

**Appendix 1.** Queries for all publications for which oxytocin was mentioned in the title and/or abstract. All queries were repeated for each of the 5-year time periods. Below, the time period ranged from 1956 to 1960. Queries were identical for all 5-year blocks with only a substitution for the specific range of years to extract the appropriate information for each time period. For example, we substituted 1961 (for 1956) and 1965 (for 1960) to advance to the next for next time period (+5 years). This was repeated until we extracted information for the final time period (2011-2016) included here.

((oxytocin[Title/Abstract]) AND ("1956"[Date - Publication] : "1960"[Date - Publication]))

**Appendix 2.** Queries for non-human and humans by treatment type. All queries were repeated for each of the 5-year time periods. Below, the time period ranged from 1956 to 1960. Queries were identical for all 5-year blocks with only a substitution for the specific range of years to extract the appropriate information for each time period. For example, we substituted 1961 (for 1956) and 1965 (for 1960) to advance to the next for next time period (+5 years). This was repeated until we extracted information for the final time period (2011-2016) included here.

**A) Nonhuman: central**

(((((intranasal[Title/Abstract]) OR central[Title/Abstract]) AND oxytocin[Title/Abstract]) AND ("1956"[Date - Publication] : "1960"[Date - Publication])) NOT (man[Title/Abstract] OR men[Title/Abstract] OR woman[Title/Abstract] OR women[Title/Abstract] OR child[Title/Abstract] OR children[Title/Abstract] OR human[Title/Abstract] OR humans[Title/Abstract] OR obstetrics[Title/Abstract] OR gynecology[Title/Abstract] OR ob/gyn[Title/Abstract]))

**B) Nonhuman: peripheral**

(((((peripheral[Title/Abstract]) OR intraperitoneal injection[Title/Abstract]) AND oxytocin[Title/Abstract]) AND ("1956"[Date - Publication] : "1960"[Date - Publication])) NOT (man[Title/Abstract] OR men[Title/Abstract] OR woman[Title/Abstract] OR women[Title/Abstract] OR child[Title/Abstract] OR children[Title/Abstract] OR human[Title/Abstract] OR humans[Title/Abstract] OR obstetrics[Title/Abstract] OR gynecology[Title/Abstract] OR ob/gyn[Title/Abstract]))

**C) Human: central**

(((((intranasal[Title/Abstract]) OR central[Title/Abstract]) AND oxytocin[Title/Abstract]) AND ("1956"[Date - Publication] : "1960"[Date - Publication])) AND (women[Title/Abstract] OR men[Title/Abstract] OR children[Title/Abstract] OR child[Title/Abstract] OR human[Title/Abstract] OR humans[Title/Abstract] OR obstetrics[Title/Abstract])) NOT (primate[Title/Abstract] OR primates[Title/Abstract] OR animal[Title/Abstract] OR animals[Title/Abstract] OR rat[Title/Abstract] OR rats[Title/Abstract] OR mouse[Title/Abstract] OR mice[Title/Abstract] OR rodent[Title/Abstract] OR rodents[Title/Abstract]))

**D) Human: peripheral**

(((((peripheral[Title/Abstract]) OR intraperitoneal injection[Title/Abstract]) AND oxytocin[Title/Abstract]) AND ("1956"[Date - Publication] : "1960"[Date - Publication])) AND (women[Title/Abstract] OR men[Title/Abstract] OR children[Title/Abstract] OR child[Title/Abstract] OR human[Title/Abstract] OR humans[Title/Abstract] OR obstetrics[Title/Abstract])) NOT (primate[Title/Abstract] OR primates[Title/Abstract] OR animal[Title/Abstract] OR animals[Title/Abstract] OR rat[Title/Abstract] OR rats[Title/Abstract] OR mouse[Title/Abstract] OR mice[Title/Abstract] OR rodent[Title/Abstract] OR rodents[Title/Abstract]))

**Appendix 3.** Queries based on behavioral type and context. All queries were repeated for each of the 5-year time periods. Below, the time period ranged from 1956 to 1960. Additional queries were identical for all 5-year blocks with only a substitution for the specific range of years to extract the appropriate information for each time period. For example, we substituted 1961 (for 1956) and 1965 (for 1960) to advance to the next for next time period (+5 years). This was repeated until we extracted information for the final time period (2011-2016) included here.

**A) Prosocial behaviors within a social context**

((oxytocin[Title/Abstract] AND ("1956"[Date - Publication] : "1960"[Date - Publication])) AND (friendship[Title/Abstract] OR friend[Title/Abstract] OR maternal[Title/Abstract] OR paternal[Title/Abstract] OR nursing[Title/Abstract] OR lactation[Title/Abstract] OR birth[Title/Abstract] OR mother[Title/Abstract] OR father[Title/Abstract] OR social bond[Title/Abstract])) AND (eye gaze[Title/Abstract] OR grooming[Title/Abstract] OR groom[Title/Abstract] OR cuddle[Title/Abstract] OR cuddling[Title/Abstract] OR huddle[Title/Abstract] OR huddling[Title/Abstract] OR cooperation[Title/Abstract] OR cooperating[Title/Abstract] OR affiliative[Title/Abstract] OR sociopositive[Title/Abstract])

**B) Antisocial behaviors within a social context**

((oxytocin[Title/Abstract] AND ("1956"[Date - Publication] : "1960"[Date - Publication])) AND (friendship[Title/Abstract] OR friend[Title/Abstract] OR maternal[Title/Abstract] OR paternal[Title/Abstract] OR nursing[Title/Abstract] OR lactation[Title/Abstract] OR birth[Title/Abstract] OR mother[Title/Abstract] OR father[Title/Abstract] OR social bond[Title/Abstract])) AND (defensive aggression[Title/Abstract] OR conflict[Title/Abstract] OR fight[Title/Abstract] OR territory[Title/Abstract] OR defense[Title/Abstract] OR defence[Title/Abstract] OR aggression[Title/Abstract] OR protection[Title/Abstract] OR outgroup[Title/Abstract] OR antisocial[Title/Abstract] OR competition[Title/Abstract] OR competitor)

**C) Prosocial behaviors within a mating context**

((oxytocin[Title/Abstract] AND ("1956"[Date - Publication] : "1960"[Date - Publication])) AND (mating behavior[Title/Abstract] OR mate choice[Title/Abstract] OR estrous[Title/Abstract] OR dating[Title/Abstract] OR marriage[Title/Abstract] OR courtship[Title/Abstract] OR monogamy[Title/Abstract] OR orgasm[Title/Abstract])) AND (eye gaze[Title/Abstract] OR grooming[Title/Abstract] OR groom[Title/Abstract] OR cuddle[Title/Abstract] OR cuddling[Title/Abstract] OR huddle[Title/Abstract] OR huddling[Title/Abstract] OR cooperation[Title/Abstract] OR cooperating[Title/Abstract] OR affiliative[Title/Abstract] OR sociopositive[Title/Abstract])

**D) Antisocial behaviors within a mating context**

((oxytocin[Title/Abstract] AND ("1956"[Date - Publication] : "1960"[Date - Publication])) AND (mating behavior[Title/Abstract] OR mate choice[Title/Abstract] OR estrous[Title/Abstract] OR dating[Title/Abstract] OR marriage[Title/Abstract] OR courtship[Title/Abstract] OR monogamy[Title/Abstract] OR orgasm[Title/Abstract])) AND (defensive aggression[Title/Abstract] OR conflict[Title/Abstract] OR fight[Title/Abstract] OR territory[Title/Abstract] OR defense[Title/Abstract] OR defence[Title/Abstract] OR aggression[Title/Abstract] OR protection[Title/Abstract] OR outgroup[Title/Abstract] OR antisocial[Title/Abstract] OR competition[Title/Abstract] OR competitor)

#### **Appendix 4.** Pilot data from field experiments manipulating oxytocin in yellow-bellied marmots

##### *Study subjects and testing arena*

Marmots regularly engage in affiliative behaviors, including greetings, play, allogrooming and maintenance of spatial associations (e.g., Blumstein et al. 2013; Smith et al. 2013). The degree to which one-year-old individuals (hereafter yearlings) engage in social interactions is an important determinant of female dispersal (Van Vuren and Armitage 1994; Blumstein et al. 2009; Armitage et al. 2011). Because the likelihood of female dispersal is strongly correlated with an individual's cohesion within its social group (Blumstein et al. 2009), understanding the proximate mechanisms mediating female decisions is of particular interest. Because the sex-specific effects of oxytocin are often strongest for females (e.g., Cho et al. 1999), we predicted that oxytocin-treated yearlings would exhibit increased sociability with the strongest effect on females.

The pilot study was conducted in and around Gothic, Colorado, USA (38°57'N, 106°59'W) at the Rocky Mountain Biological Laboratory. Individuals are regularly live-trapped, transferred to a cloth handling bag, weighed, sexed, and ear tagged for identification as part of a long-term study (Armitage 2010). This research was part of a larger experimental study on 183 individual marmots of any age whose behavioral responses to mirror image simulation (MIS) were recorded for at least one trial (for details, see Petelle and Blumstein 2014; Petelle et al. 2015). For each trial, an individual was gently shaken from the handling bag into a 92 x 92 x 92 cm testing arena made of 0.6 cm opaque PVC sheeting, with a wire mesh top. A mirror (30.5 x 61.0 cm) was secured at the base of one side of the arena and covered with an opaque sliding door. Sixteen (22.9 cm<sup>2</sup>) squares were drawn on the bottom of the arena in a grid. After a three-minute habituation period the sliding door was pulled back to reveal the mirror. The MIS lasted three minutes before the marmot was gently coaxed back into a live-trap and released at its original trap location. All trials were video recorded and behaviors were scored using JWatcher (Blumstein and Daniel 2007) using a full ethogram (Petelle and Blumstein 2014).

##### *Measure of sociability*

Sociopositive behaviors included close spatial proximity to the mirror side of the testing arena and increased tendency to interact with the mirror by pawing at the mirror or by rubbing their nose with and sitting in direct or close body contact with the mirror. A subject was scored as being in front of the mirror any time its nose was present in the two boxes on grid that were

directly in front of the mirror on the box's wall and considered to be in the half of the box with the mirror when the subject's nose was present above any of the eight squares on the half of the box with the mirror. To reduce the number of correlated traits, we used a Principal Component Analysis (PCA, Jolliffe 2002) with Varimax rotation. The first two components of the PCA accounted for 49.2 % of the variance during MIS tests. The second component, named a sociability factor was characterized by the proportion of time spent at the mirror, on the mirror half of the arena, and scratching or pawing at the mirror as well as the frequency of scratching or pawing the mirror. Because the best estimate for an individual's PCA score is generated from a large sample (Jolliffe 2002), the full distribution of 183 scores represented a larger distribution of responses to the experimental testing arena. This second factor score was used to evaluate responses for the 18 subjects who received oxytocin or saline on the third trial (see below).

#### *Oxytocin and saline manipulations*

Oxytocin manipulations focused on yearling marmots because the most socially-connected yearlings are least likely to disperse, and thus variation in sociability has demographic consequences for yearlings (Blumstein et al. 2009). Oxytocin/saline manipulations for a given yearling subject therefore included: a) habituation (trial 1), b) baseline (trial 2), and c) response (trial 3) phases. Each of the three phases involved a unique live-capture event of the same individual yearling on different days to estimate changes in the sociability factor. We administered oxytocin or a saline control intranasally over two summers (12 subjects in 2011; 6 subjects in 2012) on trial 3. A massive, natural, over-winter mortality event from 2010-2011 prevented us from studying additional yearlings (D.T. Blumstein unpublished data). Thus, of the 183 individuals tested, only of these 18 subjects were yearlings captured on the third trial and, thus, manipulated to assess the effects of oxytocin/saline on changes in sociability.

For the subset of individuals ( $N = 18$ ) subjected to the oxytocin or saline manipulation, we specifically expected oxytocin-treated marmots to show an increase in sociability from baseline. By subtracting sociability factor scores on the treatment days from those at baseline, we employed a repeated measures design on focal individuals; we report the change in sociability between the baseline measure (phase 2) and the treatment trial (phase 3) for the 18 yearlings, calculated as the PCA score for baseline trial minus the PCA score for treatment trial. Each individual marmot received only one treatment (either oxytocin or saline on the third trial). We used this repeated measures design because previous work documents consistent inter-individual

differences in endocrine (Smith et al. 2012; Blumstein et al. 2016) and behavioral (Petelle et al. 2015) responses in this species. We also investigated the changes in each individual behavior, but all approaches yielded similar results. For brevity, we only report on changes in PCA scores.

For oxytocin experiments, following standard weighing and handling methods, we transferred each individual yearling to a nearby holding area in the shade for a total of five minutes prior to testing at each of the three phases. All three trials were therefore identical in all respects with the exception that on the third capture (i.e., response phase), prior the start of the holding period, each individual was randomly assigned an oxytocin or saline treatment and, thus, injected intranasally with oxytocin or saline prior to the holding period.

Following Smith et al. (2010), we dissolved oxytocin synthesized and provided by Dr. Maurice Manning (Medical College of Ohio, University of Toledo) from its pure powdered form into sterile saline. We produced a solution with concentration of 50  $\mu\text{g}$  (~23 IU) of oxytocin (Fentocin Virbac) per 100  $\mu\text{l}$  of saline; each 1 ml contained synthetic 10 IU oxytocin and chlorobutanol (0.5% m/v) and was stored at -20 degrees C for up to 4 weeks prior to use.

Small aliquots were kept on ice in a cooler at the trapping station for no more than 2 h prior to use on the day of experimental testing. While still in the handling bag, oxytocin subjects received a mass-specific dose at the time of testing (150  $\mu\text{g}$  oxytocin per kg of subject); doses were consistent with those used previously (e.g., Parker et al. 2005; Smith et al. 2010). For example, a yearling of an average mass of  $1.8 \pm 0.1$  kg received 540  $\mu\text{l}$  of the pre-mixed solution, delivering a dose of 279  $\mu\text{g}$  of oxytocin. Control subjects received equivalent, mass-specific volumes of saline solution. On average, subjects in oxytocin ( $N = 10$ ) and control ( $N = 8$ ) groups received  $611 \pm 42$   $\mu\text{l}$  and  $612 \pm 56$   $\mu\text{l}$ , respectively; volumes were statistically equivalent between groups (Mann-Whitney U-test:  $U = 42$ ,  $P = 0.894$ ). We administered each dose in a sterile syringe by sequentially squirting half of the total volume into each nostril.

Although the exact time lag between intranasal administration of oxytocin in marmots remains unknown, neuropeptides resembling oxytocin move quickly cross the blood–brain barrier when administered intranasally, promoting a significant rise in perineural fluid within one to ten minutes and the acute effects of intranasal oxytocin are rapidly detected in humans within as little as 10 to 15 min (Hohman et al. 1985; Born et al. 2002; Vaka et al. 2009; Weisman et al. 2012). Therefore, with welfare of the animals in mind (i.e., we wished to avoid holding them for longer than absolutely required to avoid heat stress), and the goal of only capturing early peaks

in behavior, we kept yearlings in handling bags for five minutes after administration of the treatment and before starting the six-minute test. We expected this timeline to capture a rise in cerebral oxytocin while minimizing handling stress (e.g., Smith et al. 2012). Beyond ethical considerations, a prolonged handling stressor could have confounded the experiment because stressors are known to interact with oxytocin (Lang et al. 1983; Neumann 2002).

### *Statistical analysis*

Principal components were extracted in SPSS 18.0 (Chicago, Illinois, USA). We fitted a generalized linear model to examine the effects of treatment, sex and their interaction on the change in sociability (PCA scores). We included sex to account for potential sex differences in these effects. We reported absolute, rather than relative, changes between trials to assess treatment effects because only the former data conformed to a Gaussian distribution, thus allowing us to assess interaction terms. All model residuals were checked for normality visually. Models were fitted in R version 3.3.1 (R Core Team, 2016) using the package MASS (Venables and Ripley 2002). We calculated the 95% confidence interval for the effect size using Hedges'  $g$ , a corrected measure for small samples sizes (Hedges and Olkin 1980).

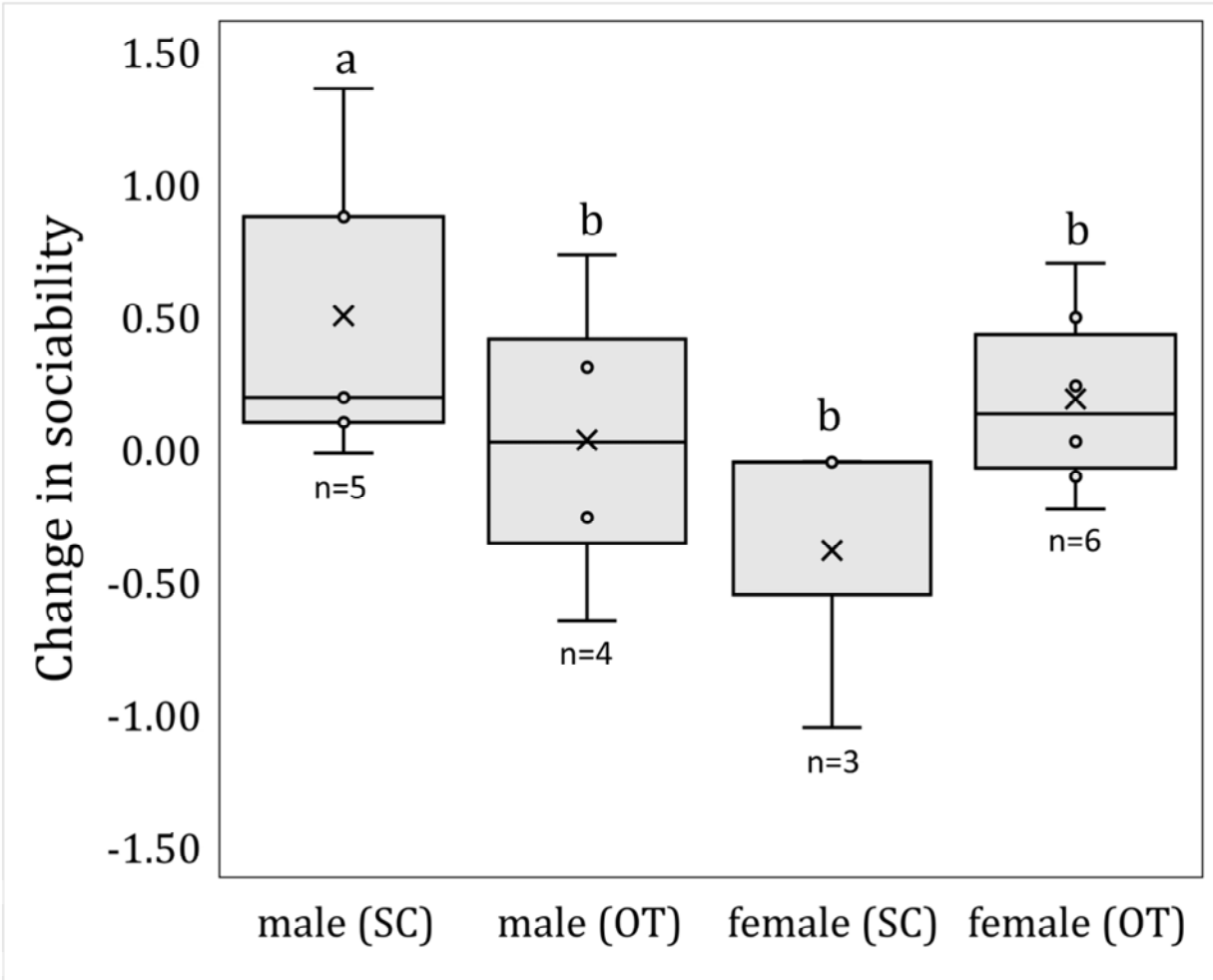
### *Ethical note*

All procedures were approved under research protocol ARC 2001-191-01 and permits issued by the Colorado Division of Wildlife. The research protocol was approved by the University of California Los Angeles Animal Care Committee on 13 May 2002 and renewed annually.

### *Behavioral responses of yellow-bellied marmots*

Behavioral changes in sociability in the mirror-image stimulation tests were highly variable among subjects and not significantly different between treatment and control groups. The interaction between treatment and sex on change in sociability failed to reach statistical significance (GMM: Treatment \* Sex:  $1.040 \pm 0.509$ ,  $t = 2.042$ ,  $P = 0.061$ , Fig. 1). When included in the model, neither the main effects of treatment (Control:  $-0.571 \pm 0.370$ ,  $t = -1.545$ ,  $P = 0.145$ ) or sex (Male:  $-0.155 \pm 0.337$ ,  $t = -0.460$ ,  $P = 0.653$ ) predicted the change in sociability (Oxytocin:  $n = 6$  females, 4 males; Saline:  $n = 3$  females, 5 males). The corrected effect sizes for males (Hedge's  $g = -0.69$ ; 95 % interval:  $-2.05$  to  $0.66$ ) and females ( $g = 0.25$ ; 95% interval:  $-1.25$  to  $1.75$ ) included zero within the confidence intervals. After removing the interaction term, the interpretation of the

results remained consistent; neither the main effect of treatment (Saline:  $-0.024 \pm 0.280$ ,  $t = -0.084$ ,  $P = 0.934$ ) nor sex (Male:  $0.301 \pm 0.278$ ,  $t = 1.083$ ,  $P = 0.296$ ) explained the change in sociability.



**Fig. 1.** Box and whisker plot showing the effects of oxytocin on the change in sociability of yearling marmots from baseline in response to a saline control (SC;  $n = 5$  males, 3 females) or oxytocin (OT;  $n = 4$  males, 6 females) intranasal injection during a mirror-simulation test. The bottom and top of each box represents the first and third quartiles for each group. The whiskers represent the minimum and maximum values and circles represent other intermediate data points. Two of the values for the female SC group are identical and, therefore, only one circle is shown to represent both data points. The bands inside of each box represent the second quartile (median), “Xs” represent the means for each group. Positive and negative sociability values, respectively, indicate increases or decreases in sociability in response to SC or OT. Different letters above plots represent statistically significant differences among treatment groups.

## References

- Armitage KB. 2010. Individual fitness, social behavior, and population dynamics of yellow-bellied marmots. In: Billick I, Price MV, editors. *The Ecology of Place Chicago*: University of Chicago Press. p. 209–247.
- Armitage KB, Van Vuren DH, Ozgul A, Oli MK. 2011. Proximate causes of natal dispersal in female yellow-bellied marmots, *Marmota flaviventris*. *Ecology* 19:218–227.
- Blumstein DT, Daniel JC. 2007. Quantifying behavior the JWatcher way. Sinauer Associates Inc.
- Blumstein DT, Keeley KN, Smith JE. 2016. Fitness and hormonal correlates of social and ecological stressors of female yellow-bellied marmots. *Anim Behav* 112:1-11.
- Blumstein DT, Lawrance KC, Smith, JE. 2013. Early play may predict later dominance relationships in yellow-bellied marmots (*Marmota flaviventris*). *Proc Roy Soc Lond B Bio Sci* 280(1759):20130485.
- Blumstein DT, Wey TW, Tang K. 2009. A test of the social cohesion hypothesis: interactive female marmots remain at home. *Proc R Soc B Biol Sci* 276:3007–3012.
- Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. 2002. Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci* 5:514–516.
- Cho MM, DeVries AC, Williams JR, Carter CS. 1999. The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (*Microtus ochrogaster*). *Behav Neurosci* 113(5):1071.
- Hedges LV, Olkin I. 1980. Vote-counting methods in research synthesis. *Psychol Bull* 88(2):359.
- Hohmann M, Künzel W, Kirschbaum M. 1985. The uterine contraction stress test with oxytocin nasal spray in the diagnosis of hypoxemia. *Z Geburtshilfe Perinatol* 190(5): 210-214.
- Jolliffe IT. 2002. *Principal Component Analysis*. Second Edition. Springer, New York.
- Lang RE, Heil JWE, Ganten D, Hermann K, Unger T, W Rascher. 1983. Oxytocin unlike vasopressin is a stress hormone in the rat. *Neuroendocrinol* 37(4):314-316.
- Neumann ID. 2002. Involvement of the brain oxytocin system in stress coping: interactions with the hypothalamo-pituitary-adrenal axis. *Prog Brain Res* 139:147-162.
- Parker KJ, Buckmaster CL, Schatzberg AF, Lyons DM. 2005. Intranasal oxytocin administration attenuates the ACTH stress response in monkeys. *Psychoneuroendocrinol* 30:924–929.
- Petelle MB, Blumstein DT. 2014. A critical evaluation of subjective ratings: Unacquainted observers can reliably assess certain personality trait. *Curr Zool* 60:162–169.
- Petelle MB, Martin, JG, Blumstein, DT 2015. Heritability and genetic correlations of personality traits in a wild population of yellow-bellied marmots (*Marmota flaviventris*). *J Evol Biol* 28(10):1840-1848.
- R Core Team. 2016. *R: A Language and Environment for Statistical Computing*. Vienna: Austria.
- Smith AS, Ågmo A, Birnie AK, French JA. 2010. Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, *Callithrix penicillata*. *Horm Behav* 57:255-262.
- Smith JE, Chung LK, Blumstein DT. 2013. Ontogeny and symmetry of social partner choice among free-living yellow-bellied marmots. *Anim Behav* 85(4):715-725.
- Smith JE, Monclús R, Wantuck D, Florant GL, Blumstein DT. 2012. Fecal glucocorticoid metabolites in wild yellow-bellied marmots: experimental validation, individual differences and ecological correlates. *Gen Comp Endocrinol* 178(2):417-426.
- Vaka SRK, Sammeta SM, Day LB, Murthy SN. 2009. Delivery of nerve growth factor to brain via intranasal administration and enhancement of brain uptake. *J Pharm Sci* 98:3640–3646.
- Van Vuren D, Armitage KB. 1994. Survival of dispersing and philopatric yellow-bellied marmots: what is the cost of dispersal? *Oikos* 69:179-181.
- Venables WN, Ripley BD. 2002. *Modern Applied Statistics with S*. Fourth Edition. Springer, New York.
- Weisman O, Zagoory-Sharon O, Feldman R. 2012. Intranasal oxytocin administration is reflected in human saliva. *Psychoneuroendocrinol* 37(9), 1582-1586.