Title: The relationship of body condition, superoxide dismutase and superoxide with sperm performance

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Running headline: Body condition, oxidative status and sperm performance

Lay summary

Damage-inducing reactive oxygen species (ROS) are a by-product of oxygen-based energy production that are quenched by energetically expensive antioxidants. Male sexual colouration requires investment of energy and resources, which may constrain allocation to other functions like antioxidant production or spermatogenesis. Here we explored whether the body condition of colourful male lizards reflected their investment in antioxidants and reduction of ROS, which may influence sperm performance. We found that drab males in better condition had more antioxidants. Colourful males had lower levels of antioxidants and their sperm performed poorly at higher ROS levels. These results suggest a trade-off between colour maintenance and sperm performance.

Abstract

Sperm competition theory predicts a negative correlation between somatic investment in traits that aid in pre- and postcopulatory sexual selection. Sperm performance is critical for postcopulatory success but are susceptible to damage by free radicals such as superoxide radicals generated during mitochondrial respiration (mtSOx). Males can ameliorate damage to spermatozoa by investing in the production of antioxidants, like superoxide dismutase (SOD), which may act as a mechanistic link for pre and postcopulatory trade-offs. Some male Australian, colour-polymorphic painted dragon lizards

(*Ctenophorus pictus*) possess a yellow throat patch (bib) that females prefer over non-bibbed males and are also more likely to win male-male contests indicating that males with bibs are better at monopolizing females. We tested whether the sperm performance in non-bibbed males was superior to that of bibbed males as predicted by sperm competition theory. We show that blood cell mtSOx levels are negatively correlated with SOD activity in the plasma in all males early in the breeding season but SOD was lower in bibbed males. Non-bibbed males maintain a positive correlation between body condition and SOD activity over time while bibbed males do not. Overall sperm performance was not different between the bib-morphs, however, higher mtSOx levels were negatively correlated with sperm performance in bibbed males, but not of non-bibbed males. Together these data suggest physiological associations between body condition, SOD activity and sperm performance are linked to the expression of a yellow gular patch, which may be related to intrinsic differences in metabolism of bibbed versus non-bibbed males.

Keywords

Sperm performance, oxidative stress, polymorphism, body condition, condition dependence, lizard

46 ## Introduction

47 Theoretical models of ejaculate investment under sperm competition predict an allocation
48 trade-off between pre- and postcopulatory traits generating a negative correlation between these
49 two types of traits (Parker, 1970; Parker et al., 2013; Parker and Pizzari, 2010; Tazzyman et al., 2009).
50 Among individuals of the same species, nutritional resources and energy allocation—quality or
51 condition—is especially likely to mediate pre- and postcopulatory trade-offs (Tuni et al., 2018).
52 Indeed, empirical results that indicate investment in precopulatory traits are often negatively
53 correlated with testes size, predominantly in species where males are able to monopolise females
54 (Dines et al., 2015; Dunn et al., 2015; Fitzpatrick et al., 2012; Kahrl et al., 2016; Lüpold et al., 2014;
55 Simmons and Emlen, 2006). However, in addition to size, other testicular adaptations may also be
56 important for postcopulatory sexual selection (Ramm and Schärer, 2014).

57 A critical function of the testis is to protect sperm from oxidative damage during
58 spermatogenesis and subsequent storage (Aitken and Roman, 2008; Ko et al., 2014; Ramm and
59 Schärer, 2014). Oxidative damage of functionally critical macromolecules (membrane lipids, cellular
60 proteins, and DNA) by reactive oxygen species (ROS) adversely affects whole organism physiological
61 performance, health and lifespan (Finkel and Holbrook, 2000). ROS are free radicals naturally and
62 principally produced in mitochondria during oxidation-reduction reactions occurring in the electron
63 transport chain (ETC) during oxidative-phosphorylation generating ATP and therefore tightly linked
64 to energy production (Murphy, 2009; Turrens, 2003). Spermatozoa are particularly vulnerable to
65 oxidative damage because their membranes are composed of a high proportion of easily oxidized
66 polyunsaturated fatty acids (Sanocka and Kurpisz, 2004). Therefore, selection on energetically
67 expensive mechanisms to reduce oxidative damage is important both for life-history evolution and
68 sexually selected traits, potentially generating condition dependent soma-germline trade-offs
69 (Dowling and Simmons, 2009; Metcalfe and Alonso-Alvarez, 2010; Monaghan et al., 2009; von
70 Schantz et al., 1999).

71 As they are terminally differentiated and transcriptionally inactive with limited antioxidant
72 defenses, spermatozoa have limited capacity to repair molecular damage (Tremellen, 2008).
73 Consequently, ROS production and damage are self-perpetuating in spermatozoa leading to reduced
74 sperm function, velocity and percent of progressively motile sperm, all of which are strongly
75 associated with male infertility in humans (Aitken et al., 1989; Aitken and Graves, 2002; Koppers et
76 al., 2008; Tremellen, 2008). Intuitively, sperm performance is conjoined with male reproductive
77 success so oxidative stress at the organismal level should have important implications in
78 postcopulatory sexual selection (Firman and Simmons, 2010; Fitzpatrick and Lupold, 2014; Møller,
79 1988; Simmons and Fitzpatrick, 2012). Sperm competition puts males in a double bind. The
80 production of more sperm requires increased metabolic activity in the testes (Gomendio et al., 2011;
81 Parapanov et al., 2008; Tourmente et al., 2011), which may then be accompanied by a concomitant
82 increase in ROS production and adaptations to ameliorate the associated costs (delBarco-Trillo and
83 Roldan, 2014; Ribou and Reinhardt, 2012). Even in species with relatively short-term sperm storage
84 and with limited opportunity for sperm competition, such as humans (Simmons et al., 2004; van der
85 Horst and Maree, 2014), oxidative damage strongly influences sperm performance and male
86 reproductive success (Aitken et al., 2014). Allocating resources for protecting sperm from oxidative
87 damage to maintain high sperm performance is even more vital in the context of sperm competition
88 (Dowling and Simmons, 2009).

89 Protection from and repairing damage caused by ROS during spermatogenesis is potentially
90 costly and may be condition dependent (Costantini, 2008, 2014; Monaghan and Costantini, 2014).
91 There is increasing evidence of the conditional dependence of sperm production (e.g. numbers and
92 morphology Dávila and Aron, 2017; Kahrl and Cox, 2015) and sperm velocity, motility and
93 fertilization success (Mitre et al., 2004; Rakitin et al., 1999). Male condition dependence of oxidative
94 status affects sperm quality, and there is increasing evidence that male colouration reflects
95 antioxidant status (Helfenstein et al., 2010; reviewed in, Svensson and Wong, 2011; von Schantz et
96 al., 1999) and studies indicate colouration and sperm quality can be correlated (Locatello et al.,

97 2006; Peters et al., 2004; Pitcher et al., 2007; Rowe et al., 2010). For example, Helfenstein et al.
98 (2010) showed that male great tits (*Parus major*) with paler carotenoid-based breast plumage
99 subjected to experimentally elevated workloads had higher lipid peroxidation (an indicator of
100 oxidative damage) as well as reduced sperm motility and 'swimming ability'; these effects were
101 ameliorated with supplementation of dietary carotenoid antioxidants. Age-related declines in the
102 innate antioxidant capacity of red junglefowl (*Gallus gallus*), was associated with an increase in
103 oxidative damage, and a decline in sperm quality (Noguera et al., 2012). Decreased endogenous
104 antioxidant expression translates to lower sperm competitiveness as Garratt et al. (2013) show that
105 mice deficient in the endogenous antioxidate superoxide dismutase (SOD) had no fertilization
106 success in sperm competition trials. Thus, the role of oxidative stress in sperm performance and/or
107 fertilization success *in vivo*, preferably in wild populations, merits attention (Dowling and Simmons,
108 2009). Such analyses would be particularly interesting in intraspecific studies on males with
109 divergent reproductive strategies characterized by trade-offs between pre- and postcopulatory
110 sexually selected traits (Lüpold et al., 2014).

111 Colour-polymorphic species can be a useful tool in determining the selective forces that
112 drive sexually selected trait expression in wild populations. Heritable colour morphs often have
113 divergent behaviours and physiology, with associated differences in reproductive tactics in animals
114 with an otherwise common genetic background (Healey et al., 2007; Huxley, 1955; Olsson et al.,
115 2007b; Olsson et al., 2009a; Pryke et al., 2007; Pryke and Griffith, 2006; Sinervo and Lively, 1996;
116 reviewed in Wellenreuther et al., 2014).

117 Here we assess the relationship between an important mitochondrial ROS—antioxidant pair,
118 a measure of resources (body condition: BCI) and sperm performance in a wild colour-polymorphic
119 lizard, the Australian painted dragon (*Ctenophorus pictus*). Primary ROS production in mitochondria
120 usually begins with the single reduction of molecular oxygen (O_2) to form superoxide ($O^{\bullet -}$), which is
121 the rate limiting step in the subsequent damaging cascade of ROS production (Andreyev et al., 2015;

122 Turrens, 2003). On its own, superoxide is a relatively weak oxidizer of macromolecules; however,
123 superoxide reduces iron (Fe^{2+}) which may then catalyse the disproportionation of hydrogen peroxide
124 (H_2O_2) to produce the hydroxyl radical ($^{\bullet}OH$), which is the strongest oxidizing agent in biological
125 systems (Fridovich, 1995; Turrens, 2003) . Superoxide that is not quenched readily exits cell-
126 membranes with high concentrations of anion channels, such as erythrocytes (Fridovich, 1995; Lynch
127 and Fridovich, 1978). So we will focus on superoxide and its elimination by the action of an
128 endogenous enzyme, superoxide dismutase (SOD), which, at sufficiently high concentrations,
129 eradicates O^{-} at the rate of diffusion (Fridovich, 1995; Turrens, 2003). Thus, although there are other
130 important endogenous antioxidants (e.g., catalase and various isoforms of glutathione peroxidase),
131 the balance between superoxide and SOD is a significant mediator of incipient oxidative stress
132 during energy (ATP) production.

133 Oxidative stress and sexual selection biology of painted dragons have been the subject of
134 extensive investigations. For example, aggressive colour-morphs have higher testosterone (T), and
135 superoxide levels than less aggressive males that tend to have lower T (Olsson et al., 2007a; Olsson
136 et al., 2009c). Although colour is in part carotenoid-based, a potential exogenous antioxidant,
137 dietary manipulation of carotenoids does not mediate a relationship between superoxide and bright
138 colouration (Olsson et al., 2008a). However, experimental reduction of superoxide levels using
139 exogenous doses of the SOD mimetic, EUK 134, does reduce colour fading over the breeding season
140 (Olsson et al., 2012b). Males in better body condition (residuals of mass-length regression) have
141 enhanced morph-specific colour maintenance despite higher net ROS levels (Friesen et al., 2017b)
142 indicating that investment in endogenous antioxidants, such as SOD, may be condition-dependent.
143 Furthermore, baseline superoxide level varies among individuals and is heritable (Olsson et al.,
144 2008b), but whether this is due to reduced production of ROS or increase quenching of ROS by
145 endogenous antioxidants in unknown. Collectively these studies suggest that there are evolutionarily
146 important links between body condition, SOD and superoxide levels, and sexual colouration in
147 painted dragons.

148 In this paper we exploit natural variation in a colour trait, the presence or absence of a
149 yellow gular patch (henceforth, bib **Figure 1**), which is an important, but costly, sexually selected
150 colour-trait in painted dragons. Bibbed male painted dragons experience greater declines in body
151 condition in the wild than non-bibbed males (Healey and Olsson, 2009a; Olsson et al., 2009a). Males
152 with bibs also have greater telomere attrition (Rollings et al., 2017) and higher resting metabolic rate
153 through the breeding season than non-bibbed males (Friesen et al., 2017a). These costs of bearing a
154 bib, must come with potential fitness benefits to explain the trait's persistence in the population.
155 Indeed, bibbed males are more attractive to females in staged preference trials and more likely to
156 win male-male competition trials when pitted against non-bibbed rivals indicating there is a
157 precopulatory mating advantage for bibbed males (McDiarmid et al., 2017b). Bibbed males are less
158 likely than non-bibbed males to lose paternity to neighbouring males in the wild (Healey and Olsson,
159 2009a; Olsson et al., 2009a), which may be due to efficient mate monopolization. McDiarmid et al.
160 (2017b), hypothesized that this precopulatory advantage of bibbed males would relax
161 postcopulatory selection on sperm performance, however sperm motility and velocity did not differ
162 between bibbed and non-bibbed males. McDiarmid et al. (2017b) did not measure superoxide or
163 SOD. We make three straightforward predictions: 1) SOD and superoxide level should be negatively
164 correlated, 2) sperm performance and superoxide levels should be negatively correlated, and 3) if
165 SOD is indeed costly, body condition should be positively correlated with SOD levels. Finally, if
166 bibbed males face a resource allocation trade-off, then bibbed males should have lower sperm
167 performance after accounting for SOD and body condition than non-bibbed males.

168 Methods

169 Animal collection/housing and mating trials

170 The Australian painted dragon (*Ctenophorus pictus*, Peters 1866) is a small (adult: mass 8 – 18 g;
171 snout-to-vent length [SVL] 55-75mm) agamid lizard native to arid parts of central New South Wales
172 to Western Australia (Cogger, 2014). All research was conducted with the approval of the University
173 of Sydney Animal Ethics Committee Project Number: 2013-2016/6050 and were collected under

174 NSW National Parks and Wildlife Service Scientific licence number SL100352. Lizards were caught at
175 Yathong Nature Reserve, in western New South Wales (145°35′; 32°35′) and were brought back to
176 holding facilities at the University of Sydney (14, October: N = 40 females; N = 56 males; 20 bibbed
177 and 36 non-bibbed males). The animals were sampled at random at the study site and, hence,
178 approximately represent natural frequencies in the wild at the time. Males were kept individually in
179 tanks (60 × 60 × 50cm) on a 12:12 h (light: dark) light regime with a basking light for
180 thermoregulation. They were watered daily, and fed crickets and mealworms to satiation every
181 second day.

182 After a three-week acclimation period, we conducted mating trials on five different days in
183 the period between 6-Nov.-4 Dec (5-8 matings per day). This period corresponds with the middle
184 third of the breeding season in the wild (late September-late December). We obtained 5-8 matings
185 on any given day which allowed for timely processing of both sperm and blood samples with minimal
186 temporal separation between them. Each male was observed continuously after the introduction of
187 a receptive female to his own cage. Males usually mated within 10 minutes of the female's
188 introduction, and trials were terminated if mating did not occur after 1 hour. Only those males that
189 mated were included in this study (N = 31 of 56 males mated; 12 bibbed and 19 non-bibbed males).
190 Although not all males mated, the ratio of bibbed/non-bibbed males was the same as those brought
191 back to the lab ($X^2_{df\ 1} = 0.077$, P = 0.781). Females were used only once during the receptive phase of
192 their 3 week reproductive cycle (Olsson et al., 2009b).

193 Subsequent to copulation, semen was collected from the female's cloaca by pulling the
194 ejaculate into a 1 mL syringe (Olsson, 2001) preloaded with 100 µL Hams F-10 medium (Cat # 99175,
195 Irvine Scientific, Santa Ana, CA, USA; 21 mM HEPES buffer, 4 mM sodium bicarbonate, 1 mM calcium
196 lactate, 0.5 mM magnesium sulfate, 5 mg mL−1 (0.5%) human albumin; e.g., (Friesen et al., 2013;
197 Friesen et al., 2014; Mattson et al., 2007; McDiarmid et al., 2017b). Sperm samples were kept at
198 35±1 °C in an incubator until they were analysed using computer-assisted sperm analysis (CASA, see

199 below) ≤ 30 minutes after collection; 35 °C is the preferred body temperature of this species
200 (Melville and Schulte II, 2001). A pilot study (N = 6) showed no significant decline in sperm motility or
201 velocity after 60 minutes, and was verified in the full data set presented in this paper (see results
202 below). We collected a blood sample in a capillary tube from a vessel in the corner of the mouth,
203 the *vena angularis*, after it was gently punctured with the tip of an 18 ga syringe needle. Each blood
204 sample was taken within 5 min of semen collection for mitochondria superoxide and plasma
205 superoxide dismutase analysis (below). Body mass and SVL were recorded the same day as mating
206 trials.

207 Sperm performance—Computer aided sperm analysis (CASA)

208 The ejaculate was diluted to $1x10^6$ / mL with Hams F-10 medium and slowly pipetted (10.0 µL) into
209 the chamber of a pre-warmed sperm analysis slide (Hamilton-Thorne 2X-CEL®; Beverly, MA, USA;
210 warmed to 35 °C on a slide warmer). Sperm were examined using CASA at a frame rate set to 60 hz
211 (Hamilton-Thorne, IVOS-II; Beverly, MA, USA). Sperm motility was determined for a minimum of 300
212 sperm per individual. In pilot work, we found it impossible to eliminate all particulates from the
213 ejaculate without negatively affecting sperm motility with excessive washing. Therefore, we
214 modified CASA factory settings to classify sperm as motile only if its velocity was ≥5 µm/s with no
215 constraints on path straightness. In addition, we visually inspected each sperm track to verify that
216 only sperm were included in the analysis and to confirm accurate tracking of multiple sperm with
217 intersecting paths at the time of analysis. In addition to providing proportion of motile sperm (MOT),
218 we used the following standard average measures of sperm kinematics data generated by CASA for
219 each sample for PCA analysis (see "statistics" below): straight-line velocity (VSL), curvilinear velocity
220 (VCL), average path velocity (VAP), amplitude of lateral head displacement (ALH), beat cross
221 frequency (BCF), linearity of movement (LIN), and proportion of progressively motile sperm (PMOT).
222 Progressively motile sperm (i.e., sperm that were 'going somewhere' and not merely convulsing or
223 turning in place) were defined as sperm with an average path velocity (VAP) of ≥50µm/s and a
224 straightness index (STR) of 70% (VSL/VAP ≥70%).

225 ### Mitochondrial Superoxide—Flow-cytometry fluorescence-activated cell sorting (FACS)

226 We followed previously published protocols for measuring mitochondrial superoxide (Ballen et al.,
227 2012; Friesen et al., 2017b; Giraudeau et al., 2016). Briefly, an aliquot of a single sample of whole
228 peripheral blood (10 µl) was diluted immediately with 9 volumes of phosphate buffered saline (PBS;
229 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH2PO4, 8 mM Na2HPO4, pH 7.4) and stored on ice prior to
230 FACS analyses that were completed within 2 hours of sampling. The remainder of the blood sample
231 was kept on ice until centrifugation for plasma collection and subsequence storage at -80°C (3 hours
232 after blood sampling) for later quantification of SOD. Prior to staining blood cells for mt-superoxide,
233 the diluted blood was further diluted 50-fold in PBS and then centrifuged (300 g for 5 min) to pellet
234 blood cells. Cells were resuspended in 100 µl of PBS containing 5 µM MitoSOX Red (MR; Molecular
235 Probes, Invitrogen, Life Technologies, Sydney, Australia). MR was added from stock solutions in
236 dimethyl sulfoxide (DMSO); the final concentration of DMSO was 0.2% (v/v) or less. Cells were
237 subsequently incubated at 35 °C for 30 min then washed with PBS by centrifugation as described
238 above and held on ice until analysed by flow cytometry; 50,000 events (cells) were acquired for all
239 samples. Flow cytometry was performed using an Accuri C6 flow cytometer (BD Accuri Cytometers,
240 Ann Arbor, MI, USA) with excitation at 488 nm and emitted fluorescence collected using band pass
241 filters of 575 ± 13 nm. Data were acquired and analysed using BD Accuri C6 Software. On the basis of
242 forward angle laser scatter and side angle laser scatter, a number of blood cell populations were
243 discerned; the results obtained were similar for all these populations. For each sample, the
244 arithmetic mean fluorescence for all 50,000 cells acquired was determined using Accuri C6 software
245 and used to compare between samples and treatments. (A pilot experiment determined that the
246 particulate matter in the semen samples precluded measurement of mitochondrial superoxide
247 production in sperm with flow cytometry).

248 ### Superoxide dismutase activity

249 We followed the same protocol described previously in this species (Olsson et al., 2012a; Olsson et
250 al., 2012b) that assayed SOD in blood plasma samples collected within five minutes of semen

251 collection. Briefly, we used the Superoxide Dismutase Assay Kit II (catalogue no. 574601,
252 Calbiochem, supplied by Merck Pty Ltd., Victoria, Australia). This kit is designed to generate
253 superoxide radicals with xanthine oxidase and hypoxanthine. The superoxide radicals are dismutated
254 by SOD in the plasma sample to molecular oxygen and hydrogen peroxide and detected by
255 tetrazolium salt. This SOD assay measures all three types of SOD (Cu/Zn-, Mn- and Fe-SOD).
256 Absorbance measurements were taken using a microplate reader (Pherastar®FSX, BMG Labtech,
257 Mornington, Victoria, Australia). The optimal plasma sample dilution was determined to be a factor
258 of 48 in previous studies (Olsson et al., 2012b), indicating that SOD is abundant in the plasma of this
259 species. Standards and samples were run in duplicate and were placed randomly on the plates for
260 intra-assay precision. The intraclass correlation coefficient (ICC) between two samples from the
261 same individual was ICC = 0.880, P < 0.001. One unit of SOD activity is defined as the amount of
262 enzyme needed to exhibit 50 % dismutation of the superoxide radical to hydrogen peroxide.

263 ## Statistical analysis

264 ### *Data preparation*

265 We calculated body condition (BCI) as the standardized residuals (mean = 0; standard deviation =1)
266 from linear regressions of ln(body mass) as a function of ln(SVL) in all males, not just those that
267 mated (Olsson et al., 2009a).

268 After inspection of normal Q-Q plots and the failure of Shaprio-Wilk tests of normality, mean
269 fluorescence values of mitochondrial superoxide (henceforth "mtSOx": W = 0.858, P < 0.001) and
270 superoxide dismutase activity (SOD: W = 0.883, P = 0.003) were ln-transformed to improve normality
271 (ln(mtSOx): W = 0.961, P = 0.317; ln(SOD): W = 0.993, P = 0.999) and reduce heteroscedasticity.
272 Hereafter, mtSOx and SOD refer to these transformed variables unless otherwise stated.

273 A priori, we wanted to assess proportions of motile (MOT) and progressively motile (PMOT)
274 sperm. MOT is one of the most commonly reported sperm performance parameters while PMOT is a
275 useful holistic indicator of efficient mitochondrial function, membrane integrity and fertilization

276 success in most species studied to date, including for example, humans, horses, ram, rabbit and
277 poultry (De Graaf et al., 2006; Froman and Feltmann, 2000; Froman and Kirby, 2005; Johinke et al.,
278 2014; Quintero-Moreno et al., 2003; Rickard et al., 2014; Simon and Lewis, 2011). PMOT captures
279 both velocity and path straightness and also scales to the relative population size of progressively
280 motile sperm in the ejaculate. However, it is unclear how these two variables and the other six
281 kinematic parameters generated by CASA are related.

282 Initial data exploration with Q-Q plots indicated MOT and PMOT were normally distributed,
283 which was also confirmed using Shaprio-Wilk tests of normality (MOT: W = 0.946, P = 0.123; PMOT:
284 W = 0.976, P = 0.684). Furthermore, MOT, VSL, VCL, VAP, ALH, BCF, LIN, and PMOT were correlated
285 (|r| range: 0.276-0.973). Rather than run multiple separate analyses with each sperm kinematics
286 parameter, following Helfenstein et al. (2010), we chose to reduce these eight dimensions into one
287 variable using principle component analysis with varimax orthogonal rotation. The first axis (PC1),
288 which we call "sperm performance" and Helfenstein et al. (2010a) call "swimming ability", explained
289 69.73% of the variance in the kinematic parameters. All kinematic parameters were significantly
290 correlated with PC1 after rotation (Listed here in order of increasing strength of positive correlation
291 with PC1: LIN, ALH, MOT, PMOT, VCL, VAP, VSL; r ranged 0.663-0.994, all p <0.0001; BCF (beat
292 frequency) had a significantly negative correlation with PC1 r = -0.578, P< 0.0001). Thus, individuals
293 with relatively large and positive values of PC1 can be described as having proportionally more
294 sperm that swim relatively fast and straight and were more efficient given the negative correlation
295 with beat frequency.

296 *Significance tests*

297 We report basic comparisons of mass, SVL and body condition between bib-morphs at time of
298 capture and at mating. We pre-planned to fit separate models including one covariate of interest
299 (BCI, mtSOx, SOD) and its interaction with bib-morph. Therefore, we chose to conduct significance
300 tests on three separate relationships using restricted maximum likelihood (REML) estimated

Generalized Linear Models (GLM; normal distribution, identity link function) with Satterthwaite approximated degrees of freedom and robust covariance estimation to manage unbalanced sample sizes in IBM® SPSS® version 24. We report Likelihood ratios and associated p-values from Omnibus tests of full versus intercept-only models for completeness but base our interpretations on parameter estimates for the pre-planned comparisons (Rutherford, 2011). All full models reported here initially included main effects of bib-morph and the covariate of interest as well as their interaction, but the interaction parameters were dropped if estimates were not significant at $\alpha > 0.05$.

Figures

We included separate regression lines for each 'morph' in all plots regardless of significance. All plots and R^2 values (when reported) for these simple linear regressions (SLR) were generated in SigmaPlot® 13.0 (Systat Software, Inc. San Jose CA, USA).

Results

Of the males that mated and were used for sperm collection, bibbed males were slightly but significantly longer (SVL: 66 mm ± 0.07 se) than non-bibbed males (SVL: 68 mm ± 0.07 se) at capture: $F_{1, 29} = 5.578$, $P = 0.025$); SVL did not change in the acclimation period. Bibbed and non-bibbed males did not differ significantly in mass or body condition at the time of capture or mating (all $P \geq 0.119$) although all males gained an average of 0.626 g ± 0.229 se or 4.7% increase in body mass in this period ($F_{1,29} = 6.820$, $P = 0.014$).

Relationship between body condition and superoxide dismutase activity

Superoxide dismutase activity, measured at the time of mating, was positively related to body condition at time of capture (SOD~ $BIC_{capture}$: $r = 0.336$, $F_{1, 29} = 4.465$, $P = 0.043$). Although BCI increased from time of capture to time of mating in the lab, SOD activity decreased slightly over the course of the mating trials (i.e., sampling period) for all males (SOD ~ date: $r = -0.355$, $F_{1,29} = 4.193$, $P = 0.050$) regardless of bib phenotype (date x Bib, $P = 0.748$). At the time of each individual male's

326 mating, BCI was not significantly correlated with SOD activity, however, SOD activity was positively
327 related to BCI in non-bibbed males, but not in bibbed males (Bib x BCI_{mating}, P =0.015; **Figure 2b**,**Table**
328 **1**), and non-bibbed males had higher SOD activity than bibbed males (P = 0.041).

329 ### Relationship between mitochondrial superoxide (mtSOx) and SOD

330 Overall, mtSOx tended to decrease with increasing SOD activity (P = 0.012) **Table 2; Figure 3**.
331 Superoxide levels increased significantly over the course of the sampling period in all males (r =
332 0.564, $F_{1, 29}$ = 13.526, P = 0.001) regardless of bib phenotype (date x Bib, P = 0.371).

333 ### Relationship between mitochondrial superoxide and sperm kinematic parameters

334 We constructed GLMs with bib, superoxide levels and their interaction as independent variables and
335 three different kinematic parameters as response variables: MOT, PMOT and PC1 or "sperm
336 performance" **Tables 3a-c** respectively. Sperm motility was significantly and negatively related to
337 superoxide levels (P = 0.001), but motility did not differ between bib-morphs (P = 0.358) **Figure 4**.
338 Sperm performance (PC1) and the proportion of progressively motile sperm (PMOT) was negatively
339 linked with superoxide levels (mtSOx: P ≤ 0.004). Progressive motility of sperm declined in both
340 morphs, but significantly more steeply in bibbed males (bib x mtSOx P = 0.036: **Figure 5a**). While
341 sperm performance (PC1) declined with increasing mtSOx levels in bibbed males (bib x mtSOx, P =
342 0.036 **Figure 5b**), no such decline occurred in non-bibbed males.

343

344 ## Discussion

345 We investigated prospective links between body condition, antioxidant defences (SOD), whether
346 antioxidant levels were associated with ROS levels (mtSOx) and finally whether the relationship
347 between ROS levels and sperm performance differ between bib- and non-bibbed male painted
348 dragon lizards. Our results suggest that somatic levels of ROS production and antioxidant protection
349 are associated with sperm performance. Mitochondrial superoxide (mtSOx), measured in blood cells
350 (somatic), is negatively associated with the proportion of motile sperm (MOT) and the proportion of

351 progressively motile sperm (PMOT) in both bib-morphs (**Figures 4 and 5a**). Similar to McDiarmid et
352 al. (2017b), we did not find bib-morph specific differences in sperm kinematic parameters per se,
353 until superoxide was included as a covariate. There were morph-specific differences in the
354 association of blood cell superoxide level with progressive sperm motility. The negative relationship
355 between superoxide levels and sperm performance was most pronounced in bibbed males
356 (represented by PMOT and PC1, **Figures 5a & 5b**). Sperm performance seems to be strongly, albeit
357 indirectly linked to oxidative status in bibbed males, but less tightly so in non-bibbed males (**Figure**
358 **5b**). This may reflect differences in superoxide dismutase (SOD) levels in plasma (i.e., somatic
359 investment), which were negatively related with mtSOx in both morphs, but significantly higher in
360 non-bibbed males. Given the susceptibility of spermatozoa to ROS damage it is not surprising that
361 there are many studies that demonstrate negative correlations between antioxidants within the
362 semen and sperm performance in humans (Aitken et al., 1989; Aitken and Graves, 2002; Aitken and
363 Roman, 2008; Sanocka and Kurpisz, 2004) (e.g., reviewed in Aitken, 2016); and a few in non-
364 mammalian models (e.g., Rojas Mora et al., 2016). Nevertheless, a holistic idea of organismal
365 function in which levels of somatic antioxidants reflects overall condition or health is also supported
366 in the literature, as reproductive senescence and many disease states in humans are also associated
367 with oxidative stress in somatic tissues and declines in sperm performance and infertility (Aitken,
368 2016; Aitken et al., 2014). The link between SOD activity and body condition we found tentatively
369 suggests a trade-off between the allocation of resources to aggressiveness and colour investment in
370 the soma of bibbed males on one hand, and the buffering against the negative effect of superoxide
371 on sperm performance in non-bibbed males on the other.

372 As predicted, SOD activity, in both bibbed and non-bibbed males, was positively related to
373 body condition (BCI) at the time of capture. SOD activity measured nearly a month after body
374 condition measures were taken had a positive relationship indicating that SOD activity may depend
375 more on the available resources at the time of SOD production, than at the time of measurement;
376 we have recently documented similar time-lagging relationships between DNA-damage repair and

377 telomere attrition in this species (Olsson et al., 2018). However, the relationship of SOD activity and
378 BCI measured at the time of mating is more complex. In non-bibbed males, BCI at mating was still
379 positively related to SOD activity, but not so in bibbed males. Indeed, SOD levels were significantly
380 lower in bibbed males than non-bibbed males even after accounting for body condition at the time
381 of mating.

382 Positive links between colour traits, oxidative stress and sperm performance have been
383 demonstrated in a few species of birds, such as: Helfenstein et al. (2010) work on great tits
384 mentioned in the introduction; the positive association between colour, immunocompetence and
385 sperm performance demonstrated by Peters et al. (2004) in mallards; and Tomášek et al. (2016) use
386 of pro-oxidant manipulations to reveal oxidative-status versus colour maintenance allocation-
387 tradeoffs in captive zebra finches. Other comprehensive studies find clear links between oxidative
388 stress, colour and sperm performance. In the zebra finch (*Taeniopygia guttata*), Tomášek et al.
389 (2017) established that sperm performance of colourfully-beaked males was no different than drab
390 males. However, dosing male zebra finch with the pro-oxidant diquat, the sperm performance of the
391 most colourful males was drastically reduced in a remarkably similar fashion as the morph-specific
392 relationship of superoxide and sperm performance in our current study (Figure 1 in Tomášek et al.,
393 2017; our Figures 5a and 5b). Rojas Mora et al. (2016) found that the ratio of oxidised to reduced
394 glutathione (an endogenous antioxidant that quenches hydrogen peroxide, a downstream product
395 of superoxide dismutation by SOD) in the ejaculate of house sparrows (*Passer domesticus*) was
396 negatively correlated with the size of a melanin-based breast badge, thus indicating a link between
397 badge size and increased ejaculate antioxidants. Nevertheless, sperm swimming performance in the
398 house sparrow was not directly related to any oxidant or antioxidant measurement in semen (Rojas
399 Mora et al., 2016). In a follow up paper, experimental increases in oxidative stress in the same
400 species of sparrow increased antioxidant levels in the ejaculate but diquat dosing still reduced sperm
401 velocity with *the least* aggressive/dominant birds being the most strongly affected, probably due to

402 socially mediated decreases in access to food resources—or condition dependence (Rojas Mora et
403 al., 2017a). In yet another study, Rojas Mora et al 2017b, semen superoxide dismutase was positively
404 correlated with blood SOD supporting our view that somatic oxidative status should relate to
405 ejaculate oxidative status. Furthermore, males that changed social status to dominate roles tended
406 to have lower quality ejaculates compared to those produced by males in the middle of the
407 hierarchy (Figure 3, Rojas Mora et al., 2017b; unless the bird started at the lowest social status who
408 then moved into higher ranks). Together Rojas Mora et al.'s comprehensive work reveals a complex
409 relationship between oxidative status, ejaculate quality and social interactions in house sparrows,
410 that we interpret broadly as pointing toward the extreme plasticity of ejaculate traits in response to
411 social status and resource allocation trade-offs that are revealed under pro-oxidant manipulations,
412 when the social system is perturbed, and when access to resources is limited by dominance related
413 social interactions.

414 Initiating aggression is a critical determinant of winning male-male contests in painted
415 dragons (Healey et al., 2007) and bibbed males are the more aggressive of the two bib-morphs
416 (McDiarmid et al., 2017b), which may partially explain why males with bibs are less likely to lose
417 paternity to neighboring rivals and males without bibs (Olsson et al., 2009a). Our results contrasts to
418 some of the results found in the wild house sparrows described above; relative superoxide levels
419 most strongly negatively associated with sperm performance of the more aggressive bibbed males,
420 who also tended to have lower SOD in unmanipulated lizards. In our study, males were not allowed
421 to interact (intense fighting occurs if males are housed together) which removes the costs of
422 aggressive interactions (on both the aggressor and their target) and the lizards were fed to satiety
423 every other day so socially mediated differential in resource limitation alone is not a likely
424 explanation of our results. But if aggressiveness is correlated with underlying physiology, such as
425 metabolic rate, costs may be incurred in addition to those due to direct interactions (Metcalfe et al.,
426 1995). For example, aggressive male phenotypes of the Atlantic salmon (*Salmo salar*) have elevated

427 metabolic rates which are beneficial in mating success, but may be deleterious during periods of low
428 food abundance and even affect the growth rate and age-related declines in performance (Fleming,
429 1996; Jonsson et al., 1991; Metcalfe et al., 1995). Thus, the cost-benefit trade-off between morph-
430 specific reproductive strategies may be linked to metabolic rate. It is possible, that such condition-
431 dependent trade-offs underpin our results.

432 The correlations we found between ROS and sperm performance were in unmanipulated
433 males, and although the relationship of body condition and SOD is only suggestive of condition-
434 dependence. In the brown anole lizard (*Anolis sagrei*), experimental food-manipulations
435 demonstrated body condition affected sperm morphology and numbers, which also translated to
436 greater fertilization success for males with higher BCI (Kahrl and Cox, 2015). Manipulative studies
437 such as those described above are necessary to fully illuminate the links we examine in this study.

438 Below we consider non-mutually exclusive hypotheses that may better explain our results
439 while also suggesting further avenues of research. First, the temporal difference in these two
440 condition dependent relationships with SOD, and the morph-specific differences in SOD may be
441 related to the decrease in SOD and increase in mtSOx over the course of our sampling period.
442 Second, morph-specific SOD turnover rates due to initial condition may have a lasting effect on SOD
443 activity that may also explain the breakdown in the relationship between BCI and SOD at the time of
444 mating. Regrettably, data on SOD turnover rates in animal taxa other than mammals (i.e., mice, rats
445 and humans) are sparse and temperature dependent rates in ectotherms is in need of further
446 research. Thermally mediated variation in SOD production and turnover rate was proposed as an
447 explanation for the slower post activity decreases in mtSOx of male painted dragons acclimated to
448 "hot" basking conditions compared with "cool" basking males (Ballen et al., 2012), but this was not
449 tested directly. Nevertheless, we can construct rough estimates of turnover rates in these dragons
450 because metabolic rate is linked to stoichiometry of protein and other macromolecular turnover
451 across taxa (Hulbert and Else, 2000).

452 Some mammalian cellular proteins like histones (H3.1) and nuclear pore complex proteins
453 (Nup205) persist nearly a year *in vivo* (Toyama et al., 2013), but most proteins have half-lives of only
454 45 minutes to 22.5 hours (Eden et al., 2011). Among proteins, superoxide dismutase is relatively
455 long-lived (Valentine et al., 2005) with a half-life of 45-80 hours for SOD expressed in mice kidneys
456 (Crisp et al., 2015; Hoffman et al., 1996). Nevertheless, the standard/basal metabolic rate of a 13g
457 mouse (e.g., *Mus spp.*) is nearly 5.5 x higher than that of a 13 g painted dragon, (0.09ml/min*60 =
458 5.4ml/hr; Friesen et al., 2017a; mouse 29.78mlO2/hr; Withers, 1992, pg 95). Furthermore, non-
459 specific protein synthesis rates in mice are ~4.8 x faster than in lizards (Sayegh and Lajtha, 1989;
460 Warne et al., 2010) and isotopic nitrogen turnover rates yield similar values (Vander Zanden et al.,
461 2015; Warne et al., 2010). Assuming that this inter-taxonomic factorial adjustment is reasonable, the
462 degradation rate of SOD in a lizard is probably 4.8-5.5 x slower than in a mouse, which translates to a
463 SOD half-life of 9-18 days in painted dragons. Given the upper end of this half-life range, BCI taken at
464 capture could plausibly influence SOD activity 21-50 days later, which might explain the effect of
465 early season condition on later SOD activity. As bibbed males have ~14% higher resting metabolic
466 rates than non-bibbed males (Friesen et al., 2017a), they are likely to have had faster degradation of
467 SOD, which could explain the disappearance of the positive relationship between SOD and BCI at
468 mating in bibbed males, but not non-bibbed males.

469 Another, although more speculative, hypothesis is that different variants of SOD or
470 mitochondrial genes may be linked to bib expression. For example, amyotrophic lateral sclerosis
471 (ALS) is associated with oxidative damage of neuronal axons (Oeda et al., 2001), and mutations in
472 SOD (>100 functional ALS mutations) are linked with 20-25% of ALS cases in humans (Valentine et
473 al., 2005). In humans, small mutations in SOD genes results in drastic 10-30 fold increases in SOD
474 levels in blood serum (Zelko et al., 2002). Thus, bibbed and non-bibbed males may have different
475 genetic variants of SOD genes that affect oxidative status and is linked to condition. Nutrition and
476 condition may also affect mitochondrial function and superoxide generation (Ballard and Youngson,
477 2015; Salin et al., 2018) and exogenous antioxidants could certainly play a role in reducing ROS levels

478 (Monaghan et al., 2009). However, in painted dragons, supplementation with vitamin E (an
479 antioxidant) does not increase longevity in the wild (Healey and Olsson, 2009b) and
480 supplementation with dietary carotenoids does not reduce the effects of ROS effects on colour
481 maintenance (Olsson et al., 2008a). Nevertheless, dietary antioxidants may affect sperm function in
482 this species and this merits further research. We did not measure colour brightness or intensity in
483 this study so we cannot directly assess the link between bib colour investment and sperm
484 performance. McDiarmid et al. (2017b), however did not find a significant relationship between bib
485 saturation and two measures of sperm function, motility (MOT) and velocity (VAP) which is
486 consistent with the meta-analysis of Mautz et al. (2013) that found only weak positive, but non-
487 significant correlations between colour traits and sperm kinematic performance. However, as Lüpold
488 et al. (2014) demonstrate, the strength and evolution of pre- and postcopulatory trade-off depends
489 on the degree of male monopolization. We might expect mate monopolization rates to be high for
490 bibbed males based on advantages in both female preference and contest success (McDiarmid et al.,
491 2017a), which seems to translate into reduced cuckoldry by neighbouring males in the wild (Olsson
492 et al., 2009a). Nevertheless, if this trade-off is mediated by an allocation trade-off rather than a strict
493 negative genetic correlation, as the relationship between SOD and body condition suggests it might,
494 negative correlations between colouration and sperm performance might not be evident in the lab
495 where resources were available ad libitum, and aggressive metabolically taxing territorial activity
496 was absent.

497 Bibbed males have shorter telomeres than non-bibbed males (Rollings et al., 2017), and
498 telomere shortening is linked with oxidative stress (Monaghan, 2010; Olsson et al., 2018; Olsson et
499 al., 2017). In the wild, male painted dragons lose mass through the breeding season which ends in
500 late December to mid-January; 90% of adults do not survive through winter 'hibernation' (beginning
501 in late May) to the next breeding season (in September) (Healey and Olsson, 2009a; Olsson et al.,
502 2007b; Olsson et al., 2009a). The increase in superoxide and decrease in SOD throughout the
503 sampling period we describe here is consistent with senescence manifest in weight loss, DNA

damage, telomere shortening, and colour fading across the breeding season in this species (Giraudeau et al., 2016; Olsson et al., 2012b; Rollings et al., 2017). We tested mtSOx in blood cells, but the mitochondrial proteins in sperm cells are the same as those in blood cells and should generate similar levels of mtSOx. Mitochondrial function and superoxide production is affected by the stress response (Manoli et al., 2007), and, if bib-morphs differ in their resilience to stress, this may explain different sensitivities of sperm to ROS. Spermatogenesis is ongoing throughout the breeding season in painted dragons, indicated by testicular enlargement persisting August through late December (Niejalke, 2006). Condition earlier in the season during spermatogenesis may have a strong influence on sperm damage and concomitant sensitivity to oxidative damage later in the season as our males were held without access to females prior to mating trials and sperm collection. This would constitute a trade-off between aggression typical of bibbed males and resilient sperm of non-bibbed males. Supplementation with a SOD mimetic, EUK 134, reduces age-related colour fading associated with oxidative stress and may also be used to experimentally tease apart the potential effects of body condition from SOD on sperm performance (Olsson et al., 2012b).

Conclusions

We found a positive relationship between body condition and blood plasma SOD activity. SOD activity, which was lower in bibbed males, was negatively related to mitochondrial superoxide of erythrocytes. SOD was measured in the plasma which precludes a direct link between these variables. Furthermore, there was a negative, morph-specific indirect link between erythrocyte mitochondrial superoxide and sperm performance. Given the correlative nature of these results, we are unable to ascribe cause or disentangle the proximate mechanisms that underlay these the linkages. Nevertheless, these results add yet another morph-specific difference in physiology and behaviour to those we have previously documented in the Australian painted dragon lizards (Friesen et al., 2017a; Friesen et al., 2017b; Healey and Olsson, 2009a; Healey et al., 2007; McDiarmid et al., 2017a; Olsson et al., 2007a; Olsson et al., 2009a; Rollings et al., 2017). Future investigations that

529 manipulate body condition and activity levels of bibbed and non-bibbed males are needed to
530 determine whether sperm performance is condition-dependent in this species.

531 Competing interests statement

532 We have no competing interests.

533 Author contributions

534 All authors contributed materially and intellectually to this experiment and manuscript.

535 Acknowledgements

536 Dr Danielle Johinke for tutorial on CASA and FACS. Dr Emily Uhrig, the Editor and three anonymous
537 reviewers for useful and sagacious comments and edits. Dr Cissy Ballen for lizard husbandry and
538 sperm collection. Dr Adele Haythornthwaite for administrative assistance. NSW National Parks
539 service for permits and access to Yathong Nature Reserve, especially E. Snaith. National Science
540 Foundation funding to CRF and Australian Research Council funding to SdG and MO. All research was
541 conducted with the approval of the University of Sydney Animal Ethics Committee Project Number:
542 2013-2016/6050.

543 Data, code and materials

544 Data will be deposited to http://datadryad.org/ upon acceptance of the manuscript.

545

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834

Figure legends

Figure 1

Male painted dragon lizards. On the left is a male without a yellow throat patch (i.e. a non-bibbed male) and on the right is a male with a yellow throat patch (i.e. a bibbed male).

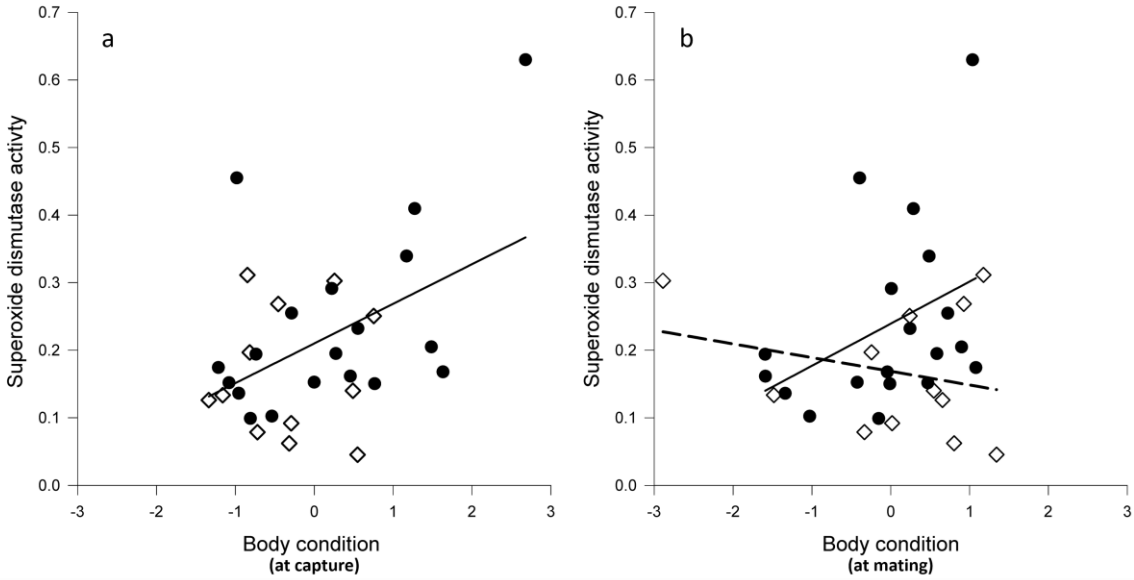
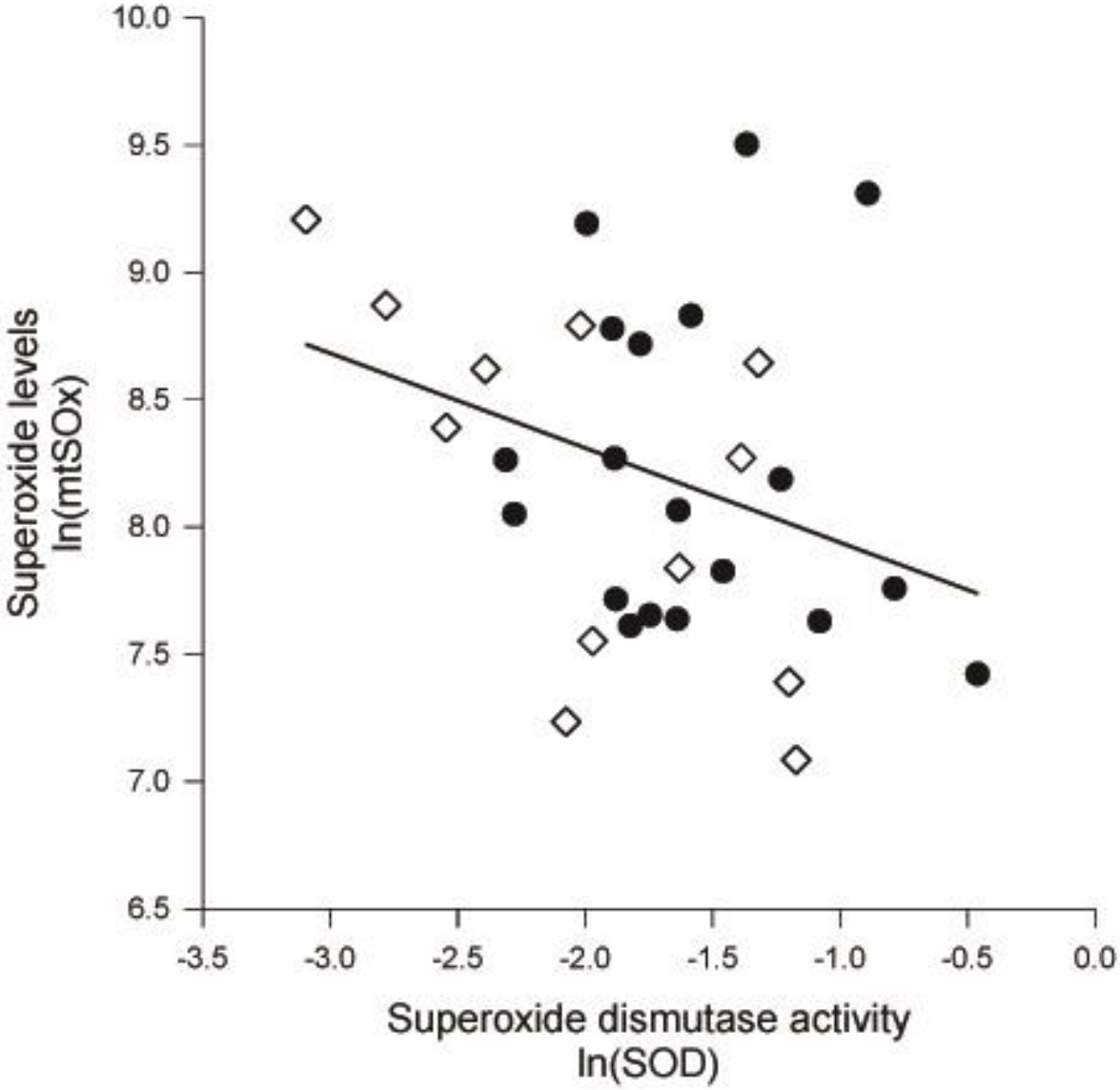


Figure 2

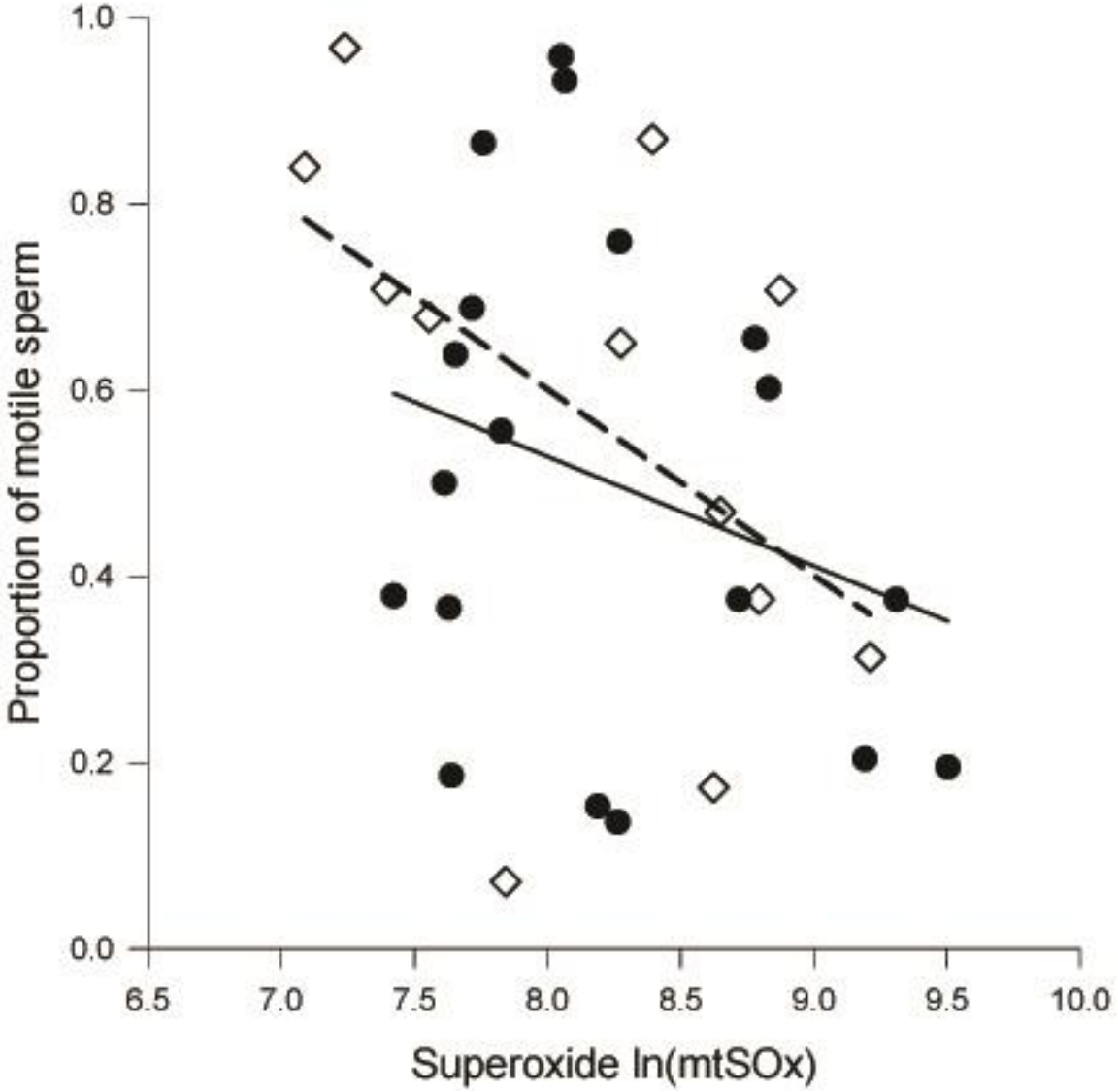
Relationship between SOD activity (untransformed data graphed) and body condition (BCI, residual ln(body mass) given ln(snout to vent length)). In each graph, solid circles (●) represent non-bibbed males and unfilled diamonds (◇) represent bibbed males. Panel **a)** SOD activity at mating as a function of body condition *at capture*: $R^2 = 0.213$, $P = 0.009$. Panel **b)** SOD activity at mating as a function of body condition at mating: separate regression lines for each morph are plotted due to the significant morph x BCI interaction ($P = 0.015$; solid line represents non-bibbed males ($R^2 = 0.149$, $P = 0.103$), dashed line represents bibbed males ($R^2 = 0.066$, $P = 0.420$).



850

851 Figure 3

852 Negative relationship between mitochondrial superoxide levels in blood cells and plasma superoxide
853 dismutase activity (R^2 = 0.107, P = 0.018). Solid circles (●) represent non-bibbed males and unfilled
854 diamonds (◇) represent bibbed males.



855

856 Figure 4

857 Overall negative relationship between sperm motility and mitochondrial superoxide levels (P =
858 0.001). We have graphed separate regressions for each bib-morph: Solid circles (●) represent non-
859 bibbed males (solid regression line, R^2 =0.080) and unfilled diamonds (◇) represent bibbed males
860 (dashed regression line, R^2 = 0.249).

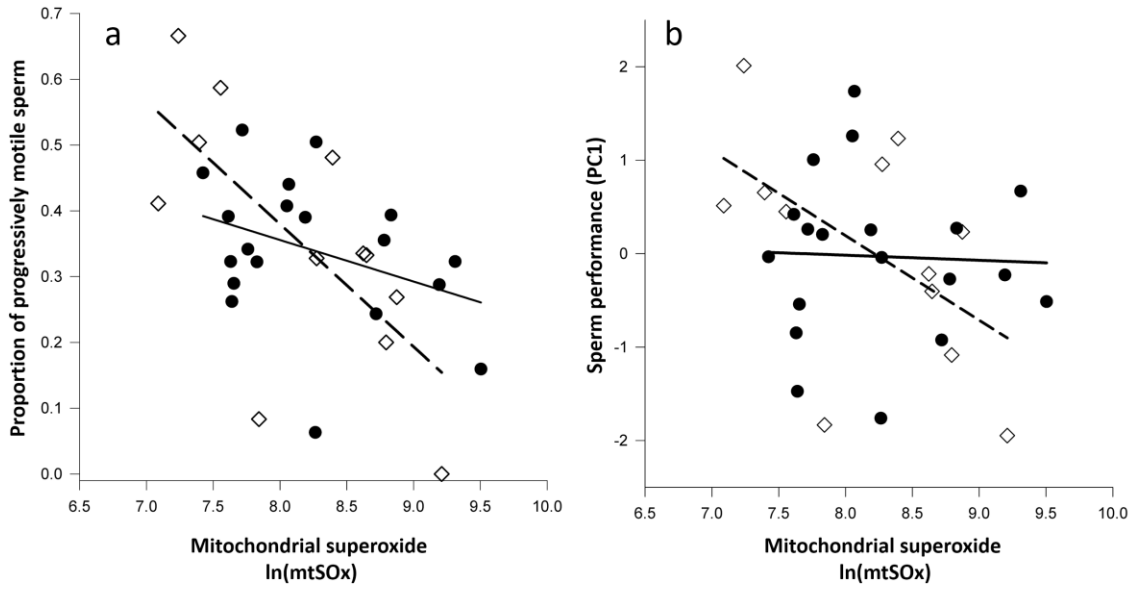


Figure 5

a) Negative relationship between the proportion of progressively motile sperm (PMOT) and mitochondrial superoxide levels in blood cells (ln(mtSOx); P < 0.001). Regression lines were calculated and plotted separately for each morph given the significant bib x ln(mtSOx) level interaction (P = 0.036). Solid circles (●) represent non-bibbed males (solid regression line: R^2 = 0.130) and unfilled diamonds (◇) represent bibbed males (dashed regression line: R^2 = 0.458).

b) Negative relationship between sperm performance (PC1) and mitochondrial superoxide levels (ln(mtSOx), P = 0.019). PC1 is positively correlated with the following sperm kinematic parameters: LIN, ALH, MOT, PMOT, VCL, VAP, VSL see text for definitions (r ranged 0.663-0.994, all p <0.0001); BCF (beat frequency) had a significantly negative correlation with PC1 (r = -0.578, P< 0.0001). Regression lines were calculated and plotted separately for each morph given the significant bib x ln(mtSOx) level interaction (P = 0.038). Solid circles (●) represent non-bibbed males (solid regression line: R^2 = 0.002) and unfilled diamonds (◇) represent bibbed males (dashed regression line: R^2 = 0.288).

Tables

Table 1: Model effects of GLM (normal, identity link function) SOD activity as a function of bib and BCI at the time of mating. Full model: ln(SOD) ~ Bib + BCI_{mating} + Bib * BCI. Likelihood ratio χ^2_{df3} = 8.9166, P = 0.043 vs intercept only model. Bib (n = 12), Non-bibbed (n = 19), a positive coefficient indicates non-bibbed greater than bibbed. Bold text indicates P ≤ 0.05.

ln(SOD)~	β(se)	95% Likelihood CI	Type III Sum of Squares

Source		(lower, upper)	Wald	Sig.
Intercept	-1.951(0.165)	-2.243, -1.658	328.891	**<0.001**
Bib	0.394(0.194)	0.021, 0.768	4.161	**0.041**
BCI_{mating}	-0.161(0.129)	-0.413, 0.90	1.570	0.210
Bib x BCI_{mating}	0.407(0.168)	0.028, 0.786	5.878	**0.015**

Table 2: Model effects of GLM (normal distribution, identity link function) mtSOx levels as a function of bib and SOD activity at the time of mating. Full model: ln(mtSOx) ~ Bib + ln(SOD) + Bib * ln(SOD): Likelihood ratio $\chi^2_{df3} = 6.345$, P = 0.096 vs intercept only model. Reduced model: mtSOx~ln(SOD) Likelihood ratio $\chi^2_{df1} = 4.646$, P = 0.031 Bib (n = 12), Non-bibbed (n = 19), a positive coefficient indicates non-bibbed greater than bibbed. Bold text indicates P ≤ 0.05.

Ln(mtSOx)~		95% Likelihood CI	Type III Sum of Squares	
Source	β(se)	(lower, upper)	Wald	Sig.
Intercept	6.786(0.470)	5.646, 7.926	445.140	**<0.001**
Bib	1.174(0.699)	0.294, 2.642	2.820	0.093
ln(SOD)	-0.701(0.194)	-1.256, -0.147	6.297	**0.012**
Bib x ln(SOD)	0.527(0.349)	-0.265, 1.318	2.276	0.131

Table 3a: Model effects of GLM (normal distribution, identity link function) sperm motility (MOT) as a function of bib and mtSOx level at the time of mating. Full model: MOT ~ mtSOx + bib + bib*mtSOx: Likelihood ratio $\chi^2_{df3} = 5.486$, P = 0.139 vs intercept only model. Reduced model: MOT ~ mtSOX Likelihood ratio $\chi^2_{df1} = 4.747$, P = 0.029. Bib (n = 12), Non-bibbed (n = 19), a positive coefficient indicates non-bibbed greater than bibbed. Bold text indicates P ≤ 0.05.

MOT~		95% Likelihood CI	Type III Sum of Squares	
Source	β(se)	(lower, upper)	Wald	Sig.
Intercept	220.008(56.382)	48.628, 391.39	21.279	**<0.001**
Bib	-73.048(79.553)	-300.85, 154.754	0.843	0.358
mtSOx	-19.993(6.822)	-40.919, -0932	11.244	**0.001**
Bib x mtSOx	-8.231(9.470)	-19.485, 35.948	0.755	0.385

Table 3b: Model effects of GLM (normal distribution, identity link function) progressively motile sperm (PMOT) as a function of bib and mtSOx level at the time of mating. Full model: PMOT ~ mtSOx

897 + bib + bib*mtSOx: Likelihood ratio χ^2_{df3} = 13.218, P = 0.004 vs intercept only model. Bib (n = 12),

898 Non-bibbed (n = 19), a positive coefficient indicates non-bibbed greater than bibbed. Bold text

899 indicates P ≤ 0.05.

PMOT~ Source	β(se)	95% Likelihood CI (lower, upper)	Type III Sum of Squares	
			Wald	Sig.
Intercept	1.872(0.448)	0.993, 2.750	17.445	**< 0.001**
Bib	-1.015(0.502)	-1.998, -0.32	4.094	**0.043**
mtSOx	-0.186(0.053)	-0.291, -0.082	12.282	**< 0.001**
Bib x mtSOx	0.013(0.003)	0.008, 0.242	4.398	**0.036**

900

901 **Table 3c**: Model effects of GLM (normal distribution, identity link function) "sperm performance"

902 (PC1) as a function of bib and mtSOx level at the time of mating. Full model: PC1 ~ mtSOx + bib +

903 bib*mtSOx: Likelihood ratio χ^2_{df3} = 6.283, P = 0.099 vs intercept only model (we did not reduce

904 model given significant parameter estimates). Bib (n = 12), Non-bibbed (n = 19), a positive coefficient

905 indicates non-bibbed greater than bibbed. Bold text indicates P ≤ 0.05.

Sperm performance (PC1) Source	β(se)	95% Likelihood CI (lower, upper)	Type III Sum of Squares	
			Wald	Sig.
Intercept	20.145(7.202)	6.030, 34.259	7.825	**0.005**
Bib	-17.744(8.617)	-34.633, -0.854	4.240	**0.039**
mtSOx	-2.456(0.863)	-4.147, -0.765	8.105	**0.004**
Bib x mtSOx	2.157(1.029)	0.141, 4.173	4.398	**0.036**

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