - Displacement (N=10).** Comparison of the displacement (running back and forth),
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5 between final training sessions and first sessions, error bars stand for the standard deviation; running
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7 back and forth, first sessions vs. last sessions (Wilcoxon test: $z = -2.028$; $p = 0.0425$).
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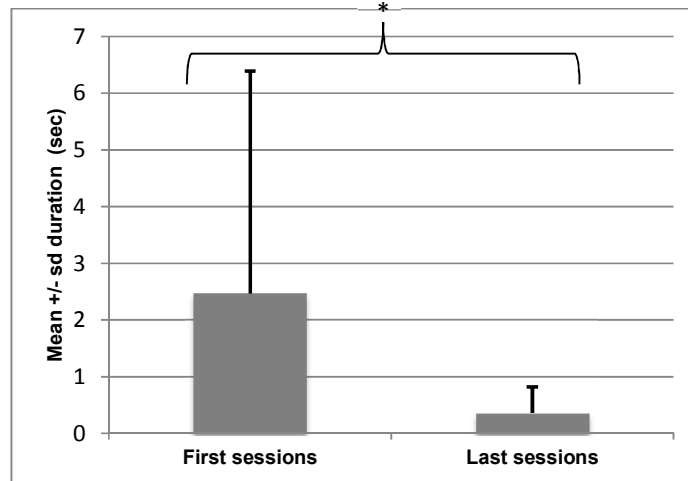
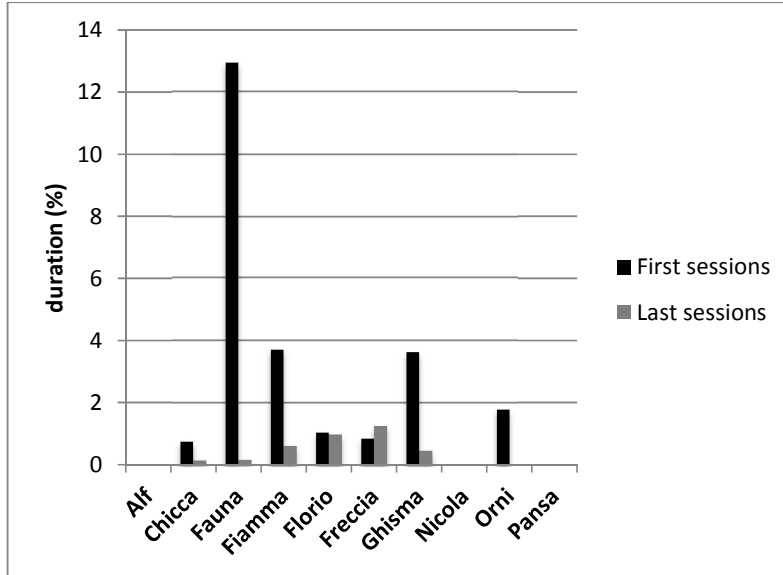


Figure 4.b - Displacement by individuals. Comparison of the displacement (running back and forth), between final training sessions and first sessions, by single individuals.



View Only

Figure 5 - Collection of rewards. Comparison between the first and last two sessions of the training programme, in terms of individuals calmly taking their own reward (Wilcoxon test: $W = 0$; $p < 0.05$).

