The Ecology of Stress: linking life-history traits with physiological control mechanisms in free-living guanacos.

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Background-Providing the context for the evolution of life-history traits, habitat features constrain successful ecological and physiological strategies. In vertebrates, a key response to life's challenges is the activation of the Stress (HPA) and Gonadal (HPG) axes. Much of the interest in stress ecology is motivated by the desire to understand the physiological mechanisms in which the environment affects fitness. As reported in the literature, several intrinsic and extrinsic factors affect variability in hormone levels. In both social and non-social animals, the frequency and type of interaction with conspecifics, as well as the status in social species, can affect HPA axis activity, resulting in changes in the reproductive success of animals. We predicted that a social environment can affect both guanaco axes by increasing the secretion of testosterone (T) and Glucocorticoid (GCs) in response to individual social interactions and the energetic demands of breeding. Assuming that prolonged elevated levels of GCs over time can be harmful to individuals, it is predicted that the HPA axis suppresses the HPG axis and causes T levels to decrease, as GCs increase.

Methods-All of the data for individuals were collected by non-invasive methods (fecal samples) to address hormonal activities. This is a novel approach in physiological ecology because feces are easily obtained through non-invasive sampling in animal populations.

Results- As expected, there was a marked adrenal (p-value= .344e-12) and gonadal (p-value= 0.002656) response due to seasonal variation in *Lama guanicoe*. No significant differences were found in fecal GCs metabolites between males/females*season for the entire study period (p-value= 0.2839). Despite the seasonal activity variation in the hormonal profiles, our results show a positive correlation (p-value= 1.952e-11,COR=0.50) between the adrenal and gonadal system. The marked endocrine (r^2 = 0.806) and gonad (r^2 = 0.7231) response due to seasonal variation in male guanaco individuals highlights the individual's energetic demands according to life-history strategies. This is a remarkable result because no inhibition was found between the axes as theory suggests. Finally, the dataset was used to build a reactive scope model for guanacos.

Discussion-Guanacos cope with the trade-off between sociability and reproductive benefits and costs, by regulating their GCs and T levels on a seasonal basis, suggesting an adaptive role of both axes to different habitat pressures. The results presented here highlight the functional role of stress and gonad axes on a critical phase of a male mammal's life—the mating period—when all of the resources are at the

disposal of the male and must be used to maximize the chances for reproductive success.

1   The Ecology of Stress: linking life-history traits with physiological control mechanisms in free-living
2   guanacos
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Abstract

Background-Providing the context for the evolution of life-history traits, habitat features constrain successful ecological and physiological strategies. In vertebrates, a key response to life's challenges is the activation of the Stress (HPA) and Gonadal (HPG) axes. Much of the interest in stress ecology is motivated by the desire to understand the physiological mechanisms in which the environment affects fitness. As reported in the literature, several intrinsic and extrinsic factors affect variability in hormone levels. In both social and non-social animals, the frequency and type of interaction with conspecifics, as well as the status in social species, can affect HPA axis activity, resulting in changes in the reproductive success of animals. We predicted that a social environment can affect both guanaco axes by increasing the secretion of testosterone (T) and Glucocorticoid (GCs) in response to individual social interactions and the energetic demands of breeding. Assuming that prolonged elevated levels of GCs over time can be harmful to individuals, it is predicted that the HPA axis suppresses the HPG axis and causes T levels to decrease, as GCs increase.

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Discussion-Guanacos cope with the trade-off between sociability and reproductive benefits and costs, by regulating their GCs and T levels on a seasonal basis, suggesting an adaptive role of both axes to different habitat pressures. The results presented here highlight the functional role of stress and gonad axes on a critical phase of a male mammal's life—the mating period—when all of the resources are at the disposal of the male and must be used to maximize the chances for reproductive success.

Key-words: Stress ecology, hormonal profiles in wildlife, *Lama guanicoe,* sociality, reproduction, non-invasive methods.

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99   **Introduction**
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101   Natural selection shapes phenotypic traits that permit organisms to respond appropriately to intrinsic and

102   extrinsic factors (Roff, 1992; Stearns, 1992; Bozinovic, 2002; Nespolo et al., 2003). Genetic variation among

103   organisms determines the resource allocation trade-offs among life-history traits (e.g. age-specific growth,

104   reproduction and survival schedules, and sociability) and are also determined by phylogeny. Environmental,

105   ecological and indirect demographic processes (extrinsic factors) create selective pressure that impinge on an

106   organism's lifetime fitness (Stearns, 1992; Zera & Harshman, 2001). Therefore, understanding life-history variation

107   (from an evolutionary perspective) among taxa requires an understanding of the physiological mechanisms that link

108   genes with phenotype and how extrinsic factors regulate trade-offs between survival and reproduction.

109   Ecophysiological, and particularly knowledge about endocrine mechanisms, is key to understanding how life-history

110   variation and trade-offs arise and are retained (Ketterson & Nolan 1999; Zera & Harshman, 2001; Crespi et al.,

111   2013).

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113   The stress response is a mechanism by which an individual of a given species copes with environmental

114   changes (stressors) (Boosntra et al., 2001; Winfield, 2005; Romero et al., 2009; McEwen and Wingfield, 2010;

115   Ovejero and Carmanchahi, 2012; Ovejero 2013). Perception of a stressful situation activates the sympathetic

116   adrenomedullary system and the hypothalamic pituitary adrenocortical (HPA) axis (Mostl and Palme, 2002).

117   Activation of the HPA axis stimulates the release of steroid hormones (glucocorticoids GCs, e.g., corticosterone,

118   cortisol). GCs in turn activate the mobilization of energy necessary to cope with adverse environmental conditions

119   (Dallman et al., 2007; Sapolsky, 2000). In the last 30 years, the word "Stress" has been strongly criticized due to the

120   ambiguity of the term and several authors have been researching allostasis and attempting to connect biomedical and

121   ecological data (McEwen & Wingfield 2003; Romero et al., 2009, 2011). Allostasis is the daily and seasonal

122   process of maintaining physiological parameters (homeostasis) within life-sustaining ranges, via allostatic mediators

123   (McEwen & Wingfield, 2003).   The allostatic model has generated criticism and Romero et al. (2009) have

124   proposed the reactive scope model, to maintain the strengths but avoid the weaknesses of the allostatic model. The

125   present work contributes to the debate about the applicability of the allostatic model to nature.

126   Extrinsic factors, like anthropogenic disturbance, biological invasions, or climate change (in terms of

127   allostasis) can negatively affect behavior, reproduction, immune function and growth through prolonged activation

128   of the HPA axis (Moberg, 1991; Boonstra and Singleton, 1993; Goymann and Wilngfield, 2004; Pride, 2005;

129   Pereira et al., 2006). Glucocorticoids (GCs) are often considered the allostatic mediators of the stress response, and

130   they have been the focus of research for decades (Sapolsky, 2000; Ovejero 2013). Much less is known, however,

131   about the role of GCs' in free-living animals (Bonier et al., 2009). The role of baseline or stress-induced GC in the

132   modulation of an individual or populations performance, is still essentially unknown. Understanding, social factors

133   and their endocrine correlates (baseline measures of GCs and T) in wild animals has potential implications for

134   wildlife management, population ecology, reproductive biology and evolution. The social environment is therefore

135   one of the primary sources of information that can induce allostatic responses (Boonstra et al., 2001; Rubenstein and

136   Shen, 2009; Creel et al., 2013. In both social and non-social animals, the frequency and type of interaction with

137   conspecifics, as well as status in social species, can affect HPA and HPG axis activity and ultimately the

138   reproductive success of animals.

139   During the breeding season of many mammals, males commonly increase testosterone levels in response to

140   social stimuli such as presence of male competitors or potential mates (Enstrom et al., 1997; Cavigelli and Parer

141   2000; Sinervo et al., 2000; Ostner and Kappeler, 2002; Soto-Gamboa et al., 2005; Schradin, 2008; Creel et al., 2013.

142   This physiological mechanism (androgen response) modulated by social environment, is likely to be mainly

143   important when considering their roles (testosterone-mediated traits) in the evolution of mating systems and life

144   histories (Wingfield et al., 1990; Knapp and Moore, 1997; Faulkes and Abbott, 1997; Hirschenhauser and Oliveira,

145   2006; Young et al., 2006; Rubenstein et al., 2009; McGlothilin et al., 2010. In addition, testosterone levels can also

146   affect female preference (Enstrom et al.,1997), thus influencing fitness through both intra and intersexual selection;

147   (Sinervo *et al.,* 2000). Although the role of short-term androgen changes has not been completely described, these

148   have long been associated with territoriality; (McGlothilin et al., 2010). The "Challenge Hypothesis" (first described

149   in bird studies) predicts that androgen levels and agonistic interactions are positively related and are socially-context

150 dependent Wingfield et al. (1997). Among mammals, these predictions have recently been tested, and a positive

151 correlation was found between social cues and testosterone levels associated with territorial defense, group

152 formation, social dominance and hierarchy formation during the breeding season Muller and Wrangham (2004);

153 Creel et al. (2013). Consequently, social stressors provide a context where the individual must respond to a series of

154 repeated stressors over time, depending on the frequency and intensity of the stimulus, and this can lead to various

155 physiological problems, Sapolsky (2005).

156 Much of the variation in life histories strategies reflects individuals' phenotypic responses to environmental

157 challenges and perceived risks. The adrenal and gonadal system strongly influence behavior, control the annual

158 cycle of development, modulate behavioral and physiological responses to the environment, and establishes

159 important incompatibilities in life stages. It provides a model for studying the connections between mammal

160 physiology and life history. Guanacos (*Lama guanicoe*) are the largest social artiodactyls of South America, and

161 these organisms have a polygynous resource-defense mating system. This wild and endemic camelid has been

162 successfully used as a study model of behavioral interactions (De Lamo et al., 1998; Franklin, 1982; Marino and

163 Baldi, 2008, Taraborelli et al. 2014), ecological studies, (Puig and Videla, 1995; Bank et al., 2003; Puig et al., 2008;

164 Acebes et al., 2010; Ovejero et al. 2011; Ovejero, 2013; Marino and Baldi, 2014; Radovani, 2004 and management

165 (Montes et al., 2006; Zapata et al., 2002; Zapata et al., 2004), but information about the guanaco's physiological

166 ecology remains scarce. More recently, guanacos have also been used as a model of stress response; a study was

167 conducted to determine how management including handling, shearing, and release induce changes in circulating

168 steroid hormones Carmanchahi et al., (2011), however this work was based on an invasive sampling method.

169 To understand how physiology mediates the relationship between life history and the environment, we

170 predict that: (A)- Social environment positively will affect the HPG and HPA axes by raising the secretion of T and

171 GCs due to individual social interactions and the energetic demands of the breeding season. (B)-Male guanacos will

172 show seasonal variation in the activity of their adrenal and gonadal systems, which perform incompatible functions

173 in different life stages (survival vs reproduction). (C)-Prolonged elevated baseline levels of GCs in male guanacos

174 due to long periods of intense social interaction will be detrimental, costly, and decrease fitness, and we would

175 expect that in this case HPA inhibits the activity of the HPG. Together, these predictions provide a description of the

176 reactive scope model and an understanding of how physiological mechanisms might constrain patterns of variation

177 in wild South-American camelid life histories. These predictions might also provide the basis for future mammalian

178 studies targeted at understanding phenotype-environment interactions.

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181 **Methods**

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183 **Ethics Statement**

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185   The present study did not warrant capturing or handling protected or endangered animals. All of the data

186 for individuals were collected by non-invasive methods (fecal samples) to address hormonal activities. This is a

187 novel approach in physiological ecology because feces are easily obtained through non-invasive sampling in animal

188 populations. This is important because the handling necessary for collection of plasma can confound estimates of

189 baseline GC measurements. The described field studies were carried out in a protected area with a permit (files:NO

190 4350-000019112/Res 238) from the DRNR (Dirección de Recursos Naturales Renovables, Mendoza).

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194 **Non-invasive methods to address hormonal activities**

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196   Fresh fecal samples were collected from 334 wild adult guanacos from La Payunia reserve (36.000S and

197 36.360S; 68.340W and 69.230W, South of Mendoza Province, Argentina). The samples were collected during 7

198 field surveys (15 days each) over the course of a year. Fresh feces samples were collected from focal individuals

199 (one sample per each indiv.) and were labeled according to the sample location, time, sex, age, social structure and

200 environment variables. To slow microbial activity and to reduce immunoreactivity problems, the samples were froze

201 immediately in liquid nitrogen and stored at -20 C° until the time of analysis (LARLAC-IMBECU-CCT CONICET-

202 MENDOZA). Fieldwork was conducted during the breeding and non-breeding months to assess seasonal variation

203 in hormone levels. Furthermore, 100% of the sampling surveys were done in the same sites. Steroids were extracted

204 from lyophylized fecal samples according to a protocol developed for fecal steroid metabolite extraction for free-

205 range ungulates (for more detail see, Ovejero 2013).The commercial ^{125}I-cortisol and ^{125}I-testosterone RIA-KITS

206 (BECKMAN, Coulter Company IM-1841/IM-1119-Immunotech-, Prague, Czech Republic) were used to quantify

207 the levels of cortisol and testosterone in the samples (for more detail see, Ovejero and Carmanchahi, 2012;Ovejero

208 2013). The analytical sensitivity of the cortisol assay is 3.075 ng / mL. The antibody used in the immunoassay is

209 highly specific for cortisol (100% cross-reaction). The cross-reactivity to other naturally occurring steroids is very

210 low, for example: aldosterone <0.1%; 8.4% corticosterone; cortisone 1.5%; 11-desoxy-cortisol 18%, etc. The

211 analytical sensitivity of the testosterone assay is 0.025 ng / mL. The antibody used in the immunoassay is highly

212 specific for testosterone (100% cross-reaction). The cross-reactivity to other naturally occurring steroids is very low,

213 for example: 0.00014% aldosterone; estrone <0.3%; 0.08 androsterone; 5-Dihydrostestosterone 10% α; 11β-

214 Hydroxytestosterone 2%; corticosterone 8.4%; 1.5% cortisone; cortisol 11-deoxy-18%, etc. In addition, all samples

215 were assayed in triplicate (extraction efficiency test) and duplicate (binding and parallelism test) and reanalyzed

216 whenever the resulting coefficient of variation exceeded 20%. Intra- and inter-assay coefficients of variation of

217 GCs-immunoassay were 6.5% and 11.2% and for testosterone immunoassays were 9.5 and 13.4%, respectively.

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221 **Statistical analyses**
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223 Welch two sample T-tests were used to evaluate the seasonal variation response of steroid hormones.

224 Furthermore, two-way ANOVAs were used to evaluate the interaction between sex and season. Generalized linear

225 models were developed (n polynomials function $= f(x) = a_nX^n + a_{n1-1}X^n - 1 + a_{n-2}X^n - 2 + \ldots$) to describe the

226 hormonal profiles and their seasonal variation in response to the environment. Moreover, a Pearson's correlation

227 was used to test the activity association between the adrenal and gonadal system throughout the year. All statistical

228 analyses were conducted using R statistical software version 3.1.0 (Development Core Team, 2012).

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230 **Results**
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233 **Ecological process and fecal-hormone profiles in wild guanacos**
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235 As expected, there was a marked adrenal (t=7.4016, df = 302.174, p-value = 1.344e-12, 1-α(0.90)=

236 7.661734-12.057391, mean =25.75864/15.89907 breeding and non-breeding season respectively) and gonadal

237 (t=3.2873, df = 28.948, p-value = 0.002656, 1-α(0.90)= 0.6213-1.9508, mean=3.209810/1.923700 breeding and

238 non-breeding season respectively for male groups) GCs and T response due to seasonal variation in *Lama guanicoe*

239 individuals. This highlights the individual's energetic demands according to life-history strategies. No significant

240 differences were found in the amounts of fecal GC metabolites between males/females*season for the entire study

241 period (F= *1.0757, df =143.529, p-value =* 0.2839; Male: ng/gr=22,42,1-α(0.90)=1.6887,std-e=0.86,

242 n=251;Female:ng/gr=20,71,1-α(0.90)=2.6079, n=76, std-e=1.33). This result was not expected as it was thought that

243 there would be a difference in the allocation of resources to cope with the different roles that that males and females

244 have in a population. The second prediction in our study is partially accepted. Despite the seasonal activity variation

245 in the hormonal profiles, our results show a positive correlation (t = 7.1524, df = 184, p-value = 1.952e-11,

246 CI=0.3457400 0.5719168, COR=0.50) between the adrenal and gonadal system; both peaks of activity concur with

247 the reproductive season. (C)-Prolonged elevated baseline levels of GCs due to long periods of intense social

248 interaction are detrimental, costly and decrease fitness; it is expected that in this context, HPA inhibits the activity of

249 the HPG. The marked endocrine (y= 0.0743x5 - 1.9677x4 + 19.136x3 - 82.442x2 + 147.4x - 58.902; r^2 = 0.806) and

250 gonad (y= 0.0041x5 - 0.1102x4 + 1.0889x3 - 4.6819x2 + 7.914x - 0.7892; r^2 = 0.7231) response due to seasonal

251 variation in male guanaco individuals (Figure 1) highlights the individual's energetic demands according to life-

252 history strategies. This is a remarkable result because no inhibition was found between the axes as theory suggests.

253 Finally, the dataset was used to build a reactive scope model for guanacos, see figure 2. Most studies measure only

254 one physiological mediator at a time; here the concentration of two mediators is placed on the y-axis for a given

255 time point. This was done because these mediators are correlated in the functional role that each one plays in the

256 normal reactive scope range. In other words, both mediators encompass responses to cope with predictable and

257 unpredictable changes in the environment. The values of each mediator are presumed to exist in four general ranges.

258 Our result shows the ranges of predictive/reactive homeostasis and homeostatic overload/failure. These ranges

259 consist of the seasonal set of point ranges for the physiological mediators.

260 **Discussion**
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262   Our results confirm the prediction that "Social environment positively affects the HPG and HPA axes by

263 raising the secretion of T and GCs due to individual social interactions and the energetic demands of the breeding

264 season." Further to this, our results suggest that social environment and breeding season are stressful scenarios for

265 male and female guanacos. These scenarios are seen to produce associated costs such as increased metabolic rate

266 and energy expenditure due to: territorial/female defense (Male-reproduction strategies), male and female social

267 dominance hierarchies, reproduction, and increased exposure to predators during the breeding season. If the data

268 value observed in March is taken as a baseline cortisol level, cortisol is seen to increase by one and a half times

269 during the non-breeding season and three times during the breeding season (Fig. 1). If the March testosterone

270 measurement is taken as the basal testosterone level, the same pattern is seen; the hormonal profile increases during

271   the non-breeding and breeding seasons (Fig.1). This highlights the importance of the functional roles of the HPA

272   and HPG axes during the reproductive period, as well as the adaptive roles they play in the seasonal pattern

273   observed, allowing the species to meet environmental challenges as well as challenges throughout its life cycle.

274   From the results gathered in this study, different immunoreactive GCs and T secretion patterns were

275   observed according to the mating system and reproduction strategies (territorial defense and female defense) that

276   guanaco males (of a migratory population) adopt during the breeding season (Ovejero 2013). Our results highlight

277   the functional role of the stress and gonad axes during the critical mating period when all of the resources at a male's

278   disposal must be used to maximize the chances of reproductive success. Generally, it appears that T is immediately

279   involved in aggression associated with reproduction, rather than with other forms of aggression (e.g., anti-predator

280   aggression), see Moyer (1968); Wingfield and Marler (1988). The data here presented support the correlation

281   between T patterns and reproductive strategies (establishment and maintenance of a breeding territory and mate-

282   guarding) proposed by Wingfield *et al.,*(1990) for male birds. However, this study extends Wingfield's finding to

283   mammals. In 1984, the same authors proposed that males of polygynous species may have higher levels of T for

284   longer periods during the breeding season than do males of monogamous species. This correlation was strengthened

285   by the finding that when T was implanted in normally monogamous males, high T levels were maintained for longer

286   than average periods than when these males became polygynous. Our results highlight the functional role that T

287   levels have in determining and maintaining the polygynous mating system in guanacos and south american camelid

288   wildlife, but we predict that this pattern could be altered in guanaco populations that coexist with human activities

289   that fragment the guanaco's normal distributional range. For example, the use of fences by rural settlers has a direct

290   impact on the space used by guanacos, and it is possible that this could promote some groups (within a population)

291   to adopt monogamous behavior.

292   On the other hand, our results highlight the notorious decrease in the activity pattern of the stress and

293   gonadal axes during the non-breeding season (Figure 1). This result was not expected because individuals must cope

294   with another critical phase of autumnal migration at this time, therefore we expected that all of the resources (in

295   terms of energy) would be available to maximize the chances for survival success during the migration trip. These

296   results confirm our prediction that "guanacos show a seasonal variation in the activity of their endocrine and gonadal

297   systems and incompatible functions (reproduction an self-maintenance) are established during different life stages."

298   In winter, food intake by birds and mammals needs to be geared towards preventing starvation while at the same

time maintaining an optimum body mass to permit rapid escape from predators. GC concentrations may be the

300 critical factor determining the size of the fat reserves and either environmental severity or predation risk; Winglfied

301 (1990). The GC hormone, corticosterone, is thought to be important in the physiological orchestration of avian

302 migration because of the identified elevated level of activity of the avian adrenal gland either prior to or during

303 migration; Naik and George (1963); John (1965); Peczely (1976). Preparation for migration and for winter requires a

304 period of hyperphagia and a laying down of fat reserves (for a review, see Holberton et al. (1999). In birds,

305 Wingfield and Silverin (1986); Astheimer et al. (1992) and mammals, Dallman et al. (1993), GCs play a vital role in

306 foraging behavior and hyperphagia, with low to moderate concentrations stimulating feeding behavior. As is noted

307 by O'Reilly and Wingfield (1995) migrating birds show increased baseline concentrations of GCs but a reduced

308 stress response. An adaptive explanation for this pattern is the migration modulation hypothesis, Holberton et al.

309 (1996, 1999). It proposes that higher than average concentrations of baseline GCs are expressed prior to and during

310 migration to facilitate migratory fattening. However, the stress response is lowered when challenged so that the

311 negative, catabolic effects of high GCs on skeletal muscle do not occur; thus, critical skeletal muscles are preserved

312 for flight during migration. As such, this hypothesis may explain the non-breeding season hormonal profiles

313 generated in this study, but more data is needed to test these assumptions. Another explanation may suggest that

314 migration (in terrestrial system) could be regulated by other endocrine mechanisms (and other hormones) involved

315 in generating fat reserves and foraging behavior such as those that involve the thyroid gland Schwabl et al. (1984).

316 Mattocks (1976) shows that gonad hormones (at least in some species) have a functional role (fat deposition) before

317 spring migration. This may suggest that the increased activities of the gonad axis compared with the stress axis in

318 guanacos could have an important role in the "migration to and from the winter range", but to confirm this

319 prediction more experimental and field studies would be needed.

320 The last prediction of this study, that "prolonged elevated baseline levels of GCs due to long periods of

321 intense social interaction are detrimental, costly and decrease fitness" was not validated by the results gathered. Our

322 interest in the relationships between social or environmental conditions and stress-gonadal hormone concentrations

323 (for both social and non-social species) is motivated by a desire to understand the physiological mechanisms by

324 which the environment affects fitness. However, understanding the relationship between hormonal profiles and

325 fitness is not straightforward, therefore this study attempts to obtain proxies that would help us begin to resolve this

326 relationship. We consider that taken together, the interplay between stress/gonad hormones across seasonal variation

327   could be an interesting proxy to "establish" causal relationships between hormonal levels and ecological correlates.

328   For example, some authors have stated that prolonged elevated baseline levels of GCs are commonly viewed to be

329   detrimental, costly and decrease fitness, Bonier et al. (2009); However, there is scarce evidence for this negative

330   association.   Part of the problem with this approach is the variation among species in terms of their

331   hormones/fitness relationships, and this variation can be attributed to a variety of demographic, social (e.g. social

332   rank, age, sex, group size) or environmental factors (e.g. resource availability). Additionally, for a variety of

333   vertebrate taxa, seasonal variation in GC levels has been reported to be due to varying food availability, temperature,

334   rainfall and tourist activity Romero (2002); Tempel and Gutierrez (2004), however it still remains unclear if this

335   holds true for mammals. The other issue is that many of the studies of HPA-fitness relationships reviewed by Bonier

336   et al. (2009) were conducted during the breeding season alone. The study herein presented provides a full set of data

337   (hormone profiles) across seasonal variation throughout the course of an entire year. As expected, guanacos cope

338   with this trade-off between sociability and reproductive benefits and costs by regulating their GCs and testosterone

339   levels on a seasonal basis like other mammals Boosntra (2005), and they may also experience fine adjustments on a

340   daily basis. We suggest that elevated levels of GCs in guanacos due to highly social interactions during the breeding

341   season may play a fundamental role in the regulation of enhanced metabolic needs during reproduction. Guanaco

342   males, like other iteroparous males, exhibit high concentrations of cortisol, a gonadal axis that is not inhibited by

343   high GC concentrations, so the last prediction presented in this work must be rejected. Furthermore, during the

344   breeding season, our results show two GC peaks of activity, one at the beginning and another at the end of the

345   breeding season. In the former, guanaco males are exposed to intense social instability caused by agonistic

346   interactions with other males due to territory establishment and hierarchies. In the latter, guanaco males are exposed

347   to female defense interactions. This suggests that for guanaco males, female defense strategies demand higher

348   energetic costs when compared to territory defense strategies. This is expected if one assumes that defending a

349   female (rather than a territory) is the principal objective (and target) of a male that wants to maximize the

350   probabilities of leaving an offspring. Moreover, birth rate data for the population was collected from 2007-2008

351   (hormonal measures)-2009, so if one expects a negative relationship between the hormonal profiles, one would also

352   expect to see a negative value for the proportion of offspring per adult. The birth rate values for those years were

353   0.14, 0.28 and 0.29 respectively; thus with these results one can expect that there is no detrimental effect on

354   individual-population fitness.

355   The lesser HPA axis activity measured during the non-breeding season confirms the prediction "that

356   reproduction and sociability may be long-term and predictable." This is likely the case because this period is

357   associated with fewer social interactions. The individual hierarchies are more relaxed because the animals are

358   preparing to migrate (Novaro pers. com.) and the species strategy at this time is to survive and to not reproduce.

359   Finally from the data collected here, a reactive scope model for guanacos has been generated, see figure 2.

360   The results show the ranges of predictive/reactive homeostasis and homeostatic overload/failure ranges; these

361   consist of the seasonal set of point ranges for the physiological mediators. For guanacos, the normal reactive scope

362   is described with two GCs and T set point ranges. The model was made to reflect the natural progression of life-

363   history stages such as breeding (figure 2). Furthermore, the minimum concentration of the mediator was presumed

364   to constitute a threshold. Subsequently, a GC and T threshold was proposed, and below this limit it is seen that the

365   individual/population enters into homeostatic failure. This threshold is predicted to indicate the minimum

366   concentration of GCs and T mediator necessary to sustain guanacos seasonal life changes (in natural conditions).

367   However, more studies are needed to test this prediction. On the other hand, above the upper end of the normal

368   reactive scope, when a physiological mediator exceeds the reactive homeostatic range, the individual/population

369   enters into homeostatic overload (pathological states). This threshold is predicted to indicate the maximum

370   concentration of GCs and T mediator necessary to sustain seasonal life changes in guanacos (in natural conditions).

371   However, more studies are needed to test this prediction especially considering that pharmacological stimulation

372   may allow individuals to reach a higher threshold though this would not be considered within the scope of natural

373   changes.

374   **Conclusions**
375
376   Knowledge of physiological and behavioral mechanisms is key to understanding how life-history variation

377   and trade-offs might arise and are maintained to cope with environmental variation. Mechanistically, trade-offs

378   result from the need to differentially allocate limited resources to traits like reproduction versus self-maintenance

379   with selection favoring the evolution of optimal allocation mechanisms. Guanacos cope with the trade-off between

380   sociability and reproductive benefits and costs by regulating their GCs and T levels on a seasonal basis. This

381   suggests the adaptive and functional role of both axes in regulating energy allocation for "predictable" life-history

382   events. During breeding and highly social interactions, elevated baseline levels of GCs and T may actually be

383   predictive of high reproductive success. Our results support the assumption that GCs via the HPA axis did not

384   suppress the HPG axis and did not diminish T levels. From this, it is proposed that guanacos anticipate stressors in

385   which the impact on the animal is acute and the duration is moderated by physiological consequences (i.e

386   reproduction). During the non-breeding season, a period with less intense social interactions, lower baseline levels

387   of GCs and T may actually be predictive of a high probability of survival. Our study provides a model for studying

388   the link between physiology and life history.

389

390   Studying both the HPA axis and the HPG axis is one of the best ways to understand the functional

391   mechanisms that an animal uses to cope with stress. Studying these two axes are of importance because the key

392   circulating steroid hormones of the stress axis, GCs, influence the expression of approximately 10% of the genome.

393   Some of the targets of GCs include genes controlling metabolism, growth, repair, reproduction and the management

394   of resource allocation Lee et al. (2005). However, both axes play fundamental roles not just when the animal is

395   experiencing stress, but during normal periods of survival and growth. These axes mediate the adaptation of the

396   organism to its environment. At the individual level, the stress axis plays a key role in allowing animals to respond

397   to the changes and challenges of both environmental certainty and uncertainty. At the species level, the stress axis

398   plays a central role in evolutionary adaptation to particular ecological pressures.

399

405

406   Figure 1. HPA & HPG axis activity patterns throughout the study period. Blue line= the seasonal baseline fecal

407   testosterone profile (defined by y= $0.0041x^5 - 0.1102x^4 + 1.0889x^3 - 4.6819x^2 + 7.914x - 0.7892$; $r^2 = 0.7231$), Pink line=

408   the seasonal baseline fecal cortisol profile (defined by y= $0.0743x^5 - 1.9677x^4 + 19.136x^3 - 82.442x^2 + 147.4x - 58.902$; r^2

409    $= 0.806$). There was marked adrenal and gonadal activity during the breeding and non-breeding (CI = 7.661734-

410   12.057391, mean =25.75864/15.89907 respectively) season respectively for the male groups, the GCs and T response was

411   due to seasonal variation in the guanacos highlighting the individual's energetic demands according to life-history

412   strategies.

413

414 Figure 2. Most studies measure only one physiological mediator at a time, here the concentration of two mediators

415 (cortisol-C /testosterone.T) is placed on the y-axis at a given time point. The mediators are correlated in terms of the

416 functional role that each one plays in the normal reactive scope range. In other words, both mediators encompass

417 responses for coping with predictable and unpredictable changes in the environment. The values of each mediator

418 are presumed to exist in four general ranges. The minimun (5-10 Ng.gr for C;1-2 Ng.gr for T) and maximun (20-35

419 Ng.gr for C;3-4 Ng.gr for T) concentration of the mediator was presumed to constitute a threshold. The results show

420 the ranges (double arrows) of predictive/reactive homeostasis (PH/RH) and homeostatic overload/failure, the stress-

421 gonadal and feedback response (gray circles) indicates the max-min activity patterns for HPA & HPG axis during

422 one year. The C activity patterns in guanacos shows two peak of RH (dotted lines) in the reproductive season,

423 between Ag./O. males adopts a territory-defense strategies at the beginning of the reprod. season;   during Ja./F.

424 males changes to a female-defense strategies at the ends of the reprod. season. These results consist of the seasonal

425 set of point ranges for the physiological mediators.

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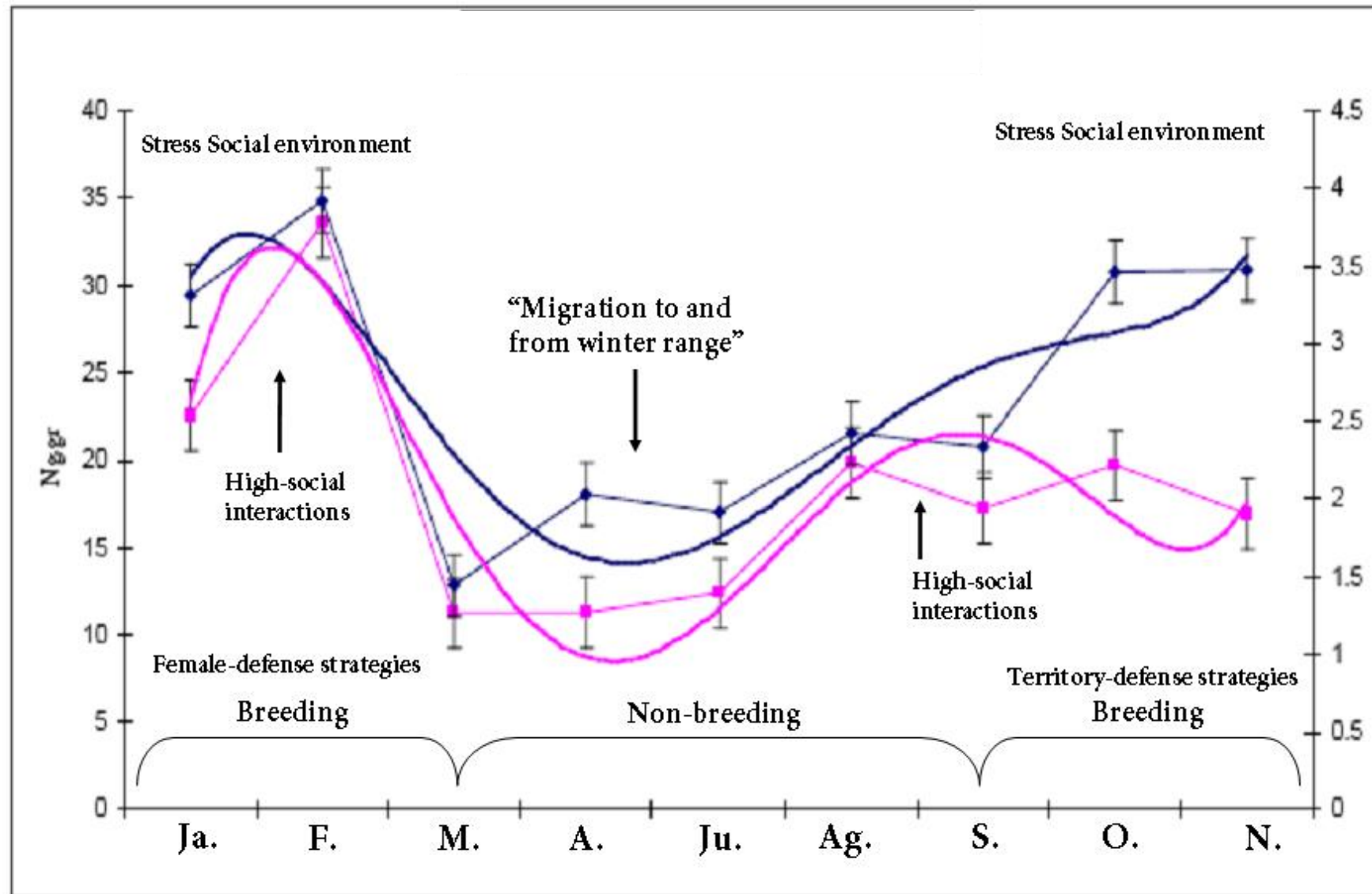716
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Figure 1(on next page)

HPA & HPG axis activity patterns throughout the study period

Figure 2(on next page)

The reactive scope model for guanacos