1 **Interplay between group size, huddling behavior and basal metabolism: an**

2 **experimental approach in the social degus**

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4 Monica Nuñez-Villegas[1], Francisco Bozinovic[2] and Pablo Sabat[1*]

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6 [*]Pablo Sabat (corresponding author)

7 [1]Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile,

8 Casillla 653, Santiago, Chile. psabat@uchile.cl

9 [2]Departamento de Ecología, LINC-Global, MIII, Facultad de Ciencias Biológicas,

10 Pontificia Universidad Católica de Chile, Santiago 6513677, Chile.

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Abstract

24 Mammals exposed to low temperatures increase their metabolic rate to maintain constant

25 body temperature and thus compensate heat loss. This high and costly energetic demand

26 can be mitigated through thermoregulatory behavior such as social grouping or huddling,

27 which helps to decrease metabolic rate as function of the numbers of individuals grouped.

28 Sustained low temperatures in endothermic animals produce changes over time in rates of

29 energy expenditure, by means of phenotypic plasticity. However, the putative modulating

30 effect that huddling exerts on the flexibility of the basal metabolic rate (BMR) due to

31 thermal acclimation remains unknown. We determined BMR values in *Octodon degus*,

32 an endemic Chilean rodent, after being acclimated either to 15 °C or 30 °C during 60

33 days, both alone and in groups of 3 and 5 individuals. At 15 ºC, BMR of huddling

34 individuals was 40 % lower than that of animals housed alone. Moreover, infrared

35 thermography revealed a significant increase in local surface temperatures in huddled

36 animals. Furthermore, individual thermal conductance was lower in individuals

37 acclimated to 15 °C than at 30 °C, but no differences were observed between single and

38 grouped animals. Our results indicate that huddling prevent an increase in BMR when

39 animals are acclimated to cold conditions and that this effect is proportional to the number

40 of animals grouped.

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Introduction

50 **Introduction**

51 Endothermy is defined as the ability to produce endogenous heat, allowing individuals to

52 maintain a positive temperature differential with the environment and remain in

53 homeothermic condition (Hill et al., 2004). Heat production may be modulated through

54 behavioral and physiological changes at different scales and across a wide range of

55 ambient temperatures (Gilbert et al., 2010). For example, animals exposed to temperatures

56 below the thermoneutral zone (TNZ) must compensate heat losses by increasing their

57 metabolic rate in order to remain homeothermic (Canals, 1998b). Thus survival of small

58 mammals at low temperatures may depend on their ability to reduce heat loss and/or to

59 increase metabolic rate, which in many cases involves a large energy cost (Kauffman et

60 al., 2003).

61   To compensate for the increased energy expenditure caused by exposure to low

62 temperatures individuals may exhibit behavioral responses such as social grouping or

63 huddling (Canals, 1998b; Gilbert et al., 2010). Recently Gilbert et al. (2012) documented

64 that local heating is crucial in reducing the extent of the cold challenge in huddling rabbit

65 pups. Through thermal images they demonstrated that at 14 ºC, the mean surface

66 temperature of the huddle was higher than the mean temperature of isolated pups. This

67 study demonstrated that local heating when huddle provided each pup with an ambient

68 ''public warmth'' in the cold. Thus, huddling behavior reduces energy costs by reducing

69 the metabolic rate and average thermal conductance of each individual in the group, due

70 mainly to the reduction of surface area and altering the thermal environment experienced

71 by animals exposed to the cold. In this sense, however, whilst in some species the benefit

72 is shared in an equitable manner, in others it has been reported that some individuals may

73 benefit more than others when huddling (Bustamante et. al., 2002). Apparently, this

74 asymmetry would be the result that some animals would preferably occupy the best

75 location in the group (i.e., the center), while others would be relegated to occupy a larger

76 proportion of time to the periphery (Shank and Alberts, 1997). Moreover, this reduction is

77 proportional to the number of individuals in the group to the power of -0.33 (Gilbert et al.,

78 2010; Canals and Bozinovic, 2011). For example, for *Octodon degus*, a rodent that dwells

79 in semi-arid areas of northern and central Chile, the huddling effectiveness (He, the

80 maximum energy saving during huddling, Canals et al. 1997) when these organisms are

81 exposed to temperatures of 0, 5 and 10 °C reaches 43%, which constitutes a significant

82 fraction of the body's energy budget (Canals et al., 1998a). Therefore, at temperatures at

83 least 5 °C below the lower limit of the TNZ the He is constant, a phenomenon that was

84 also reported for other mammal species. However, with increasing temperature above this

85 critical value He tends to decrease; therefore huddling effectiveness decreases at high

86 temperatures (Canals et al., 1997).

87   Thus the capacity of endotherms inhabiting seasonal environments depends on their

88 ability to develop physiological and behavioral mechanisms allowing physiological

89 homeostasis (Pigliucci, 2001, Piersma and van Gils 2011). In this vein physiological

90 flexibility, the ability to change and modify physiological traits in response to

91 environment cues, is crucial for maintaining homeostasis in changing environments

92 (Piersma and Drent, 2003; McKechnie et al., 2007) and has been demonstrated to occur in

93 response to seasonal variations (Bozinovic and Contreras, 1990; Bacigalupe et al., 2004)

94 and in laboratory thermal acclimation experiments in several mammal species (Nespolo

95 and Rosenmann, 1997; Nespolo et al., 2002). This physiological flexibility, a particular

96 case of phenotypic plasticity (see Garland and Adolph, 1991; Piersma and Drent , 2003),

97 can cause variations in thermal insulation as well as changes in basal (BMR) and

98 maximum cold- induced metabolic rates (M_{sum}). Thus, changes in any of these parameters

99 may be indicative of changes in energy expenditure rates (Piersma et al., 1996; Nespolo et

100 al., 1999). In particular, BMR represents the minimum rate of energy necessary to

101 maintain homeostasis and reflects the cost of maintaining high levels of sustained activity.

102 This metabolic trait exhibits high flexibility, which has been demonstrated in experiments

103 in the laboratory by thermal acclimation (Nespolo and Rosenmann, 1997; Speakman,

104 2000). The reaction norm of BMR (i.e. a function that describes the changes of BMR as a

105 function of acclimation temperature) exhibits a negative association, that is, endotherms

106 acclimated to low temperatures commonly exhibited an increase in their BMR. Such

107 increase in BMR is thought to be related to the increase in maintenance costs of

108 metabolically active organs when animals are faced with the high energy requirements of

109 thermoregulation in the cold (see Cruz-Neto et al., 2003; McKechnie et al., 2007).

110   Experimental evidence has shown the existence of i) decreased rates of individual

111   energy expenditure (e.g. resting metabolic rate) in grouped organisms when exposed to

112   temperatures lower than the TNZ and ii) a remarkable physiological flexibility of rates

113   of energy expenditure when acclimated to different temperatures. In the former case

114   huddling allows energy savings during the grouping behavior, whereas physiological

115   flexibility modifies the rates of energy expenditure in the medium term (e.g. weeks) in

116   order to cope with the different thermoregulatory needs. However, to date there is no

117   evidence that the use of huddling may affect medium- to long-term rates of energy

118   expenditure in endotherms, i.e., that huddling behavior affects the phenotypic response

119   of individuals acclimated to different temperatures. The aim of this study was to

120   estimate the effect of social grouping on flexibility in BMR, TEWL and thermal

121   conductance in *Octodon degus* or degu, a social rodent that exhibits huddling behavior

122   both in captivity and in the wild (Ebensperger and Wallen, 2002) and dwells in highly

123   seasonal environments of central Chile (Di Castri and Hajek, 1976). We test the

124   hypothesis that the presence of huddling and group size plays a modulating role in the

125   acclimation capacity of BMR in adults. Specifically, we predict that huddling will

126   decrease or prevent an increase in BMR when animals are acclimated to cold

127   conditions and that this effect is proportional to the number of animals grouped.

128

129   **Results**

130   After thermal acclimation, we found a significant effect of body mass on total BMR. The

131   allometric equation relating BMR with body mass (bm) was: $BMR = 7.9*bm^{0.53}$ ($r^2 = 0.21$,

132    $F_{(1, 25)} = 13.88$, $p = 0.009$). We also found a significant effect of the acclimation

133   temperature ($F_{1, 25} = 6.98$, $P = 0.014$), the number of individuals grouped ($F_{2, 25} = 9.27$, P

134    $= 0.001$) and the interaction between these two factors on mass-adjusted BMR ($F_{2, 25} =$

135   6.92, $P = 0.004$). The *post hoc* test revealed that mass-adjusted BMR of rodents

136   acclimated individually at 15 °C was greater than that observed in rodents acclimated

137   individually at 30 °C (Fig.1). In groups of three, *O.degus* decreased mass-adjusted BMR

138   by 15% and 7% when acclimated at 15 °C and 30 °C, respectively (Figure 1).

139   Additionally, cold-acclimated animals in groups of three exhibited higher mass-adjusted

140   BMRs than those of warm-acclimated animals in groups of three, but this difference

141   appeared to be smaller than that observed in individual acclimated groups. Compared with

142   those housed alone, in groups of five the decrease in mass-adjusted BMR was

143   approximately 40% for degus acclimated to 15 °C. However, there were no significant

144   energy reduction for degus acclimated to 30 °C. Finally, mass-adjusted BMRs of animals

145   in groups of five individuals did not present significant differences between warm and

146   cold-acclimated groups. Furthermore, the mass-adjusted BMR of cold-acclimated animals

147   in groups of three was similar to that of cold-acclimated animals in groups of five

148   individuals. The same was true for warm-acclimated animals for these two groups. Also,

149   we found a significant effect of acclimation temperature on thermal conductance in *O.*

150   *degus* (ANOVA $F_{1, 29} = 6.47$, P = 0.016, Table 1). Specifically, we found that the average

151   individual conductance of animals was greater in rodents acclimated to 30 °C than at 15 °

152   C (Fig. 2). However, we found no effect of the group size ($F_{2, 29} = 1.56$, p = 0.22) or the

153   interaction between factors ($F_{2, 29} = 0.90$, P = 0.41) on thermal conductance.

154   We found significant differences in measured temperatures between treatments

155   ($F_{9,71} = 165.9$, p < 0.0001). The a posteriori analyses showed that maximum surface

156   temperature and mean contour temperatures were higher in grouped than isolated animals

157   at 15ºC, but not at 30ºC. Moreover, at 15ºC the temperature of contact was higher than the

158   contour temperatures of grouped and isolated animals (Figure 2). We also found that the

159   spatial location of individuals in the group was different between individuals ($X^2 = 16.94$,

160   d.f. = 4; P = 0.02). Some individuals spent more than 5 times in the center of the group

161   than others. The 95% confidence intervals of the frequencies that each animal spent in the

162   center of the group were: [0.06-0.24], [0.07-0.26], [0.03-0.17], [0.17-0.40] and [0.26-

163   0.51]. Finally, we found solitary animals consumed significantly more food than grouped

164   animals at 15 ºC which in turn consumed more food than animals maintained at 30ºC

165   (Kruskall-Wallis ANOVA: $H_{3,20} = 16.63$, P = 0.001). In descending order the food intake

166   per animal were the follows: solitary animals at 15 ºC (29.98 ± 4.87 g/day per animal) >

167   grouped at 15 ºC (19.86 ± 2.35) > grouped at 30 ºC (10.47 ± 2.17) = solitary at 30ºC

168   (8.02 ± 1.70).

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Discussion

Our results confirm the previously demonstrated effect of short-term thermal acclimation on energy expenditure rates in endotherms. In fact, the difference in BMR between cold- and warm solitary acclimated animals can reach ca 30%, which is in the range of the acclimation magnitudes reported for other rodent species in comparable studies (e.g., Novoa et al. 2005; Nespolo et al., 2001). However, our results demonstrate for the first time that huddling behavior exerts a modulatory effect on thermal acclimation of BMR in endotherms. Increasing the number of individuals to three animals per group and allowing them to huddle during acclimation led to a reduction in the difference in BMR between temperatures. When animals were allowed to form groups of five individuals, BMR was lower than solitary animals when acclimated at 15 ℃, suggesting that degus were acclimated to different effective temperatures. In fact, huddling promotes local heating and reduces the cold challenge, because single degus experienced colder conditions compared with degus huddling in a group of five (Fig. 2), a phenomenon that has been also recently demonstrated in rabbit pups (Gilbert et al. 2012). It is therefore likely that the individual minimum energy requirement decreases in degus which huddle for long periods, as occurs with the energy expenditure of this species when grouped (Canals et al., 1989; Kotze et al., 2008). In this vein, the observed reduction in the acclimation response of individual BMR in grouped degus (ca 40%) is comparable with the reduction of metabolic rate by means of huddling of grouped individuals in other mammal species (range: 11-50%, see Canals et al., 1989; Canals et al., 1998, Gilbert et al., 2010 and references therein). Moreover, the fact that individuals acclimatized at 30 °C and maintained in groups of five individuals did not save significant amounts of energy agrees with the idea proposed by Canals et al. (1998a), which states that the huddling efficiency decreases at temperatures near the TNZ of the animal, which for *O. degus* corresponds to the range between 24 °C and 32 °C (Rosenmann, 1977).

Thus huddling allows energy savings over at least two time scales. First, huddling induces metabolic depression of animals while grouped, as has been demonstrated previously (e.g., Canals et al., 1997) and second, our results strongly suggest that in degus acclimated to low temperatures and in groups of three or more

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200 individuals, individual energy expenditure (BMR) decreases compared to degus subjected

201 to the same temperatures but acclimated individually. Huddling therefore helps mitigate

202 the excessive expenditure of energy to maintain homeothermy, buffering the decreasing

203 ambient temperature by decreasing its thermogenic response. This is also supported by the

204 observed decrease in food consumption when animals were grouped at low temperatures.

205 Interestingly, the low food consumption may lead to a decrease of the masses of digestive

206 organs (not measured), which suggests the possibility that BMR changes reflect changes

207 in the mass of metabolically active organs. Accordingly, some studies have shown a

208 significant increase in size of metabolically active organs such as the heart and liver when

209 exposed to cold (Hammon et al., 2001; Naya et al., 2010). This possible proximal

210 mechanism, not addressed in this paper, could be considered in future studies.

211   Energy economy may be beneficial for rodents inhabiting arid or semi-arid

212 environments, where animals are exposed to large temperature variations and

213 heterogeneous distribution of food (Scantlebury et al., 2006a; Scantlebury et al., 2006b).

214 The energy savings observed in the laboratory probably can also have consequences in the

215 field. Thus, decreasing thermogenic response by means of huddling may lead to a

216 decreases in food needs and thus to allocate time to activities other than foraging.

217 Alternatively, since energy is limited for animals, the energy saved by huddling may be

218 allocated to perform other biological functions or activities, while acting as a selective

219 pressure important for life in groups in rodents (Gilbert et. Al 2012; Ebensperger and

220 Wallen, 2002; Shradin et al., 2006). For example, in *O. degus* some activities associated

221 with foraging may be energetically costly, such as digging in dry areas with low humidity

222 (Ebensperger and Bozinovic, 2000), exploration behavior in open spaces at low

223 temperatures after foraging and building burrows (Ebensperger, 2000; Quispe et al.,

224 2009). All these activities may increase the biological fitness of *O. degus*, particularly in

225 periods of high-energy demand such as winter and during the breeding season.

226   In this way individuals can regulate the levels of energy expenditure to maintain their

227 functional capabilities in variable thermal environment through plastic and adaptive

228 changes in metabolic rate, and reassign the energy saved to other activities (Nespolo,

229 2000). However, Bozinovic et al. (2004) reported that the BMR in *O. degus* in the wild is

230 not altered by seasonality in a Mediterranean environment of central Chile. Indeed, these

231 authors found that the BMR remains unchanged along the year. Interestingly, the average

232 BMR values reported for wild degus are very similar to the BMR of cold-acclimated

233 degus in groups of three and five individuals. As *O. degus* is a social rodent that lives in

234 groups of four or more individuals sharing the same burrow system and performs

235 huddling in nature, one might infer that thermoregulatory behavior such as huddling and

236 its long-term effects could be a compensatory mechanism that helps maintain this rodent

237 BMR relatively stable over the year.

238   There are several factors that can affect metabolic expenditure of animals performing

239 huddling. Among the most important are decreasing body area exposed to the

240 environment (Canals et al., 1998) and the local microclimate (Hayes et al., 1992). In

241 agreement with that, our results revealed that the surface temperature of the entire group

242 exposed to 15 ºC was higher than the surface temperature of solitary degus and that such

243 difference is coupled with the increased temperature of the boundary of each animal when

244 huddle (Fig. 2). An additional hypothesis the so-called socio-physiological effect

245 (Speakman and Rossi, 1999), states that there would be a decrease in metabolic rate of

246 animals grouped since they tend to decrease their levels of anxiety when perceiving

247 conspecifics (Martin et al., 1980). However, the socio-physiological factor did not have a

248 significant effect in this study, because individuals grouped in five and acclimated at 30

249 °C did not exhibit a significant reduction compared to the BMR observed in solitary

250 individuals (Fig.1).

251   Furthermore, the decrease in energy expenditure individually within the group

252 conducting huddling also has a per capita cost since the fuel consumed by thermogenesis

253 is generated individually, although the benefits are shared by the group (Haig, 2007). In

254 this vein, it is possible that some individuals could benefit more than others in huddling.

255 Our video record revealed that some degus of our group treatments may have benefited

256 more than others and thus decreased their BMR more, which is consistent that the

257 coefficient of variation in BMR seems to be higher in grouped than in non-grouped

258 acclimated degus. According to Bustamante et al. (2002), small individuals of the rodent

259 *Phyllotis darwini* are most favored by the larger individuals huddling, especially at

260 temperatures below the TNZ. Further experimental studies will need to assess whether all
261 individuals in the group performing huddling receive the same benefits, as for example,
262 individuals who are dominant competitors and can occupy the best location in the center
263 of the group (Shank and Alberts, 1997). In this vein, experiments of Bautista et al. (2008)
264 reported that the offspring of rabbits (*Orytolagus cuniculus*) share the thermal advantages
265 by moving continuously within the group. It would be interesting to study the effect of
266 body size, ontogeny and social hierarchy within the group on the energy benefits in the
267 medium term (acclimation effect) of huddling in adult and juvenile of *O. degus*.
268 Furthermore, from the viewpoint of comparison it would also be interesting to perform
269 acclimation experiments of other species that exhibit different degrees of sociability.

270   Individual thermal conductance of *O. degus* was lower only in individuals acclimated
271 at 15 °C, irrespective of the group size. Thus the results of this study suggest that
272 compared to the temperature factor, the grouping effect would not be so important. It is
273 possible that the metabolic changes due to huddling are less rigid than the changes that
274 occur at the morphological level such as increased length and density of the fur, which
275 could in turn restrict their movement (Cutrera and Antinuchi, 2004). Future studies will
276 then be necessary to determine the mechanisms that explain the association between
277 metabolic changes and thermal conductance acclimatization *O. degus.*

278   The convergence of huddling behavior among animals is a well established evolutionary
279 event, being emperor penguins a notable case (Gilbert et al 2010). In this vein it is
280 possible that this phenomena might also occur in other endotherms including birds, which
281 has been demonstrated can reduce energy expenditure by comparable amounts when
282 huddle (MacKechnie and Lovegrove 2001, Wojciechowski et al. 2011). Summarizing,
283 during huddling animals group together and maintain close bodily contact, being
284 particularly important for decreasing thermoregulatory costs and for increasing survival
285 times when environmental conditions are harsh. Huddling induces metabolic depression
286 without hypothermia and is mainly attributed to the reduced surface area/volume ratio of
287 the huddling group and to the increase of effective temperature. The novelty of our
288 results are that huddling not only decreases metabolic rate in individuals while grouped,
289 but also provides significant energy savings at the individual level that persists after

290 huddling. Finally, further studies are necessary to assess if huddling behavior could

291 modulate the thermal acclimation in other species of endotherms.

292

293 **Methods & Techniques**

294 Thirty-two adult individuals of *O. degus* were trapped with Shermann traps in autumn-

295 winter 2011 in the Quebrada de la Plata, Chile (33º 28'S, 70º 54'W). Individuals were

296 transported to the laboratory in Santiago, Chile where they were kept in cages of 2.0 m x

297 2.5 m x 2.0m (length x width x height) with food and water *ad libitum* for one month, to

298 minimize the possible effects of the previous thermal experience (see Nespolo and

299 Rosenmann, 1997). Then individuals were randomly divided into two groups of 16

300 individuals. One group was acclimatized at 15 °C and the other at 30 °C for 2 months,

301 with water and food *ad libitum* and with a photoperiod of 12L: 12D. Each acclimation

302 group was divided into three treatments: solitary individuals (n = 5), two groups of 3 (n =

303 6) and one group of 5 (n = 5) individuals in plastic cages 87 cm x 33 cm x 32 cm (length x

304 width x height). We decided to use a maximum size group of 5 since five individuals

305 exhibited the largest energy savings by huddling in this species (Canals et al., 1989). All

306 observations and measurements were performed on adult males and females with a body

307 mass (mean ± SD) of 173 ± 25.7 g (males) and 147.92 ± 23.76g (females). No differences

308 in body mass between sexes (p=0.1) or between groups of different size were found

309 (p=0.09).

310

311 *Respirometry*

312 Following acclimation, BMR was measured as the oxygen consumption rate using a

313 computerized open-flow respirometry system (Sable Systems, Henderson, NV). For BMR

314 determinations, post-absorptive animals were placed individually in stainless steel

315 metabolic chambers (5000 mL) for 12 h in the rest phase (night) in the dark at 30.0 ± 0.5

316 °C, which is within the thermoneutral zone of this species (Rosenmann, 1977). To

317 calculate thermal conductance we determined oxygen consumption at two temperatures

318 below TNZ (20 and 10 ± 1 °C) for at least 2 h or until a visual inspection of the

319   recorded data allowed us to determine when steady- state conditions had been

320   achieved. With these values of oxygen consumption we estimated heat loss using the

321   following equation: $J = \frac{M}{\Delta T}$, (eqn. 1) were C is in J g^{-1} h^{-1} $°C^{-1}$. Values of oxygen

322   consumption were transformed into Joules using caloric equivalent of oxygen of 20.17 J

323   per mL O_2. Briefly, external air (700 mL min^{-1}) was drawn into the metabolic chamber by

324   negative pressure created by a downstream vacuum pump controlled by a Sierra mass

325   flowmeter/ controller (Sierra Instruments, Monterey, CA), which was calibrated monthly

326   with a volumetric flowmeter. Before arriving in the chamber the air was dried using

327   Drierite desiccant and passed through Bev-A-Line tubing (Thermoplastic Processes). The

328   excurrent air from the metabolic chamber passed through Drierite, Baralyme, and Drierite

329   to remove water vapor and CO_2 gas before being passed through the O_2 analyzer (model

330   FoxBox, Sable Systems). The open-flow respirometry system was calibrated with a

331   known mixture of oxygen (20%) and nitrogen (80%) that was certified by

332   chromatography (INDURA, Chile). Because CO_2 and water vapour was scrubbed before

333   entering the O_2 analyzer, oxygen consumption was calculated according to Withers (1977

334   p. 122):

335    $$VO_2 = FR * 60 * \frac{Fi\,O2 - Fe\,O2}{1 - Fi\,O2},$$ (eqn. 2) where FR is the flow rate

336   (mL min^{-1}) and Fi and Fe are the fractional concentrations of O_2 entering and leaving the

337   metabolic chamber, respectively. In order to confirm that animals were euthermic after the

338   metabolic trials (Tb = 37-38 ºC), we recorded their rectal body temperature (Tb) with a

339   Cole Palmer 24-gauge copper-constantan thermocouple attached to a Digisense

340   thermometer (model 92800- 15). Outputs from the oxygen analyzer (%) were digitized

341   using a Universal Interface II (Sable Systems) and recorded on a personal computer using

342   data acquisition software (EXPEDATA, Sable Systems). Our sampling interval was 5 s.

343   We averaged O_2 concentration of the excurrent air stream over an entire record period of

344   30 min after the lower steady state was reached (following Bozinovic et al., 2009). To

345   assess if changes in thermal/huddling conditions were accompanied with changes in

346   energy intake, we measured food consumption of grouped (5) and solitary animals at 15

347   and 30 ºC. At the end of the acclimation period, animals were placed in metabolic cages

348   to estimate food intake in 48-h trials. We fed animals with a known amount of pellet

349   (rabbit food, Champion®) and after 48 h we collected the uneaten food. Samples were

350   dried at 90 °C to constant weight (± 0.001 g), and the intake was calculated by subtracting

351   the uneaten to the total offered food.

352

353   *Thermography and behavior*

354   In a second experiment, the surface temperature of solitary and degus in groups of 5, was

355   recorded at the end of acclimation periods using a thermal imaging camera FLIRi40

356   calibrated at FLIR Systems Brasil (2011,wwwFLIR.com). Images were recorded at a

357   height of 1 m above the chamber and at two ambient temperatures of 15 and 25 °C .

358   Thermal images were analyzed using the ad hoc software FLIR QuickReport 1.3 SP1;

359   with a fur emissivity 0.98. Surface temperatures were averaged from 10 images for each

360   treatment taken at the end of the respirometry periods. The contour surface temperature of

361   degus was determined by fitting a polygon around the individual animal in the case of

362   isolated pups and around the entire huddle for grouped animals by using the option

363   "isotherm" of the software. This isotherm is the average temperature along the polygon,

364   which defines the limit between above and below temperatures observed in the thermal

365   image. The maximum temperature of degus was determined by using the options area of

366   the software. After fitting the area of huddled or solitary animals, a unique maximum

367   temperature value was recorded for each thermography. Furthermore, we determined the

368   temperature of contact between pairs of individuals in grouped animals using the option

369   mobile, by fitting a line of contact between paired animals, dividing it in five sections and

370   using the four points inside the sections in order to get the average surface temperature of

371   contact between two animals. Then we averaged those temperatures to calculate the

372   average temperature of contact of the entire group. Finally, in order to assess whether

373   grouped animals constantly changed positions, we determine the proportion of time that

374   each animal spent in various positions within the group. For this, we marked each

375   individual with hair dye on the back and performed 10 two-hour films each in a group of

376   5 animals kept at 15 ° C. The measurements were performed at 1 m height using a Sony

377   NP-FV50. The films were analyzed on a personal computer by recording the time each

13

378 individual spent in the center, on the periphery of the group or isolated while doing

379 huddling in groups of five individuals. In order to maximize the time that each animal was

380 analyzed and because the groups are not always formed with the maximum number of

381 individuals (i.e., 5), video recordings were analyzed only when the animals spent more

382 than 15 min clustered in groups of at least 4 individuals. The proportion of time that

383 animals was in such behavior approached 87%.

384

385 *Statistical Analysis*

386 We performed a General lineal Model (GLM) to compare BMR between treatments using

387 Log BMR and thermal conductance as the dependent variables, and sex, temperature and

388 the number of individuals in the group as fixed factors and log body mass as covariate.

389 Because our analyses exhibited a non-significant effect of sex ($F_{1,19} = 0.84$, $r^2 = 0.01$, $p =$

390 0.77 and $F_{1,19} = 0.84$, $r^2 = 0.01$, $p = 0.77$ respectively) this term was dropped from the

391 models. Our analysis also revealed that thermal conductance was not related to body mass

392 in degus ($F_{1,30} = 0.99$, $r^2 = 0.03$, $p = 0.33$), thus, we only performed an ANOVA to test

393 differences among experimental groups. Then a *post hoc* Fisher test was performed to

394 determine significant differences in mass-adjusted BMR (least square means calculated

395 from ANCOVA) and thermal conductance between specific groups. To analyze the

396 association between physiological variables and body mass, a simple linear regression

397 was performed. All the data met the assumptions of the ANOVA. Surface and contour

398 temperatures of the different experimental conditions (grouped and solitary at 15 and 30

399 ºC) were compared with one-way ANOVAs and then a post-hoc Tukey test was

400 performed to check for specific differences among treatments. Proportion of time each

401 animal spent in the center of the group was evaluated by means of counting the times each

402 animal was viewed in this position in random analyses. Each tape record was analyzed

403 through random selection of five periods of 6 min by trial (two hour each). We hence

404 analyzed a total of 50 records for which we obtained 239 individual observations. We

405 count as positive when an animal were surrounded by at least two animals and negative

406 when where the subject was located at the periphery of the group. Then, we calculated the

407 observed frequency of times that each animal spent in the center of the group and the

408 expected frequency by chance (i.e., total number of observations divided by the number of

409 animals). A Chi-square test was used to estimate significant differences among

410 individuals. Food intake was evaluated by means of a nonparametric Kruskall-Wallis test

411 and then a multiple comparison of mean rank was performed. All results are reported as

412 mean ± SE. All analyses were performed using Statistica 7.0 software (StatSoft Inc.,

413 Tulsa, OK, USA).

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References

Bacigalupe, L.D., Nespolo, R.F., Opazo, J.C and F, Bozinovic, F. (2004). Phenotypic flexibility in a novel thermal environment: Phylogenetic inertia in thermogenic capacity and evolutionary adaptation in organ size. *Physiol. Biochem. Zool.,* **77** (5), 805-815.

Bautista, A., García-Torres, E., Martínez-Gómez, M and Hudson, R. (2008). Do newborn domestic rabbits *Oryctolagus cuniculus* compete for thermally advantageous positions in the litter huddle? *Behav. Ecol. Sociobiol.*, **62**, 331 – 339.

Bozinovic, F and Contreras L.C. (1990). Basal rate of metabolism and temperature regulation of two desert herbivorous octodontid rodents: *Octomys mimax* and *Tympanoctomys barrerae*. *Oecologia*, **84**, 567-570.

Bozinovic, F., Bacigalupe, L.D., Vasquez, R.A., Henk Visser, G., Veloso, C and Kenagy, G.J. (2004). Cost of living in free-ranging degus (*Octodon degus):* seasonal dynamics of energy expenditure. *Comp. Biochem. Physiol. A* **137**, 597-604.

Bozinovic, F., Rojas, J.M., Broitman, B.R and Vásquez, R.A. (2009). Basal metabolic rate is correlated with habitat productivity among populations of degus (*Octodon degus*). *Comp. Biochem. Physiol. A* **152**, 560–564.

462

463 **Bustamante, D., Nespolo, R., Rezende, E and Bozinovic, F.** (2002). Dynamic Thermal

464 Balance in the Leaf-Eared Mouse: The Interplay among ambient temperature, body size,

465 and behavior, *Physiol. Biochem. Zool.* **75** (4), 396-404.

466

467 **Canals, M., Rosenmann, M and Bozinovic, F.** (1989). Energetics and geometry of

468 huddling in small mammals. *J. Theor. Biol.*, **141**,181-189.

469

470 **Canals, M., Rosenmann, M and Bozinovic, F.** (1997). Geometrical aspects of the

471 energetic effectiveness of huddling in small mammals. *Acta Theriol.*, **42**, 321-328.

472

473 **Canals, M., Rosenmann, M., Novoa, F.F and Bozinovic, F.** (1998a). Modulating factors

474 of energetic effectiveness of huddling in small mammals. *Acta Theriol.*, **43**, 337-348.

475

476 **Canals, M**. (1998b). Thermal ecology of small animals. *Biol. Res.*, **31**, 367-374.

477

478 **Canals, M and Bozinovic, F.** (2011). Huddling behavior as critical phase transition

479 triggered by low temperaturas. *Complexity*, **17**, 35-43.

480

481 **Clarke, A.** (2003). Costs and consequences of evolutionary temperatures adaptation.

482 *Trends Ecol. Evol.*, **18**, 573-579.

483

484 **Contreras, L.C.** (1984). Bioenergetics of huddling Test of a psycho-social hypothesis. *J.

485 Mammal.,* **65**, 56-262.

486

487 **Cruz- Neto, A.P., Briani, D.C and Bozinovic, F.** (2003). La tasa metabolica basal:

488 una variable unificadora en energética animal? In: Fisiología y ecología evolutiva. Teoría

489 y casos de estudios en animales (ed. F. Bozinovic), pp. 379-395. Ediciones Universitaria

490 Universidad Católica de Chile, Santiago, Chile.

491

492 **Cutrera, A.P and Antinuchi, C.A.** (2004). Fur changes in the subterranean rodent

493 *Ctenomys talarum*: possible thermal compensatory mechanism. *Rev. Chil. Hist. Nat.*, **77**,

494 235-242.

495

496 **Dawson, W.R and Olson, J.M.** (2003). Thermogenic capacity and enzymatic activities in

497 the winter-acclimatized dark-eyed junco (*Junco hyemalis*). *J. Ther. Biol.*, **28**, 497–508.

498

499 **Di Castri , F. and Hayek, E.** (1976). Bioclimatología de Chile. Ediciones Universidad

500 Católica, Santiago.

501

502 **Ebensperger, L.A and Bozinovic, F.** (2000). Communal burrowing in the hystriconath

503 rodent, *Octodon degus*: a benefit of sociality? *Behav. Ecol. Sociobiol.*, **47**, 365 -369.

504

505 **Ebensperger, L.A.** (2001). A review of evolutionary causes of rodent group-living. *Acta*

506 *Theriol.*, **46**, 115-144.

507

508 **Ebensperger, L.A and Wallen, P.K.** (2002). Grouping increases the ability of social

509 rodent, *Octodon degus*, to detect predators when using exposed microhabitats. *Oikos*, **98**,

510 491-497.

511

512 **Garland Jr.T and Adolph, S.C**. (1991). Physiological differentiation of vertebrate

513 populations. *Annu. Rev. Ecol. Evol. S.*, **22**, 193–228.

514

515 **Gilbert, C., McCafferty, D., Le Maho, Y., Martrette, J.M., Giroud, S., Blanc, S and**

516 **Ancel, A.** (2010). One for all for one: the energetic benefits of huddling in endotherms.

517 *Biol. Rev.*, **85**, 545-569.

518 **Gilbert, C., McCafferty, D.J., Giroud, S., Ancel, A., Blanc, S**. (2012) Private Heat for

519 Public Warmth: How Huddling Shapes Individual Thermogenic Responses of Rabbit

520 Pups. PLoS ONE 7(3): e33553. doi:10.1371/journal.pone.0033553

521

522 **Haig, D**. (2007). Huddling: Brown Fat, Genomic Imprinting and the Warm Inner Glow.

523 *Curr. Biol.*, **18** (4), R172-174.

524

525 **Hammond, K.A., Szewczak, J and Krol, E.** (2001). Effects of altitude and temperature

526 on organ phenotypic plasticity along an altitudi0nal gradient. *J. Exp. Biol.*, **204**, 1991–

527 2000.

528

529 **Hayes, J.P., Speakman, J.R and Racey, P.** (1992). The contributions of local heating

530 and reducing exposed surface-area to the energetic benefits of huddling by short-tailed

531 field voles (*Microtus agrestis*). *Physiol. Zool.,* **65**, 742-762.

532

533 **Hill, R.W.,Wyse, G.A and Anderson, M.** (2004). Animal Physiology. pp. 214-228.

534 Sinauer Asssociates, Inc. Publishers Sunderland, Massachusetts U.S.A.

535

536 **Kauffman, A.S., Paul, M.J., Butler, M.P and Zucker, I.** (2003). Huddling, locomotor,

537 and nest-building behaviors of furred and furless Siberian hamsters. *Physiol. Behav.,* **79**,

538 247-256.

539

540 **Kotze, J., Bennet, N.C and Scantlebury, S.** (2008). The energetic of huddling in two

541 species of mole-rat (Rodentia: Bathergidae). *Physiol. Behav.*, **93**, 215-221.

542

543 **Martin, R.A, Fiorentini, M and Connors, F.** (1980). Social facilitation of reduced

544 oxygen consumption in *Mus musculus* and *Meriones unguiculatus*. *Comp. Biochem.*

545 *Physiol.*, A **65**, 519-522.

546 **McKechnie A.E., Lovegrove, B.G**. (2001). Thermoregulation and the energetic

547 significance of clustering behavior in the white-backed mousebird (Colius colius). *Physiol*

548 *Biochem Zool* **74**, 238–249.

549

550 **McKechnie, A.E., Chetty, K and Lovegrove, B.G**. (2007). Phenotypic flexibility in the

551 basal metabolic rate of *Laughing Doves*: responses to short-term thermal acclimation. *J.*

552 *Exp. Biol.*, **210**, 97-106.

553

554 **Naya, D.E., Veloso, C., Sabat, P and Bozinovic, F.** (2010). Seasonal flexibility in organ

555 size in the Andean lizard *Liolaemus moradoensis. J. Morphol.*, **271**, 1440-1445.

556

557 **Nespolo, R.F and Rosenmann, M.** (1997). Thermal history in Chilean rodents: an

558 experimental approach. *Rev. Chil. Hist. Nat.,* **70**, 363-370.

559

560 **Nespolo, R.F., Opazo, J.C., Rosenmann, M and Bozinovic, F** (1999). Thermal

561 acclimation, maximum metabolic rate and nonshivering thermogenesis in *Phyllotis*

562 *xanthopygus* (Rodentia) inhabiting the Andean range. *J. Mammal.,* **80**, 742-748.

563

564 **Nespolo, R.F.** (2000). Physiological performance, seasonality and phenotypic plasticity in

565 small mammals: microevolution of change capacity in thermoregulatory characters. *Rev.*

566 *Chil. Hist. Nat.,* **73**, 553-563.

567

568 **Nespolo, R.F., Bacigalupe, F., Rezende, E.L., and Bozinovic, F.** (2001). When non

569 shivering thermogenesis equals maximum metabolic rate: thermal acclimation and

570 phenotypic plasticity of fossorial *Spalacopus cyanus* (Rodentia). *Physiol Biochem Zool*

571 **74**, 325–332.

572

573 **Nespolo, R.F., Bacigalupe, L.D and Bacigalupe, F.** (2003). The influence of heat

574 increment of feeding on basal metabolic rate in *Phyllotis darwini* (Muridae*). Comp.*

575 *Biochem. Physiol.*, Part A **134**, 139-145.

576

577 **Novoa, F.F., Rivera-Hutinel, A., Rosenmann, M. and Sabat, P.** (2005).Intraspecific

578 differences in metabolic rate of *Chroeomys olivaceus* (Rodentia: Muridae): the effect of

579 thermal acclimation in arid and mesic habitats. Rev. Chil. Hist. Nat. **78**, 207-214.

580 **Piersma, T., Bruinzeel, L., Drent, R., Kersten, M., Van der Meer, J and Wiersma, P.**

581 (1996). Variability in basal metabolic rate of a long distancemigrant shorebird (red knot,

582 *Calidris canutus*) reflects shifts in organ sizes. *Physiol. Zool.,* **69**, 191–217.

583

584 **Piersman, T and Drent, J.** (2003). Phenotypic flexibility and the evolution of organismal

585 desing. *Trends Ecol. Evol.,* **18**, 228-233.

586

587 **Pigliucci, M.** (2001). Phenotypic plasticity: beyond nature and nurture. Syntheses in

588 Ecology and Evolution (ed. S.M. Sheiner), pp. 315. Johns Hopkins Press, Baltimore,

589 Maryland, USA.

590

591 **Puchalski, W., Bockler, H., Heldmaier, G and Langefeld, M**. (1987). Organ blood flow

592 and brown adipose tissue oxygen consumption during noradrenaline-induced

593 nonshivering thermogenesis in the djungarian hamster. *J. Exp. Zool.,* **242**, 263–271.

594

595 **Quispe, R., Villavicencio, C.P., Cortés, A and Vásquez, R**. (2009). Inter-Population

596 variation in hoarding behavior in Degus, *Octodon degus*. *Ethology,* **115**, 465-474.

597

598 **Rosenmann, M.** (1977). Regulacion térmica en *Octodon degus*. *Medio ambiente* (Chile),

599 **3**, 127-131.

600

601 **Scantlebury, M., Bennett J.R., Speakman, N., Pillay, N and C, Schradin, C.** (2006a).

602 Huddling in groups leads to daily energy savings in free-living African Four-Striped

603 Grass Mice, *Rhabdomys pumilio*. *Funct. Ecol.*, **20**, 166-173.

604

605 **Scantlebury, M., Pillay, N., Speakman, J.R., Bennett, N.C and Schradin, C.** (2006b).

606 The energetics of huddling in group-living African four-striped field mice *Rhabdomys*

607 *pumilio. Funct. Ecol,* **20**, 166–173.

608

609 **Schank, J.C and Alberts, J.R.** (1997). Self-organized huddles of rat pups modeled by

610 simple rules of individual behaviour. *J. Theoret. Biol.,* **189**, 11 – 25.

611

612 **Schradin, C., Schubert, M and Pillay, N.** (2006). Winter huddling in the striped mouse.

613 *Can. J. Zool.*, **84**, 693-698.

614

615 **Speakman, J.R and Rossi, F**. (1999). No support for socio-physiological suppression

616 effect on metabolism of paired white mice (*Mus sp*.). *Funct. Ecol.*, **13**, 373-382.

617

618 **Speakman, J.R.** (2000). The cost of living: field metabolic rate of small mammals. *Adv.*

619 *Ecol. Res.,* **30**, 178- 297.

620

621 **StatSoft, Inc.** (2004). Stadistica (data analysis software system) version 7.

622 www.statsoft.com.

623

624 **Withers, P.C.** (1977). Measurements of metabolic rate, V_{CO_2}, and evaporative water loss

625 with flow through mask. *J. Appl. Physiol.*, **42**, 120–123.

626 **Wojciechowski, M.S., Jefimow, M. and Pinshow B.** (2011). Heterothermy, and the

627 energetic consequences of huddling in small migrating passerine birds. *Integ. Comp. Biol.*

628 *51, 409-418.*

629

Zhang, L., Wang, R., Zhu, W.L.,Liu, P.F., Cai, J.H., Wang, Z.K., Sivasakthivel, S and Lian, X. (2011). Adaptative thermogenesis of liver in a tree shrew (*Tupaia belangeri*) during cold acclimation. *Anim. Biol., 61*, 385-401.

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Figure legend

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Figure 1. Mass-adjusted basal metabolic rate in *Octodon degus* as a function of the number of individuals grouped after a thermal acclimation of two months to two temperature conditions. The black bars represent the mean ± SE of BMR animals acclimated to 15 ° C, and the white to 30 ℃. Similar letters denote non-significant difference between treatments by a Fisher *a posteriori* test.

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Figure 2. Lower panel: Surface temperatures of the back (maximum), perimeter and contact of isolated and huddling *Octodon degus*, at 236C and 15 ℃ and 30 ℃. Similar letters denote non-significant difference between treatments by a Fisher a posteriori test. Upper panel: Thermal images are shown for isolated and huddling degus, exposed to an ambient temperature of 15 ℃. Examples of measurements of the temperature of the perimeter (Tp), maximum temperature (Tm) and temperature of the contact between individuals (Tc) are shown.

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664 Table 1. Body mass (bm), whole animal BMR and wet thermal conductance (Ct) of

665 *Octodon degus* acclimated (Ta) either at 15 or 30 °C for two months in groups of 3, 5 and

666 solitary individuals. Data are presented as mean ± SD. In parenthesis is shown the

667 coefficient of variation in percentage.

Ta	Group size	bm (g)	BMR (kJ h^{-1})	Ct (J g^{-1} h^{-1} °C^{-1})
	1	152.27 ± 12.20	2.78 ± 0.24 (8.7)	0.67 ± 0.25
15 °C	3	176.55 ± 9.80	2.59 ± 0.25 (9.6)	1.25 ± 0.84
	5	148.37 ± 17.70	1.96 ± 0.45 (23.1)	1.30 ± 0.38
	1	192.27 ± 10.37	2.60 ± 0.23 (8.2)	1.50 ± 0.33
30 °C	3	157.44 ± 8.16	1.92 ± 0.36 (18.8)	1.72 ± 0.17
	5	146.33 ± 13.60	2.19 ± 0.32 (14.6)	1.97 ± 0.42

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