

# Physiological consequences of social descent: studies in *Astatotilapia burtoni*

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## Abstract

In many species, social interactions regulate reproductive capacity, although the exact mechanisms of such regulation are unclear. Since social stress is often related to reproductive regulation, we measured the physiological signatures of change in reproductive state as they relate to short-term stress and the stress hormone cortisol. We used an African cichlid fish, *Astatotilapia burtoni*, with two distinct, reversible male phenotypes: dominant (territorial, T) males that are larger, more brightly colored, more aggressive, and reproductively competent and non-dominant males (non-territorial, NT) that are smaller, camouflage colored, and have regressed gonads. Male status, and hence reproductive competence, depends on social experience in this system. Specifically, if a T male is placed among larger male fish, it quickly becomes NT in behavior and coloration, but complete regression of its reproductive axis takes *ca.* 3 weeks (White *et al.* 2002). Reproduction in all vertebrates is controlled by the

hypothalamic–pituitary–gonadal axis in which the key signaling molecule from the brain to the pituitary is GnRH1. Here, we subjected T males to territory loss, a social manipulation which results in status descent. We measured the effects of this status change in levels of circulating cortisol and testosterone as well as mRNA levels of GnRH1 and GnRH receptor-1 (GnRH-R1) in the brain and pituitary, respectively. Following short-term social suppression (4 h), no change was observed in plasma cortisol level, GnRH1 mRNA expression, GnRH-R1 mRNA expression, or plasma testosterone level. However, following a somewhat longer social suppression (24 h), cortisol and GnRH1 mRNA levels were significantly increased, and testosterone levels were significantly decreased. These results suggest that in the short run, deposed T males essentially mount a neural ‘defense’ against loss of status.

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## Introduction

How do social conditions interact with physiological processes to influence behavior? Although a complete answer to this question remains unknown, it is clear that behavioral output results from complex interactions that cannot be understood simply as stimulus–response systems, but depend on more complex combinatorial mechanisms. Two key elements influencing behavior in a social system are social context and circulating hormone levels (Fernald 2002, Oliveira *et al.* 2002, McEwen & Wingfield 2003, Sapolsky 2005). To understand how social conditions influence physiology, we analyzed hormone levels, social context, and reproductive physiology following defined social stress in semi-natural conditions using an African cichlid fish, *Astatotilapia burtoni*.

*A. burtoni* has a two-tiered social system in which males can be either dominant (territorial males, T males) or non-dominant (NT males), and importantly, these states are reversible. T males are brightly colored, physically larger, aggressively defend a territory, and have fully spermiated testes, as contrasted with non-territorial males (NT males), which are camouflaged, smaller, school with female fish, and are not reproductively

active (Fernald & Hirata 1977). Corresponding to these substantial behavioral differences are a suite of physiological and molecular differences (reviewed in Fernald 2002).

In *A. burtoni*, as in all the vertebrates, reproduction is ultimately controlled by the brain via the hypothalamic–pituitary–gonadal (HPG) axis. In the hypothalamus, neurons release gonadotropin-releasing hormone 1 (GnRH1), a signaling peptide that stimulates the release of gonadotropins from the pituitary, which in turn causes the gonads to mature and produce androgens. Expression of GnRH1 and its receptor in the pituitary is strongly up-regulated in T males as compared to NT males (White *et al.* 2002, Au *et al.* 2006). *A. burtoni* males change between T and NT states depending on the social environment. When a T male is placed with significantly larger fish, it assumes NT male status quickly in both behavior and coloration. However, the loss of reproductive competence takes *ca.* 3 weeks after loss of territory (White *et al.* 2002). In contrast, NT males ascending socially to become T males will show an eyebar and aggressive behavior almost immediately (Burmeister & Fernald 2005), and will shift physiologically to territorial status in *ca.* 1 week (White *et al.* 2002).

Several investigators have identified a direct link between social status and the stress hormone cortisol (Blanchard *et al.* 1993), which is one output of the hypothalamic–pituitary–adrenal axis. When an animal is under stress, the hypothalamus releases corticotropin-releasing hormone to the pituitary, which in turn releases adrenocorticotropic hormone (ACTH) into the blood stream. At the adrenal cortex, ACTH causes the release of cortisol through which it mobilizes energy stores, increases heart rate, slows digestion and stimulates gluconeogenesis (reviewed in Becker *et al.* 1992). Thus, the activity of cortisol directs metabolic energy away from long-term physiological projects such as reproduction and toward the short-term goal of combating the stressor (Sapolsky 1993a). Animals under chronic stress may have these long-term, metabolically expensive activities inhibited indefinitely, corresponding to reproductive regression. This regression is observed in T males following the loss of a territory and ensuing descent in status.

The status differences observed in *A. burtoni* and other species with social stratification correspond to chronic social stress experienced by lower as compared with higher ranking individuals (Manogue *et al.* 1975, De Goeij *et al.* 1992, Blanchard *et al.* 1993, Sapolsky 1993b). However, the relationship of cortisol to social status in *A. burtoni* appears to depend not only on the status of the individual, but also on the social community and maturational state of the animals. For example, when males are housed in T–NT social pairs, GnRH neuronal soma size differences are significantly up-regulated in Ts vs NTs and differences in cortisol are statistically significant in older fish that lived as stable pairs for 2 weeks (Fox *et al.* 1997). Among younger fish and in the first week of such pairings, cortisol levels were significantly more variable and showed only a trend towards higher values in NT males. However, when animals are placed into a novel group setting, significant, stable T–NT cortisol differences are clearly evident at approximately 7 weeks, once the community has stabilized (Fox *et al.* 1997). Thus, in the short run, stress profiles and GnRH neuronal soma sizes may be uncoupled in some situations. T–NT dyads, a context that produces extreme behavioral status differences, may indicate other regulatory systems related to individual social experience not directly tied to cortisol.

Here, we subjected individual T males to very short (4 h) and longer (1 day) stress of territory loss that ultimately produces a status shift from T to NT. We measured circulating androgen and cortisol levels and GnRH1 and GnRH receptor-1 (GnRH-R1) mRNA levels in the brain and pituitary respectively at 4 and 24 h after social descent. Our data suggest that physiological changes occurring during the first day of status descent differ from those observed in the long term, implying an immediate response to territory loss that is not sustained.

## Materials and Methods

### Subjects

We used 16 *A. burtoni* T males, originally derived from wild-caught stock in Lake Tanganyika, East Africa (Fernald &

Hirata 1977). Prior to the experiment, fish were kept in aquaria under environmental conditions similar to those in nature (28 °C, pH 8 and a 12 h light:12 h darkness cycle with full spectrum illumination). Fish were fed cichlid pellets and flakes (AquaDine; Healdsburg, CA, USA) each morning *ad libitum*. Gravel covered the bottom and terracotta pots were placed in each aquarium to facilitate establishment of territories. Each community tank held 2–4 T males, 2–4 NT males, and 5–12 females. Individual animals were tagged with a combination of colored beads attached with a thin nylon string just below the dorsal fin to allow individuals to be identified for behavioral observations. Animals were treated at all times in accordance with Stanford's Administrative Panel for Laboratory Animal Care approved animal treatment policies.

### Procedure

Each subject in the group tank was observed for 3 min, three times per week for at least 1 week prior to selection. Behavioral patterns characteristic of T or NT males were recorded, including establishment and defense of a territory, coloration, and aggressive and sexual behavioral displays, in order of importance for this study (Fernald & Hirata 1977). Aggressive behaviors include: border threat displays (gill flaring and swimming rapidly in the direction of another male across an apparent territory border), carouseling (biting and attacking another male fish during a fight), and chasing or biting other fish. Sexual behaviors include courting of females (moving tail and end of body in solicitation), digging (each instance in which the male picks up a gravel in its mouth and spits it out), and spawning. Behaviors that indicated the NT status were fleeing from an attack and schooling with females. Subjects were assigned a status according to these behavioral observations. Due to dynamic social conditions, the T males often defend poorly defined or variable territories. Thus, to ensure that males were of full T status, only those defending a physically identifiable territory were considered territorial.

Eight of these T males were randomly assigned to the group to be sacrificed after a short time (e.g., 4 h: four experimental males and four control males) and eight T males assigned to the group to be sacrificed after a longer time (e.g., 24 h; four experimental males and four control males). On the evening prior to subjecting the T male to social descent, an individual male (focal male) was moved to the test tank for habituation. The test tank was bisected by an opaque barrier, creating two equal-sized hemi-tanks. In one hemi-tank, each focal male was housed with two females. For animals assigned to experimental conditions, an exceptionally large T male (stimulus male) was housed with six females on the opposite side of the opaque barrier. At the time of exposure (at 2400 h (4 h group) or 1600 h (24 h group)), the focal male was transferred into the territory of the stimulus male by being placed into the other hemi-tank. Being placed in this context with a large T male, it mimicked the loss of the focal male's territory. In the control condition, no T male was housed in

the opposite tank, leaving only six females. Since no dominating male was present, this did not mimic a territory loss. For comparison, this group acts as a control, allowing the measurement of the effects of the transfer without the sustained stress of territory loss, so that we might be sure that the stress experienced by the experimental group is not merely from the transfer. Following the exposure time (4 or 24 h), plasma was drawn and the animal sacrificed.

#### *Circulating hormone levels*

Immediately before sacrifice, blood samples (between 50 and 100 µl) were taken from each male using heparinized capillary tube butterfly needle. Blood was collected from the first caudal vein and centrifuged for 3 min at 13 000 r.p.m. to separate plasma from red blood cells. Blood samples were obtained within the first 3 min following capture to ensure that any acute stress associated with the blood draw itself did not affect the obtained cortisol levels (Fox *et al.* 1997). Plasma was then isolated and stored at  $-80^{\circ}\text{C}$  until assayed. After blood collection, subjects were immediately returned to their community tanks.

#### *Cortisol assay*

Cortisol concentration was measured in the plasma samples using an ELISA (Cortisol Correlate-EIA kit; Assay Designs, Inc., Ann Arbor, MI, USA). Plasma samples were diluted in assay buffer at the ratio of 1:30 and the kit protocol strictly followed. The reactions were performed in 96-well plates, which were read with a conventional plate reader at 405 nm (Vmax Microplate Reader, Molecular Devices, Sunnyvale, CA, USA). To normalize our cortisol data distribution, we transformed measurements of the circulating cortisol using the natural logarithm function.

#### *Testosterone assay*

Plasma testosterone concentration was measured using an ELISA (Testosterone Correlate-EIA kit; Assay Designs, Inc.). Plasma samples were thawed and diluted in a 1:30 solution with assay buffer and placed in normal 64-well plates. The ELISA kit protocol was then strictly followed with the exclusion of heating the plate to  $37^{\circ}\text{C}$  and placing it on a plate shaker during the second incubation instead of letting it sit at room temperature. In validation trials with *A. burtoni* plasma, this step was found to greatly reduce interwell variability. Plates were read at 405 nm (Vmax Microplate Reader; Molecular Devices). To normalize circulating testosterone distribution, we transformed the data using a square root function before statistical testing.

Though 11-ketotestosterone (11-KT) is typically more abundant than testosterone, we have demonstrated that in this species, testosterone levels in dominant versus submissive males mirror 11-KT levels (Parikh *et al.* 2006), and so here we use the testosterone level as a proxy for all circulating androgen levels.

A review of teleost androgens reports that in salmonids, 11-KT and testosterone both peak in the breeding season (Borg 1994).

#### *Gene expression measurement*

Levels of GnRH1 mRNA in the brain and GnRH-R1 mRNA in the pituitary were assayed using quantitative real-time RT-PCR (quantitative RT-PCR).

#### *Tissue preparation*

Tissue was homogenized in Trizol reagent (1 ml for brain and 850 µl for pituitaries; Invitrogen) using a Tissue Tearor (Biospec Products, OK, USA) for approximately 1 min, or until no visible tissue debris existed in solution. RNA was extracted from this homogenate using a volume of chloroform, 0.2 times the volume of Trizol used. Isopropyl alcohol was used to precipitate the RNA in the amount of 0.5 times the amount of Trizol used. The RNA was then washed with an amount of 75% ethanol equal to the original Trizol amount. The RNA pellet was re-suspended in RNase-free water (20 µl for pituitary and 50 µl for brain) and stored at  $-80^{\circ}\text{C}$  for not more than 5 days. The re-suspended RNA was treated with Turbo DNase kit (Ambion, Austin, TX, USA) to remove any DNA from the solution. The concentration of the RNA in this solution was determined using spectrophotometric readings at 260/280 nm (Beckman). To make cDNA, either 0.3, 0.5, or 1 µg RNA was used with iScript cDNA synthesis kit (BioRad).

#### *Gene expression levels: real-time PCR*

Primers for GnRH1 (GenBank accession CN469235) and GnRH-R1 (GenBank accession AY705931) were designed from full-length sequences (Chen & Fernald 2006, Grens *et al.* 2005) using Oligo 6.7 (MBI, Cascade, CO, USA) and were synthesized commercially (Invitrogen). GnRH1 primers were: upper 5'-CAG-ACA-CAC-TGG-GCA-ATA-TG-3' and lower 5'-GGC-CAC-ACT-CGC-AAG-A-3'. GnRH-R1 primers were: upper 5'-GCG-TGC-TCA-GTT-CCG-AGT-T-3' and lower 5'-CGC-ATC-ACC-ACC-ATA-CCA-CT-3'. As an internal control for differences in cDNA synthesis, tissue volume and loading during the PCR, each template was also tested for the presence of actin mRNA transcripts, as actin is a ubiquitously expressed housekeeping gene, which serves as an adequate control. Actin primer sequences were: upper 5'-GGC-CCA-GAG-CAA-GAG-AGG-TAT-C-3' and lower 5'-GAT-GCC-AGA-TCTCT-TCT-CCA-TGT-CAT-CC-3'.

First, primers were diluted with nuclease-free water to 10 mM concentration and the cDNA sample was diluted with nuclease-free water (Ambion) at a ratio of 1:3 for 0.3 µg, 1:5 for 0.5 µg, and 1:20 for 1 µg preparations. Quantitative RT-PCR was performed using 30 µl triplicate reactions with 1× IQ SYBR Green Supermix (Bio-Rad), 0.5 µM of each primer, and 0.5 ng/µl cDNA for each sample and each gene on the MyIQ Single-Color Real-Time PCR Detection

System (BioRad). The PCR was run with the following PCR parameters: 5 min at 95 °C, 45 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C, followed by melt curve analysis. We detected the fluorescence at 490 nm at the start of the annealing step (60 °C) in each cycle. Melt curves for PCR products showed two distinct peaks in each case, indicating purity of primers for both genes. In the case of GnRH-R1, only one peak appeared, which is to be expected, given the highly similar melting points of the GnRH-R1 and actin primers.

Raw fluorescent data (background subtracted data) provided by the MyIQ software (BioRad) were analyzed using the real-time PCR Miner program (Zhao & Fernald 2005), which uses the resultant PCR efficiency and fractional cycle number of the threshold (CT) for gene quantification. All data are expressed as a ratio of gene of interest expression to actin expression.

#### Statistical analysis

A two-tailed *t*-test was used to compare means for all measures, and statistical significance set as  $P < 0.05$ . In the case of plasma hormone concentration, standard transformations were applied to the raw data to make their distributions approximately normal. Levene's test for heterogeneity of variance was used to assess the data distribution before and after transformation. This was done to ensure that a *t*-test could be used to reliably assess the statistical significance of data. For transformed data, none of the Levene's tests was significant at level  $P = 0.05$ , so we have no reason to expect large deviations from a normal distribution. For plasma cortisol level, the natural logarithm function was used to transform the data, and for plasma testosterone level, the square root function was used to transform data.

## Results

#### Short-term social suppression (4 h)

As seen in Fig. 1, there were no significant differences between experimental and control subjects, although GnRH-R1 mRNA expression showed a trend towards increase in experimental animals ( $P = 0.12$ ) (two-tailed *t*-test). A regression analysis shows a trend suggesting that increased sample size might reveal up-regulation of GnRH-R1 expression in the experimental group ( $R^2 = 0.60$ ).

#### Longer-term social suppression (24 h)

After 24 h of sustained social stress, there is significant up-regulation of circulating cortisol ( $P = 0.01$ ) and GnRH1 mRNA expression ( $P = 0.02$ ) as compared with controls (Fig. 2A and B).

For GnRH-R1 mRNA expression, a regression analysis revealed a trend suggesting that increased sample size might

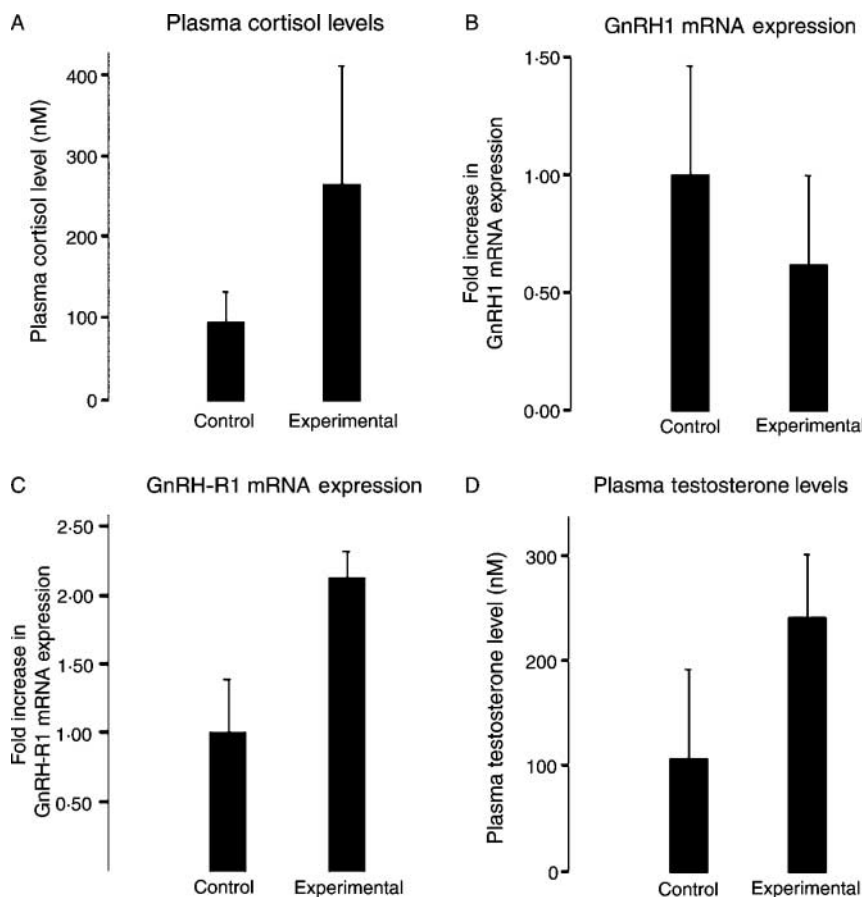
reveal a down-regulation of GnRH-R1 mRNA in the experimental group ( $P = 0.12$ ,  $R^2 = 0.37$ ; Fig. 2C). A two-tailed *t*-test showed that experimental subjects had significantly lower levels of circulating testosterone than did control subjects ( $P = 0.008$ ; Fig. 2D). There is also a positive correlation between circulating testosterone level and GnRH-R1 mRNA expression ( $R^2 = 0.76$ ; Fig. 3) as well as a positive correlation between plasma cortisol concentration and GnRH1 mRNA expression ( $R^2 = 0.70$ ; Fig. 4).

## Discussion

*A. burtoni* males have distinct behavioral and physiological phenotypes characterizing them as either T or NT males. Several different experiments in semi-natural conditions showed that these male phenotypes differ physiologically, particularly with respect to parameters of their reproductive systems. For example, T males in stable conditions have low cortisol (Fox *et al.* 1997) and have higher testosterone and 11-KT levels (Parikh *et al.* 2006) as compared with NT males. In the HPG axis, T males have higher levels of the key signaling peptide, GnRH1 and its mRNA (White *et al.* 2002), higher levels of GnRH-R1 mRNA (Au *et al.* 2006), and larger gonads (Davis & Fernald 1990) than NT males. Moreover, T male gonads contain mature sperm in contrast to NT males whose gonads contain largely immature sperm (Fraleigh & Fernald 1982). Experiments designed to follow the time-course of transition between these two phenotypes revealed that ascent and descent each had different temporal trajectories for several of these parameters (White *et al.* 2002). Specifically, animals appear to ascend much faster than they descend in social status as reflected in their physiology and behavior. Here, we examined the physiological and behavioral consequences of imposed social descent from T to NT over a significantly shorter time period and found a novel physiological consequence of forced social descent.

After a 4-h social stress, we found that none of the output measures was significantly changed in socially suppressed males. However, in animals that experienced a somewhat longer sustained social stress for 24 h, we found several differences. As expected, the circulating cortisol level increased significantly, while the circulating testosterone level dropped significantly, both of which are consistent with the steady-state differences measured between the T and NT phenotypes in longer-term experiments (Fox *et al.* 1997, Parikh *et al.* 2006). GnRH1 gene expression surprisingly increased significantly. Assuming that mRNA expression is a reliable marker of protein production, this change would ultimately produce a countervailing effect on the HPG axis compared with the regression in HPG physiology evident in decreased testosterone output. This could be interpreted as an attempt to counteract HPG regression, possibly via an alternate pathway.

Forcing a T male into the NT status has important consequences. When an animal is subjected to a change in its



**Figure 1** Effects of short-term (4 h) social suppression on plasma levels of (A) cortisol, (D) testosterone, and mRNA levels of (B) hypothalamic GnRH1, and (C) pituitary GnRH receptor-1 (GnRH-R1), none of which showed any significant difference between control and experimental animals. All mRNA data are expressed as fold increase with respect to control after normalization to actin mRNA expression. There is a trend toward an increase in GnRH-R1 expression (see Results). All data are represented as mean  $\pm$  s.e.m. (A) Experimental,  $n=4$ ; control,  $n=4$ ;  $P=0.47$  (two-tailed  $t$ -test); (B) experimental,  $n=4$ ; control,  $n=4$ ;  $P=0.55$  (two-tailed  $t$ -test); (C) experimental,  $n=2$ ; control,  $n=3$ ;  $P=0.12$  (two-tailed  $t$ -test); (D) experimental,  $n=3$ ; control,  $n=4$ ;  $P=0.18$  (two-tailed  $t$ -test).

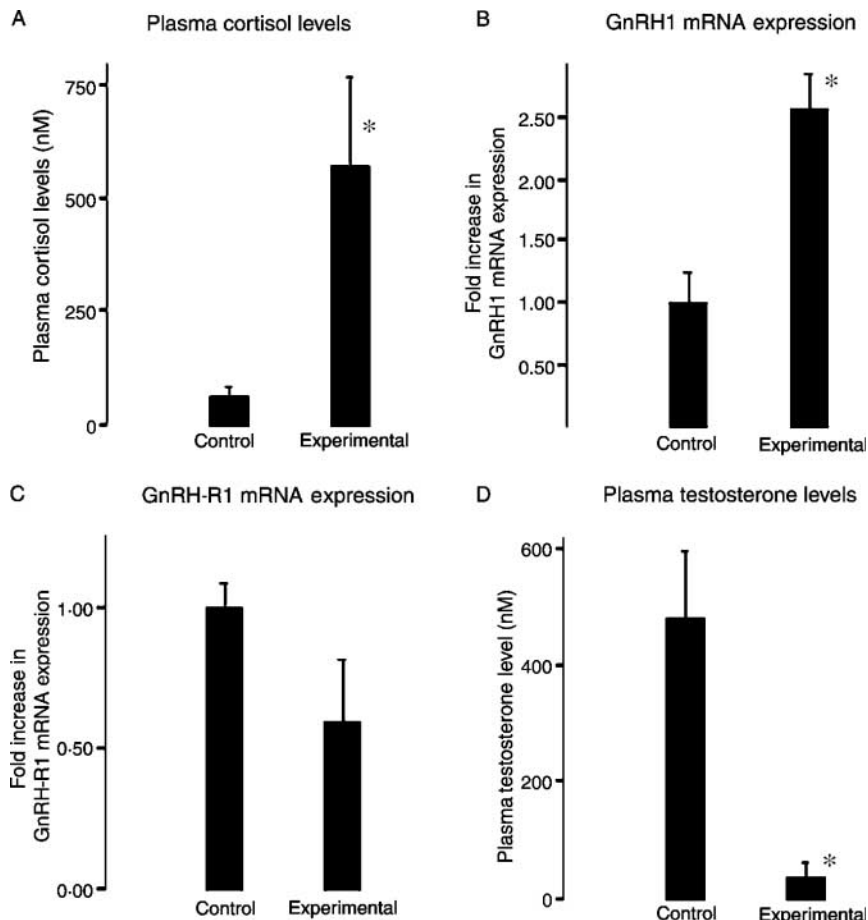
dynamic homeostatic equilibrium, whether social or physiological, it expends energy in an attempt to restore the original steady state, responding in one of three ways. The body can regain normal equilibrium once the stressor has ended, or it can enter an over- or under-aroused state if the stress is sustained. In the experimental paradigm used here, animals cannot regain normal equilibrium because the stressor persists and so these observations document the response of animals to an ongoing short-term stressor without respite.

One of the hallmarks of the response to stress is an attempt to maintain stability through change, termed allostasis (McEwen & Wingfield 2003). The concept of allostasis refers to attempts to maintain homeostatic balance through accommodations in response to ongoing challenges. However, when the energetic costs of this readjustment outweigh energy intake (e.g., 'type 1' allostatic overload; McEwen & Wingfield 2003), the animal must adjust by removing itself

from its present life-cycle stage and retreat into a 'survival state.' Status loss in *A. burtoni*, moves the animal from a reproductive life-cycle phase to a non-reproductive state.

Glucocorticoids have been implicated as physiological and behavioral indicators of this change. For example, glucocorticoid response has been shown to be modulated by perceived control over a situation (Weiss 1971, Weiss *et al.* 1981). In particular, attention has been focused on cortisol for mediating behavioral effects, because this steroid hormone is released in response to numerous stressors and can cross the blood-brain barrier to influence behavior, whereas epinephrine cannot (Wendelaar Bonga 1997). Moreover, there are cortisol receptors in the brains of many species, including *A. burtoni* (Greenwood *et al.* 2003), implicating cortisol in behavior modulation in this system.

The unexpected finding here is the increase in GnRH1 mRNA levels together with the rise in circulating cortisol after

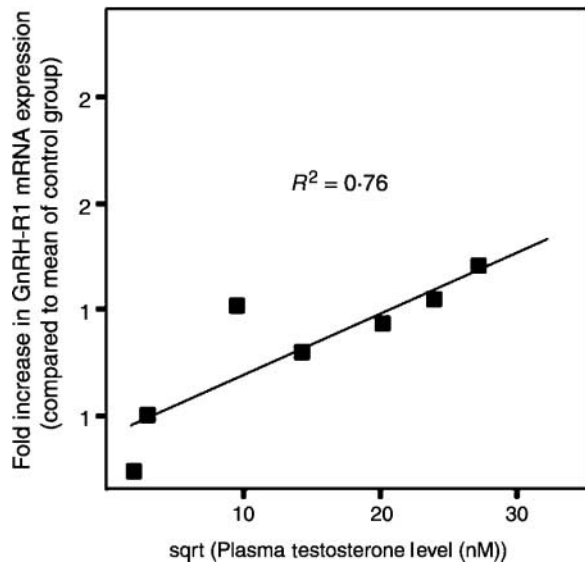


**Figure 2** Effects of longer-term (24 h) social suppression on (A) plasma levels of cortisol, (D) testosterone, and mRNA levels of (B) hypothalamic GnRH1, and (C) pituitary GnRH receptor-1 (GnRH-R1). Mean plasma cortisol level and GnRH1 mRNA level were significantly increased, mean plasma testosterone level decreased and there was no significant change in GnRH-R1 mRNA level. All mRNA data are expressed as fold increase with respect to control after normalization to actin mRNA expression. All data are represented as mean  $\pm$  s.e.m. (A) Experimental,  $n=2$ ; control,  $n=4$ ;  $P=0.01$  (two-tailed  $t$ -test); (B) experimental,  $n=2$ ; control,  $n=4$ ;  $P=0.02$  (two-tailed  $t$ -test); (C) experimental,  $n=3$ ; control,  $n=4$ ;  $P=0.12$  (two-tailed  $t$ -test); (D) experimental,  $n=3$ ; control,  $n=4$ ;  $P=0.008$  (two-tailed  $t$ -test). \* indicates statistical significance (see text).

24 h of social stress (Fig. 2A). Further, 24 h after the onset of social stress, plasma cortisol is positively correlated to GnRH1 mRNA expression (Fig. 4). Cortisol has been shown in cell culture systems to down-regulate gene expression of both GnRH (Chandran *et al.* 1994) and GnRH receptor mRNAs (Maya-Nunez & Conn 2003) in mammals. Moreover, in *A. burtoni*, in the steady state, the up-regulation of cortisol is correlated with down-regulation of GnRH1 (Fox *et al.* 1997) and GnRH1 mRNA (White *et al.* 2002). Thus, in the early stages of status descent, the up-regulation of GnRH1 mRNA suggests that the animals may be mounting a defense against social descent at the level of production of GnRH1 mRNA, the essential signal to the pituitary. Interestingly, testosterone output is down-regulated, reflecting a negation of the up-regulation

of GnRH1. Since this GnRH1 effect is at the level of transcriptional regulation, we do not know whether there is a corresponding effect at the level of the synthesized protein.

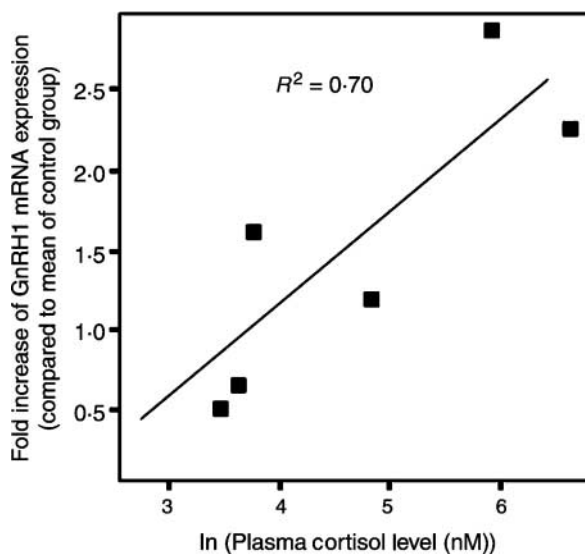
In analyzing these data, there are two important considerations. First, there is growing evidence that fish can and do distinguish amongst social stressors. For example, animals observing other animals engaged in conflict show an increase in cortisol and testosterone (Oliveira *et al.* 1998, 2001), yet fish engaged in fighting with their own image in a mirror do not show a testosterone response (Oliveira *et al.* 2005). Further, in *A. burtoni*, social status correlates with different behavioral responses in an aggressor than in a subordinate. Social status and cortisol response may interact to produce direct versus displaced aggression in response to that



**Figure 3** Correlation between plasma testosterone and GnRH-R1 mRNA levels in 24-h social suppression.

aggressor (Clement *et al.* 2005). This suggests that fish may have distinct behavioral coping strategies depending both on their own status and that of the aggressor, and that these may be reflected in physiology.

Secondly, dominant *A. burtoni* males mount a physiological defense against loss of status by increasing the mRNA levels for the key signaling peptide, GnRH1, while displaying high cortisol levels. This immediately suggests that the typical negative feedback effect of high cortisol on GnRH1 is countered and even reversed in T males during the initial hours following social descent. This reversal may reflect an



**Figure 4** Correlation between GnRH1 mRNA level and plasma cortisol in 24-h social suppression.

attempt to preserve the HPG axis, in case social opportunity arises again. It has been postulated that such resistance to the negative effects of social stress as measured in the HPG axis might make sense in some cases (Wingfield & Sapolsky 2003). Animals with limited time to reproduce, such as *A. burtoni*, for example, would mount a defense against social descent to extend reproductive opportunities. Other experiments have shown that changes in social hierarchy occur regularly in *A. burtoni* (Hofmann *et al.* 1999), so it is possible that animals resist the down-regulation of GnRH1 mRNA in anticipation of a possible quick return to social dominance. In some species, there are distinct coping strategies such as displaced aggression that can reduce the stress response (e.g. Houston 1972, Davis & Levine 1982, Clement *et al.* 2005) although the mechanisms of these are unknown.

During the social descent produced in this experiment, many physiological systems are pitted against one another in the service of homeostasis. As the animal spends energy to bring its behavioral and physiological systems under control, it may be attempting to preserve the *status quo*, in case there is a chance for return to high status. Given this perspective, assuming that gene translation mirrors gene transcription, it is surprising that testosterone output was decreased, as this reflects a neutralization of the GnRH1 expression up-regulation. We do not know whether the physiological defense we observe is maintained for more than 24 h. Further, there are several possible mechanisms through which the effect of cortisol on GnRH1 gene expression might operate; however, understanding how this occurs at the cellular level will require further experiments. It seems likely that this glimpse of a physiological defense may be part of a complex response to social descent mounted by the animal.

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