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Plasma concentrations of progesterone and estradiol and the relation to reproduction in Galápagos land iguanas, *Conolophus marthae* and *C. subcristatus* (Squamata, Iguanidae)

Michela Onorati[a,f], Giulia Sancesario[b], Donatella Pastore[c], Sergio Bernardini[d], Jorge E. Carrión[e], Monica Carosi[a], Leonardo Vignoli[a], Davide Lauro[c], Gabriele Gentile[f,*]

[a] *Department of Science, University Roma Tre, Rome, Italy*
[b] *Department of Clinical and Behavioral Neurology IRCC S. Lucia-Rome, Italy*
[c] *Department of Systems Medicine, University of Rome Tor Vergata, Italy*
[d] *Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, Italy*
[e] *Direction of the Galápagos National Park, Puerto Ayora, Santa Cruz Island, Galápagos Islands, Ecuador*
[f] *Department of Biology, University of Rome Tor Vergata, Italy*

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ABSTRACT

In a combined approach, endocrine and ultrasonic analyses were performed to assess reproduction of two syntopic populations of terrestrial Galápagos iguanas the *Conolophus marthae* (the Galápagos Pink Land Iguana) and *C. subcristatus* on the Volcán Wolf (Isabela Island). The ELISA methods (enzyme-linked immunosorbent assay) were used to measure plasma concentrations of progesterone (P4) and 17β-estradiol (E2) from samples collected over the course of three different seasons: July 2010, June 2012–2014. As for *C. subcristatus*, the large number of females with eggs in 2012 and 2014 were associated with increased plasma P4 concentrations and the corresponding absence of females with eggs in July 2010 when concentrations of both hormones levels were basal indicating reproduction was still ongoing in June and had ended in July. In *C. marthae*, even though there was a positive relationship between egg-development stages and hormone concentrations, P4 concentrations were basal through the three years that samples were collected, with some females having a lesser number of eggs compared with *C. subcristatus*. In *C. marthae* P4 and E2 patterns did not allow for defining a specific breeding season.

1. Introduction

Reproductive activity in reptiles is associated with an essential variation in circulating concentrations of the primary sex steroid hormones: progesterone (P4) and 17β-estradiol (E2) (Jones and Guillette, 1982; Crews and Silver, 1985; Wibbels et al., 1992; Edwards and Jones, 2001; Taylor et al., 2004; Norris and Lopez, 2010). Reproductive rhythms, sexual behaviors, physiological processes correlated to reproduction such as mating, gestation and oviposition are under a complex endocrine control which involves the activity and regulation of the hypothalamic-pituitary-gonadal axis (HPG) on sex steroid hormone production (Licht, 1979; Crews and Silver, 1985).

* Corresponding author at: University of Rome Tor Vergata, Department of Biology, Via della Ricerca Scientifica 1, 00133, Rome- Italy.
E-mail address: gabriele.gentile@uniroma2.it (G. Gentile).

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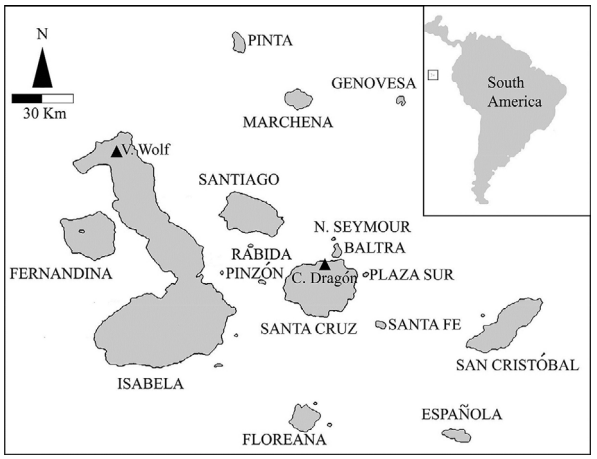

Fig. 1. The Galápagos archipelago.

In reptiles, surveys focused on sex steroid hormones indicate the importance of P4 in regulating the oocyte maturation and in maintaining gestation (Callard et al., 1992; Custodia-Lora and Callard, 2002). P4 has a role in determining the timing of oviposition (Norris, 2007), in inhibiting oviductal contractility (Guillette and Jones, 1985; Edwards and Jones, 2001), and in maintaining pregnancy (Guillette et al., 1991).

The E2 is the primary estrogenic steroid hormone in reptiles (Norris, 2007). Ovarian development and the vitellogenesis process (yolk production) are usually associated with elevated plasma concentrations of E2 in squamates (Bonnet et al., 1994), turtles (Ho et al., 1981), alligators (Guillette et al., 1997) and iguanas (Uribe et al., 1996). Vitellogenesis is clearly an estrogen-dependent process; E2 regulates the synthesis of vitellogenin by the liver and the yolk protein accumulation in blood and oocytes (Licht, 1979; Ho, 1987). Moreover, E2 has an important role in inducing sexual behavior during the mating period (Whittier and Tokarz, 1992; Rhen and Crews, 2000); indeed, exogenous administration of estrogen stimulates female sexual receptivity in some species of lizards (Crews, 1975; Valenstein and Crews, 1977). Thus, investigating circulatory concentrations of sexual hormones can be very informative of the reproductive status of wild reptiles and may prove very useful when conservation is also an issue, especially when the duration of field investigations, that allow direct observations, is strongly limited by logistic constraints.

This is the case of the pink iguana from the Galápagos *Conolophus marthae*, a species recently discovered (Gentile and Snell, 2009; Gentile et al., 2009) and listed as Critically Endangered in the IUCN Red List (Gentile, 2012). The species inhabits only the upper portion and northwest slopes of the Volcán Wolf (0.046944°, −91.362205° – Isabela Island), the highest peak (1,707 m) and one of the most remote and difficult field sites in the Galápagos archipelago (Fig. 1). Threats to this species include small population size, extremely limited population distribution, introduced predators and possible competition with a syntopic population of *C. subcristatus* (Gentile et al., 2016). Unlike the pink iguana, *C. subcristatus* is widely distributed across the archipelago, including the Isabela Island and Wolf volcano.

Little is known about the reproductive biology of these species. The available information is incomplete and only exists for *C. subcristatus* for which previous studies indicated that clutch size and mating season vary across islands (Werner, 1983; Snell et al., 1984). Information on the reproductive biology and ecology of the *C. marthae* is limited to circumstantial observations.

As the two syntopic species may compete for nesting sites, understanding times and modes of reproduction is important particularly as to whether the two populations have overlapping reproductive seasons. Unfortunately, no previous studies of sexual hormones of the *Conolophus* species exist. Sexual steroids were, however, investigated in the marine iguana *Amblyrhynchus cristatus* (Rubenstein and Wikelski, 2005; Vitousek et al., 2010; Vitousek and Romero, 2013), the sister taxon of *Conolophus* (Rassmann et al., 1997). Such studies focused primarily on how baseline patterns of sex steroids vary during the breeding season in relation to female aggression (Rubenstein and Wikelski, 2005), receptivity (Vitousek et al., 2010), and mate selection (Vitousek and Romero, 2013). In *A. cristatus,* physiological changes in circulating hormones affect reproductive biology. The P4 and E2 concentrations were reported to be associated with different reproductive processes and there were distinct hormonal patterns during mating and nesting periods. The P4 concentration was elevated at the beginning of mating period but decreased towards the end, increased again at the beginning of nesting period, and subsequently there was a sustained decrease throughout the nesting phases (Rubenstein and Wikelski, 2005). On the contrary, E2 apparently stimulated mate attraction and receptivity of female marine iguanas; plasma concentration of E2 was basal during all nesting phases but peaked during the mating period when stimulation of the vitellogenesis process occurred and appeared to modulate aggressive behavior (Rubenstein and Wikelski, 2005).

Considering the background information available for marine iguanas and the sister taxon relationship between *Amblyrhynchus* and *Conolophus*, in the present study *A. cristatus* were used as a reference biological system with a combined approach of biometric, endocrine and ultrasonic analyses to examine and explain baseline plasma concentrations of P4 and E2 in the two syntopic populations of terrestrial Galápagos iguanas, *C. marthae* and *C. subcristatus*.

2. Materials and methods

2.1. Ethics statement

Animal manipulation and blood sampling were performed according to a protocol that minimized animal stress, in accordance with the European Community guidelines and with the approval of the Galápagos National Park. Samples were exported and imported under the CITES permits 101/BG and IT/IM/2015/MCE/01711.