How does a viviparous semifossorial lizard reproduce? *Ophiodes intermedius* (Squamata: Anguidae) from subtropical climate in the Wet Chaco region of Argentina

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ABSTRACT

The best predictors of reproductive patterns are commonly associated with climate factors, but evolutionary history also plays an important role. The semifossorial and viviparous lizard *Ophiodes intermedius* from the Wet Chaco region of Argentina showed an annual cycle with asynchrony between males and females and an unusual pattern for subtropical climates, with vitellogenesis beginning in autumn, ovulation and copulation in spring, and births occurring in summer. Males exhibited annual variation of testicular size associated with spermatogenic activity, reaching their maximum gonadal activity in late summer (March), but sperm storage in the epididymis and/or deferent duct occurred throughout the year. Females showed an extended reproductive cycle beginning in mid-autumn (May) with vitellogenesis and finishing with births from late spring to mid-summer (December to February). Litter size varied from 4 to 9 offspring. Females reached sexual maturity at a larger snout–vent length and, overall, showed greater body size than males, while males exhibited larger heads than females. Fat body cycles indicated that females use lipid reserves to support vitellogenesis and embryo development, while males allocate lipid resources to the search for females, courtship and copulation rather than to gametogenesis. *Ophiodes intermedius* differed from other species of the genus in litter size, gestation period, timing of birth and the minimum size at sexual maturity, probably as a result of the influence of ecological, historical and phylogenetic factors.

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1. Introduction

A diversity of reproductive patterns have been registered among Squamates (Fitch, 1970, 1982; Vitt, 2015), ranging from continuous reproductive cycles (Vitt, 1983; Mojica et al., 2003; Watling et al., 2005; Lozano et al., 2015), characteristic of the tropics (Brown and Shine, 2006; Vitt, 2015), to seasonal reproductive patterns, present in a variety of habitats, from tropical to temperate areas (Fitch, 1970, 1982; Fitzgerald et al., 1999; Brown and Shine, 2006; Medina and Ibargüengoytía, 2010). Oviparous species frequently reproduce during spring and summer months (Fitch, 1982; Vitt, 2015), while for some viviparous lizards, gametogenesis, ovulation and mating occur in autumn and births during spring, representative of an autumn reproductive cycle (Guillette and Casas-Andreu,

1980, 1987; Guillette and Méndez-de la Cruz, 1993; Ramírez-Pinilla et al., 2009). There is a third pattern, the "partial autumn reproductive cycle", in which vitellogenesis begins in autumn, ovulation and copulation occur in spring, and births take place in summer (Ramírez-Pinilla, 1992, 1995; Medina and Ibargüengoytía, 2010; Méndez-de la Cruz et al., 2013). The autumn reproductive patterns are frequently observed in species that inhabit high elevations in tropical and subtropical latitudes (Guillette and Casas-Andreu, 1980, 1987; Guillette and Méndez-de la Cruz, 1993; Ramírez-Pinilla, 1991, 1992, 1995; Ramírez-Bautista et al., 2014) or high latitudes at low elevations in Patagonia, Argentina (Boretto and Ibargüengoytía, 2009; Boretto et al., 2014).

The reproductive phenotypes are shaped by the interplay between the evolutionary history of a lineage (James and Shine, 1985; Dunham et al., 1988; Mesquita and Colli, 2010) and other biotic and abiotic factors (Vitt and Caldwell, 2014). For example, among abiotic factors, the most relevant are: temperature, photoperiod (Licht, 1971, 1973) and rainfall (Marion, 1982; Colli,

1991); and among biotic factors: food availability (Ballinger, 1977; Dunham, 1982) and the resulting energy storage as fat body masses (Hahn and Tinkle, 1965; Derickson, 1976; Bonnet et al., 1998; Warne et al., 2012), predation risk (Brown and Shine, 2006) and social structure (Ibargüengoytía and Cussac, 1999). However, the way these different factors affect fossorial lizards is still understudied (How and Shine, 1999; Resende and Nascimento, 2015).

Ophiodes Wagler, 1828 [RS1] is a Neotropical genus of the family Anguidae (Pyron et al., 2013) with only five recognized species (Cacciali and Scott, 2015), characterized by cryptozoic habits, a long, limbless cylindrical body, and a viviparous reproductive mode (Cei, 1993; Pough et al., 2016). *Ophiodes intermedius* Boulenger, 1894 [RS2] is distributed in Bolivia, Paraguay, and the northern and central parts of Argentina (Cei, 1993; Herrera et al., 2001), where it is present in grasslands (Cei, 1993) and also in urban areas (Herrera et al., 2001). It feeds on insects and spiders (Cabrera, 2009), but little is known about its reproductive biology, probably due to its cryptozoic habits which impede any ecological study (Pizzatto, 2005). The investigation of the reproductive biology of *Ophiodes fragilis* (Pizzatto, 2005) and *Ophiodes* cf. *striatus* (Montechiaro et al., 2011) from the southeast of Brazil, and of *Ophiodes fragilis* (formerly *O. striatus*) from the Atlantic Forest of Brazil (Barros and Teixeira, 2007) revealed an annual reproductive cycle for females (Pizzatto, 2005; Montechiaro et al., 2011) and a continuous male cycle with sperm storage in the deferent duct until the mating season (Pizzatto, 2005; Montechiaro et al., 2011).

Here we present the male and female reproductive and fat body cycles, minimum adult size, litter size, and sexual dimorphism of *Ophiodes intermedius* from the Wet Chaco region of Argentina (subtropical climate). The reproductive biology of this semifossorial lizard is discussed in relation to environmental variables and compared with other Neotropical congeneric and sympatric species in order to better understand the possible evolutionary causes of the reproductive pattern observed.

2. Materials and methods

2.1. Study area and climate

O. intermedius was studied in the grasslands of Chaco and Corrientes provinces (Argentina), in the eastern district of the Chaco phytogeographic province known as Wet Chaco (Cabrera and Willink, 1973; Cabrera, 1976; Carnevali, 1994).

The climate in the northeast of Argentina is mainly warm and subtropical; there is no dry season although winter rainfall is significantly low (Cabrera, 1976 Bruniard, 1997). The average annual rainfall varies from 1000 to 1500 mm. The mean annual temperatures vary from 20 to 23 °C; the mean minimum temperatures occur in July and vary from 13 to 16 °C, while the mean maximum temperatures are recorded in January and vary from 26 to 28 °C (Cabrera, 1976; Carnevali, 1994 Bruniard, 1997). Frosts are uncommon and only registered in winter (Carnevali, 1994).

2.2. Sampling and laboratory methods

Males ($n = 16$) and females ($n = 25$) were collected from October 2009 until March 2014, with different capture techniques, such as visual search and capture by hand, pitfall traps with drift fences and artificial shelters. In addition, in order to complete our sample, we used 4 adult males (UNNEC: 0849, 6426, 6835, 7237), 5 adult females (UNNEC: 5031, 5714, 6794, 1185, 10056) and 1 juvenile (UNNEC: 5163) from the herpetological collection of the Universidad Nacional del Nordeste at Corrientes (UNNEC). The specimens were collected in the following localities: Rincon Chico Ombu, Ituzaingó Department (27°16′S, 56°25′W), Paso de

la Patria (27°19′20″S, 58°34′47″W) and Santa Ana de los Guácaras (27°21′S, 58°39′W), San Cosme Department, Mburucuyá, Mburucuyá Department (28°03′S, 58°14′W), Concepción, Concepción Department (28°24′S, 57°53′W), San Cayetano (27°33′22″S, 58°40′33″W) and Corrientes city (27°28′50″S, 58°44′24″W), Capital Department of Corrientes province, and Campo Largo, Independencia Department (27°26′49″S, 58°38′43″W) of Chaco province.

Lizards were sexed and then weighed (body mass, BM) to the nearest 0.01 g with a digital balance (Ohaus traveler scale TA320; Ohaus, Parsippany, NJ, USA) before euthanasia. Sexual dimorphism was studied considering the following variables measured with a digital vernier caliper to the nearest 0.01 mm: snout–vent length (SVL), head length (HL), head width (HW) and head height (HH) at the level of the interparietal scale, neck width (NW), hip width (HipW) measured as body width at the insertion of the vestigial hind legs, and maximum body width (BW). Specimens were euthanised by intraperitoneal administration of articaine and L-adrenaline, and the testes and ovaries were dissected out to be measured and processed. After fixation in Bouin's solution for 24 h, lizards were stored in 70% ethanol and deposited at the herpetological collection of the Universidad Nacional del Nordeste (UNNEC), Corrientes province (UNNEC: 10576, 10577, 13013-13051).

2.3. Male reproductive cycle

The male gonadal cycle was determined based on macro- and microscopic observations. Testes size (TS) was measured as the antero–posterior diameter using a digital caliper (± 0.1 mm). Gonads were dehydrated in a graded ethanol series and embedded in paraffin for 24 h in the oven at 52 °C. Following a histological conventional protocol, sections of 5 μm were cut with a rotary microtome (Arcano KD-1508A; Zhejiang Jinhua Kedi Instrumental Equipment Co., Jinhua, China) and stained with hematoxylin and eosin.

Spermatogenic stages were determined based on the most advanced cell type present in the seminiferous tubules following Mayhew and Wright (1970), and cell types were recognised based on Uribe (2002). Five spermatogenic stages were defined: (I) only spermatogonia, (II) primary and secondary spermatocytes, (III) round spermatids and spermatids in the process of elongation, (IV) spermatozoa in the seminiferous tubules, and (V) regression with cellular debris and scarce spermatozoa in the tubular lumen (modified from Mayhew and Wright, 1970). The presence or absence of spermatozoa in the epididymis and/or ductus deferens was also registered. Minimum SVL at sexual maturity in males was determined based on the smallest specimen with spermatogenic activity (stages II–V) or spermatozoa in the epididymis and/or ductus deferens.

2.4. Female reproductive cycle

Female gonadal cycles were defined by the presence and number of vitellogenic follicles, oviductal embryos and corpora lutea. The sizes of follicles and/or embryos were defined as the diameter of the largest follicle and/or embryo for each female (Ibargüengoytía and Cussac, 1998) and measured with an ocular micrometer to the nearest 0.1 mm using an Olympus SZX9 stereoscopic microscope (Olympus, Tokyo, Japan). Pregnant females were classified (sensu Leyton et al., 1980) based on embryonic development as initial (from cleavage to somatic embryos), medium (curvate trunk, outlines of ears and eyes, and abundant yolk), and advanced (foetus with scales and pigmented skin). Litter size was determined by counting the number of ovulated oocytes or embryos in uterus (Ibargüengoytía and Cussac, 1998). Minimum SVL at sexual maturity for females was determined by considering the smallest female containing enlarged vitellogenic follicles, embryos in utero or cor-

pora lutea. Females without vitellogenic follicles and with oviducts with either no folds or only small folds were classified as juveniles. This method follows the definition of Ibargüengoytía and Cussac (1998): "Uterus type 1: folds, if present, are small and never spread over the entire uterus."

2.5. Fat body cycles

Fat bodies were excised after the necropsy of each adult lizard, and weighed to the nearest 0.01 g using a digital balance (Ohaus traveler scale TA320).

2.6. Statistical analyses

Statistical analyses were conducted using Infostat (version 2011), SPSS (version 17.0), and Sigmaplot (version 10.0). Assumptions of normality and homogeneity of variance were tested with the Shapiro–Wilk test and with the Levene test, respectively. Dependence between variables was tested performing simple regression and Pearson correlation. When correlation between independent variables and SVL was found, residuals of the linear regression were used to perform further analysis (Ramírez-Bautista and Vitt, 1997).

To analyse the sexual dimorphism, we performed a Student's t-test to determine the difference between female and male SVL. Residuals of the regression of the ln-transformed morphological variables (BM, HL, HW, HH, HipW, and BW) and ln-transformed SVL were used in the stepwise discriminant analysis (based on the p value with $\alpha = 0.05$ and $\alpha = 0.10$ as input and output significance levels) to determine the variables that best explain the differences between the sexes. The significance level used was $P < 0.05$ for all statistical tests and results are presented as means ± standard deviation (SD).

3. Results

3.1. Body size, sexual maturity and sexual dimorphism

The minimum adult size for males was 94.5 mm SVL (corresponding to a specimen with spermatocytes in the seminiferous tubules = stage II). In females, the minimum adult size was 126.9 mm SVL (corresponding to an individual with vitellogenic follicles). SVL of adult males ranged from 94.5 to 177.0 mm (mean = 136.7 ± 22.2, $n = 20$), and BM from 2.73 to 16.71 g (mean = 9.9 ± 4.36 g, $n = 14$), while SVL of adult females ranged from 126.9 to 205.8 mm (mean = 160.5 ± 18.7, $n = 28$), and BM from 7.6 to 20.58 g (mean = 14.78 ± 3.77 g, $n = 24$). SVL of juveniles ranged from 55.6 to 118.9 mm (mean = 95.5 ± 22.2, $n = 6$). Adult females were larger than adult males (SVL, Student's t−test; $T = −4.02$, $P < 0.001$, $n = 48$; Fig. 1), while males exhibited larger heads (HL, discriminant analysis; $\lambda = 0.684$, $X^2 = 9.695$, df = 3, $P < 0.002$, $n = 48$).

3.2. Male reproductive cycle

The relationship between male SVL and testicular size was positive (linear regression; $r^2 = 0.45$, $F_{1,18} = 14.78$, $P = 0.001$, $n = 20$). Therefore, adjusted testicular size increased at the end of spring (December), and reached the highest value in late summer (March), and for some males it remained high until mid-autumn (May; Fig. 2). Histological analyses showed that males exhibit different spermatogenic stages throughout the year. Males with primary and secondary spermatocytes (stage II, $n = 6$) were captured from late summer (March) to mid-spring (November), and males with round and elongated spermatid phases (stage III, $n = 7$) were captured from mid-winter (August) to late spring (December). Males with spermatozoa in the seminiferous tubules (stage IV, $n = 3$) were
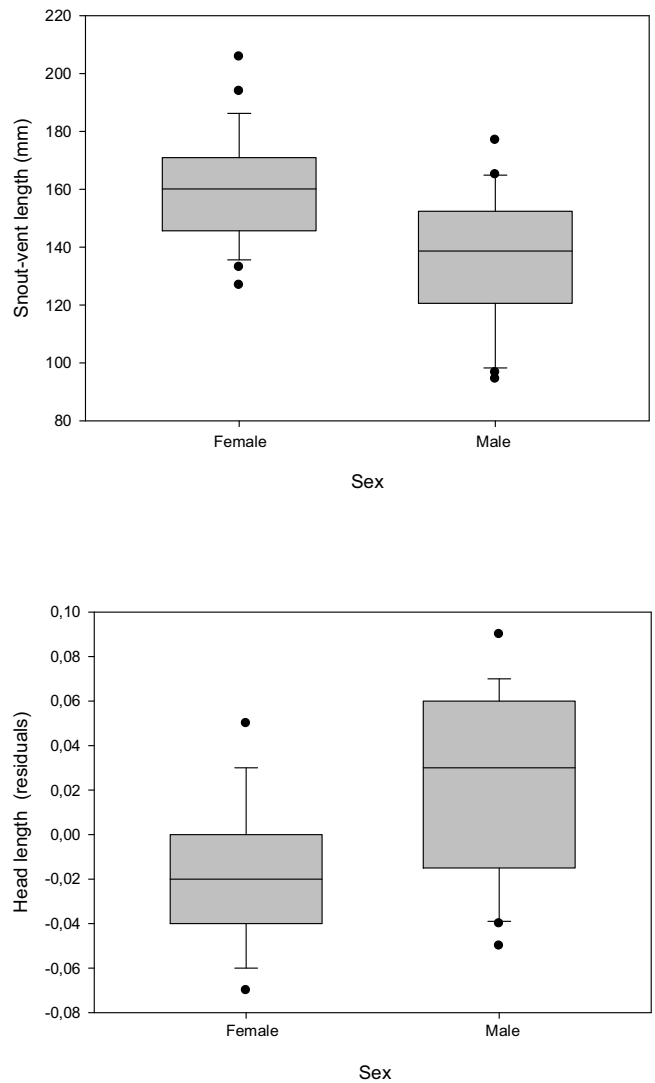

**Fig. 1.** Box plot of the significant dimorphic traits ($P < 0.05$) of female and male *Ophiodes intermedius*.

found in late summer (March) and mid-autumn (May), while males with testicular regression (stage V, $n = 4$) were captured in spring (October, December) and in mid-summer (February; Fig. 3). 80% (16–20) of adult males with testes in different spermatogenic stages (stage II–V) exhibited spermatozoa in the epididymis and/or ductus deferens throughout the year.

### 3.3. Follicular size, litter size and female reproductive cycle

The vitellogenic cycle begins in mid-autumn (May), extending to early spring (October; Fig. 4). Pregnant females with early stage embryos (stage 1) were collected in early spring (September and October), with embryos at a later stage of development (stage 2) from early spring to early summer (October to January), and only one female with late stage embryos (stage 3) was captured (on December 13th). Additionally, one female with corpora lutea was collected in early summer (January), and adult non-reproductive females ($n = 11$) were captured throughout the year. Litter size ranged from 4 to 9 embryos (mean = 6.4 ± 1.44, $n = 12$) and was not correlated with maternal SVL (Pearson correlation; $r = 0.32$, $P = 0.31$). The smallest lizard with a visible umbilical scar (neonate) measured 55.6 mm SVL and was captured in January.
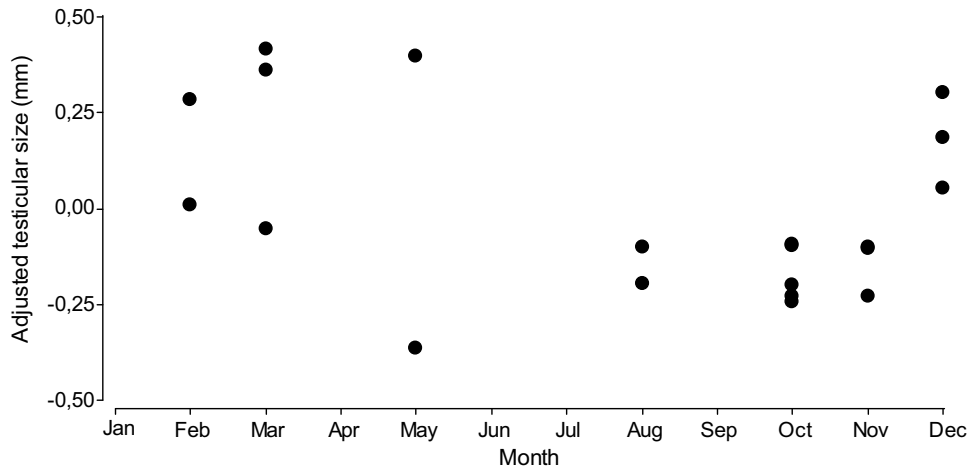
Fig. 2. Variation in adjusted testicular size of adult males of *Ophiodes intermedius* throughout the year. Solid circles correspond to the residuals from snout–vent length versus testicular diameter regression.
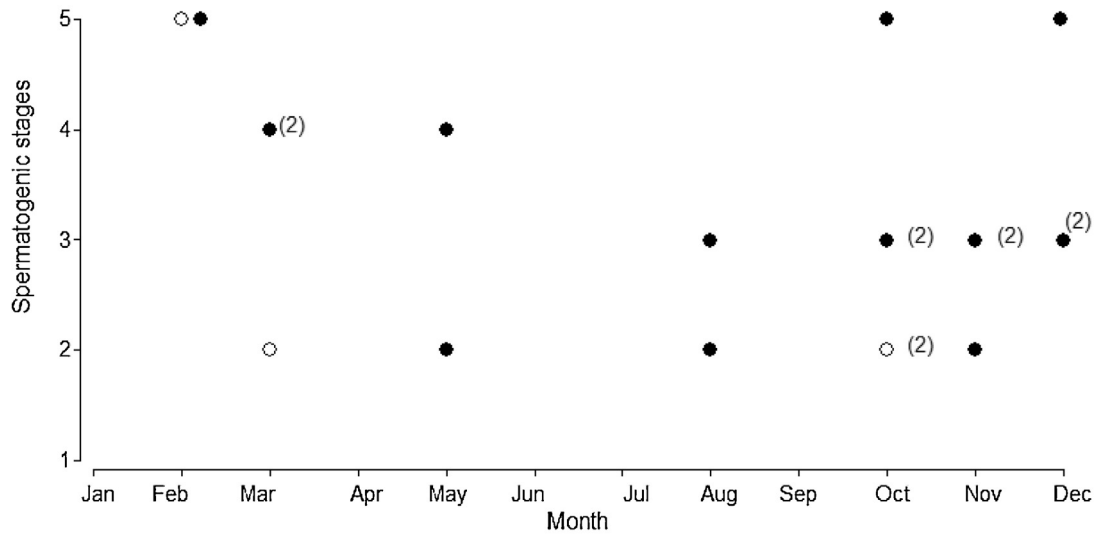
Fig. 3. Spermatogenic stages of *Ophiodes intermedius* versus data. Absence (white circles) and presence (solid circles) of spermatozoa in the epididymis and/or deferent duct are indicated. The numbers in brackets are the numbers of observations.
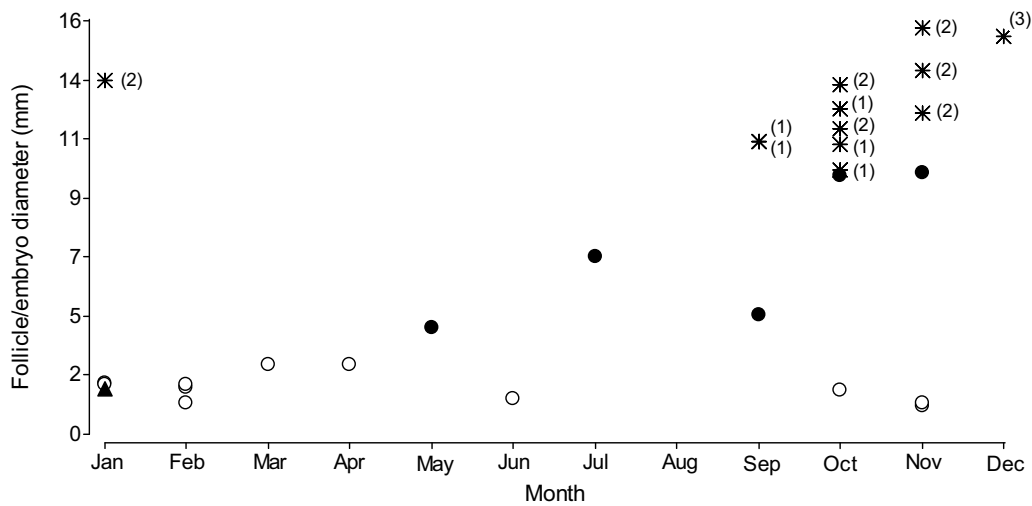
Fig. 4. Female reproductive cycle of *Ophiodes intermedius*. The largest diameters of the ovarian follicles or embryos in adult females are represented: nonvitellogenic follicles (white circles), vitellogenic follicles (solid circles), nonvitellogenic follicles and corpora lutea (solid triangles), and embryos (asterisks). The numbers in brackets indicate the embryonic development stage as initial (1), medium (2), and advanced (3).

3.4. Fat body cycles

Male fat body mass showed a significant relationship with SVL (linear regression; $r^2 = 0.42$, $P = 0.006$, $n = 16$), but in females this relationship was not significant ($r^2 = 0.05$, $P = 0.312$, $n = 22$). There was also a positive and significant correlation between fat body mass and testicular diameter ($r^2 = 0.30$, $P = 0.028$, $n = 16$; Fig. 5A). In contrast, females exhibited a negative correlation between fat body mass and the sizes of follicles or embryos ($r^2 = -0.27$, $P = 0.014$, $n = 22$; Fig. 5B).

4. Discussion

O. intermedius from the subtropical climate of the Wet Chaco region shows an unusual reproductive pattern characterized by vitellogenesis starting in autumn and ending in spring. Male gametogenesis exhibits asynchrony with females but the storage of sperm in their reproductive tracts probably allows them to coordinate courtship and copulation in spring. The timing of reproductive events and the asynchrony between males and females observed in *O. intermedius* are common in viviparous lizards that live at high altitudes (Leyton et al., 1980; Guillette and Casas-Andreu, 1980; Méndez-de la Cruz and Villagrán, 1998; Ramírez-Pinilla, 1991, 1992; Villagrán-Santa Cruz et al., 2009; Ramírez-Bautista et al., 2014) and latitudes (Jones et al., 1997; Boretto and Ibargüengoytía, 2009; Boretto et al., 2014).

The possible benefits of the autumn reproductive activity in *O. intermedius* reside in the early births (spring–summer) during the rainy season, when temperature and food availability are high. Early births would allow newborns to grow and store energy before the next winter, increasing their survival probability as has been suggested for other viviparous species living in harsh environments (Guillette and Bearce, 1986; Boretto and Ibargüengoytía, 2006, 2009; Ramírez-Pinilla et al., 2009; Boretto et al., 2007, 2014; Cabezas Cartes et al., 2010). In addition, in viviparous species, embryonic development during spring and summer months can be linked to the need for suitable environmental temperatures for the normal development of embryos (Almeida-Santos and Salomão, 2002), since temperature stability during development is considered a factor in preventing abnormalities and shortening the development period (Hubert, 1985).

The asynchronous gametogenic activity between males and females, as observed in *O. intermedius,* has been attributed to the different timing needs for the maturation of male and female gonads, the different energy requirements for gametogenesis of each sex, and the need to complete the reproductive cycle in short activity seasons (Jones et al., 1997; Boretto and Ibargüengoytía, 2006; Boretto et al., 2007, 2015; Cabezas Cartes et al., 2010; Mouton et al., 2012). In asynchronous cycles, sperm storage is obligatory (Mouton et al., 2012), either in the reproductive tract of females (Ramírez-Bautista et al., 1998; Sever and Hopkins, 2004; Ortega-León et al., 2009) or of males (Guillette and Casas-Andreu, 1980; Jones et al., 1997; Boretto et al., 2007; Cabezas Cartes et al., 2010).

The presence of a reproductive pattern characteristic of cold temperate environments in *O. intermedius* that lives in a subtropical climate in the Wet Chaco region could be a result of the retention of an ancestral reproductive pattern evolved in a cold climate, as has been reported for a diversity of snake species from tropical and subtropical North America (Quinn, 1979; Jackson and Franz, 1981; Johnson et al., 1982) and South America (Almeida-Santos and Salomão, 2002; Almeida-Santos et al., 2006; Marques et al., 2013; Barros et al., 2012). In addition, even though *O. intermedius* lives in a warmer environment than its ancestors, the male gonadal cycle and the temporally dissociated mating may be a result of their semifossorial habit if moist and cool conditions prevail in their microhabitat during the coldest months of the year.

According to molecular data, the genus *Ophiodes* may have originated about 21–22 million years ago (mya), in the early Miocene (Wiens and Slingluff, 2001). The evidence suggests that orogenic and climatic conditions were very unstable around the Oligocene–Miocene boundary (approximately 23 mya). The concentration of oxygen isotopes recorded in the early Miocene makes evident a short period of glaciation after which the climate became warmer until the mid-Miocene (17–15 mya), when a gradual drop of global temperatures began (Miller et al., 1991; Zachos et al., 2001). In South America, the cooling and aridification processes from the mid to late Miocene were associated with global climatic and local orogenic events (uplifting of the Andes), and a marine transgression that covered vast plains of South America (Janis, 1993; Webb, 1995; Ortiz-Jaureguizar and Cladera, 2006; Rossetti et al., 2013). These events led to the expansion of open habitats (grasslands and savannas) at the expense of woodlands (Retallack, 2001; Strömberg, 2011). In this relatively cool and dry scenario that may have prevailed in the past, the species of the genus *Ophiodes* may have developed their viviparous reproductive mode and their present reproductive pattern. The origin and evolution of viviparity in Squamata have been commonly associated with cold environments of high latitudes or altitudes, gradually developed from an oviparous ancestor by the progressive increase of the retention period of the egg, shell reduction, and development of some kind of primitive placenta for the exchange of water and gases (Tinkle and Gibbons, 1977; Blackburn, 1982, 2005, 2015; Shine, 1985; Guillette, 1993). Pyron and Burbrink (2014) suggested that viviparity could be an ancestral condition in squamate reptiles; however, more recent reviews mention a variety of morphological, physiological and ecological factors that refute this hypothesis (Griffith et al., 2015), and the reversal from viviparity to oviparity could only have occurred in a few squamate species (King and Lee, 2015). *O. intermedius* females of the Wet Chaco produce oocytes with a large amount of yolk, resulting, after fertilization, in macrolecithal eggs. The embryos are surrounded by a thin transparent membrane sac, without the calcareous deposits of oviparous species. The abundant deposition of yolk in the oocytes suggests that embryo nutrition depends mainly on egg yolk, which is consistent with other species of this genus (Pizzatto, 2005; Barros and Teixeira, 2007). However, we cannot discard the possibility that there is also an exchange of organic and inorganic substances as has been found in other lizards (Stewart and Castillo, 1984; Stewart and Thompson, 1993, 2000; Crocco et al., 2008).

Since ovulation in *O. intermedius* occurs in September, one would expect to find storage of sperm not only in males, but also in females, given the fact that we recorded bite marks with a "V" shape, typically produced during mating (Anderson and Vitt, 1990), on the head of *O. intermedius* females captured in May, July and October. These marks indicate that mating occurs during the months of vitellogenic activity (May to November), when males show spermatocytes and spermatids in testes. It has been suggested that storage of sperm in males may indicate a prolonged period of mating, and may be obligatory if the optimal time for spermatogenesis and oogenesis occurs at different times of the year (Mitchell and Zug, 1984; Birkhead and Møller, 1993; Almeida-Santos et al., 2006), while sperm storage in females could promote sperm competition, causing them to be inseminated by several males (Birkhead and Møller, 1993; Adams et al., 2005; Barros et al., 2012). The storage of sperm in the ductus deferens seems to be a character shared by males of the genus *Ophiodes*; however, in *O. intermedius* from the Wet Chaco, the spermatogenic activity is seasonal, while the male cycle in *O. fragilis* and *O.* cf. s *triatus* from subtropical regions of the Atlantic Forest in Brazil is continuous (Pizzatto, 2005; Montechiaro et al., 2011).
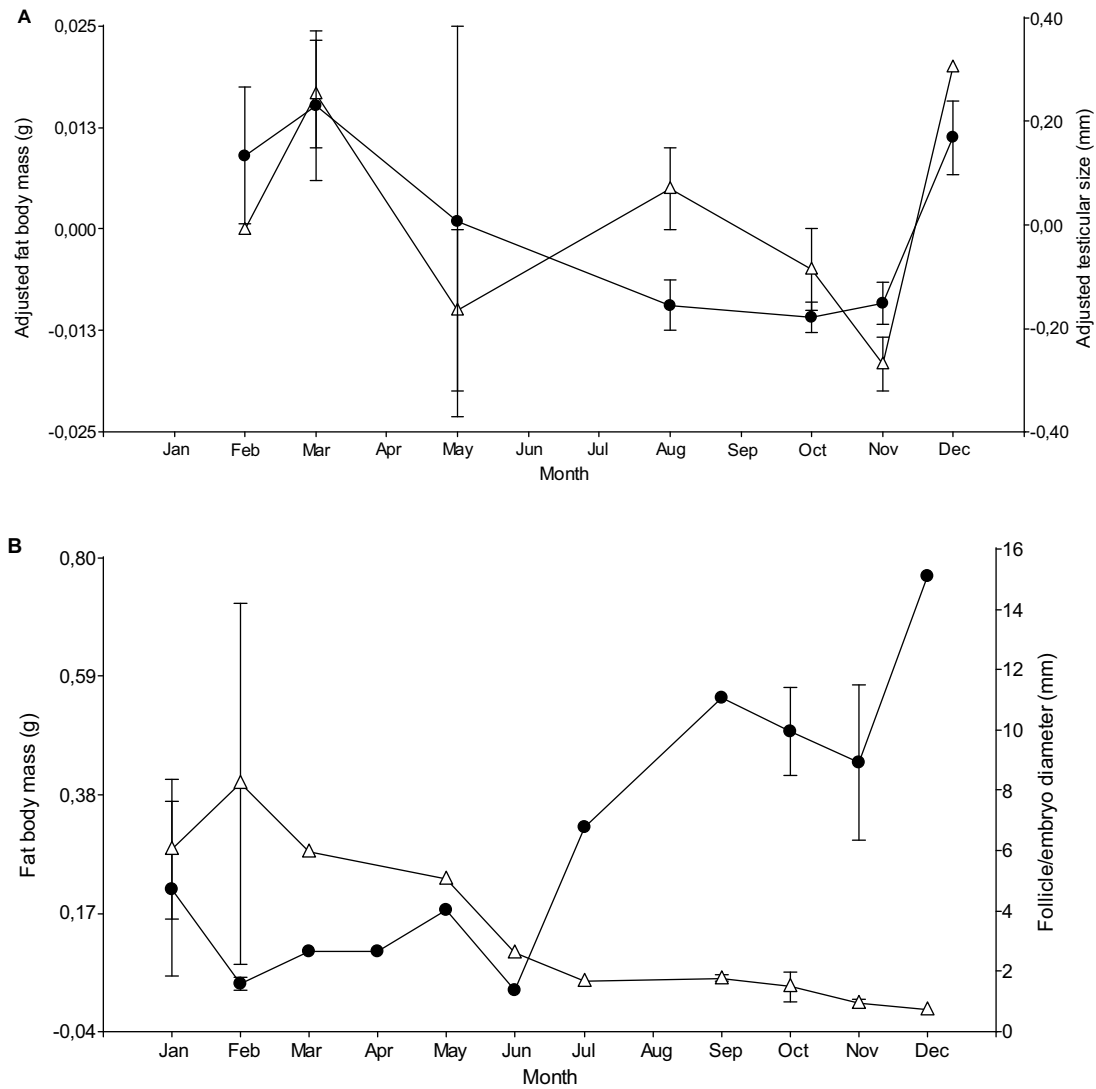
Fig. 5. Fat body annual cycle of *Ophiodes intermedius* adults. (A) Monthly means of the residuals obtained from the linear regressions between fat body mass and snout–vent length (triangles), and between means of testicular diameter and snout–vent length (circles). (B) Monthly means of fat bodies (triangles), and means of the largest follicles or embryos (circles) are also indicated. Bars indicate the standard error.

Reproductive asynchrony between males and females might favour the development of dimorphic traits that increase mating ability (Ramírez-Bautista et al., 2002). *O. intermedius* males exhibited a head size proportionately larger than females, which could be related to intrasexual competition. This could mean an advantage over other males during agonistic encounters for the access to females (Vitt and Cooper, 1985; Anderson and Vitt, 1990; Andersson, 1994), and might also relate to the advantage of better apprehension of the female during copulation (Anderson and Vitt, 1990; Hews, 1990). Probably, this is the case in *O. intermedius,* as evidenced by the presence of bite marks of copulation in females. Yet, larger heads in males have also been linked to niche partition between the sexes (Schoener, 1967; Schoener and Gorman, 1968; Presst, 1994; Herrel et al., 1999). Meanwhile, females showed a greater SVL, as has been observed in other *Ophiodes* species (Pizzatto, 2005; Barros and Teixeira, 2007; Montechiaro et al., 2011), probably as a result of fecundity selection, as has been reported in other species (Shine, 1988). However, while in other *Ophiodes* species the litter size depends on female SVL (*O. fragilis*, Santa Teresa population, Barros and Teixeira, 2007; *O.* cf. *striatus*, Montechiaro et al., 2011), litter size was independent of female body size in *O. intermedius* (present study) and *O. fragilis* from the

southeast of Brazil (Pizzatto, 2005). Nonetheless, other factors can interfere with the relationship between litter size and maternal body size. For example, the nutritional conditions and the amount of lipid storage in females can also influence litter and/or offspring size (Doughty and Shine, 1998; Bonnet et al., 2001).

Our study of fat body mass in *O. intermedius* revealed a pattern of energy allocation linked to reproduction, as has been described in a diversity of lizard species (Rocha, 1992; Guillette and Bearce, 1986; Ramírez-Bautista et al., 2009). The increment of fat body masses with spermatogenesis suggests that males probably allocate more resources to the search for females, courtship and copulation during the mating period than to the spermatogenic activity (energetically less expensive), while females probably use lipid reserves to support vitellogenesis and embryonic development, as previously documented for other lizards (Avery, 1970; Goldberg, 1972; Guillette and Casas-Andreu, 1981; Guillette and Bearce, 1986; Ramírez-Bautista et al., 2009).

Female cycles and reproductive parameters of the species of the genus *Ophiodes* show variations in the minimum size at sexual maturity, the vitellogenic cycle, the timing of ovulation and births, and the embryonic development period (Table 1). In particular, as latitude increases, the timing of births in *Ophiodes* species

Table 1
Overview of reproductive characteristics of the investigated *Ophiodes* species. Comparisons among reproductive season, female size at sexual maturity, mean adult female size, litter size, and birthing period in different biomes and localities. Mean values are presented with ±1 SD, and with ranges in parentheses.

Species	Biome	Reproductive period	Female SVL (mm) at sexual maturity	Female mean size (SVL, mm)	Litter size	Birthing period	Locality	References
Ophiodes intermedius	Wet Chaco	May–January	126.9	157.0 ± 21.56 (112.7–205.8)	6.4 ± 1.44 (4–9)	December–January	Chaco and Corrientes, northeastern Argentina	Present study
Ophiodes fragilis	Atlantic Forest	February–November	156.0	192.5 ± 28 (56–300)	7.5 ± 2.3 (5–13)	August–November	São Paulo and Paraná, southeastern Brazil	Pizzatto (2005)
Ophiodes fragilis	Atlantic Forest	March–November	161.0	179.7 ± 25.5 (111.0–235.0)	6.0 ± 2.0 (3–11)	November	Espiritu Santo, southeastern Brazil	Barros and Teixeira (2007)
Ophiodes cf. *striatus*	Atlantic Forest	February–December	107.0	161.0 ± 25.7 (107–220)	7.9 ± 3.8 (2–14)	December	Río Grande do Sul, Santa Catarina and Paraná, southeastern Brazil	Montechiaro et al. (2011)
Ophiodes striatus	Pampa	–	145	218.0 ± 38.0 (145–252)	10.5 ± 7.6 (5–24)	December	Río Grande do Sul, southeastern Brazil	Leitão (1973)

shifts from early spring to early or mid-summer (Table 1), probably related to temperature and rainfall patterns (Montechiaro et al., 2011). *O. intermedius* deviates from the usual reproductive pattern of this lineage, showing an asynchrony between male and female reproductive cycles, which is an unusual pattern in subtropical climates. This reproductive style differs from that of sympatric lizard species previously studied, like *Tropidurus catalanensis* (formerly *T. torquatus*, Ortiz et al., 2014) and *Kentropyx viridistriga* (Ortiz et al., 2016). The comparative analysis showed that *O. intermedius* from the Wet Chaco exhibits reproductive characteristics and sexual dimorphism that reveal the influence of ecological, historical and phylogenetic factors.

Acknowledgements

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