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Stress axis regulation during social ascension in a group-living cichlid fish

Brett M. Culbert[a,*], Kathleen M. Gilmour[b], Sigal Balshine[a]

[a] Department of Psychology, Neuroscience & Behaviour, McMaster University, 1280 Main St W, Hamilton, Ontario L8S 4K1, Canada
[b] Department of Biology, University of Ottawa, 20 Marie Curie Private, Ottawa, Ontario K1N 6N5, Canada

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ABSTRACT

Animals living in groups often form social hierarchies, with characteristic behaviours and physiologies associated with rank. However, when social opportunities arise and a subordinate ascends into a dominant position, quick adjustments are necessary to secure this position. Such periods of social transition are typically associated with elevated glucocorticoid production, but the precise regulation of the stress axis during these occasions is not well understood. Using the group-living cichlid, *Neolamprologus pulcher*, the effects of social ascension on the stress axis were assessed. Ascenders rapidly filled experimentally created vacancies, adopting a dominant behavioural phenotype within 72 h—elevating aggression, activity, and workload, while receiving high rates of affiliative behaviours from their group members. Despite assuming behavioural dominance within their groups, ascenders displayed higher cortisol levels than dominants three days post-ascension. Additionally, compared to subordinates, ascenders had increased transcript abundance of steroidogenic acute regulatory protein (*star*) and cytochrome *p*450 side-chain cleavage enzyme (*p450scc*) in the head kidney, indicating activation of the stress axis. Cortisol levels were lowest in ascenders that displayed low rates of aggression, potentially reflecting the reestablishment of social stability in these groups. Increased transcript abundance of both glucocorticoid receptors (*gr1* and *gr2*) in the brain's preoptic area (POA) of ascenders compared to dominants suggested an enhanced capacity for cortisol regulation via negative feedback. Our results reveal a regulatory cascade of behavioural and physiological interactions and highlight the importance of investigating the underlying mechanisms regulating the stress axis.

1. Introduction

Living in a social group can provide a number of advantages, such as increased vigilance (Evans et al., 2016; Roberts, 1996), improved food acquisition (Evans et al., 2016; Ward and Zahavi, 1973) and workload sharing and load lightening (Ausband et al., 2016; Balshine et al., 2001; Dornhaus et al., 2008). Thus, by living in a group, individuals can save time and energy. However, social life also has costs. Conflicts within groups often arise over access to food, shelter, and reproductive opportunities (Milinski and Parker, 1991; Stockley and Bro-Jørgensen, 2011). Hierarchy formation, a common phenomenon in social groups, is thought to have evolved to reduce conflict over such limited resources. The most competitive individuals typically attain dominant positions, and secure primary access to resources, while less competitive individuals are subordinate and typically have less access to resources. Consequently, dominants and subordinates often differ considerably in terms of behaviour, physiology, health, and fitness (Sapolsky, 2004, 2005; Silk et al., 2003).

Levels of stress frequently vary with social rank and these rank-related physiological phenotypes have been well investigated (Creel, 2001; Creel et al., 2013; Goymann and Wingfield, 2004). However, the majority of studies only measure glucocorticoid levels, and not the mechanisms regulating glucocorticoid production. Moreover, the relationship between social rank and stress is often complex, influenced by a variety of factors. For example, during periods of social instability, challenges in rank order often are associated with elevated glucocorticoid levels (Sapolsky, 1992) but as rank is established glucocorticoids levels usually decrease (Engh et al., 2006; Van Meter et al., 2009). To date, few studies have focused on the mechanisms regulating stress axis dynamics during periods of social instability, but such knowledge would strongly enhance our understanding of the factors influencing social stress.

To study the mechanisms regulating stress during social transitions, we used *Neolamprologus pulcher*, a cooperatively breeding cichlid fish that lives in permanent social groups. Groups consist of a dominant breeding pair and 1–20 subordinate helpers within a hierarchy (Wong and Balshine, 2011). Dominants are more aggressive (Fitzpatrick et al., 2008), more active (Sopinka et al., 2009), and perform more territory

* Corresponding author.
E-mail address: culbertb@mcmaster.ca (B.M. Culbert).

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defense (Desjardins et al., 2008) compared to subordinates. When a dominant position becomes available, a subordinate can ascend to the dominant position (Balshine-Earn et al., 1998; Bergmüller et al., 2005; Fitzpatrick et al., 2008). The effects of social transition on cortisol production as yet have not been assessed in *N. pulcher*, although in *Astatotilapia burtoni*—a closely related cichlid, where transitions between territorial and non-territorial status occur repeatedly and reversibly—social ascension is associated with a rapid increase in circulating cortisol levels (< 30 min; Maruska, 2015) that can persist for 3 or more days (Huffman et al., 2015). However, the mechanisms underlying these changes in glucocorticoid production remain poorly understood.

We tested the hypothesis that periods of social ascension activate the entire stress axis, from the preoptic area (POA) of the brain where stress responses are initiated via release of corticotropin-releasing factor (CRF) to the head kidney, where cortisol is produced by interrenal cells. We removed dominant males from social groups, creating an opportunity for subordinate males to ascend in social rank and assume the dominant position. We predicted that ascending males would rapidly adopt a dominant behavioural phenotype, and that this period would be associated with increased activation of the stress axis. Specifically, we measured and compared circulating cortisol levels as well as transcript abundance of stress axis genes in stable dominant, stable subordinate, and ascending males. In the POA, we targeted CRF (*crf*), which initiates activation of the stress axis following a stressor (Aguilera, 1998). We also measured transcript levels of the glucocorticoid receptors, which contribute to the regulation of cortisol production via negative feedback (Dallman et al., 1994). In most teleost fish, including *N. pulcher* (O'Connor et al., 2013), two isoforms of the glucocorticoid receptor exist (GR1 and GR2; Stolte et al., 2006), and therefore we measured both *gr1* and *gr2*. In the head kidney (analogous to the adrenal in mammals and birds), we measured transcript abundance of melanocortin 2 receptor (*mc2r*), steroidogenic acute regulatory protein (*star*), and cytochrome P450 side-chain cleavage enzyme (*p450scc*). We chose these genes because cortisol synthesis in steroidogenic cells is initiated when MC2R is activated by adrenocorticotropic hormone (Fridmanis et al., 2017), and is rate-limited by the conversion of cholesterol to pregnenolone, involving transfer of cholesterol across the mitochondrial membrane, which is regulated by StAR (Tokarz et al., 2015), and its cleavage to pregnenolone, which is catalyzed by P450scc (Payne and Hales, 2004).

2. Materials and methods

2.1. Experimental animals

The experiment was conducted from November 2016 – April 2017 at McMaster University in Hamilton, Ontario, Canada. Fish were laboratory-reared descendants of wild-caught *Neolamprologus pulcher* from Lake Tanganyika, Africa. Social groups consisting of a dominant breeding male-female pair, 1–3 large helpers (standard length (SL) > 4.5 cm), and 1–4 small helpers (SL < 4 cm) were held within 189 L aquaria. All social groups (n = 20) had been together for at least a month and had produced young prior to any experimental manipulation. Each fish was given a unique dorsal fin clip for identification, which does not adversely affect behaviour (Stiver et al., 2004). Each aquarium contained two large sponge filters, a heater, 3 cm of coral sand for substrate, two terracotta flowerpot halves, two mirrors, and two PVC tubes as shelter. Water was kept at 27 °C and a 13 L:11D photoperiod was maintained throughout 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 were in compliance with the guidelines of the Canadian Council on Animal Care (CCAC) regarding the use of animals in research and teaching.

2.2. Experimental protocols

Thirty-two focal fish were targeted in this experiment. At the beginning of the experiment (Day 0), all individuals within a group were weighed and measured, and each group was randomly designated as control (N = 8; average of 6.75 ± 0.45 group members) or treatment (N = 12; average of 6.83 ± 0.42 group members). Behavioural observations (see Section 2.3) were recorded using a video camera (Canon VIXIA HF S200) on Days 10, 11, 13, and 14, and later scored. In treatment groups, dominant males were removed and sampled (mass = 7.45 ± 0.35 g, SL = 6.73 ± 0.18 cm, mean ± SEM; N = 12) on the morning of Day 11—providing an opportunity for subordinate males within these groups to ascend to the dominant position. On the morning of Day 14, ascending males were removed and sampled (mass = 5.21 ± 0.38 g, SL = 5.86 ± 0.17 cm; N = 8). In four of the twelve treatment groups, a clear dominant male had not emerged by Day 14 and therefore target ascending males were not collected from these groups. In control groups, subordinate males were removed and sampled on the morning of Day 11 (mass = 3.79 ± 0.31 g, SL = 5.23 ± 0.12 cm; N = 12). Note that in four control groups, two stable subordinate males were sampled.

2.3. Behavioural analyses

Fish were given a 5 min acclimation period following placement of the camera in front of their tank. Focal fish were then continuously recorded for 10 min and all behaviours performed or received were scored (see Sopinka et al., 2009 for a detailed species-specific ethogram). A dominance index (see Fig. 1A) was determined for each focal fish 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 ($DI = (Agg_{Given} + Sub_{Rec}) - (Agg_{Rec} + Sub_{Given})$, see Fitzpatrick et al., 2008). The total number of affiliative acts (follows, parallel swims, and soft touches) received from all group members was also determined (see Fig. 1B). A workload index (see Balshine et al., 2001) for each fish was assigned 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; see Fig. 1C), and defensive aggressive acts performed towards a mirror and neighbours. To assess locomotor activity, tanks were visually split into 12 quadrats using a grid and the number of times each fish crossed between quadrats was counted (see Fig. 1D). Behaviours are reported as averages of the two observation periods (i.e. Days 10/11 or Days 13/14). Following the initial behavioural recordings on Days 10 and 13, an unfamiliar male conspecific (SL = 5.82 ± 0.14 cm) was placed in the centre of the tank within a clear perforated plastic tube (see Fig. 1E) and the number of aggressive defense acts performed towards the intruding conspecific was recorded over a 10 min period.

2.4. Tissue sampling

Fish were rapidly netted and killed via terminal anaesthesia (0.5 g L^{-1} ethyl-*p*-aminobenzoate; Sigma-Aldrich, Oakville, ON, Canada), and mass and standard length were recorded. All fish were sampled between 0800 h and 1100 h to avoid diurnal fluctuations in

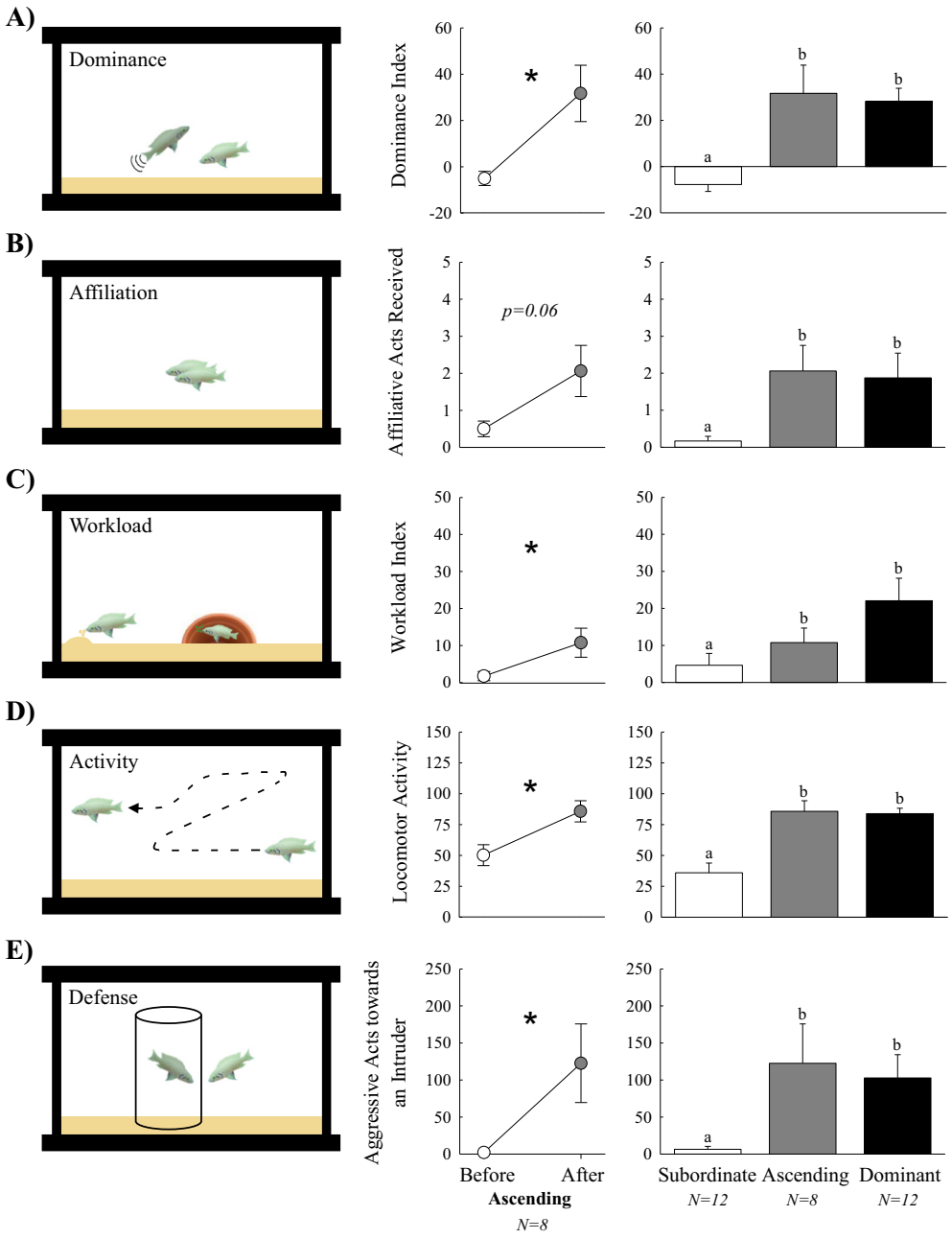

Fig. 1. Illustrations in the first column depict the behavioural assays used to measure dominance (A); affiliation received (B); workload (C); locomotor activity (D); and defense against an intruder (E). The second column refers to changes in behaviour as a result of ascension. The third column shows the associated differences in behaviours of subordinate, ascending, and dominant males. Behaviours were recorded over 10 min. Affiliative acts received, aggressive acts, and locomotor activity (lines crossed) refer to counts of these behaviours over the 10 min period. Values are means ± SEM. Treatment groups that share a letter are not significantly different from one another. An asterisk indicates a difference with ascension (see text for additional details).

cortisol production. Blood was collected within 2–3 min via caudal severance into heparinized micro-hematocrit capillary tubes (Thermo-Fisher Scientific, Ottawa, ON, Canada) and centrifuged (4750*g* for 3 min). Plasma was flash frozen in liquid nitrogen and stored at −80 °C for later analysis of cortisol concentrations. Sex was confirmed via examination of the gonads, and the head kidney and POA were dissected out, flash frozen, and stored at −80 °C for later analysis of transcript abundance.

2.5. Cortisol quantification

Circulating cortisol levels were measured using an enzyme-linked immunosorbent assay (EIA; Neogen, Lexington, KY, USA) following the manufacturer's protocol. The kit has a detection limit of 0.04 ng mL^{-1}. Based on preliminary trials, plasma samples were diluted 50× with Milli-Q water (EMD Millipore, Etobicoke, ON, Canada) prior to analysis. Samples were assayed in duplicate with an intra-assay variation of 2.8% (% CV). We were unable to collect blood from three subordinates, and therefore plasma cortisol was not determined for these fish.

2.6. Transcript abundance analysis by real-time RT-PCR

Tissues were homogenized on ice in TRIzol reagent (Invitrogen, Burlington, ON, Canada) using a sonicator (Sonic Dismembrator Model 100; Thermo-Fisher Scientific) and total RNA was extracted according to the manufacturer's protocol. Extracted RNA was quantified (NanoDrop 2000c UV–vis Spectrophotometer; Thermo-Fisher Scientific) and complementary DNA (cDNA) was generated using a QuantiTech Reverse Transcription Kit (Qiagen, Toronto, ON, Canada) following the manufacturer's protocol.

Gene specific primers (Table 1) were used to assess changes in transcript abundance by semi-quantitative real-time RT-PCR. Previously published primers were used when available. Primers for target genes in the head kidney (*p450scc*, *star*, and *mc2r*) were designed using

Table 1
Gene specific primers used for real-time RT-PCR.

Gene	Primer sequence (5′ to 3′)	Amplicon size (bp)	Efficiency (%)	Annealing temperature (°C)	Accession number	Reference
18s	F: ACAAGAAGAGACCTTCACCTGG R: CTCAATCTCGTGTGGCTGAA	146	POA: 93 HK: 90	56 60	AF337051	O'Connor et al., 2013
crf	F: ATCACCTTCCATCTTCAACAG R: CTGGACATCTCCATCATCTC	204	POA: 93	60	JX134406	Taborsky et al., 2013
gr1	F: GCTGATCAAGATGAAAGTGC R: AGAGTAGACATGAGCCGTGA	198	POA: 95	60	EF661652	Taborsky et al., 2013
gr2	F: TCGTTCCAACAATGTTATCC R: GCAGAGTCATCTGATCATCC	204	POA: 111	60	EF661651	Taborsky et al., 2013
p450scc	F: AAGTCAGGGAGACTTGCTGG R: GGATGCAACCTGAGTGTTTCC	81	HK: 97	60	XM_006789280	
star	F: TCAGTGTCCGATGTGCCAAG R: TTTCCGCTCTGACAACACCC	105	HK: 94	60	XM_006805871	
mc2r	F: CATATACGCCTTCCGCATCG R: CCAGTTAAATCAGCAGAGCTTCC	89	HK: 97	60	XM_006795993	

18s, 18S ribosomal RNA; *crf*, corticotropin-releasing factor; *gr1*, glucocorticoid receptor 1; *gr2*, glucocorticoid receptor 2; *p450scc*, cytochrome P450 side-chain cleavage enzyme; *star*, steroidogenic acute regulatory protein; *mc2r*, melanocortin 2 receptor.

Primer-BLAST (NCBI; Ye et al., 2012) based on predicted sequences for *Neolamprologus brichardi*. *N. brichardi* is considered to be a subspecies of *N. pulcher* (Taborsky and Grantner, 1998), and genetic evidence suggests that *N. brichardi* and *N. pulcher* may be the same species (Duftner et al., 2007). To confirm primer specificity, pooled PCR products were purified using a QIAquick PCR purification kit (Qiagen) and sequenced (StemCore Laboratories, Ottawa, ON, Canada).

All real-time RT-PCR reactions were carried out in duplicate using a Rotor-Gene SYBR Green PCR Kit (Qiagen) and a Rotor-Gene Q real-time PCR system (Qiagen), following the manufacturer's protocol with the exception that reaction volumes were scaled to 10 μL from 25 μL. Each reaction contained 5 μL SYBR 2× PCR mix, 1 μL of combined forward and reverse primers (10 μM of each), 3 μL of RNase/DNase free water, and 1 μL of cDNA template. Cycling parameters consisted of a 5 min activation step at 95 °C, followed by 40 cycles consisting of a 5 s denaturation step at 95 °C, and a combined 10 s annealing and extension step. Standard curves were developed for each primer set using serial dilutions (4×) of pooled cDNA from each individual, and conditions were adjusted to optimize the efficiency of each reaction. Negative controls, including no template controls (where cDNA was replaced with water) and no reverse transcriptase controls (where RNA was treated as per other cDNA reactions but reverse transcriptase was replaced with water) were included. Melt curves were performed at the end of each run to confirm the presence of a single product, as well as the absence of primer dimers. Transcript abundance was calculated relative to the subordinate group using the modified delta-delta Ct method (Pfaffl, 2001), normalizing to mRNA abundance of the reference gene 18S, which did not vary among groups.

2.7. Statistical analysis

Statistical analyses were performed using R (version 3.3.2, R Core Team, 2014). All data are presented as means ± 1 standard error of the mean (SEM) 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 transformed, or if the data could not be transformed to meet the assumptions, then equivalent non-parametric analyses were performed. To assess behavioural changes in ascending fish prior to, and after removal of the dominant male, paired Student's *t*-tests or Wilcoxon signed-ranks tests were performed. To investigate behavioural and physiological differences across dominant, subordinate and ascending fish, general linear mixed models (GLMM) were fit using the lmer function in the 'lme4' package (Bates et al., 2015). To account for non-independence of animals sampled from the same group, group id was included as a random factor in all models. When overall differences were detected using the Anova function in the 'car' package (Fox and Weisberg, 2011), Tukey's HSD post-hoc analysis was performed using the glht function in the 'multcomp' package (Hothorn et al., 2008).

3. Results

3.1. Behaviour

Following dominant removal, ascending males became more aggressive and less submissive, resulting in higher dominance index scores (Fig. 1A; Paired *t*-test, $t_7 = 2.96$, $p = 0.02$). Ascenders also tended to receive more affiliative acts from other group members (Fig. 1B; Wilcoxon signed-rank test, $W_7 = 15.00$, $p = 0.06$), increased their workload (Fig. 1C; Paired *t*-test, $t_7 = 2.93$, $p = 0.02$), displayed higher locomotor activity (Fig. 1D; $t_7 = 2.78$, $p = 0.03$), and were more aggressive towards an intruding conspecific after dominant removal (Fig. 1D; $t_7 = 5.17$, $p = 0.001$).

Dominance index scores of stable dominants and ascending males did not differ from one another, but both were higher than those of subordinate males (Fig. 1A; GLMM, $X^2 = 56.61$, df = 2, $p < 0.001$). Similarly, dominant and ascending males received more affiliative acts from group members (Fig. 1B; $X^2 = 19.85$, df = 2, $p < 0.001$), had higher workloads (Fig. 1C; $X^2 = 22.43$, df = 2, $p < 0.001$), higher locomotor activity (Fig. 1D; $X^2 = 37.57$, df = 2, $p < 0.001$), and performed more aggressive acts towards an intruding conspecific (Fig. 1E; $X^2 = 36.50$, df = 2, $p < 0.001$) than subordinate males.

3.2. Circulating cortisol levels

Circulating cortisol levels varied across social ranks with subordinates displaying the highest plasma cortisol levels (Fig. 2B; GLMM, $X^2 = 11.22$, df = 2, $p = 0.004$). Dominants had lower cortisol levels than subordinates ($p = 0.008$) and ascenders ($p = 0.02$), and ascending males did not differ from subordinates ($p = 0.89$). Circulating cortisol levels of ascenders strongly and positively correlated with both the dominance index (Pearson's correlation, $r = 0.765$, $p = 0.03$) and aggression towards an intruding conspecific ($r = 0.885$, $p < 0.001$), with the most aggressive ascenders displaying the highest levels of cortisol.

3.3. Stress axis transcript levels

Transcript levels of key components of the HPI axis were elevated in ascending fish. In the POA, ascending fish had higher mRNA abundance of *gr1* (Fig. 2C; GLMM, $X^2 = 6.23$, df = 2, $p = 0.04$) and *gr2* (Fig. 2D; $X^2 = 12.31$, df = 2, $p = 0.002$) than dominants. No differences in *crf* transcript abundance were detected (Fig. 2E; $X^2 = 3.67$, df = 2,

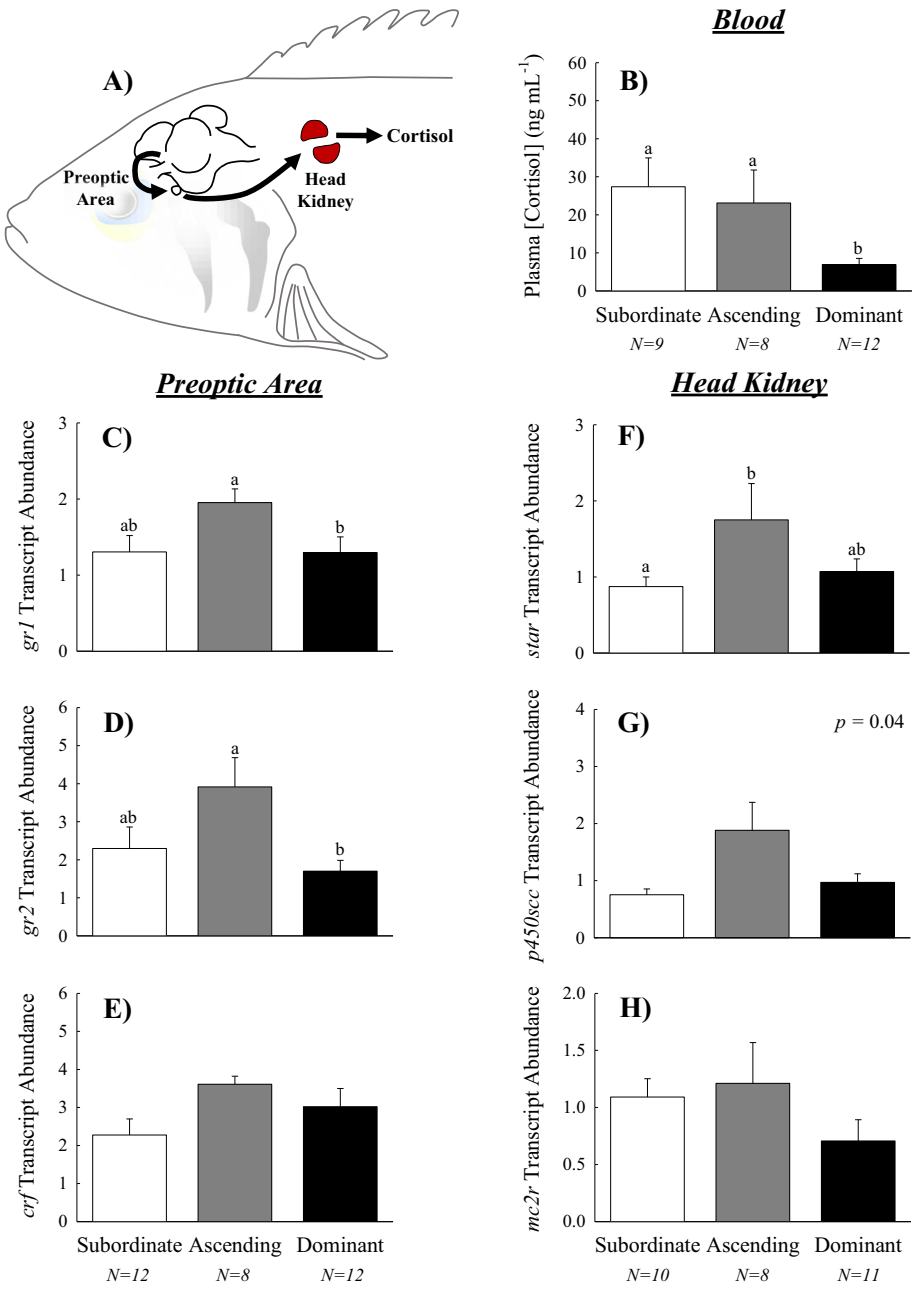

Fig. 2. Regulation of stress axis activity by social status. In response to a stressor, corticotropin-releasing factor (CRF) from the preoptic area stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary; ACTH circulates to the head kidney and stimulates cortisol production (A). Circulating plasma cortisol levels (B); transcript abundance of *gr1* (C), *gr2* (D), and *crf* (E) in the preoptic area; and transcript abundance of *star* (F), *p450scc* (G), and *mc2r* (H) in the head kidney of subordinate, ascending, and dominant males. Ascending males were sampled 72 h after being provided the opportunity to ascend. Values are means ± SEM. Treatment groups that share a letter are not significantly different from one another (see text for further details). Post-hoc analysis failed to identify the source of the significant differences in Panel G.

$p = 0.16$). Interestingly, *crf* transcript abundance did not correlate with circulating cortisol levels (Pearson's correlation, $r = -0.04$, $p = 0.82$), but instead correlated positively with both activity ($r = 0.45$, $p = 0.01$) and dominance ($r = 0.37$, $p = 0.04$).

In the head kidney, mRNA abundance of the cholesterol transport protein *star* was higher in ascenders (Fig. 2F; GLMM, $X^2 = 7.46$, df = 2, $p = 0.02$) than subordinates ($p = 0.02$), but not dominants ($p = 0.14$). Similarly, transcript levels of the steroidogenic enzyme *p450scc* were affected by social status (Fig. 2G; $X^2 = 6.19$, df = 2, $p = 0.04$) with ascenders tending to have higher levels than both dominants ($p = 0.06$) and subordinates ($p = 0.09$). No differences in *mc2r* transcript levels were detected across social ranks (Fig. 2H; $X^2 = 1.79$, df = 2, $p = 0.40$).

4. Discussion

Periods of social transition and instability are typically considered to be both dangerous and stressful. Here, we show for the first time that social ascension modulates the stress axis at multiple levels; specifically, at the site of cortisol synthesis—the head kidney—and in the POA of the brain. Transcriptional changes at the head kidney indicate an enhanced capacity for cortisol production as a result of stress incurred during ascension to the dominant position. However, social hierarchies stabilized rapidly within each group, as indicated by ascenders displaying increased levels of aggression, activity, and work, while receiving more affiliative acts—all characteristics associated with dominance in *N. pulcher* (Wong and Balshine, 2011). As hierarchies stabilized, ascenders began to suppress their cortisol levels, aided by an enhanced capacity for negative feedback of the stress axis in the POA.

Periods of within-group social instability are associated with high levels of aggression among group members and elevated glucocorticoid levels, as has been documented in mammals (e.g. olive baboons, *Papio anubis*; Sapolsky, 1992) and birds (e.g. Japanese Quail, *Coturnix coturnix japonica*; Guibert et al., 2010). However, as social order stabilizes, rates of aggression decrease and glucocorticoid levels typically fall (e.g. Maruska, 2015). In the lekking cichlid *Astatotilapia burtoni*, social

change can cause acute (< 30 min; Maruska, 2015), or even chronic elevation of cortisol levels (3 days; Huffman et al., 2015). Similarly, increased levels of aggression and cortisol production were observed during an attempted dominance takeover within the hierarchy of a wild group of female Chacma baboons (*Papio hamadryas ursinus*; Engh et al., 2006). We found that, relative to stable dominants, ascending males displayed elevated circulating cortisol levels, but there was considerable variability in the levels measured. Ascenders displaying the highest cortisol levels were the most aggressive, with the highest dominance index scores and high levels of defense displayed towards intruders. Such high levels of aggression may reflect continuing social instability, with ascenders attempting to solidify their new rank through aggressive interactions. In turn, social uncertainty coupled with a high frequency of aggressive encounters may have resulted in higher levels of stress and hence circulating cortisol levels. Overall, these findings suggest that the hierarchies within some social groups had not yet stabilized 72 h following disruption, resulting in elevated levels of stress and aggression in ascenders from these groups.

Dominant males had relatively low circulating cortisol levels. This finding contrasts with a previous study where cortisol levels were higher in dominant than subordinate *N. pulcher* (Mileva et al., 2009). Dominants with higher cortisol levels are common in many cooperatively breeding species that have high reproductive skew, reflecting an elevated allostatic load associated with the need to reproductively suppress subordinates and maintain dominance (Creel, 2001; Creel et al., 2013; Goymann and Wingfield, 2004). However, allostatic load can also be influenced by the number of individuals in a group (Rubenstein and Shen, 2009), with larger groups translating to higher allostatic loads for dominants. Compared to Mileva et al. (2009), the social groups used in our study contained fewer individuals per group (6.83 ± 0.42 vs 10.8 ± 0.8 individuals in Mileva et al., 2009), meaning that dominants in our study had fewer subordinates to police. This factor may have reduced their allostatic load and hence stress level associated with dominance, offering a possible explanation for the difference between studies.

Most previous studies on stress axis regulation during social transitions have focused exclusively on glucocorticoid levels. In contrast, we investigated the mechanisms underlying social regulation of the stress axis and show that ascending males exhibit elevated transcript abundance of *star*, with a similar trend for *p450scc*, in the head kidney compared to that of subordinate males. Cortisol production is rate limited by p450scc and StAR (Payne and Hales, 2004; Tokarz et al., 2015), thus increased transcript abundance suggests activation of the stress axis and enhanced capacity for cortisol synthesis. Similarly, elevated transcript abundance of *p450scc* and *star* in the head kidney of dominant rainbow trout (*Oncorhynchus mykiss*; Jeffrey et al., 2012) was attributed to the acute stress of hierarchy formation (Øverli et al., 1999), serving to facilitate responses to additional stressors. Hence, steroidogenic capacity at the head kidney (analogous to the adrenal in mammals and birds) appears to be socially regulated.

Ascending males also exhibited elevated *gr* transcript abundance in the POA compared to dominant males. Negative feedback of cortisol production via glucocorticoid receptor signalling in the brain occurs in mammals (Dallman et al., 1994), birds (Cornelius et al., 2018; Dickens et al., 2009) and fish (Alderman et al., 2012; Kiilerich et al., 2018). Dominants typically downregulate their stress response more quickly than subordinates (Jeffrey et al., 2014; Sapolsky, 1983), and our results suggest that this capacity may be even greater in ascenders. In an unstable social environment, it may be beneficial not only to be prepared to mount an immediate cortisol response to mobilize energy to overcome social challenges, but also to be able to rapidly downregulate the stress response, thereby avoiding the costs of chronically elevated cortisol (McEwen, 2008). Similarly, Taborsky et al. (2013) found that *N. pulcher* raised in the absence of a social group displayed elevated transcript abundance of *gr1* in the brain. *N. pulcher* typically live their entire lives as part of a social group, therefore, development in the absence of a group constitutes chronic social instability. Taken together, these results suggest that enhanced negative feedback of the stress axis may serve an important regulatory role during periods of socially instability.

Although CRF is a critical component of the stress axis—stimulating adrenocorticotropic hormone production in the pituitary (Aguilera, 1998)—its involvement in the regulation of social stress remains unclear. While CRF expression increases in response to acute stressors, there is mounting evidence that the CRF system habituates during chronic social stress (Backström and Winberg, 2013). In mammals, chronic stress causes a shift from CRF to arginine vasopressin as the primary secretagogue of ACTH (Ma et al., 1997; Ma and Lightman, 1998; Pinnock and Herbert, 2001). Social ascension in the closely related cichlid, *A. burtoni*, is associated with increased whole brain transcript abundance of arginine vasotocin (AVT; the teleost homologue of arginine vasopressin) as well as elevated circulating cortisol levels (Huffman et al., 2015), suggesting that a similar switch from CRF- to AVT-driven ACTH production may occur during periods of social transition. However, production of CRF is not limited to the POA (Alderman and Bernier, 2007; Pepels et al., 2002), and not all CRF neurons originating in the POA project onto corticotropes (Zupanc et al., 1999). Thus, further research into the regulatory role of CRF during ascension is warranted. In addition to its role in stimulating ACTH production, CRF is also implicated in the regulation of a wide range of behaviours (Hostetler and Ryabinin, 2013), including aggression (Carpenter et al., 2009; Mele et al., 1987) and decision-making (Bryce and Floresco, 2016; Summers et al., 2017). Modulation of such behaviours is likely important as social order is re-established following hierarchy disturbances. However, central injections of CRF also increase locomotion in many vertebrates (Lowry and Moore, 2006), including fish (Clements et al., 2002). Consistent with these observations, we observed a positive relationship between activity and *crf* transcript abundance in the POA. Therefore, CRF appears to be playing an immediate and central role in the regulation of behaviour during social ascension.

In conclusion, our findings provide novel insight into regulation of the stress axis during social ascension. Specifically, cortisol levels during social transitions reflect the impact of perceived stability within a group on stress axis activity. In turn, activity of the stress axis is tuned to allow such dynamic adjustments of cortisol, with enhanced capacity for cortisol production at the head kidney as well as enhanced capacity for negative feedback regulation at the POA. These results illustrate the importance and benefit of integrative studies investigating the mechanisms underlying social regulation of stress.

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Competing interests

The authors declare no competing interests.

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Data accessibility

Supporting data are available on Mendeley Data.

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