Comparative Biochemistry and Physiology, Part A xxx (xxxx) 111126

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Antioxidant capacity differs across social ranks and with ascension in males of a group-living fish

Brett M. Culbert [a, 1, *], Peter D. Dijkstra [b], Kathleen M. Gilmour [c], Sigal Balshine [a]

[a] Department of Psychology, Neuroscience & Behaviour, McMaster University, 1280 Main St W, Hamilton, Ontario L8S 4K1, Canada
[b] Department of Biology, Central Michigan University, 1200 S Franklin St, Mount Pleasant, MI 48859, USA
[c] Department of Biology, University of Ottawa, 30 Marie Curie Private, Ottawa, Ontario, Canada, K1N 6N5

ARTICLE INFO

Editor: Michael Hedrick

Keywords:
11-ketotestosterone
Androgens
Dominance
Neolamprologus pulcher
Oxidative stress
Social instability
Social rank

ABSTRACT

Animals that live in groups often form hierarchies in which an individual's behaviour and physiology varies based on their social rank. Occasionally, a subordinate can ascend into a dominant position and the ascending individual must make rapid behavioural and physiological adjustments to solidify their dominance. These periods of social transition and instability can be stressful and ascending individuals often incur large metabolic costs that could influence their oxidative status. Most previous investigations examining the link between oxidative status and the social environment have done so under stable social conditions and have evaluated oxidative status in a single tissue. Therefore, evaluations of how oxidative status is regulated across multiple tissues during periods of social flux would greatly enhance our understanding of the relationship between oxidative status and the social environment. Here, we assessed how antioxidant capacity in three tissues (brain, gonad, and muscle) varied among dominant, subordinate, and ascending males of the group-living cichlid fish, *Neolamprologus pulcher*. Antioxidant capacity in the brain and muscle of ascending males was intermediate to that of dominant (highest levels) and subordinate males (lowest levels) and correlated with differences in social and locomotor behaviours, respectively. Gonad antioxidant capacity was lower in ascending males than in dominant males. However, gonad antioxidant capacity was positively correlated with the size of ascending males' gonads suggesting that ascending males may increase gonad antioxidant capacity as they develop their gonads. Overall, our results highlight the widespread physiological consequences of social ascension and emphasize the importance of tissue-specific measures of oxidative status.

1. Introduction

Individuals living in groups typically benefit from reduced predation risk (Cresswell and Quinn, 2011; Roberts, 1996), increased foraging success (Evans et al., 2016; Ward and Zahavi, 1973) and lighter workloads (Clutton-Brock et al., 2002; Ulrich et al., 2018). However, group-living does not benefit all individuals equally because groups are often despotic with dominants using aggression, intimidation, or simply their higher status to monopolize resources (Isbell, 1991; Milinski and Parker, 1991; Stockley and Bro-Jørgensen, 2011). Additionally, dominants commonly suppress subordinate reproduction and somatic growth (Ang and Manica, 2010; Kokko and Johnstone, 1999; Lukas and Clutton-Brock, 2014). These rank-based differences are thought to be major contributors to the widespread physiological differences observed across social ranks, including variation in metabolic rates (Senar et al., 2000; Sloman et al., 2001), the size of energy reserves (Gilmour et al., 2012; Hellmann et al., 2016; Witter and Swaddle, 1995), and the activity of enzymes that regulate metabolic pathways (Gilmour et al., 2017; Hammond et al., 2000; Regan et al., 2015). However, while metabolic differences associated with social rank have been well described in several taxa, fewer studies have evaluated the relationship between an individual's social environment and their oxidative status.

An individual's oxidative status is determined by the relative production of reactive oxygen species (ROS) compared to the neutralization capacity of antioxidants (Monaghan et al., 2009; Sies et al., 2017). ROS are naturally produced during the oxygen-related redox reactions that drive aerobic metabolism (Dowling and Simmons, 2009; Sies et al., 2017) and if ROS production exceeds the neutralization capacity of antioxidants then oxidative damage to lipids, proteins, and nucleic acids can occur (Finkel and Holbrook, 2000; Monaghan et al., 2009). There-

* Corresponding author.
E-mail address: culbertb@uoguelph.ca (B.M. Culbert).
[1] Current Address: Department of Integrative Biology, University of Guelph, 50 Stone Rd E, Guelph, Ontario, Canada, N1G 2W1.

https://doi.org/10.1016/j.cbpa.2021.111126
Received 27 August 2021; Received in revised form 7 December 2021; Accepted 7 December 2021

fore, antioxidants are critical in preventing oxidative stress and maintaining protein function, DNA integrity, and overall cellular efficiency (Pamplona and Costantini, 2011; Pisoschi and Pop, 2015). Rank-based differences in oxidative status are generally thought to reflect differences in the metabolic demands associated with a given social rank and/or status-based differences in access to resources (e.g., Border et al., 2019; Cram et al., 2015; Losdat et al., 2019; Mendonça et al., 2020; Silva et al., 2018). However, most of these previous studies have evaluated differences between ranks under stable social conditions; with only a very small number of studies investigating the regulation of oxidative status during periods of social instability (e.g., Beaulieu et al., 2014; Border et al., 2019; Fialkowski et al., 2021; Georgiev et al., 2015; Mora et al., 2017). Periods of social instability are generally associated with elevated levels of stress and heightened metabolic demands (Culbert et al., 2019; Sapolsky, 1983; Van Meter et al., 2009), meaning that adjustments in the regulation of oxidative status are likely. Additionally, the handful of studies that have investigated the regulation of oxidative stress during periods of social flux have generally evaluated oxidative status in blood, which may not be representative of changes in other tissues (Argüelles et al., 2004; Garratt et al., 2012; Veskoukis et al., 2009; but see Margaritelis et al., 2015). Therefore, cross-tissue measurements of oxidative status during periods of social instability would greatly enhance our understanding of how oxidative status is affected by an individual's social environment.

To address these gaps in our knowledge we made use of the African cichlid, *Neolamprologus pulcher*, a small fish that lives in permanent social groups consisting of a dominant male-female breeding pair and up to 20 subordinates that "help" by performing brood care, territory maintenance, and territory defense (Taborsky and Limberger, 1981; Wong and Balshine, 2011a). Subordinate *N. pulcher* occasionally have the opportunity to ascend into a dominant position (Balshine-Earn et al., 1998; Bergmüller et al., 2005a; Fitzpatrick et al., 2008; Stiver et al., 2006) and ascending males become more aggressive and active, and increase their workload, growth rate, and reproductive capacity (Culbert et al., 2018, 2019; Fitzpatrick et al., 2008; Sopinka et al., 2009). Consequently, ascension is a metabolically demanding (Culbert et al., 2019) and stressful period (Culbert et al., 2018) that is accompanied by physiological adjustments across many tissues. For example, ascending individuals often enlarge their gonads and/or their body size when they transition to a more dominant position because these changes increase their probability of mating (Culbert et al., 2019; Fitzpatrick et al., 2006). Additionally, larger gonads provide a greater capacity to synthesize androgens, which are often associated with aggression and dominance (Hirschenhauser and Oliveira, 2006; Rosvall et al., 2020; Wingfield et al., 1990). Indeed, circulating levels of 11-ketotestosterone (11-KT)—a primary androgen in teleost fish (Borg, 1994; Oliveira et al., 2002)—increase when male *N. pulcher* become dominant (Fitzpatrick et al., 2008; Taves et al., 2009). However, these changes are fuelled, at least in part, by the catabolism of energy reserves in key metabolic tissues (e.g., liver and muscle; Culbert et al., 2019). It is therefore likely that oxidative status will vary across different tissues during social ascension, but few studies to date have assessed how periods of social flux affect oxidative status in different tissues. Here, we assessed how antioxidant capacity varied across brain, muscle, and gonad of dominant, subordinate, and ascending male *N. pulcher*.

We hypothesized that social ascension elicits tissue-specific changes in antioxidant capacity which relate to the different behavioural and/or physiological functions served by each tissue (see Fig. 1 for a visual representation of our predictions). Specifically, in the brain we hypothesized that that antioxidant levels may be related to the cognitive load associated with dominance (Flack et al., 2005). Therefore, we predicted that brain antioxidant capacity would be positively related with how dominant an individual was within their group and that it would increase as males ascended to the dominant position. In muscle, we hypothesized that antioxidant levels reflect the elevated metabolic demands associated with dominance in *N. pulcher* (Culbert et al., 2019; Hellmann et al., 2016; Sopinka et al., 2009). Accordingly, we predicted that muscle antioxidant capacity would increase during ascension and would be positively related with an individual's locomotor activity, growth rate and/or workload. Lastly, we hypothesized that gonad antioxidant levels would be higher in ascenders to combat elevated ROS production (Covarrubias et al., 2008; Hernández-García et al., 2010) associated with the extensive growth and development of the gonads that occurs during ascension (Fitzpatrick et al., 2008; Maruska, 2014; Scaia et al., 2020). Therefore, we predicted that gonad antioxidant capacity would be highest in ascending males and would be positively related to the size of their gonads and/or circulating levels of 11-KT (a proxy for changes in rates of androgen synthesis).

2. Experimental methods

2.1. General housing

The experiment was conducted between November 2016 and April 2017 at McMaster University in Hamilton, Ontario, Canada. All fish were laboratory-reared descendants of wild-caught *N. pulcher* from Lake Tanganyika, Africa. Twenty social groups were used, each consisting of a dominant breeding male-female pair, 1–3 large subordinates of mixed sex (standard length (SL) > 4.5 cm), and 1–4 small subordinates of mixed sex (SL < 4 cm). Groups were held within 189 L aquaria containing two large sponge filters, a heater, 3 cm of coral sand for substrate, and two terracotta flowerpot halves that served as brood chambers (where the dominant breeding pair lay eggs and rear young). In addition, each tank contained two mirrors on either side of the tank to reduce aggression between group members (*N. pulcher* readily interact with their reflections as they would an intruding conspecific (Balzarini et al., 2014; Hotta et al., 2018; Reddon et al., 2012)), and two PVC tubes as shelter. Group members could also interact across the aquarium glass with members of neighbouring social groups in adjacent aquaria (one on either side of each aquaria). All groups had been together for at least a month and had produced young prior to experimentation. Each fish received unique dorsal fin clips for identification; these clips do not adversely affect behaviour (Stiver et al., 2004). Tank water was held at 27 °C and a 13 L:11D photoperiod was maintained through-

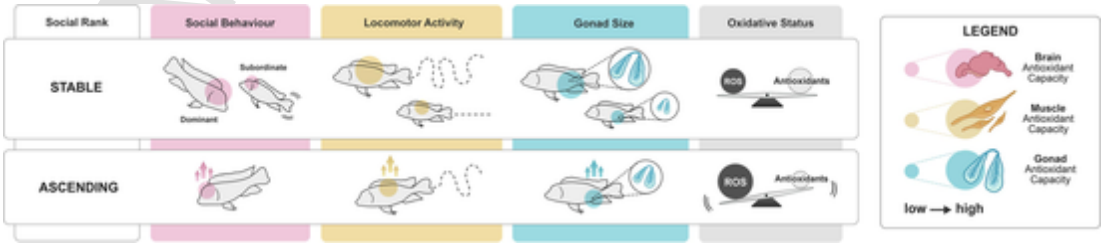

Fig. 1. Predictions of how antioxidant capacity in brain, muscle, and gonad varies across dominant, subordinate, and ascending male *Neolamprologus pulcher*, as well as the predicted behavioural/physiological factors related to these differences. Note that reactive oxygen species (ROS) were not measured in our study and antioxidant levels do not necessarily reflect oxidative status.

out the experiment. Fish were fed 1% combined group body weight daily with NorthFin floating cichlid pellets (1 mm; Canadian Aquatic Feed Inc., Toronto, ON, Canada). All experimental protocols were approved by the Animal Research Ethics Board of McMaster University (Animal Utilization Protocol No. 14–02-05) and followed the guidelines of the Canadian Council on Animal Care (CCAC) regarding the use of animals in research and teaching.

2.2. Social ascension protocol

At the beginning of the experiment (Day 0), all individuals within each social group were quickly netted and placed into 2 L containers filled with water from their home tank. Fish were then placed on a damp towel where their length was measured with calipers (to the nearest 0.01 mm). Next, fish were transferred into a pre-weighed container of water to determine their mass (to the nearest mg) and then were returned to their respective tank. This entire process took less than 1 min per fish (no fish was out of water for longer than 5 s during this period) and fish resumed normal behaviour within ~2 min of being returned to their tank. We randomly designated each social group as either a control group (N = 8; mean ± SEM of 6.8 ± 0.5 group members) or a treatment group (N = 12; mean ± SEM of 6.8 ± 0.4 group members). Ten days later (the morning of Day 11), all fish in each group were recaptured and measured as described above. At the same time, we removed and terminally sampled all large subordinate males (1–2 per group) from the control groups (N = 12; mass = 3.79 ± 0.31 g, SL = 5.23 ± 0.12 cm) and the dominant male from each treatment group (N = 12; mass = 7.45 ± 0.35 g, SL = 6.73 ± 0.18 cm). Note that two subordinate males were sampled from a single group in four control groups. All other fish were returned to their respective tanks. The removal of the dominant male from treatment groups provided the opportunity for a subordinate male in these groups to ascend to the dominant position. Three days later (the morning of Day 14), males that had ascended to the dominant position were captured and terminally sampled (N = 8; mass = 5.21 ± 0.38 g, SL = 5.86 ± 0.17 cm). In three groups, the large subordinate male (N = 3; mass = 3.97 ± 0.85 g, SL = 5.32 ± 0.29 cm) did not ascend to the dominant position by Day 14 (i.e., their dominance index scores remained negative and/or did not increase following removal of the dominant male; see Section 2.3), and so these males were not collected from these groups (see Supplementary Material for a comparison of the morphology and behaviour of ascending versus non-ascending males). Note that in one group a large subordinate female was miscategorized as a male at the start of the experiment and therefore no large subordinate male was present to ascend to the dominant position in this group.

2.3. Behavioural analyses

All social groups were videorecorded twice (on Days 10 and 11) to assess the behaviour of all males. Additionally, treatment groups were recorded twice after dominant males had been removed (on Days 13 and 14) to assess the behaviour of ascending males. On Days 11 and 14, groups were recorded immediately before males were caught and sampled. A video camera (Canon; VIXIA HF S200) was placed approximately 0.5 m from the front of each tank, which allowed us to view the entire tank and easily identify group members based on their unique fin clips. The first 5 mins of each recording served as an acclimation period following the placement of the camera and was therefore not scored. Following the acclimation period, we scored all behaviours that were performed or received by all dominant and subordinate/ascending males over the subsequent 10 min (see Sopinka et al., 2009 for a full detailed species-specific ethogram). A dominance index was determined for each male by subtracting the combined number of aggressive acts (chases, bites, rams, opercular flares, aggressive postures, and lateral displays) received and submissive acts (flees, and submissive postures and displays) given from the total number of aggressive acts given and submissive acts received [Dom Index = (Agg_{Given} + Sub_{Rec}) − (Agg_{Rec} + Sub_{Given})]. A workload index (see Balshine et al., 2001) for each fish was determined by combining the number of visits to the brood chamber, the number of territory maintenance behaviours (digs and carries—the act of picking up and moving substrate with their mouths), and defensive aggressive acts (i.e., those performed towards a mirror or fish in neighbouring tanks). Locomotor activity was also assessed by measuring the proportion of time that males were moving during each 10 min observation period. Behaviours are reported as averages across the two observation periods for each fish (i.e., Days 10/11 for dominant and subordinate males or Days 13/14 for ascending males).

2.4. Tissue sampling

Following removal, fish were immediately killed via terminal anaesthesia (0.5 g L^{-1} ethyl-p-aminobenzoate), their mass and standard length were recorded, and blood was collected (within 2–3 min of approaching the tank) into heparinized micro-hematocrit capillary tubes (Thermo-Fisher Scientific) following caudal severance. Plasma was collected after centrifugation (4750 *g* for 3 min), flash frozen in liquid nitrogen, and stored at −80 °C for later analysis of 11-KT concentrations. Gonads were removed and weighed after which they—along with the brain and a piece of white muscle—were flash frozen and stored at −80 °C for later analysis of antioxidant capacity.

2.5. Quantification of antioxidant capacity

Total antioxidant capacity (TAC) was measured in tissue lysates via an Oxygen Radical Absorbance Capacity (ORAC) assay (Border et al., 2019; Ou et al., 2001; Wilson et al., 2012). TAC is a global measure of antioxidant activity that evaluates the combined contributions of both small molecule (e.g., dietary) and enzymatic antioxidants.

Frozen tissue samples were transferred into 0.25 mL of lysis buffer (20 mM Tris–HCl, 137 mM NaCl, 1% NP-40, 10% glycerol, 2 mM EDTA) and homogenized using an Omni Tissue Master (Omni International). Following centrifugation (17,000 *g* for 10 min at 4 °C), the resulting supernatant was collected, and protein concentration was measured using a bicinchoninic acid assay kit (Pierce). Fluorescence was measured with an excitation wavelength of 485 nm, an emission wavelength of 515 nm, and a cut-off filter wavelength of 495 nm. Assays were carried out at 37 °C in black-sided 96-well plates and wells on the edges of each plate were filled with 200 μL of distilled water to increase assay stability. All other wells contained 120 μL fluorescein in 75 mM potassium phosphate (3.82 μM final concentration), and 20 μL of tissue lysate (protein concentration standardized to ~80 μg mL^{-1} with a final 1:2 dilution of lysate:PBS), blank (75 mM potassium phosphate, pH 7.4), or standard [6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid in 75 mM potassium phosphate (Trolox); 0–400 μM initial concentration]. All samples were run in duplicate. Each plate was initially incubated at 37 °C for 30 min, then 60 μL of 2.2′-azobis (2-amidinopropane) dihydrochloride in 75 mM potassium phosphate (AAPH, 79.83 mM final concentration) was rapidly added to each well and fluorescence was measured every 35 s for 60 min using a plate reader (Spectramax M3; Molecular Devices). The area under the curve (AUC) was calculated for each well by dividing the fluorescence at each time point by the initial fluorescence for that well and summing the results for all data points. Net AUC for samples and standards was determined by subtracting the AUC of the blank, and the TAC of each sample (reported as μmol Trolox equivalents (TE) per μg protein) was determined using the standard curve. Mean inter- and intra-assay coefficients of variation were 5.0% and 1.6% in brain, 4.8% and 1.3% in muscle, and 9.0% and 1.1% in gonad, respectively. We were unable to measure TAC in two samples of brain (one dominant and one subordinate

male) and in two samples of gonad (one subordinate and one ascending male).

2.6. 11-Ketotestosterone (11-KT) quantification

To avoid potential interference by steroid binding proteins (Bobe et al., 2010; Hammond, 2011), steroids were extracted from plasma using C18 solid phase extraction (SPE) columns (Cleanert C18-N-SPE 100 mg mL^{-1}; Agela Technologies) that had been primed with 1 mL of methanol and 1 mL of ultrapure water. Plasma samples (5 μL) from each fish were diluted with 1 mL of acetate buffer (40 mM glacial acetic acid, 9 mM sodium acetate; pH 4.0) and passed through the SPE columns. Following this step, 1 mL of ultrapure water followed by 1 mL of hexanes were added to the columns, and the eluates were discarded. Steroids were eluted from the SPE columns with 2 mL of ethyl acetate (containing 1% methanol) and collected in glass tubes. Samples were dried under air at room temperature and reconstituted in 500 μL of enzyme-linked immunoassay (EIA) buffer (Cayman Chemicals). Circulating 11-KT levels were measured using a commercially available EIA (Cayman Chemicals) following the manufacturer's instructions. Our protocol yielded an extraction efficiency of 97%, and serial dilution of a pooled sample ran parallel to the standard curve. Samples were assayed in duplicate (final dilution of 1:1000) with mean inter- and intra-assay coefficients of variation of 9.2% and 7.5%, respectively. We were unable to collect blood from three subordinates, and therefore circulating 11-KT levels were not determined in these fish.

2.7. Statistical analyses

Specific growth rates were calculated as $[\ln(SL_{final}) - \ln(SL_{initial})] \times 100/D$, where *SL* is the standard length of the fish in millimeters and *D* is the number of days that elapsed between measurements (Ricker, 1975). Growth rates were calculated using measurements taken on Days 0 and 11 for dominant and subordinate males, and measurements taken on Days 11 and 14 for ascending males.

Statistical analyses were conducted using R (version 3.6.3; R Core Team, 2021) and a significance level (α) of 0.05 was used for all tests. When data did not meet the assumptions of normality and/or equal variance, data were log-transformed to meet these assumptions. To investigate whether antioxidant capacity (in brain, gonad, or muscle), gonad mass, or plasma 11-KT levels varied across social ranks, we fitted general linear mixed models (LMMs) with social rank (dominant, subordinate, or ascending males) as a fixed factor and group ID as a random factor using the lmer function in the "lme4" package (Bates et al., 2015). Although body size is inherently associated with social rank in *N. pulcher* because these fish form size-based dominance hierarchies (Hamilton et al., 2005; Reddon et al., 2011; Wong and Balshine, 2011a), we did not find any relationship (all $p > 0.2$) between body mass or body length and any of the physiological parameters measured in this study (antioxidant capacity in each tissue or plasma 11-KT levels) within a given social rank (dominant, subordinate, or ascending males). Therefore, to avoid overfitting the models, we only included body mass as a covariate when assessing differences in gonad mass between statuses and when assessing the relationship between gonad antioxidant capacity and gonad mass (to control for the relationship between body mass and gonad mass). We also assessed whether brain, muscle, and gonad antioxidant capacities were related to individual differences in cognitive (dominance index), metabolic (locomotor activity, growth, workload), and reproductive demands (gonad mass and 11-KT levels), respectively. We used LMMs when models included fish that originated from the same group (analyses that included all fish or only subordinate males) and general linear models (LMs) when models included fish that all originated from different social groups (only dominant males or only ascending males). When overall differences were detected using the Anova function in the "car" package (Fox and Weisberg, 2011), post hoc Tukey's tests were performed using the emmeans function in the "emmeans" package (Lenth, 2016). Effect sizes for overall models were estimated by calculating partial eta-squared values (η^2) using the 'sjstats' package (Lüdecke, 2020) and by calculating Cohen's d values (d) for post hoc contrasts using the using the 'emmeans' package (Lenth, 2016).

3. Results

3.1. Antioxidant capacity across tissues

Antioxidant capacity varied across social ranks in brain (Fig. 2A; $X^2_{2,27} = 8.20$, $p = 0.02$, $\eta^2 = 0.26$), muscle (Fig. 2B; $X^2_{2,29} = 12.00$, $p = 0.003$, $\eta^2 = 0.34$), and gonad (Fig. 2C; $X^2_{2,27} = 8.76$, $p = 0.01$, $\eta^2 = 0.30$). Dominant males had antioxidant capacities that were 15% and 13% higher in their brain and muscle, respectively, compared to subordinates (brain: $t = 2.72$, $p = 0.03$, d = 1.23; muscle: $t = 3.28$, $p = 0.008$, d = 1.53). Antioxidant capacities in the brain and muscle of dominant males were not higher than those of ascending males (brain: $t = 1.58$, $p = 0.28$, d = 0.75; muscle: $t = 1.83$, $p = 0.19$, d = 0.87). In contrast, the antioxidant capacity of dominant male gonad was 27% higher than the gonadal antioxidant capacity of ascending males ($t = 2.83$, $p = 0.03$, d = 1.41) but was not different from subordinate males ($t = 1.56$, p = 0.28, d = 0.76). The antioxidant capacities of subordinate and ascending males did not differ across any of the tissues that were assessed (muscle: $t = 1.27$, $p = 0.43$, d = 0.66; brain: $t = 0.96$, $p = 0.61$, d = 0.47; gonad: $t = 1.15$, $p = 0.49$, d = 0.65).

3.2. Relationships between brain & muscle antioxidant capacities and behaviour & growth

In brain, antioxidant capacities were greatest in males that were the most behaviourally dominant, as reflected by their dominance index score (Fig 3A; $X^2_{1,28} = 9.93$, $p = 0.002$, $\eta^2 = 0.26$). In muscle, antioxidant capacity was positively related to activity levels (Fig 3B; $X^2_{1,28} = 4.97$, $p = 0.03$, $\eta^2 = 0.18$) but only when two subordinates that were unusually inactive (active during <35% of the observation periods vs >49% for other fish) were excluded (as opposed to $X^2_{1,30} = 2.43$, $p = 0.12$, $\eta^2 = 0.08$ when all fish were included). Antioxidant capacity in muscle also tended to be positively related to workload index scores (Fig 3C; $X^2_{1,30} = 3.19$, $p = 0.07$, $\eta^2 = 0.10$), but this relationship did not quite reach significance. Muscle antioxidant capacity was unrelated to specific growth rates (data not shown; $X^2_{1,30} = 0.77$, $p = 0.38$, $\eta^2 = 0.03$).

3.3. Relationships between gonad antioxidant capacity, reproductive physiology, and behaviour

The size of males' gonads varied across social ranks (Fig 4A; $X^2_{2,28} = 12.26$, p = 0.002, $\eta^2 = 0.30$) and increased with overall body mass ($X^2_{1,28} = 4.55$, $p = 0.03$, $\eta^2 = 0.14$). Dominant males had ~50% larger gonads than either subordinate ($t = 2.97$, $p = 0.02$, d = 2.42) or ascending males ($t = 3.16$, $p = 0.01$, d = 1.96) after controlling for body mass, but gonad mass did not differ between subordinate and ascending males ($t = 0.83$, $p = 0.69$, d = 0.46). Circulating levels of 11-KT also varied across social ranks (Fig. 4B; $X^2_{2,26} = 22.67$, $p < 0.001$, $\eta^2 = 0.47$), with ascending males having ~6× and 2× higher 11-KT concentrations compared to subordinate ($t = 4.54$, p < 0.001, d = 2.31) and dominant ($t = 2.80$, p = 0.03, d = 1.30) males, respectively. Dominant males also tended to have ~4× higher 11-KT levels than subordinate males, but this difference did not reach statistical significance ($t = 2.20$, $p = 0.09$, d = 1.01). To evaluate whether these differences in reproductive state were related to gonad antioxidant ca-

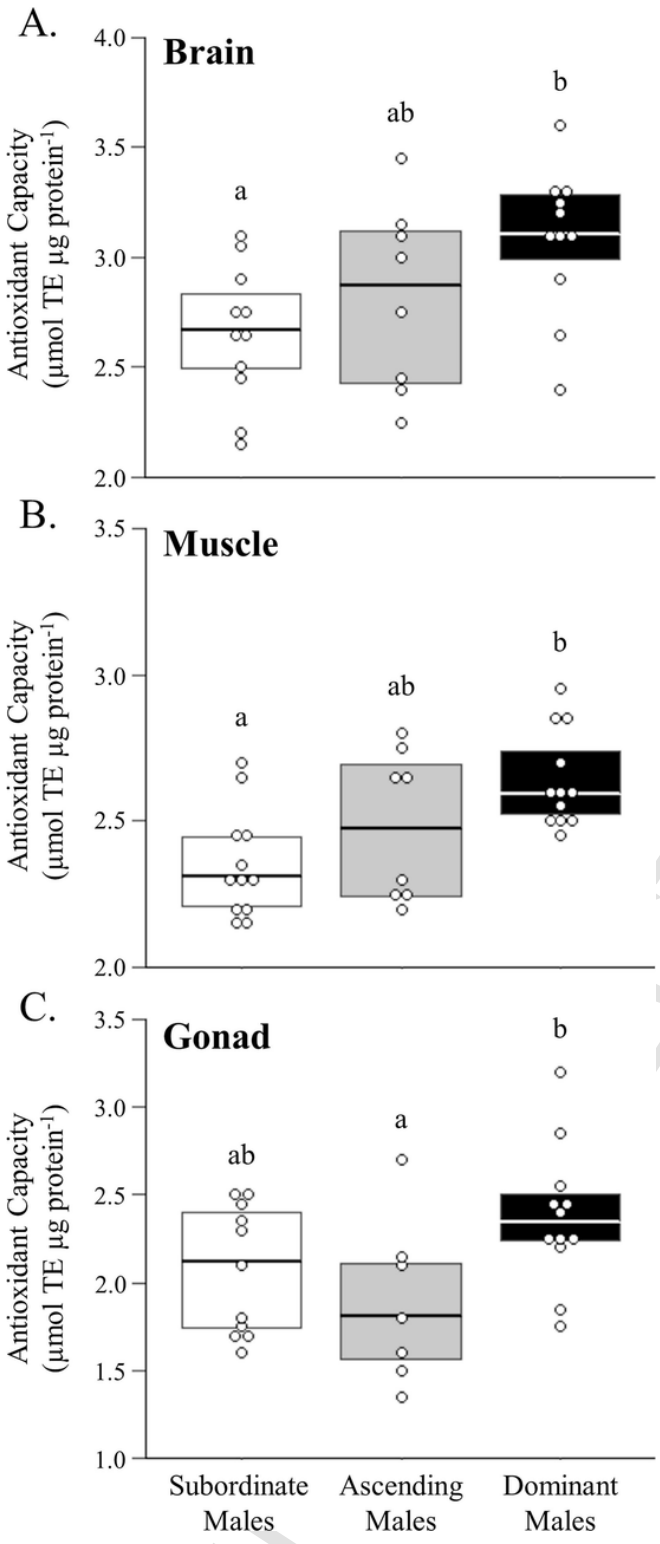

Fig. 2. Total antioxidant capacity in brain (A), muscle (B), and gonad (C) of subordinate (white), ascending (grey), and dominant (black) male *Neolamprologus pulcher*. Values are presented as boxplots where the line through a box represents the median and the limits of the box represent the 1st and 3rd quartiles; points represent individual values. Treatment groups that share a letter are not statistically different from one another (see text for detailed statistical results). TE: trolox equivalents.

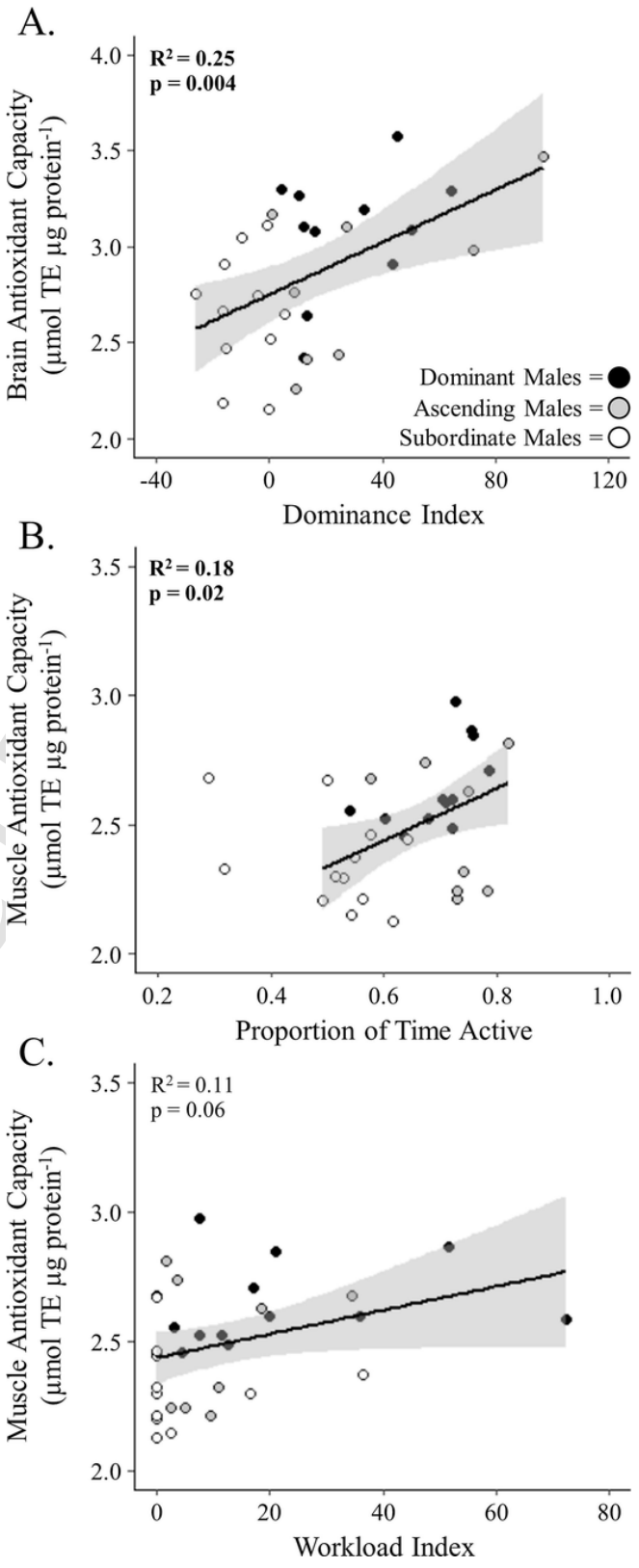

Fig. 3. Relationships between (A) dominance scores and antioxidant capacity measured in brain; and the relationships between (B) activity rates and (C) workload scores and antioxidant capacity in muscle for subordinate (white), ascending (grey), and dominant (black) male *Neolamprologus pulcher*. All graphs depict the behaviour of ascending males after the removal of the previous dominant male. Linear regressions were fit (see text for detailed statistical

◀ results) and the shaded area shows the 95% confidence interval of the regression line. Two subordinate outliers were not included in the linear regression shown in Panel B. TE: trolox equivalents.

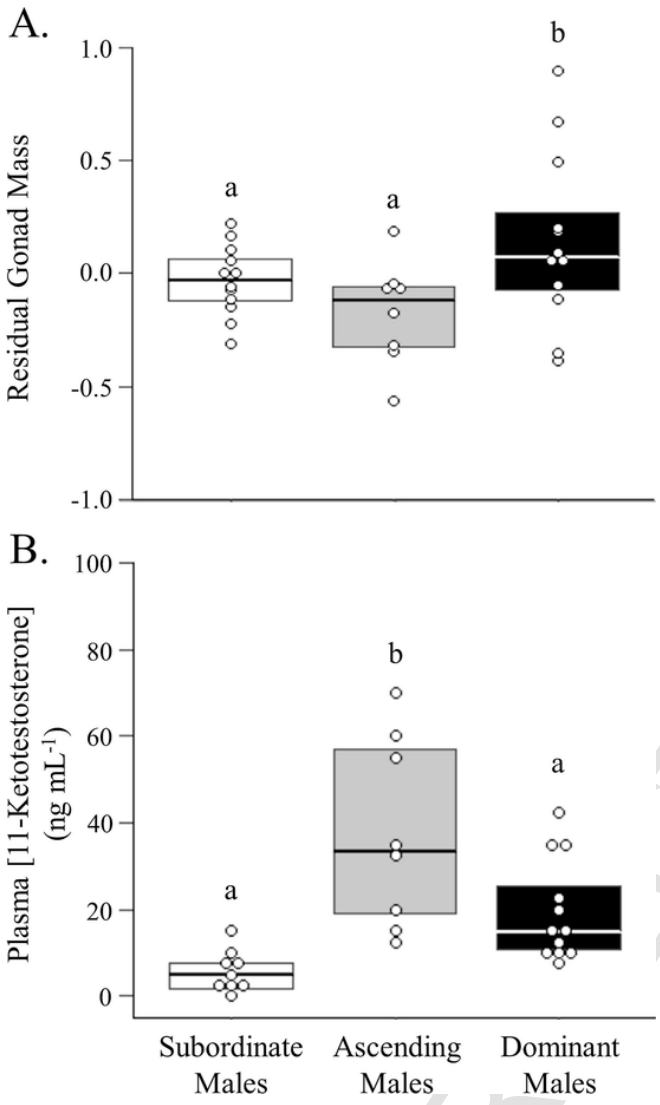

Fig. 4. Residual gonad masses (A) and circulating levels of 11-ketotestosterone (B) in subordinate (white), ascending (grey), and dominant (black) male *Neolamprologus pulcher*. Values are presented as boxplots where the line through a box represents the median and the limits of the box represent the 1st and 3rd quartiles; points represent individual values. Treatment groups that share a letter are not statistically different from one another (see text for detailed statistical results). Residuals were determined based on the log-log relationship of gonad mass versus body mass.

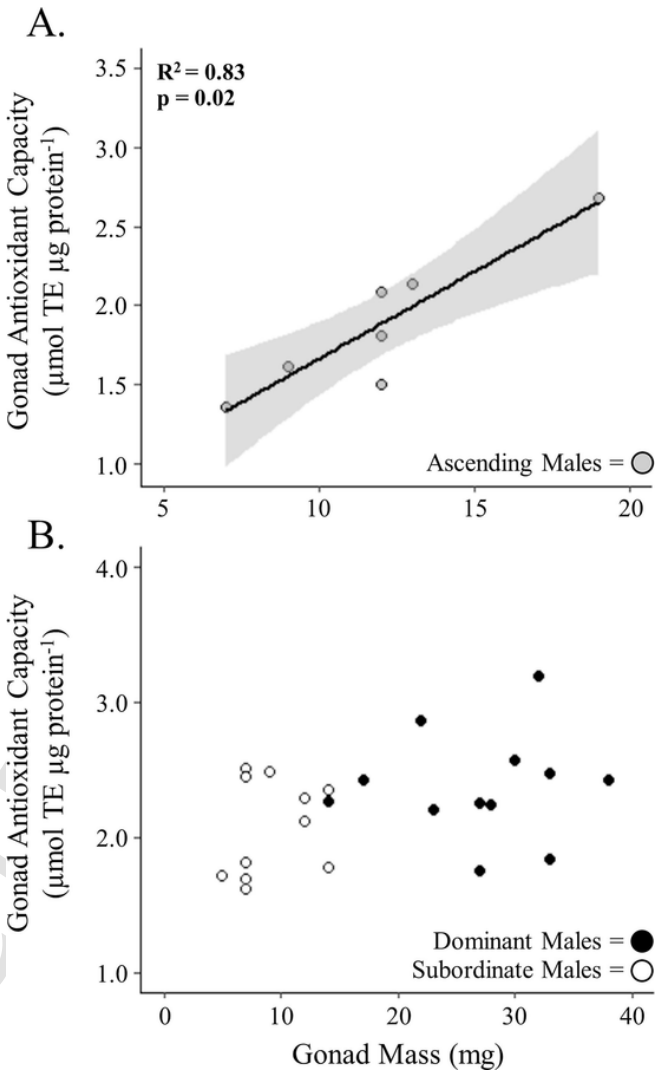

Fig. 5. Relationship between gonad antioxidant capacity and gonad mass in (A) ascending (grey) or (B) in dominant (black) and subordinate (white) male *Neolamprologus pulcher*. A linear regression was fit for the significant relationship only (see text for detailed statistical results) and the shaded area shows the 95% confidence interval of the regression line. TE: trolox equivalents.

pacity, we performed analyses to determine relationships between these measures.

Gonad antioxidant capacity was positively related to gonad mass in ascending males (Fig. 5A; $F_{1,4} = 29.57$, $p = 0.006$, $\eta^2 = 0.91$), but was not related to their body mass (data not shown; $F_{1,4} = 4.40$, $p = 0.10$, $\eta^2 = 0.52$). The relationship between gonad mass and antioxidant capacity was specific to ascending males, as neither dominant (Fig. 5B; $F_{1,9} = 0.01$, $p = 0.97$, $\eta^2 = 0.01$) nor subordinate (Fig. 5B; $X^2_{1,8} = 0.14$, $p = 0.71$, $\eta^2 = 0.02$) males showed a similar relationship.

Circulating levels of 11-KT in ascending males were not related to gonad antioxidant capacity (data not shown; $F_{1,5} = 1.46$, $p = 0.28$, $\eta^2 = 0.23$). However, circulating 11-KT levels were positively correlated with dominance index scores across all males ($F_{1,27} = 12.50$, $p < 0.001$, $\eta^2 = 0.32$). This relationship was mainly driven by variation within ascending males (Fig. 6A; $F_{1,6} = 8.47$, $p = 0.03$, $\eta^2 = 0.59$) and not dominant (Fig. 6B; $F_{1,10} = 2.90$, $p = 0.12$, $\eta^2 = 0.22$) or subordinate males (Fig. 6B; $X^2_{1,7} = 0.56$, $p = 0.46$, $\eta^2 = 0.07$).

4. Discussion

Social instability has widespread effects on the behaviour and physiology of individuals (Culbert et al., 2018; Maruska, 2015; Sapolsky, 1983; Wong and Balshine, 2011b), including changes in the regulation of oxidative status (Beaulieu et al., 2014; Fialkowski et al., 2021; Mora et al., 2017). Unlike previous studies that have primarily examined the effects of social instability on indices of oxidative status in the blood, our study assessed oxidative responses across different tissues. Consistent with our hypotheses, we observed tissue-specific differences in antioxidant capacities of ascending male *N. pulcher* compared to dominant and subordinate males. In brain and muscle of ascending males, antioxidant capacities were intermediate to those of subordinate (lowest levels) and dominant males (highest levels), and reflected differences in

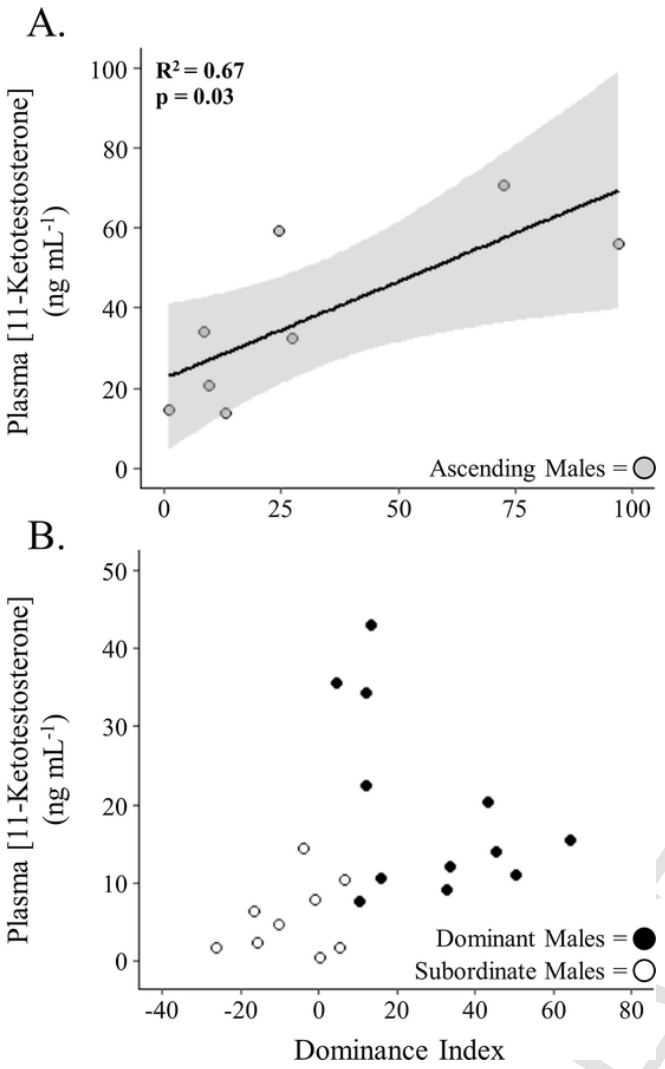

Fig. 6. Relationship between plasma 11-ketotestosterone levels and dominance scores in (A) ascending (grey) or (B) dominant (black) and subordinate (white) male *Neolamprologus pulcher*. The behaviour of ascending males depicted in Panel A was scored after the removal of the previous dominant male. A linear regression was fit for the significant relationship only (see text for detailed statistical results) and the shaded area shows the 95% confidence interval of the regression line.

social and locomotor behaviours, respectively. Whereas ascending males had lower gonad antioxidant capacity than dominant males, ascenders with larger gonads had higher gonad antioxidant capacity; potentially, to minimize oxidative damage in the gonads during this period of tissue remodelling.

Enhanced somatic growth during social ascension is critical for ascending individuals (Bergmüller et al., 2005b; Buston, 2003; Dengler-Crish and Catania, 2007; Huchard et al., 2016; Thorley et al., 2018). Larger differences in body size between group members are often associated with lower rates of aggression and conflict (Ang and Manica, 2010; Wong et al., 2007); therefore, enhanced growth by ascending males is thought to help re-stabilize hierarchies. Accordingly, ascending males in the current study grew ~2-3× faster than either dominants or subordinates (Culbert et al., 2019). Periods of enhanced growth are often associated with increased oxidative damage (Almroth et al., 2012; Nussey et al., 2009; Stier et al., 2014), which can be caused by reductions in antioxidant capacity (Almroth et al., 2012; Alonso-Alvarez et al., 2007; De Block and Stoks, 2008; Hsu et al., 2019; but see Smith et al., 2016). In teleosts, changes in somatic growth are generally reflective of changes in muscle growth because body mass is largely comprised of muscle (Weatherley et al., 1988). However, antioxidant capacity in muscle did not appear to be directly related to differences in growth in the current study. The muscle of ascending males (the fish that grew the most; Culbert et al., 2019) had intermediate antioxidant capacity compared to values for dominant and subordinate males, and growth rates did not correlate with muscle antioxidant capacity. Instead, antioxidant capacity in muscle was positively correlated with individual activity levels and workloads, both of which increased as males ascended (Culbert et al., 2018, 2019). Therefore, antioxidant capacity in muscle appeared to reflect the metabolic costs associated with energetically demanding behaviours and not the cost of somatic growth.

Similarly, differences in antioxidant capacity in the brain appeared to reflect behavioural differences across social ranks. Dominant males had higher antioxidant capacities in their brains compared to subordinate males and we detected a positive relationship between antioxidant capacity and dominance index scores across all males. Like other cooperative animals (Cant et al., 2014; Flack et al., 2005), dominant *N. pulcher* police subordinate behaviour and maintain social order within their group (Dey et al., 2013; Schürch et al., 2010). Dominants may thus face greater neurological and cognitive demands than subordinates because they must be more attentive and responsive to changes in their social landscape and keep all the members of their group in line. Accordingly, rates of neurogenesis are typically higher in dominant individuals compared to subordinates across mammals (reviewed by Holmes, 2016) and fishes (Johansen et al., 2012; Maruska et al., 2012; Sørensen et al., 2012; Tea et al., 2019). Additionally, rates of neurogenesis in *Astatotilapia burtoni*—an African cichlid that is closely related to *N. pulcher*—increased as males were in the process of ascending to dominance (24 h post dominant removal; Maruska et al., 2012). Neurogenesis is often associated with increased ROS production (Walton et al., 2012; Yuan et al., 2015) and oxidative stress owing to increased ROS production can have neurodegenerative effects (Andersen, 2004; Kim et al., 2015). Therefore, dominance-related differences in the antioxidant capacity of the brain may serve to minimize oxidative stress in the brain.

Antioxidant capacity in the gonads of ascending males was lower than that of dominant males. In social groups, dominants often use aggression and intimidation to supress reproduction by same-sex subordinates (Arnold and Dittami, 1997; Faulkes and Bennett, 2001; Montgomery et al., 2018). Consequently, subordinates typically have smaller gonads and/or a reduced reproductive potential compared to dominants (Dengler-Crish and Catania, 2007; Fitzpatrick et al., 2006; Stiver et al., 2006; Young et al., 2006). However, when a subordinate is released from this reproductive suppression (e.g., when they are able to ascend to dominance), they quickly begin to increase their reproductive potential (Fitzpatrick et al., 2008; Maruska, 2015; Russell et al., 2004; Thorley et al., 2018). We found that three days after removal of the dominant male, ascending males had higher circulating levels of 11-KT than dominant or subordinate males suggesting that their endocrine reproductive axis was upregulated compared to males of stable social ranking. Androgens can directly affect oxidative defenses in mammalian testes (Aydilek et al., 2004; Chainy et al., 1997; Ghosh et al., 2002; Peltola et al., 1996; Zini and Schlegel, 2003), but 11-KT levels were not correlated with gonad antioxidant capacity in the cichlid fish used in the current study. Instead, gonad antioxidant capacity in ascending males appeared to reflect the relative size of their gonads. Although ascending males had smaller gonads than dominant males (relative to their body size), gonad antioxidant capacities were higher in ascending males that had comparatively larger gonads. This relationship suggests that ascending males may have increased investment in gonad antioxidants as their gonads grew; potentially to minimize local levels of oxidative stress associated with the rapid development/growth of their gonads (likely associated with increased ROS production; Covarrubias et al., 2008; Hernández-García et al., 2010). In *A. burtoni*,

it takes at least a week for the gonads of ascending males to reach a size comparable to those of dominants (Maruska, 2015). However, changes in the cellular composition and spermatogenic potential of the testes occur during the first few days of ascension (Huffman et al., 2012; Maruska and Fernald, 2011). Therefore, while the gonads of ascending *N. pulcher* males in the current study remained smaller than those of dominants, it is likely that ascenders' gonads had already begun to develop and grow. Overall, differences in antioxidant levels in the gonads of ascending males appeared to be directly related to differences in tissue development/growth and not androgen synthesis.

Androgens are often associated with agonistic behaviour and dominance across vertebrates (Hirschenhauser and Oliveira, 2006; Rosvall et al., 2020; Wingfield et al., 1990). Consistent with this relationship, we found that circulating levels of 11-KT in ascenders were ~ 6× higher than those of subordinates and ~ 2× higher than those of dominant males. Additionally, dominance scores were positively correlated with circulating 11-KT levels in ascending males. Therefore, elevated levels of circulating 11-KT in ascending males are likely related to changes in agonistic behaviours associated with ascension.

It can be difficult to evaluate whether differences observed during periods of social ascension are primarily due to the actual act of ascending to dominant status (only experienced by the ascending individual) or due to the more general social instability induced by changes in the social structure of a group (experienced by all group members). Both occurred simultaneously in the present study. However, previous studies support the notion that the patterns we observed for antioxidant capacity were likely caused specifically by social ascension and not general social instability. For example, both short term (72 h; Border et al., 2019) and long-term (22 weeks; Border et al., 2021) periods of instability have minimal effect on measures of oxidative stress in male *A. burtoni*, which included measures of antioxidant capacity in the gonads and muscle. Therefore, it appears that antioxidant capacity is largely resilient to social instability in the absence of ascension. However, it would be beneficial if future studies could include a control group that experiences social instability in the absence of social ascension to confirm this assertion for *N. pulcher*.

In our study, antioxidant capacity in several tissues varied between dominant, subordinate, and ascending males, but the behavioural, morphological, and/or physiological factors driving these differences varied across tissues. Future studies should use larger sample sizes that will facilitate the detection of subtle differences between social ranks and it would also be advantageous to incorporate additional oxidative measures (see Monaghan et al., 2009) to provide a more comprehensive evaluation of how oxidative state is affected by the social environment. Additionally, in the future it would be useful to determine whether a subordinate's oxidative status influences their propensity to ascend to dominance when the opportunity arises. Despite these omissions, overall, the results expand our knowledge of the widespread changes that occur during periods of social transition and highlight the important role that the social environment has on an individual's physiology.

Funding

This work was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants provided to KMG (RGPIN-2017-05487) and SB (RGPIN-2016-05772). BMC was supported by an Ontario Graduate Scholarship and a NSERC Master's Canadian Graduate Scholarship (CGS-M).

CRediT authorship contribution statement

Brett M. Culbert: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Peter D. Dijkstra:** Investigation, Methodology, Resources, Writing – review & editing. **Kathleen M. Gilmour:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. **Sigal Balshine:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Shana Border for performing the antioxidant capacity assays, Jessica Qiu for creating Fig. 1, Dr. Nicholas Bernier for assistance with the steroid extraction protocol and Carol Best for helpful discussions while writing this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2021.111126.

References

Almroth, B.C., Johnsson, J.I., Devlin, R., Sturve, J., 2012. Oxidative stress in growth hormone transgenic coho salmon with compressed lifespan a model for addressing aging. Free Radic. Res. 46, 1183–1189. https://doi.org/10.3109/10715762.2012.698009.

Alonso-Alvarez, C., Bertrand, S., Faivre, B., Sorci, G., 2007. Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. Funct. Ecol. 21, 873–879. https://doi.org/10.1111/j.1365-2435.2007.01300.x.

Andersen, J.K., 2004. Oxidative stress in neurodegeneration: cause or consequence? Nat. Rev. Neurosci. 10, S18. https://doi.org/10.1038/nrn1434.

Ang, T.Z., Manica, A., 2010. Aggression, segregation and stability in a dominance hierarchy. Proc. R. Soc. B 277, 1337–1343. https://doi.org/10.1098/rspb.2009.1839.

Argüelles, S., García, S., Maldonado, M., Machado, A., Ayala, A., 2004. Do the serum oxidative stress biomarkers provide a reasonable index of the general oxidative stress status? Biochim. Biophys. Acta 1674, 251–259. https://doi.org/10.1016/j.bbagen.2004.06.023.

Arnold, W., Dittami, J., 1997. Reproductive suppression in male alpine marmots. Anim. Behav. 53, 53–66. https://doi.org/10.1006/anbe.1996.0277.

Aydilek, N., Aksakal, M., Karakilçik, A.Z., 2004. Effects of testosterone and vitamin E on the antioxidant system in rabbit testis. Andrologia 36, 277–281. https://doi.org/10.1111/j.1439-0272.2004.00618.x.

Balshine, S., Leach, B., Neat, F.C., Reid, H., Taborsky, M., Werner, N., 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). Behav. Ecol. Sociobiol. 50, 134–140. https://doi.org/10.1007/s002650100343.

Balshine-Earn, S., Neat, F.C., Reid, H., Taborsky, M., 1998. Paying to stay or paying to breed? Field evidence for direct benefits of helping behavior in a cooperatively breeding fish. Behav. Ecol. 9, 432–438. https://doi.org/10.1093/beheco/9.5.432.

Balzarini, V., Taborsky, M., Wanner, S., Koch, F., Frommen, J.G., 2014. Mirror, mirror on the wall: the predictive value of mirror tests for measuring aggression in fish. Behav. Ecol. Sociobiol. 68, 871–878. https://doi.org/10.1007/s00265-014-1698-7.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Beaulieu, M., Mboumba, S., Willaume, E., Kappeler, P.M., Charpentier, M.J.E., 2014. The oxidative cost of unstable social dominance. J. Exp. Biol. 217, 2629–2632. https://doi.org/10.1242/jeb.104851.

Bergmüller, R., Heg, D., Peer, K., Taborsky, M., 2005a. Extended safe havens and between-group dispersal of helpers in a cooperatively breeding cichlid. Behaviour 142, 1643–1667. https://doi.org/10.1163/156853905774831800.

Bergmüller, R., Heg, D., Taborsky, M., 2005b. Helpers in a cooperatively breeding cichlid stay and pay or disperse and breed, depending on ecological constraints. Proc. R. Soc. B 272, 325–331. https://doi.org/10.1098/rspb.2004.2960.

Bobe, J., Guiguen, Y., Fostier, A., 2010. Diversity and biological significance of sex hormone-binding globulin in fish, an evolutionary perspective. Mol. Cell. Endocrinol. 316, 66–78. https://doi.org/10.1016/j.mce.2009.09.017.

Border, S.E., Deoliveira, G.M., Janeski, H.M., Piefke, T.J., Brown, T.J., Dijkstra, P.D., 2019. Social rank, color morph, and social network metrics predict oxidative stress in a cichlid fish. Behav. Ecol. 30, 490–499. https://doi.org/10.1093/beheco/ary189.

Border, S.E., Piefke, T.J., Funnel, T.R., Fialkowski, R.F., Sawecki, J., Dijkstra, P.D., 2021. Experimental evidence that social instability influences rank-specific patterns in oxidative stress in a cichlid fish. J. Exp. Biol. jeb. 237172. https://doi.org/10.1242/jeb.237172.

Borg, B., 1994. Androgens in teleost fishes. Comp. Biochem. Physiol. Part C Comp. 109, 219–245. https://doi.org/10.1016/0742-8413(94)00063-G.

Buston, P.M., 2003. Social hierarchies: size and growth modification in clownfish. Nature 424, 145–146. https://doi.org/10.1038/424145a.
Cant, M.A., Nichols, H.J., Johnstone, R.A., Hodge, S.J., 2014. Policing of reproduction by hidden threats in a cooperative mammal. Proc. Natl. Acad. Sci. U. S. A. 111, 326–330. https://doi.org/10.1073/pnas.1312626111.
Chainy, G.B.N., Samantaray, S., Samanta, L., 1997. Testosterone-induced changes in testicular antioxidant system. Andrologia 29, 343–349. https://doi.org/10.1111/j.1439-0272.1997.tb00328.x.
Clutton-Brock, T.H., Russell, A.F., Sharpe, L.L., Young, A.J., Balmforth, Z., McIlrath, G.M., 2002. Evolution and development of sex differences in cooperative behavior in meerkats. Science 297, 253–256. https://doi.org/10.1126/science.1071412.
Covarrubias, L., Hernández-García, D., Schnabel, D., Salas-Vidal, E., Castro-Obregón, S., 2008. Function of reactive oxygen species during animal development: passive or active? Dev. Biol. 320, 1–11. https://doi.org/10.1016/j.ydbio.2008.04.041.
Cram, D.L., Blount, J.D., Young, A.J., 2015. Oxidative status and social dominance in a wild cooperative breeder. Funct. Ecol. 29, 229–238. https://doi.org/10.1111/1365-2435.12317.
Cresswell, W., Quinn, J.L., 2011. Predicting the optimal prey group size from predator hunting behaviour. J. Anim. Ecol. 80, 310–319. https://doi.org/10.1111/j.1365-2656.2010.01775.x.
Culbert, B.M., Gilmour, K.M., Balshine, S., 2018. Stress axis regulation during social ascension in a group-living cichlid fish. Horm. Behav. 103, 121–128. https://doi.org/10.1016/j.yhbeh.2018.06.007.
Culbert, B.M., Balshine, S., Gilmour, K.M., 2019. Physiological regulation of growth during social ascension in a group-living fish. Physiol. Biochem. Zool. 92, 211–222. https://doi.org/10.1086/702338.
De Block, M., Stoks, R., 2008. Compensatory growth and oxidative stress in a damselfly. Proc. R. Soc. B Biol. Sci. 275, 781–785. https://doi.org/10.1098/rspb.2007.1515.
Dengler-Crish, C.M., Catania, K.C., 2007. Phenotypic plasticity in female naked mole-rats after removal from reproductive suppression. J. Exp. Biol. 210, 4351–4358. https://doi.org/10.1242/jeb.009399.
Dey, C.J., Reddon, A.R., O'Connor, C.M., Balshine, S., 2013. Network structure is related to social conflict in a cooperatively breeding fish. Anim. Behav. 85, 395–402. https://doi.org/10.1016/J.ANBEHAV.2012.11.012.
Dowling, D.K., Simmons, L.W., 2009. Reactive oxygen species as universal constraints in life-history evolution. Proc. R. Soc. B Biol. Sci. 276, 1737–1745. https://doi.org/10.1098/rspb.2008.1791.
Evans, J.C., Votier, S.C., Dall, S.R.X., 2016. Information use in colonial living. Biol. Rev. 91, 658–672. https://doi.org/10.1111/brv.12188.
Faulkes, C.G., Bennett, N.C., 2001. Family values: group dynamics and social control of reproduction in African mole-rats. Trends Ecol. Evol. 16, 184–190. https://doi.org/10.1016/S0169-5347(01)02116-4.
Fialkowski, R., Aufdemberge, P., Wright, V., Dijkstra, P., 2021. Radical change: temporal patterns of oxidative stress during social ascent in a dominance hierarchy. Behav. Ecol. Sociobiol. 75. https://doi.org/10.1007/s00265-021-02981-x.
Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.
Fitzpatrick, J.L., Desjardins, J.K., Stiver, K.A., Montgomerie, R., Balshine, S., 2006. Male reproductive suppression in the cooperatively breeding fish *Neolamprologus pulcher*. Behav. Ecol. 17, 25–33. https://doi.org/10.1093/beheco/ari090.
Fitzpatrick, J.L., Desjardins, J.K., Milligan, N., Stiver, K.A., Montgomerie, R., Balshine, S., 2008. Female-mediated causes and consequences of status change in a social fish. Proc. R. Soc. B 275, 929–936. https://doi.org/10.1098/rspb.2007.1449.
Flack, J.C., de Waal, F.B.M., Krakauer, D.C., 2005. Social structure, robustness, and policing cost in a cognitively sophisticated species. Am. Nat. 165, E126–E139. https://doi.org/10.1086/429277.
Fox, J., Weisberg, S., 2011. An R Companion to Applied Regression, 2nd ed. Sage Publications, Thousand Oaks, CA.
Garratt, M., Mcardle, F., Stockley, P., Vasilaki, A., Beynon, R.J., Jackson, M.J., Hurst, J.L., 2012. Tissue-dependent changes in oxidative damage with male reproductive effort in house mice. Funct. Ecol. 26, 423–433. https://doi.org/10.1111/j.1365-2435.2011.01952.x.
Georgiev, A.V., Thompson, M.E., Mandalaywala, T.M., Maestripieri, D., 2015. Oxidative stress as an indicator of the costs of reproduction among free-ranging rhesus macaques. J. Exp. Biol. 218, 1981–1985. https://doi.org/10.1242/jeb.121947.
Ghosh, D., Das, U.B., Ghosh, S., Mallick, M., Debnath, J., 2002. Testicular gametogenic and steroidogenic activities in cyclophosphamide treated rat: a correlative study with testicular oxidative stress. Drug Chem. Toxicol. 25, 281–292. https://doi.org/10.1081/DCT-120005891.
Gilmour, K.M., Kirkpatrick, S., Massarsky, A., Pearce, B., Saliba, S., Stephany, C.-É., Moon, T.W., 2012. The influence of social status on hepatic glucose metabolism in rainbow trout *Oncorhynchus mykiss*. Physiol. Biochem. Zool. 85, 309–320. https://doi.org/10.1086/666497.
Gilmour, K.M., Craig, P.M., Dhillon, R.S., Lau, G.Y., Richards, J.G., 2017. Regulation of energy metabolism during social interactions in rainbow trout: a role for AMP-activated protein kinase. Am. J. Phys. Regul. Integr. Comp. Phys. 313, R549–R559. https://doi.org/10.1152/ajpregu.00341.2016.
Hamilton, I.M., Heg, D., Bender, N., 2005. Size differences within a dominance hierarchy influence conflict and help in a cooperatively breeding cichlid. Behaviour 142, 1591–1613. https://doi.org/10.1163/156853905774831846.
Hammond, G.L., 2011. Diverse roles for sex hormone-binding globulin in reproduction. Biol. Reprod. 85, 431–441. https://doi.org/10.1095/biolreprod.111.092593.
Hammond, K.A., Chappell, M.A., Cardullo, R.A., Lin, R.S., Johnsen, T.S., 2000. The mechanistic basis of aerobic performance variation in red junglefowl. J. Exp. Biol. 203, 2053–2064. https://doi.org/10.1242/jeb.203.13.2053.
Hellmann, J.K., Ligocki, I.Y., O'Connor, C.M., Reddon, A.R., Farmer, T.M., Marsh-Rollo, S.E., Balshine, S., Hamilton, I.M., 2016. The influence of status and the social environment on energy stores in a social fish. J. Fish Biol. 88, 1321–1334. https://doi.org/10.1111/jfb.12890.
Hernández-García, D., Wood, C.D., Castro-Obregón, S., Covarrubias, L., 2010. Reactive oxygen species: a radical role in development? Free Radic. Biol. Med. 49, 130–143. https://doi.org/10.1016/j.freeradbiomed.2010.03.020.
Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. Anim. Behav. 71, 265–277. https://doi.org/10.1016/J.ANBEHAV.2005.04.014.
Holmes, M.M., 2016. Social regulation of adult neurogenesis: a comparative approach. Front. Neuroendocrinol. 41, 59–70. https://doi.org/10.1016/j.yfrne.2016.02.001.
Hotta, T., Komiyama, S., Kohda, M., 2018. A social cichlid fish failed to pass the mark test. Anim. Cogn. 21, 127–136. https://doi.org/10.1007/s10071-017-1146-y.
Hsu, B.Y., Doligez, B., Gustafsson, L., Ruuskanen, S., 2019. Transient growth-enhancing effects of elevated maternal thyroid hormones at no apparent oxidative cost during early postnatal period. J. Avian Biol. 50, 1–10. https://doi.org/10.1111/jav.01919.
Huchard, E., English, S., Bell, M.B.V., Thavarajah, N., Clutton-Brock, T., 2016. Competitive growth in a cooperative mammal. Nature 533, 532–534. https://doi.org/10.1038/nature17986.
Huffman, L.S., Mitchell, M.M., O'Connell, L.A., Hofmann, H.A., 2012. Rising StARs: behavioral, hormonal, and molecular responses to social challenge and opportunity. Horm. Behav. 61, 631–641. https://doi.org/10.1016/j.yhbeh.2012.02.016.
Isbell, L.A., 1991. Contest and scramble competition: patterns of female aggression and ranging behavior among primates. Behav. Ecol. 2, 143–155. https://doi.org/10.1093/beheco/2.2.143.
Johansen, I.B., Sørensen, C., Sandvik, G.K., Nilsson, G.E., Höglund, E., Bakken, M., Øverli, Ø., 2012. Neural plasticity is affected by stress and heritable variation in stress coping style. Comp. Biochem. Physiol. Part D. Genom. Proteom. 7, 161–171. https://doi.org/10.1016/j.cbd.2012.01.002.
Kim, G.H., Kim, J.E., Rhie, S.J., Yoon, S., 2015. The role of oxidative stress in neurodegenerative diseases. Exp. Neurobiol. 24, 325–340. https://doi.org/10.5607/en.2015.24.4.325.
Kokko, H., Johnstone, R.A., 1999. Social queuing in animal societies: a dynamic model of reproductive skew. Proc. R. Soc. B 266, 571–578. https://doi.org/10.1098/rspb.1999.0674.
Lenth, R.V., 2016. Least-squares means: the R package lsmeans. J. Stat. Softw. 69, 1–33. https://doi.org/10.18637/jss.v069.i01.
Losdat, S., Mora, A.R., Bellut, C., Chargé, R., Falchi, V., Glauser, G., Vallat, A., Helfenstein, F., 2019. Social dominance, but not parasite load, affects sperm quality and sperm redox status in house sparrows. J. Exp. Biol. 222, jeb200675. https://doi.org/10.1242/jeb.200675.
Lüdecke, D., 2020. sjstats: Statistical Functions for Regression Models. https://CRAN.R-project.org/package=sjstats.
Lukas, D., Clutton-Brock, T., 2014. Costs of mating competition limit male lifetime breeding success in polygynous mammals. Proc. R. Soc. B Biol. Sci. 281, 20140418. https://doi.org/10.1098/rspb.2014.0418.
Margaritelis, N.V., Veskoukis, A.S., Paschalis, V., Vrabas, I.S., Dipla, K., Zafeiridis, A., Kyparos, A., Nikolaidis, M.G., 2015. Blood reflects tissue oxidative stress: a systematic review. Biomarkers 20, 97–108. https://doi.org/10.3109/1354750X.2014.1002807.
Maruska, K.P., 2014. Social regulation of reproduction in male cichlid fishes. Gen. Comp. Endocrinol. 207, 2–12. https://doi.org/10.1016/J.YGCEN.2014.04.038.
Maruska, K.P., 2015. Social transitions cause rapid behavioral and neuroendocrine changes. Integr. Comp. Biol. 55, 294–306. https://doi.org/10.1093/icb/icv057.
Maruska, K.P., Fernald, R.D., 2011. Plasticity of the reproductive axis caused by social status change in an african cichlid fish: II. Testicular gene expression and spermatogenesis. Endocrinology 152, 291–302. https://doi.org/10.1210/en.2010-0876.
Maruska, K.P., Carpenter, R.E., Fernald, R.D., 2012. Characterization of cell proliferation throughout the brain of the African cichlid fish *Astatotilapia burtoni* and its regulation by social status. J. Comp. Neurol. 520, 3471–3491. https://doi.org/10.1002/cne.23100.
Mendonça, R., Vullioud, P., Katlein, N., Vallat, A., Glauser, G., Bennett, N.C., Helfenstein, F., 2020. Oxidative costs of cooperation in cooperatively breeding Damaraland mole-rats. Proc. R. Soc. B 287, 20201023. https://doi.org/10.1098/rspb.2020.1023.
Milinski, M., Parker, G.A., 1991. Competition for resources. In: Krebs, J.R., Davies, N.B. (Eds.), Behavioural Ecology. Blackwell Scientific, pp. 137–168.
Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol. Lett. 12, 75–92. https://doi.org/10.1111/j.1461-0248.2008.01258.x.
Montgomery, T.M., Pendleton, E.L., Smith, J.E., 2018. Physiological mechanisms mediating patterns of reproductive suppression and alloparental care in cooperatively breeding carnivores. Physiol. Behav. 193, 167–178. https://doi.org/10.1016/j.physbeh.2017.11.006.
Mora, A.R., Meniri, M., Gning, O., Glauser, G., Vallat, A., Helfenstein, F., 2017. Antioxidant allocation modulates sperm quality across changing social environments. PLoS One 12, 1–21. https://doi.org/10.1371/journal.pone.0176385.
Nussey, D.H., Pemberton, J.M., Pilkington, J.G., Blount, J.D., 2009. Life history correlates of oxidative damage in a free-living mammal population. Funct. Ecol. 23, 809–817. https://doi.org/10.1111/j.1365-2435.2009.01555.x.
Oliveira, R.F., Hirschenhauser, K., Carneiro, L.A., Canario, A.V.M., 2002. Social modulation of androgen levels in male teleost fish. Comp. Biochem. Physiol. Part B Biochem. Mol. Biol. 132, 203–215. https://doi.org/10.1016/S1096-4959(01)00523-1.
Ou, B., Hampsch-Woodill, M., Prior, R.L., 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J. Agric. Food Chem. 49, 4619–4626. https://doi.org/10.1021/

jf010586o.
Pamplona, R., Costantini, D., 2011. Molecular and structural antioxidant defenses against oxidative stress in animals. Am. J. Phys. Regul. Integr. Comp. Phys. 301, R843–R863. https://doi.org/10.1152/ajpregu.00034.2011.
Peltola, V., Markku, I., Huhtaniemi, A., 1996. Induction of lipid peroxidation during steroidogenesis in the rat testis. Endocrinology 137, 105–112. https://doi.org/10.1210/endo.137.1.8536600.
Pisoschi, A.M., Pop, A., 2015. The role of antioxidants in the chemistry of oxidative stress: a review. Eur. J. Med. Chem. 97, 55–74. https://doi.org/10.1016/j.ejmech.2015.04.040.
R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL: http://www.R-project.org/.
Reddon, A.R., Voisin, M.R., Menon, N., Marsh-Rollo, S.E., Wong, M.Y.L., Balshine, S., 2011. Rules of engagement for resource contests in a social fish. Anim. Behav. 82, 93–99. https://doi.org/10.1016/j.anbehav.2011.04.003.
Reddon, A.R., O'Connor, C.M., Marsh-Rollo, S.E., Balshine, S., 2012. Effects of isotocin on social responses in a cooperatively breeding fish. Anim. Behav. 84, 753–760. https://doi.org/10.1016/j.anbehav.2012.07.021.
Regan, M.D., Dhillon, R.S., Toews, D.P.L., Speers-Roesch, B., Sackville, M.A., Pinto, S., Bystriansky, J.S., Scott, G.R., 2015. Biochemical correlates of aggressive behavior in the Siamese fighting fish. J. Zool. 297, 99–107. https://doi.org/10.1111/jzo.12259.
Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can 191, 1–382.
Roberts, G., 1996. Why individual vigilance declines as group size increases. Anim. Behav. 51, 1077–1086. https://doi.org/10.1006/ANBE.1996.0109.
Rosvall, K.A., Bentz, A.B., George, E.M., 2020. How research on female vertebrates contributes to an expanded challenge hypothesis. Horm. Behav. 123, 104565. https://doi.org/10.1016/j.yhbeh.2019.104565.
Russell, A.F., Carlson, A.A., McIlrath, G.M., Jordan, N.R., Clutton-Brock, T., 2004. Adaptive size modification by dominant female meerkats. Evolution 58, 1600–1607. https://doi.org/10.1111/j.0014-3820.2004.tb01739.x.
Sapolsky, R.M., 1983. Endocrine aspects of social instability in the olive baboon (*Papio anubis*). Am. J. Primatol. 5, 365–379. https://doi.org/10.1002/ajp.1350050406.
Scaia, M.F., Cavallino, L., Pandolfi, M., 2020. Social control of spermatogenesis and steroidogenesis in cichlid fish: a comparative approach. Reproduction 159, R31–R43. https://doi.org/10.1530/REP-18-0650.
Schürch, R., Rothenberger, S., Heg, D., 2010. The building-up of social relationships: behavioural types, social networks and cooperative breeding in a cichlid. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 365, 4089–4098. https://doi.org/10.1098/rstb.2010.0177.
Senar, J.C., Polo, V., Uribe, F., Camerino, M., 2000. Status signalling, metabolic rate and body mass in the siskin: the cost of being a subordinate. Anim. Behav. 59, 103–110. https://doi.org/10.1006/anbe.1999.1281.
Sies, H., Berndt, C., Jones, D.P., 2017. Oxidative stress. Annu. Rev. Biochem. 86, 715–748. https://doi.org/10.1007/978-3-540-69361-1_27.
Silva, L.R., Lardy, S., Ferreira, A.C., Rey, B., Doutrelant, C., Covas, R., 2018. Females pay the oxidative cost of dominance in a highly social bird. Anim. Behav. 144, 135–146. https://doi.org/10.1016/j.anbehav.2018.08.006.
Sloman, K.A., Taylor, A.C., Metcalfe, N.B., Gilmour, K.M., 2001. Effects of an environmental perturbation on the social behaviour and physiological function of brown trout. Anim. Behav. 61, 325–333. https://doi.org/10.1006/anbe.2000.1567.
Smith, S.M., Nager, R.G., Costantini, D., 2016. Meta-analysis indicates that oxidative stress is both a constraint on and a cost of growth. Ecol. Evol. 6, 2833–2842. https://doi.org/10.1002/ece3.2080.
Sopinka, N.M., Fitzpatrick, J.L., Desjardins, J.K., Stiver, K.A., Marsh-Rollo, S.E., Balshine, S., 2009. Liver size reveals social status in the African cichlid *Neolamprologus pulcher*. J. Fish Biol. 75, 1–16. https://doi.org/10.1111/j.1095-8649.2009.02234.x.
Sørensen, C., Nilsson, G.E., Summers, C.H., Øverli, Ø., 2012. Social stress reduces forebrain cell proliferation in rainbow trout (*Oncorhynchus mykiss*). Behav. Brain Res. 227, 311–318. https://doi.org/10.1016/j.bbr.2011.01.041.
Stier, A., Delestrade, A., Zahn, S., Arrivé, M., Criscuolo, F., Massemin-Challet, S., 2014. Elevation impacts the balance between growth and oxidative stress in coal tits. Oecologia 175, 791–800. https://doi.org/10.1007/s00442-014-2946-2.
Stiver, K.A., Dierkes, P., Taborsky, M., Balshine, S., 2004. Dispersal patterns and status change in a co-operatively breeding cichlid *Neolamprologus pulcher*: evidence from microsatellite analyses and behavioural observations. J. Fish Biol. 65, 91–105. https://doi.org/10.1111/j.0022-1112.2004.00427.x.
Stiver, K.A., Fitzpatrick, J.L., Desjardins, J.K., Balshine, S., 2006. Sex differences in rates of territory joining and inheritance in a cooperatively breeding cichlid fish. Anim. Behav. 71, 449–456. https://doi.org/10.1016/j.anbehav.2005.06.011.
Stockley, P., Bro-Jørgensen, J., 2011. Female competition and its evolutionary consequences in mammals. Biol. Rev. 86, 341–366. https://doi.org/10.1111/j.1469-185X.2010.00149.x.
Taborsky, M., Limberger, D., 1981. Helpers in fish. Behav. Ecol. Sociobiol. 8, 143–145. https://doi.org/10.1007/BF00300826.
Taves, M.D., Desjardins, J.K., Mishra, S., Balshine, S., 2009. Androgens and dominance: sex-specific patterns in a highly social fish (*Neolamprologus pulcher*). Gen. Comp. Endocrinol. 161, 202–207. https://doi.org/10.1016/j.ygcen.2008.12.018.
Tea, J., Alderman, S.L., Gilmour, K.M., 2019. Social stress increases plasma cortisol and reduces forebrain cell proliferation in subordinate male zebrafish (*Danio rerio*). J. Exp. Biol. 222, jeb194894. https://doi.org/10.1242/jeb.194894.
Thorley, J., Katlein, N., Goddard, K., Zöttl, M., Clutton-Brock, T., 2018. Reproduction triggers adaptive increases in body size in female mole-rats. Proc. R. Soc. B 285, 20180897. https://doi.org/10.1098/rspb.2018.0897.
Ulrich, Y., Saragosti, J., Tokita, C.K., Tarnita, C.E., Kronauer, D.J.C., 2018. Fitness benefits and emergent division of labour at the onset of group living. Nature 560, 635–638. https://doi.org/10.1038/s41586-018-0422-6.
Van Meter, P.E., French, J.A., Dloniak, S.M., Watts, H.E., Kolowski, J.M., Holekamp, K.E., 2009. Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. Horm. Behav. 55, 329–337. https://doi.org/10.1016/j.yhbeh.2008.11.001.
Veskoukis, A.S., Nikolaidis, M.G., Kyparos, A., Kouretas, D., 2009. Blood reflects tissue oxidative stress depending on biomarker and tissue studied. Free Radic. Biol. Med. 47, 1371–1374. https://doi.org/10.1016/j.freeradbiomed.2009.07.014.
Walton, N.M., Shin, R., Tajinda, K., Heusner, C.L., Kogan, J.H., Miyake, S., Chen, Q., Tamura, K., Matsumoto, M., 2012. Adult neurogenesis transiently generates oxidative stress. PLoS One 7, e35264. https://doi.org/10.1371/journal.pone.0035264.
Ward, P., Zahavi, A., 1973. The importance of certain assemblages of birds as "information-centres" for food-finding. Ibis 115, 517–534. https://doi.org/10.1111/j.1474-919X.1973.tb01990.x.
Weatherley, A.H., Gill, H.S., Lobo, A.F., 1988. Recruitment and maximal diameter of axial muscle fibres in teleosts and their relationship to somatic growth and ultimate size. J. Fish Biol. 33, 851–859. https://doi.org/10.1111/j.1095-8649.1988.tb05532.x.
Wilson, S.M., Gravel, M.A., Mackie, T.A., Willmore, W.G., Cooke, S.J., 2012. Oxidative stress associated with paternal care in smallmouth bass (*Micropterus dolomieu*). Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 162, 212–218. https://doi.org/10.1016/j.cbpa.2012.02.023.
Wingfield, J.C., Hegner, R.E., Dufty, A.M., Ball, G.F., 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am. Nat. 136, 829–846. https://doi.org/10.1086/285134.
Witter, M.S., Swaddle, J.P., 1995. Dominance, competition, and energetic reserves in the European starling, *Sturnus vulgaris*. Behav. Ecol. 6, 343–348. https://doi.org/10.1093/beheco/6.3.343.
Wong, M.Y.L., Balshine, S., 2011a. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. Biol. Rev. 86, 511–530. https://doi.org/10.1111/j.1469-185X.2010.00158.x.
Wong, M.Y.L., Balshine, S., 2011b. Fight for your breeding right: hierarchy re-establishment predicts aggression in a social queue. Biol. Lett. 7, 190–193. https://doi.org/10.1098/rsbl.2010.0639.
Wong, M.Y.L., Buston, P.M., Munday, P.L., Jones, G.P., 2007. The threat of punishment enforces peaceful cooperation and stabilizes queues in a coral-reef fish. Proc. R. Soc. B 274, 1093–1099. https://doi.org/10.1098/rspb.2006.0284.
Young, A.J., Carlson, A.A., Monfort, S.L., Russell, A.F., Bennett, N.C., Clutton-Brock, T., 2006. Stress and the suppression of subordinate reproduction in cooperatively breeding meerkats. Proc. Natl. Acad. Sci. U. S. A. 103, 12005–12010. https://doi.org/10.1073/pnas.0510038103.
Yuan, T.F., Gu, S., Shan, C., Marchado, S., Arias-Carrión, O., 2015. Oxidative stress and adult neurogenesis. Stem Cell Rev. Rep. 11, 706–709. https://doi.org/10.1007/s12015-015-9603-y.
Zini, A., Schlegel, P.N., 2003. Effect of hormonal manipulation on mRNA expression of antioxidant enzymes in the rat testis. J. Urol. 169, 767–771. https://doi.org/10.1016/S0022-5347(05)64010-4.