Relationship between pre- and post-copulatory traits in *Salvator rufescens* (Squamata: Teiidae)

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Understanding pre- and post-copulatory mechanisms of sexual selection can provide insights into the evolution of male reproductive strategies. The phenotype-linked fertility hypothesis postulates that male sperm quality and secondary sexual characteristics will positively co-vary, whereas the sperm competition hypothesis predicts a negative association between those traits. Male reproductive traits often show variation throughout the reproductive period, suggesting that the relationship between pre- and post-copulatory sexual selection may vary temporally. Here, we evaluated the relationship between secondary sexual character and sperm traits and its temporal variation in *Salvator rufescens,* a south American lizard. We observed a negative relationship between jaw muscle and principal piece length of sperm and a variation in the relationship between pre- and post-copulatory traits throughout the reproductive period. Collectively, our results evidenced a trade-off between pre- and post-copulatory traits and a strong seasonal flexibility of male reproductive strategies in this lizard species. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **00**, 000–000.

KEYWORDS: lizards – secondary sexual character – sexual selection – sperm traits.

INTRODUCTION

Disentangling the factors that regulate the relationship between traits conferring a reproductive advantage before and after copulation is crucial to understanding how sexual strategies evolve (Cornwallis & Birkhead, 2007; Evans, 2010; Engqvist, 2011; Fitzpatrick *et al.*, 2012; Kvarnemo & Simmons, 2013). Males have a fixed energy budget to invest in reproduction during a given reproductive episode; hence, male reproductive success is determined by the interaction between the ability to access and choose females of the highest reproductive quality and the ability to outcompete the ejaculates of rival males after copulation (Cornwallis & Birkhead, 2007; Parker, Lessells & Simmons, 2012; Kvarnemo & Simmons, 2013; Lüpold *et al.*, 2014). Predicting the

directionality of the relationship between sperm quality metrics and secondary sexual characters is difficult. Two different hypotheses have been proposed to make different predictions. The phenotype-linked fertility hypothesis (Sheldon, 1994) suggests that male sperm quality and secondary sexual character elaboration will positively co-vary. Concordant patterns of pre- and post-copulatory sexual selection have been described in species in which competitive fertilization success favours highly attractive males (Evans *et al.*, 2003; Hosken *et al.*, 2008). Conversely, the sperm competition hypothesis (Parker, 1998) predicts a negative association between investment in sperm quality and secondary sexual character (Parker *et al.*, 2012).

Species with alternative mating strategies exhibit evidence of a trade-off between the investment in secondary sexual characteristics to afford pre-copulatory sexual selection and the investment in ejaculate

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quality to afford sperm competition (Beausoleil *et al.*, 2012). Dominant males, which are exposed to lower sperm competition risk, produce ejaculates of lower quality than subordinate males, which continually face sperm competition (Cornwallis & Birkhead, 2007; Locatello *et al.* 2007; Pitcher *et al.*, 2009; Beausoleil *et al.*, 2012). However, some studies have reported a lack of relationship between pre- and post-copulatory traits (Hettey & Roberts, 2006; Lifjeld *et al.*, 2012; Mautz, Møller & Jennions, 2013). In a recent study, Lüpold *et al.* (2014) proposed that the degree of female monopolization within a species should be a key factor to explain the variation of the relationship among sexual traits observed in different species. They found that species in which males monopolize females, testes size is inversely related to traits important in male–male contest competition. By contrast, these relationships were positive in taxa in which female monopolization is rare or absent (Lüpold *et al.*, 2014).

Secondary sexual traits evolve through the process of sexual selection, either via female choice, male–male competition or a combination of both (Berglund, Bisazza & Pilastro, 1996; Malo *et al.*, 2005; Karsten *et al.*, 2009). There are diverse hypotheses about the meaning of secondary sexual character. Some studies have suggested that this characteristic indicates the male health condition (Darwin, 1871; Andersson, 1994; McCullough & Emlen, 2013), the reproductive condition or reproductive potential (Lailvaux & Irschick, 2006; Naretto *et al.*, 2014), and the outcome of aggressive interactions (Huyghe *et al.*, 2005; Lailvaux & Irschick, 2006). Moreover, secondary sexual characteristics could be honest signals of a male's ejaculate quality (Malo *et al.*, 2005).

Furthermore, males must be able to produce a sufficient amount of sperm, thereby influencing the evolution of testicular size and function (Ramm & Schärer, 2014) and sperm of good quality to ensure fertilization (Fitzpatrick & Lüpold, 2014). Several ejaculate traits have been proposed as determinant in fertilization success. Sperm number increases with sperm competition risk and body size. In particular, in large species, males need a large number of spermatozoa to increase the chances of fertilization and to avoid sperm dilution inside the female reproductive tract (Lüpold & Fitzpatrick, 2015). The size of different sperm components has been considered important in ejaculate quality because it contributes differentially with diverse sperm functions (Gomendio & Roldan, 2008; Fitzpatrick *et al.*, 2009; Lüpold *et al.*, 2009; Tourmente, Gomendio & Roldan, 2011a; Blengini *et al.*, 2014). A positive contribution of principal piece length or flagellum length to sperm velocity has been reported for several taxa (Gomendio & Roldan, 2008; Fitzpatrick *et al.*, 2009; Lüpold *et al.*,

2009; Tourmente *et al.*, 2011a). Moreover, an increase of midpiece length or sperm neck may reflect an increase in the energetic reserves for the sperm (Ruiz-Pesini *et al.*, 2007). Moreover, an elongation of head size may play an important role during sperm storage, contributing to sperm–female interactions (Pitnick, Wolfner & Suarez, 2009a), and reducing the drag experienced by the sperm cell (Malo *et al.*, 2006). Finally, sperm velocity is known to be a major determinant of male fertility (Birkhead *et al.*, 1999; Gomendio & Roldan, 2008; Beausoleil *et al.*, 2012).

Male reproductive traits often show variation throughout the reproductive period (Lüpold, Brikhead & Westneat, 2012). Naretto *et al.* (2014) showed that secondary sexual characteristics in lizards are dynamic; moreover, in several species there is evidence of changes in sperm traits during the breeding season (Lüpold *et al.*, 2012; Cramer *et al.*, 2013). Hence, we could also expect variation in the relationship between pre- and post-copulatory traits throughout the reproductive period.

Here, we focus on a lizard species, *Salvator rufescens* (Günther, 1871) (formerly *Tupinambis rufescens*), because males express a secondary sexual characteristic, the pterygoideus muscle of the head (jaw muscle). Sexual dimorphism in the pterygoideus muscle is produced by an increase in muscle mass during the reproductive period. Moreover, the relative jaw muscle mass is related to relative testis mass and to the presence of sperm, suggesting that the relative size of the jaw muscle acts as an honest signal of the reproductive condition of males (Naretto *et al.*, 2014). However, the relationship between jaw muscle mass and sperm quality has not been studied in this and in other lizard species. In *Salvator* lizards, females can copulate with different males, even on the same day (Lopes & Abe, 1999). Moreover, follicular development is completed ~20 days after mating, suggesting sperm retention in female genital ducts (Garcia Valdez *et al.*, 2011). Thus, there is ample opportunity for sperm competition to occur in these species. Indeed, Blengini *et al.* (2014) confirmed that *S. rufescens* is exposed to high levels of sperm competition risk and evidenced a large among-male and within-male variation in sperm morphometric and dynamic traits, suggesting that males vary in sperm competitive ability. Variation in secondary sexual characteristics, relative testis mass and length of sperm component was observed between populations of *S. rufescens*, suggesting differences in the allocation strategy among pre- and post-copulatory traits (Naretto *et al.*, 2016a). In the present study, we focused on the temporal variation in male reproductive strategies by studying the relationship between the expression of a secondary

sexual characteristic (jaw muscle) and sperm traits throughout the reproductive season in S. *rufescens*.

METHODS

SPECIES AND STUDY AREA

Salvator rufescens is a Teiidae lizard that occurs in the southernmost area of genus distribution in South America (Lanfri *et al.*, 2013). At adulthood, it can exceed 50 cm in snout-to-vent length, or SVL (130 cm total length) and weigh up to 4.7 kg (Naretto *et al.*, 2014). This species is included in the appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (Cites, Convention on International Trade in Endangered Species, 2008) and is under legal commercial harvesting in Argentina (Porini, 2006). *S. rufescens* overwinters in burrows, emerges during hot seasons and is seasonal breeder that reproduces from October to December (Mercolli & Yanosky, 1990; Fitzgerald, Cruz & Perotti, 1993). Specimens used in this study were collected from a wild population in central Argentina.

DATA COLLECTION

Forty-three *Salvator rufescens* individuals were caught by local authorized hunters from wild populations in central Argentina (29°35′W, 64°10′S to 31°10′W, 63°15′S) throughout the reproductive season (Naretto *et al.*, 2014). We were authorized by the government environmental agencies for scientific capture, and we selected and accompanied local hunters to standardize the sampling procedure with the aim of avoiding size bias in capture rates. Each animal was sacrificed for the legal skin trade, in accordance with AVMA Guidelines on Euthanasia [AVMA (American Veterinary Medical Association), 2007]. The body was immediately weighed (BM), and testes (TM) and superficial pterygoideus muscle mass (PMM) were obtained by dissection and then weighed using an electronic balance with a precision of 0.1 g (Traveler TA302; OHAUS, NJ, USA), according to Naretto *et al.* (2014). Only sexually active males, with sperm in epididymides, were included in this study.

SPERM SAMPLING PROCEDURE

In the laboratory, spermatozoa were obtained from the terminal portion of the epididymis. Time elapsed between animal death and sperm analyses ranged from 2 to 3 h. During this period, sperm samples were not affected, in that $96.03 \pm 3.72\%$ of all sperm were progressively motile and over 80% were viable (Blengini *et al.*, 2014). Sperm samples were collected in a 1.5 mL plastic tube containing 90 μL of phosphate-buffered saline (PBS). Sperm concentration was determined using a Neubauer chamber and the samples were diluted to a concentration of 1×10^6 cells mL^{-1} in Biggers, Whitten, and Wittingham culture medium (Biggers, Whitten & Whittingham, 1971) supplemented with 4% bovine serum albumin (Tourmente, Giojalas & Chiaraviglio, 2011b).

SPERM MORPHOMETRY

Aliquots of sperm samples were fixed for photography in 2% formaldehyde (Blengini *et al.*, 2014) and stained with Blue Brilliant Coomassie (Firman & Simmons, 2010). The samples were examined at ×400 magnification under a phase contrast Nikon eclipse Ti microscope (Nikon Instruments Inc, Tokyo, Japan). Microphotographs were taken using Nikon DS-Qi1Mc digital camera with a controller DS-U2 (Nikon Instruments Inc). Total sperm length and length of the sperm head, midpiece and principal piece were determined in 50 spermatozoa per individual using the software ImageJ version 1.43 μ (NIH, Bethesda, MD, USA). Then the ratio between midpiece and principal piece length (PPL:MPL) was determined. All measurements were taken by the same person to reduce potential inter-observer variability.

SPERM VELOCITY

Sperm aliquots (500 μL) were incubated at 25 °C in a thermally stable water bath for 30 min (Tourmente *et al.*, 2011b; Blengini *et al.*, 2014). Sperm suspension (20 μL) was placed in a plastic observation chamber and covered with a coverslip. Dynamic parameters were measured at room temperature (25 °C) using a video microscopy system composed of a phase contrast microscope (CX41; Olympus, Tokyo, Japan) equipped with a video camera (ICAM 1500; Labomed, Fremont, CA, USA).

Videos were captured using Virtualdub v.1.6.16 (Avery Lee); spermatozoa were recorded at ×100 magnification for 4 min, in different fields that were randomly changed every 5 s. Subsequently, 45 sperm tracks from each individual were followed for 3 s and transformed to a matrix of Cartesian coordinates using ImageJ version 1.43 μ (NIH) and its plug-in MtrackJ v. 1.1.0 (Eric Meijering). Straight line velocity (VSL; μm s^{-1}) was calculated by means of Spermtrack v. 4.2 (Universidad Nacional de Cordoba, Argentina) (Blengini *et al.*, 2011, 2014).

STATISTICAL ANALYSES

To test whether post-copulatory traits were associated with secondary sexual characteristics, a

multiple regression analysis was performed using pterygoideus muscle mass (PMM) as a dependent variable and testis mass (TM), body mass (BM), sperm concentration, head length, midpiece length, principal piece length and sperm velocity as predictors. The co-linearity of sperm morphometric traits was discarded previously by Spearman's (r_s) correlation test (all $P > 0.22$). Then, a reduced model was performed using pterygoideus muscle mass (PMM) as a dependent variable and body mass (BM), midpiece length, principal piece length and sperm velocity as predictors based on the importance of these variables in the full model. Furthermore, a multiple regression analysis was also performed with jaw muscle mass as dependent variable and body mass and midpiece:principal piece length ratio (MPL: PPL) as predictors. This multiple regression analysis was performed using a sequential (Type I) sum of squares, in which the predictor variables were added to the model in the following order: body mass (BM), midpiece:principal piece length ratio. Testes mass, jaw muscle mass, body mass and VSL were log10-transformed.

To obtain a measure of lizard's jaw muscle investment, the residual scores from the general linear regression of log-transformed jaw muscle mass to log-transformed body mass were calculated (Madsen & Shine, 1999) ($F = 362.39$; $P < 0.0001$). Then, two categories of males: males with high investment (positive residuals) and males with low investment (negative residuals) in jaw muscle were determined.

To determine the variation of reproductive traits throughout the breeding season, a 3-month period (October, November and December) was considered. Differences in the frequency of male categories according to jaw muscle investment among months

of the reproductive period were estimated by chi square test. Monthly variation in male reproductive traits, taking account jaw muscle mass, body mass, midpiece length, principal piece length and VSL, were evaluated by a principal component analysis, in a multivariate way by MANOVA. Spearman's (r_s) correlation was used to analyze the relationships between the sexual traits and the principal component. Finally, temporal variation in the jaw muscle mass and the sperm traits were evaluated separately by ANCOVA, using body mass as covariate, and nested-ANOVA respectively. Statistical analysis was performed using InfoStat software (version 2012; Universidad Nacional de Cordoba, Argentina).

RESULTS

Firstly, the relationship between secondary sexual character and post-copulatory traits was studied. A negative relationship was found between relative pterygoideus muscle mass and principal piece length (Table 1 and Fig. 1) and a positive relationship was found between relative jaw muscle mass and the midpiece: principal piece length ratio ($F_{1,35}$: 5.22; R: 0.85; $P < 0.03$, Fig. 1).

No differences were observed in the relative frequency of the two categories of males, based on jaw muscle investment, over the 3 months of the reproductive period (χ^2: 1.63, d.f. = 2; $P < 0.5$; Fig. 2). The principal component analyses performed on the original variables yielded two principal factors (Table 2). The scores of the PC1 and PC2 explained 73% of the variation in the data. Pterygoideus muscle mass and body mass made a similar contribution to PC1.

Table 1. Relationship between pterygoideus muscle mass (PMM) and testis mass (TM) and sperm traits

Model	Dependent variable	Predictor	Slope	R^2	F	P
Full model				0.84		
$N = 28$	Log$_{10}$ PMM	Log$_{10}$ BM	1.81		95.97	< 0.0001
		Log$_{10}$ TM	0.04		0.88	0.3594
		Sperm concentration	0.000008		0.57	0.4404
		Head length	0.01		0.003	0.9574
		Midpiece length	0.08		2.01	0.1719
		Principal piece length	−0.03		3.12	0.0928
		Log$_{10}$ VSL	−0.14		0.21	0.6527
Reduced model				0.85		
$N = 35$	Log$_{10}$ PMM	Log$_{10}$ BM	1.97		161.6	< 0.0001*
		Midpiece length	0.09		3.4	0.0751
		Principal piece length	−0.04		4.86	0.0353*
		Log$_{10}$ VSL	0.08		0.1	0.7545

BM, body mass; *N*, number of individuals; TM, testis mass; VSL, linear velocity; *significant relationship.
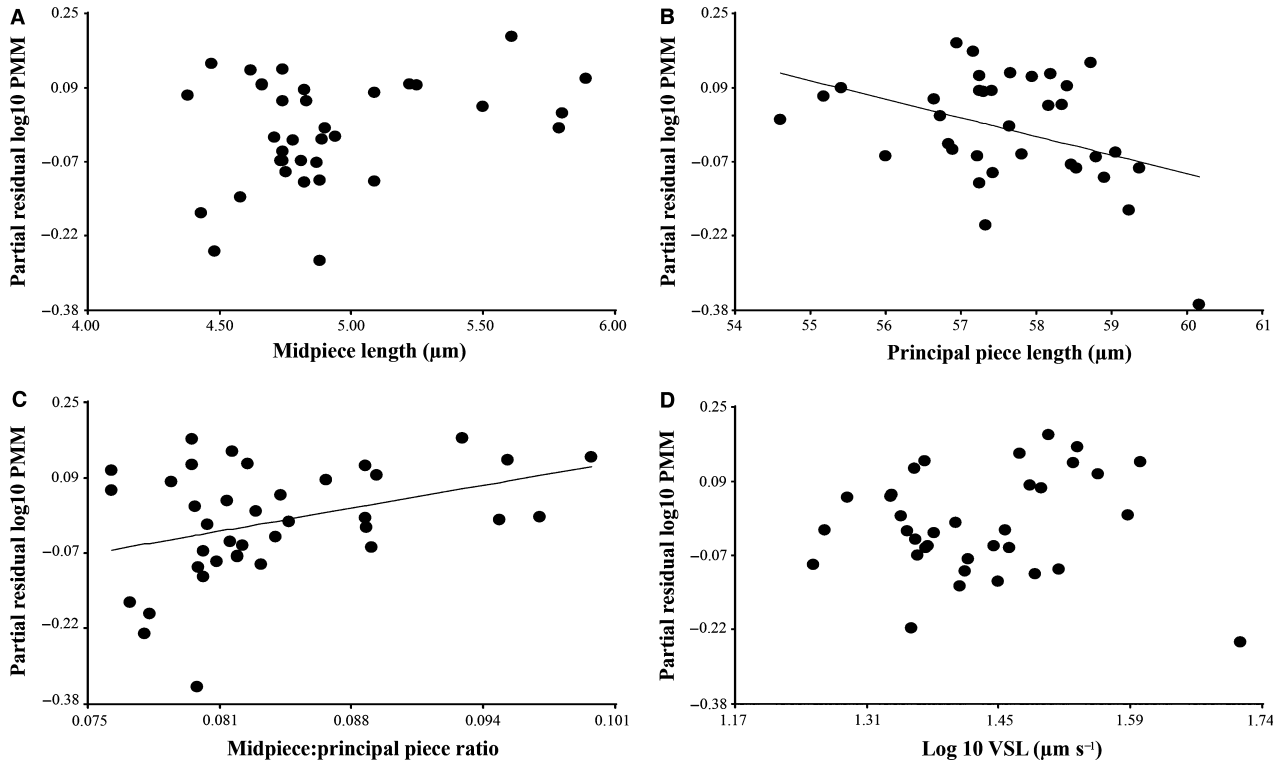
Figure 1. Relationship between pterygoideus muscle mass (PMM) and sperm traits. A, midpiece length; (B) principal piece length; (C) midpiece: principal piece ratio; (D) linear velocity (VSL).
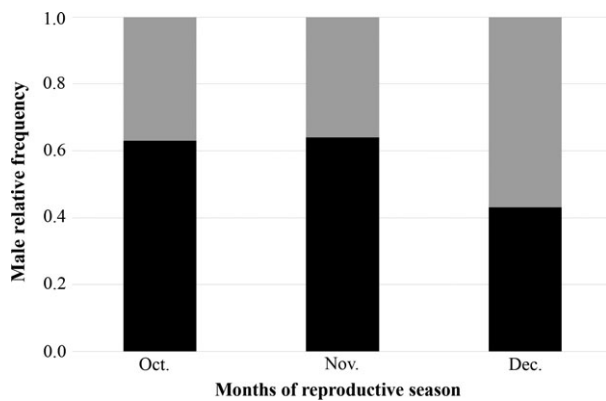

Figure 2. Temporal variation of different male categories based on pterygoideus muscle mass in *Salvator rufescens*. Grey: males with low investment in pterygoideus muscle mass; black: males with high investment in pterygoideus muscle mass.

According to PC2, male reproductive variance was also explained by principal piece length and sperm velocity (Table 2 and Fig. 3). Spearman's correlation revealed that PMM and BM are highly correlated with PC1 and VSL are highly correlated with PC 2 ($r_s > 0.75$) (Table 3).

Table 2. First and second axes resulting from principal component analysis performed on reproductive traits

	PC1	PC2
Eigenvalue	2.03	1.62
Variation explained	0.41	0.32
Log $_{10}$ PMM	0.58	−0.41
Log $_{10}$ BM	0.64	−0.27
Log $_{10}$ VSL	0.25	0.62
Midpiece length (μm)	−0.36	−0.35
Principal piece length (μm)	0.26	0.5

BM, body mass; PC, principal component; PMM, pterygoideus muscle mass; VSL, linear velocity.

Reproductive traits varied significantly among months, based on the scores of PC 2 (Table 4 and Fig. 3). At the beginning of the reproductive period (October), males presented on average larger pterygoideus muscle mass and longer sperm midpiece length than males of the late reproductive period (November and December) (Fig. 3). Moreover, the latter presented longer principal piece length and faster sperm than the former (Fig. 3). Moreover, when each reproductive trait was analyzed
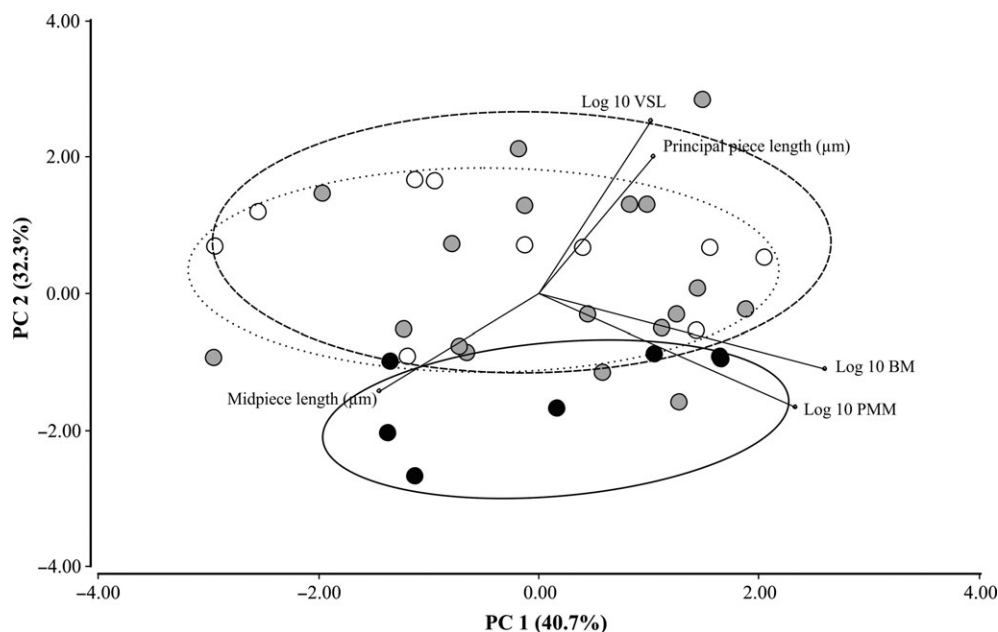
Figure 3. Principal component analysis of male reproductive traits. Predictive ellipses are indicated (October: Black dots and solid line; November: grey dots and dashed line; December: white dots and dotted line). PMM, pterygoideus muscle mass; BM, body mass; VSL, linear velocity.

Table 3. Spearman's correlation coefficients for correlations between sexual traits and principal components in males of *Salvator rufescens*

	PC 1	PC 2
Log_{10} PMM	0.81***	−0.5**
Log_{10} BM	0.91***	−0.36**
Log10 VSL	0.35**	0.78***
Midpiece length	−0.5**	−0.34**
Principal piece length	0.35**	0.59**

BM, body mass; PMM, pterygoideus muscle mass; VSL, linear velocity.
**$P < 0.05$; ***$P < 0.001$.

separately, variation among reproductive months were observed in sperm velocity (Nested-ANOVA: $F_{2,35}$: 7.28; $P < 0.0001$), and principal piece length although was not significant (Nested-ANOVA: $F_{2,37}$: 2.28; $P < 0.112$). In both cases, larger values were found in November and December (Fig. 4). In contrast, no variation among reproductive months in pterygoideus muscle mass (ANCOVA: $F_{2,35}$: 0.51; $P < 0.7$) and midpiece length was found (nested-ANOVA: $F_{2,37}$: 0.86; $P < 0.5$) (Fig. 4).

DISCUSSION

Understanding the relationship between male traits conferring a reproductive advantage before and after

Table 4. Monthly phenotypic variation males of *Salvator rufescens* based on scores on PC1 and PC2 (MANOVA)

Component	Month	N	Mean	SD	Median	F	P
PC1	October[a]	7	0.1	1.38	0.17	4.07	0.0054*
	November[b]	18	0.15	1.32	0.51		
	December[b]	10	−0.34	1.71	−0.53		
PC2	October[a]	7	−1.45	0.7	−1		
	November[b]	18	0.22	1.24	−0.27		
	December[b]	10	0.63	0.83	0.68		

Different superscript letters indicate significant differences between months (*a posteriori* Hotelling's test); *Significant differences.
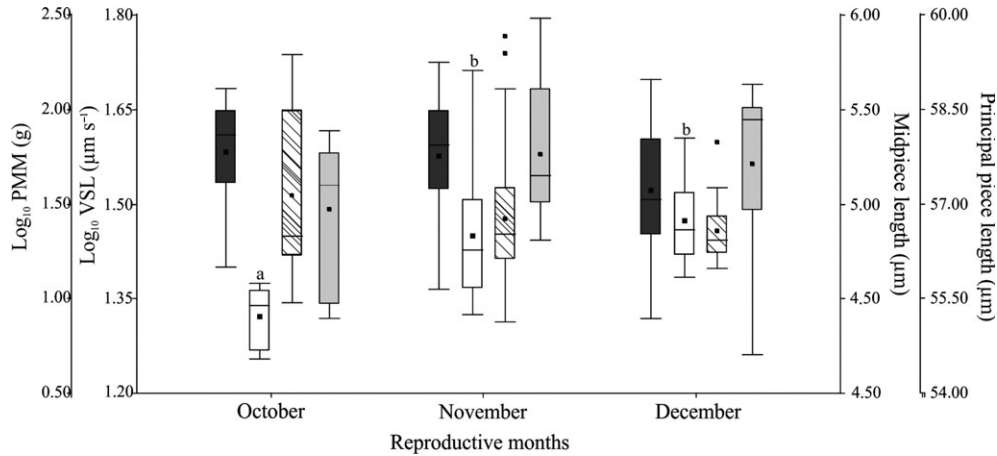
Figure 4. Temporal variation of reproductive traits in *Salvator rufescens*. Black: Pterygoideus muscle mass (PMM); white: sperm velocity (VSL); lined: midpiece length; grey: principal piece length. Lowercase letter indicates significant differences among months.

copulation is crucial to elucidating how sexual strategies evolve (Mautz *et al.*, 2013; Lüpold *et al.*, 2014). Here, we show evidence of a negative relationship between pre- and post-copulatory traits in a lizard, consistent with the hypothesis of sperm competition. Males with lower investment in pterygoideus muscle mass had sperm with longer principal piece than males with high muscle mass investment. Moreover, we highlight the variability of male reproductive traits in *Salvator rufescens,* showing variation throughout the reproductive period.

Maximizing the investment in pre- and post-copulatory traits could be difficult because both can be energetically demanding (Olsson, Madsen & Shine, 1997; Emlen, 2001; Allen & Levinton, 2007). There is evidence that supports the two hypotheses proposed to explain the relationship between secondary sexual character and sperm quality: the phenotype-linked fertility (Evans *et al.*, 2003; Malo *et al.*, 2005) and sperm competition (Parker *et al.*, 2012). The results presented here show a negative relationship between relative pterygoideus muscle mass and principal piece length and a positive relationship with midpiece: principal piece ratio in *S. rufescens*. However, we did not find a relationship between pterygoideus muscle mass and sperm velocity. Pterygoideus muscle mass was proposed as an honest signal of reproductive condition in *Salvator* lizards (Naretto *et al.*, 2014); however, it is not clear his role as indicator of sperm quality. Even though, there are evidences about the relationship between sperm traits and fertilization success in other taxa (Gomendio & Roldan, 2008; Pitnick, Hosken & Birkhead, 2009b; Pitnick *et al.*, 2009a), this has not been studied in lizards. Further studies are needed to understand the

importance of sperm morphology and sperm velocity in the reproductive success in *S. rufescens* and other lizard species. A trade-off between secondary sexual characters and sperm traits could promote evolution of alternative mating strategies; males compensate for their inability of female monopolization by investing in competitive ejaculates (Oliveira, Taborsky & Brockmann, 2008). In lizards, there are few studies about the link between pre- and post-copulatory traits, males often display pre-copulatory dominance behaviour and limit the opportunity of other males to mate with females (Keogh *et al.*, 2013). Accordingly, males with low jaw muscle investment could produce sperm with longer principal piece than males with high jaw muscle investment.

A potential mechanism that may mediate the negative relationship between jaw muscle and sperm traits is the effect of circulating testosterone levels on an individual's morphology and physiology. Sex hormones are thought to play a crucial role in the expression of male sexual traits, including secondary sexually selected traits, as well as sperm production and maturation (Husak & Moore, 2008; Cox *et al.*, 2009; Shen *et al.*, 2015). Naretto *et al.* (2014) discuss that the development of jaw muscle mass in *Salvator* could be related to testosterone levels, because there is evidence in *Gallotia galloti* that an increase in testosterone caused an increase in jaw adductor muscle mass (Huyghe *et al.*, 2010). In birds, changes in sperm morphometry were related to within-individual changes in testosterone levels, males with elevated testosterone levels had significantly increased midpiece lengths and decreased principal piece lengths (Immler *et al.*, 2010). Then we need further studies to elucidate if the testosterone is being

responsible for the negative relationship between secondary sexual character and sperm traits in *Salvator* lizard.

Furthermore, sexual selection on reproductive traits depends on social environments (Shine *et al.*, 2006; Procter, Moore & Miller, 2012). Variation in reproductive traits often persists because the phenotype that will maximize fitness of an individual often depends upon the condition of that individual, its social interactions, and the phenotypes expressed by other individuals (Oliveira *et al.*, 2008; Bergmüller & Taborsky, 2010). Reproductive traits may vary throughout the reproductive period according to competition pressure, and availability and quality of partners and competitors (Gasparini, Serena & Pilastro, 2013). Hence, we could expect a flexible relationship between pre- and post-copulatory traits. In *S. rufescens*, the frequency of both categories of males based on jaw muscle investment is the same throughout the reproductive period: almost 50:50 in each month. However, in a multivariate analysis, taking into account jaw muscle and sperm traits together, a temporal variation of male reproductive traits was found. These results were partially supported when each trait was analyzed separately, in particular the sperm velocity shows variation among reproductive months. At the beginning of the reproductive period (October), the observed males exhibited high jaw muscle mass and produced sperm with a long midpiece, whereas in the middle and at the end of the reproductive period (November and December), males present exhibited low jaw muscle mass and produced sperm with a long principal piece and high velocity. Indeed, at the beginning of the reproductive period, males with high jaw muscle investment might have access to females of high quality (Cardozo *et al.*, 2015) and produce sperm with a long midpiece. Sperm with a long midpiece would contribute with an increase in the energetic reserves for the sperm because it contains mitochondria that are essential for energy metabolism (Ruiz-Pesini *et al.*, 2007) and the fibrous sheath in which occurs the anaerobic glycolysis, a complementary metabolic pathway for the production of sperm energy, allowing energy transmission along the principal piece (Ruiz-Pesini *et al.*, 2007; Tourmente *et al.*, 2015a). Moreover, there is some evidence linking sperm midpiece size with ATP production (Vladic, Afzelius & Bronnikov, 2002; Rowe *et al.*, 2013; Tourmente *et al.*, 2015a). It is important to note that the fibrous sheath is present both in the midpiece and principal piece in lizards (Tavares-Bastos *et al.*, 2002). Thus, enhanced energy production could influence the capability of sperm to survive longer inside female reproductive tracts (Parker & Begon, 1993), being important in this case because females are still not ready to ovulate in that moment

(Fitzgerald *et al.*, 1993; Naretto *et al.*, 2016a, b). In a sister species, *S. merianae*, the average time between mating and oviposition was 22 days, ranging from 7 to 33 days and suggesting variation in the period of sperm retention in female genital ducts (Garcia Valdez *et al.*, 2011). Conversely, in the middle and at the end of the reproductive period, we found males that produced sperm with a long principal piece and high velocity that allowed them to reach the fertilization site faster. As Blengini *et al.* (2014) showed, principal piece and sperm velocity are positively associated in this species and ATP concentration has been proposed to predict sperm velocity in other taxa (Tourmente *et al.*, 2015a, b) then, a long principal piece could ensure also higher sperm velocity by increasing ATP production in the fibrous sheath by anaerobic glycolysis (Ruiz-Pesini *et al.*, 2007; Tourmente *et al.*, 2015a, b). This pattern could be related to variation in female quality, as in a sister species, *S. merianae*, temporal variations of female phenotype and therefore female quality have been observed, expressing behavioral components in relation to sexual and social contexts of mate preferences (Cardozo *et al.*, 2015). The pattern of variation in male reproductive strategies could be associated with changes in social context, not only in terms of female quality but also of competitor quality throughout the reproductive period.

ETHICAL STANDARDS

The authors declare that this paper complies with the current laws of Argentina.

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AUTHORS' CONTRIBUTION

CSB collected field data and carried out the laboratory work, participated in data analysis, in the design of the study and drafted the manuscript; SN collected field data and participated in the design of the drafted manuscript; GC collected field data and participated in the design of the drafted manuscript; LCG helped with laboratory work and participated in the design of the manuscript; MC conceived, designed, and coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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