Color change as a potential behavioral strategy

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ABSTRACT

Within species, color morphs may enhance camouflage, improve communication and/or confer reproductive advantage. However, in the male cichlid *Astatotilapia burtoni*, body color may also signal a behavioral strategy. *A. burtoni* live in a lek-like social system in Lake Tanganyika, Africa where bright blue or yellow territorial (T) males (together ~10–30% of the population) are reproductively capable and defend territories containing food with a spawning site. In contrast, non-territorial (NT) males are smaller, cryptically colored, shoal with females and have regressed gonads. Importantly, males switch between these social states depending on their success in aggressive encounters. Yellow and blue morphs were thought to be adaptations to particular habitats, but they co-exist both in nature and in the laboratory. Importantly, individual males can switch colors so we asked whether color influences behavioral and hormonal profiles. When pairing territorial males with opposite colored fish, yellow males became dominant over blue males significantly more frequently. Moreover, yellow T males had significantly higher levels of 11-ketotestosterone than blue T males while only blue NT males had higher levels of the stress hormone cortisol compared to the other groups. Thus color differences alone predict dominance status and hormone profiles in T males. Since T males can and do change color, this suggests that *A. burtoni* may use color as a flexible behavioral strategy.

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Introduction

In animal populations, multiple mechanisms contribute to the maintenance of color polymorphisms, most of which are not labile (Gray et al., in press; Gray and McKinnon, 2007). In some vertebrate species, morphs vary geographically (Bond, 2007; Resnick and Jameson, 1963) and in others, the trade-off between conspicuousness and crypsis has led to specific color adaptations (Endler, 2006) or annual color changes. Color morphs may have significance for particular agonistic and/or mating preferences (Knapton and Falls, 1983; Roulin, 2004; Sinervo and Zamudio, 2001; Watt et al., 1984). This is also the case for a number of fish species (Baer et al., 1995; Barlow, 1973; Kingston et al., 2003) such as the pygmy swordtail fish (*Xiphophorus pygmaeus*) where 13–25% of males are gold and the rest are blue (Baer et al., 1995). Blue morphs are preferred by females while gold males dominate agonistic interactions (Kingston et al., 2003). In contrast, in Midas cichlids (*Theraps citrinellum*), gold males (<10% of the population) are more aggressive giving them an advantage in agonistic interactions over gray morphs. However, no associated female preference for either gold or grey morphs has been found (Barlow, 1973, 1983). In one case, a Tanganyikan cichlid which chases its prey, *Lepidiolamprologus perofundicola*, individuals are either brown-black and hunt in shady areas or are creamy white and hunt in open water (Kohda and Hori, 1993). Regulation of color morphs by such external factors led us to examine the cause(s) of color morphs in *Astatotilapia burtoni*.

East African cichlids offer examples of color morphs that can influence natural selection. For example, female choice based on male coloration has been shown experimentally to be essential for reproductive isolation in lake Victoria where decreased visibility significantly alters mate choice (Seehausen et al., 1997). The rapid radiation of cichlid species is thought to be a consequence of sexual selection acting on male coloration. In addition, there have been rapid trophic specializations and corresponding changes in jaw morphology (Galis and Metz, 1998) facilitating occupation of new ecological niches. Male–male competition has also been hypothesized to play a role in the evolution of these cichlid species. Specifically agonistic male–male interactions biased by nuptial coloration appear to have contributed to speciation and subsequent stabilization of species (Dijkstra et al., 2006; Seehausen and Schluter, 2004) as exemplified by male–male competition which facilitated invasion of new color morphs of the genus *Pundamilia* into existing populations in Lake Victoria (Dijkstra et al., 2005). Previous studies on agonistic preferences in color polymorphic cichlids has revealed that males prefer to interact aggressively with males of the same color (Seehausen and Schluter, 2004) however, this preference depends on the frequency of each morph in the population (Dijkstra et al., 2007b). Early work on animals in the haplochromine genus suggested

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it was selectively neutral with respect to mate choice among different color morphs (Seehausen and Van Alphen, 1998), but more recently, Maan et al. (2006a,b) showed that for some haplochromines, female choice was associated with parasite load that correlates with color intensity and territory size.

A. burtoni, the cichlid species studied here, is endemic to Lake Tanganyika where they live in lek-like social systems in which territorial (T) males defend a site where they feed and spawn. Non-territorial (NT) males mimic female coloration and behavior to gain access to food. These two phenotypes differ in behavior, body color, reproductive capacity, and in levels of hormones related to reproduction and stress (Clement et al., 2005; Fernald, 1977; Fernald and Hirata, 1977; Fox et al., 1997; Parikh et al., 2006a; White et al., 2002). T males interact aggressively to defend territories using a suite of behaviors ranging from threatening postures to direct aggressive contact, i.e., biting neighboring T males. T males also attack and chase NT males and females which always flee. T males have higher circulating levels of both testosterone and a fish specific androgen,11-ketotestosterone, than do NT males (Parikh et al., 2006b), both of which are correlated with aggression and reproductive competence in fish (Bender et al., 2006; Oliveira et al., 2002). Conversely, levels of the stress hormone, cortisol, are significantly higher in NT males than T males, suggesting that cortisol could inhibit reproductive capacity of NT males (Fox et al., 1997). Importantly, males can rapidly switch between T and NT phenotypes depending on social opportunity and/or environmental conditions (Fernald, 1977; Fernald and Hirata, 1977).

Territorial males express bright blue or yellow body colors while NT males are pale and females exhibit a pale brown/gray cryptic pattern. Prior research in the field and with models in aquaria has demonstrated the importance of bright coloration for winning territories and eliciting aggression from conspecifics (Fernald, 1977; Fernald and Hirata, 1977; Fernald and Liebman, 1980). Because blue and yellow morphs were originally observed in murky shore pools and the open lake respectively, body color was hypothesized to be an adaptation to particular light regimes (Fernald and Liebman, 1980). Furthermore, in contrast to the early field studies where all observed territorial males in shore pools were blue (Fernald and Hirata, 1977), later research showed that juveniles raised isolation will exhibit aggression when viewing males of either color (Fernald and Liebman, 1980). Yellow and blue territorial *A. burtoni* male morphs are approximately equally represented in aquarium populations with identical feeding and lighting, suggesting that any adaptive value of the colors had been lost when the fish were removed from the wild (Robison, 2000). We recently showed, however, that the two morphs display distinctly different behavioral phenotypes, indicating that body color is an important social signal in this species. Yellow T males initiate more aggressive attacks towards blue T males than against their yellow T neighbors, whereas blue T males attack and chase NT and females more than neighboring yellow T males (Korzan and Fernald, 2007). When given a choice of opponents in a novel environment, both yellow and blue T males preferred to interact agonistically with neighbors of the opposite color. Moreover when these males were allowed to compete physically for the same territory, yellow T males became dominant in the majority of trials (Korzan and Fernald, 2007).

A. burtoni males can, however, change body coloration from yellow to blue and vice-versa (Korzan and Fernald, 2007; Robison, 2000; Fernald pers. obs. 1977), which may signal a change of internal state and/or behavioral strategy. The greater social success of yellow males in aggressive encounters raises the question: Why don't all males turn yellow? Are there also advantages to being blue not revealed by earlier experiments? Do hormonal profiles distinguish these morphs? Based on the behavioral profiles of blue and yellow morphs, we predicted that both male social status and color would correspond to specific hormone level profiles. From previous work, we knew that NT males

of both colors would have lower levels of both androgen types and higher levels of the stress hormone, cortisol, than T males so we hypothesized that different T color morphs would have different levels of testosterone, 11-ketotestosterone and cortisol. We tested this hypothesis, by placing size-matched T males in a novel environment and allowing formation of stable social relationships. We showed that opponent color predicted both hormone and growth profiles suggesting that body color is an important signal in this species. Since body color can change, it is possible these fish manipulate color as a signal.

Methods

Animals

A. burtoni bred from an original population of >900 wild caught individuals for ca. 50 generations were housed in aquaria under conditions mimicking their natural habitat: 29 °C; pH 8; 12:12 light:dark cycle with full spectrum illumination (Fernald, 1977). A layer of gravel (~3 cm) covered the bottom of the aquaria and terracotta pots in each tank facilitated the establishment and maintenance of territories by males. We chose male *A. burtoni* that had maintained discrete blue or yellow color for 1 month. Animals were fed ad libitum every morning with cichlid pellets and flakes (AquaDine, Healdsburg, CA). All animals were treated in accordance with the Stanford University Institutional Animal Care and Use Committee (IACUC) guidelines.

Pairing of males

Reproductively active adult *A. burtoni*, were housed in groups of 7–9 females and 7–9 males in each of 16 aquaria (85 l×53.7 w×30 d cm). Animals were identified individually using randomized combinations of colored beads (excluding yellow and blue beads) attached just beneath the dorsal fin. Males selected for this experiment were from aquaria that housed 2–4 yellow and 2–4 blue territorial males. Each T male was observed for 10 min at 9 am, 3 and 9 pm over four consecutive days to verify social status and record body color. Two pairs of size-matched T males ($n=56$) from different aquaria were introduced to a new experimental aquarium with three females, identical to their former aquarium except that a perforated divider separated the tank into two equal sections. A pair of T males and 3 females were housed on each side of the divider (two pairs of males in each aquarium, 28 pairs) and kept for 30 days (Fox et al., 1997). Within minutes of being transferred to the aquaria, each pair of T males began agonistic behaviors that resulted in one male maintaining his prior T status and the other becoming NT. In each pairing males were only used once.

Aggressive behaviors by T males directed towards females and NT males of the pair as well as towards T, NT males and females across the clear divider were identified and recorded using the following categories (Fernald, 1977):1) Bite = bite an opponent; 2) Side threat (lateral display) = sideward presentation of spread opercula, fins and distended chin to an opponent; 3) Border fight = confrontation between two T males at their common border; 4) Approach = swim quickly toward then stop near another fish 5) Displaced aggression = biting, approaching and chasing of females and non-territorial males. Slight modifications of the category of aggressive behaviors were used to score behavior of T males separated by the divider because the animals could not physically contact one another. Specifically, a bite was scored when both fish were face to face against the divider, opened their mouths and nipped at the divider. An approach was scored when an animal swam rapidly towards an opponent but stopped short of the clear divider. Other behaviors recorded included spawning and shelter entries. Observation protocol was replicated for 28 experiments (5 blue vs. blue, 7 yellow vs. yellow and 15 yellow vs. blue fish) with one pair (blue vs. blue) lost before collection.

As noted above, social dominance was established between each pair of animals by the end of the first day and remained consistent for the duration of the experiment for all pairs. Behavior of the paired males and the neighboring territorial opponent was recorded with a digital video camera (MiniDV, JVC) for 30 min 6 h after lights on each day for the three consecutive final days of the experiment (days 1–4). The first and second day of recording, status and color were recorded. On the third day, behavior was analyzed as described above. For each behavioral recording session the first 10 min after onset of behavioral interaction was scored. Aggressive behavior for each male was ranked using a dominance index which is the sum of aggressive behavioral acts (e.g. bites and chases) minus subordinate behavioral acts (e.g. flee) performed per 10 min recording (White et al., 2002).

On the fourth day, immediately after the final observation, both pairs of T and NT males in a tank were killed and gonads, brains and plasma were collected. Plasma was collected using a hypodermic needle (25 ga) coated with heparin and inserted on the midline ~5 mm behind the anal fin into the caudal vein (Fox et al., 1997). Approximately 200 μl of blood was collected and centrifuged for 3 min ($12×10^3$ rpm) to separate plasma from blood cells. The plasma was then transferred into a clean collection tube (1.5 ml). Plasma and brains were immediately frozen on dry ice and stored at −80 °C. Body standard length and weight and gonad weight were recorded and used to calculate a gonado-somatic index (GSI: [Ratio of body weight to gonad weight]×100) computed to assess the male's reproductive capacity (White et al., 2002).

Steroid hormone measurements

Testosterone, 11-ketotestosterone and cortisol levels in *A. burtoni* plasma were measured using commercially available reagents (testosterone and cortisol; Assay Design, Ann Arbor, MI; 11-ketotestosterone Cayman Chemical Ann Arbor, MI) following protocols established by Parikh et al. (2006a,b). Plasma cortisol concentrations were measured in triplicate using a standard competitive immunoassay from blood plasma. Plasma (10 μL) was added to 290 μL of diluent, of which 100 μL was put into each of three wells in the assay plate together and a range of known concentrations was placed in other wells. 50 μL of cortisol EIA conjugate was added to each well, followed by 50 μL of cortisol antibody. The plate was then incubated at 25 °C for 2 h on a plate shaker, wells emptied and washed three times with wash buffer and emptied, and 200 μL of pNpp substrate was added to each well. The samples and standards were then incubated at 25 °C for 1 h, and 50 μL of 'stop' solution added. Cortisol levels were measured by reading the absorbance of samples at 405 nm (Molecular Devices Microplate Reader) and calculating the concentration from the standard curve. Similar protocols were used for 11-ketotestosterone and testosterone quantification with the exception of an additional extraction step utilizing diethyl ether and ethyl acetate/hexane respectively to remove the hormone from the whole plasma.

Data analysis

The percentage of victorious yellow or blue males that became dominant in yellow and blue territorial male pairs was compared using two-tailed paired *t*-tests (Figure not shown). Dominance index, GSI, change in body weight and standard length as well as testosterone, 11-ketotestosterone and cortisol levels were compared between yellow and blue T and NT males using one-way analysis of variance followed by *post hoc* examination using Duncan's multiple range test. A nonparametric rank test (Mann–Whitney test) was used to compare testosterone and 11-ketotestosterone of yellow NT males paired with blue or yellow T males. The level of significance was $P < 0.05$ (SPSS 13.0, SPSS Inc., Chicago, Ill.).

Results

Color, agonistic behavior, growth and reproductive capacity

In all pairs, males formed stable dominant-subordinate relationships on the first day of the experiment and newly established T males frequently interacted agonistically with neighboring territorial males through the clear barrier. Territorial males exhibited similar agonistic and dominance behavior towards NT males and females independent of their opponent's color as shown by their similar dominance indices (Fig. 1) so experimental results are presented according to status and color of each individual for statistical comparisons.

Both yellow and blue T males' dominance indices were significantly higher than those of NT males (Fig. 1 [$F(7, 27) = 11.11$, $P < 0.0001$]).
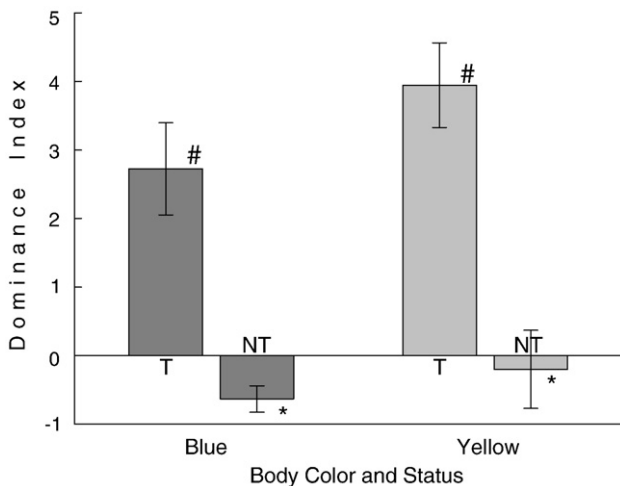

Fig. 1. Territorial males exhibit more aggressive behavior than do non-territorial males as shown by dominance index (see Methods). Color and status are shown on the ordinate axis and the mean (±SEM) score of dominance index for T and NT males plotted as a function of body color. Mean values that share or do not have a superscript symbol are not significantly different, and those with no common superscript symbols are significantly different ($p < 0.05$, see Methods).
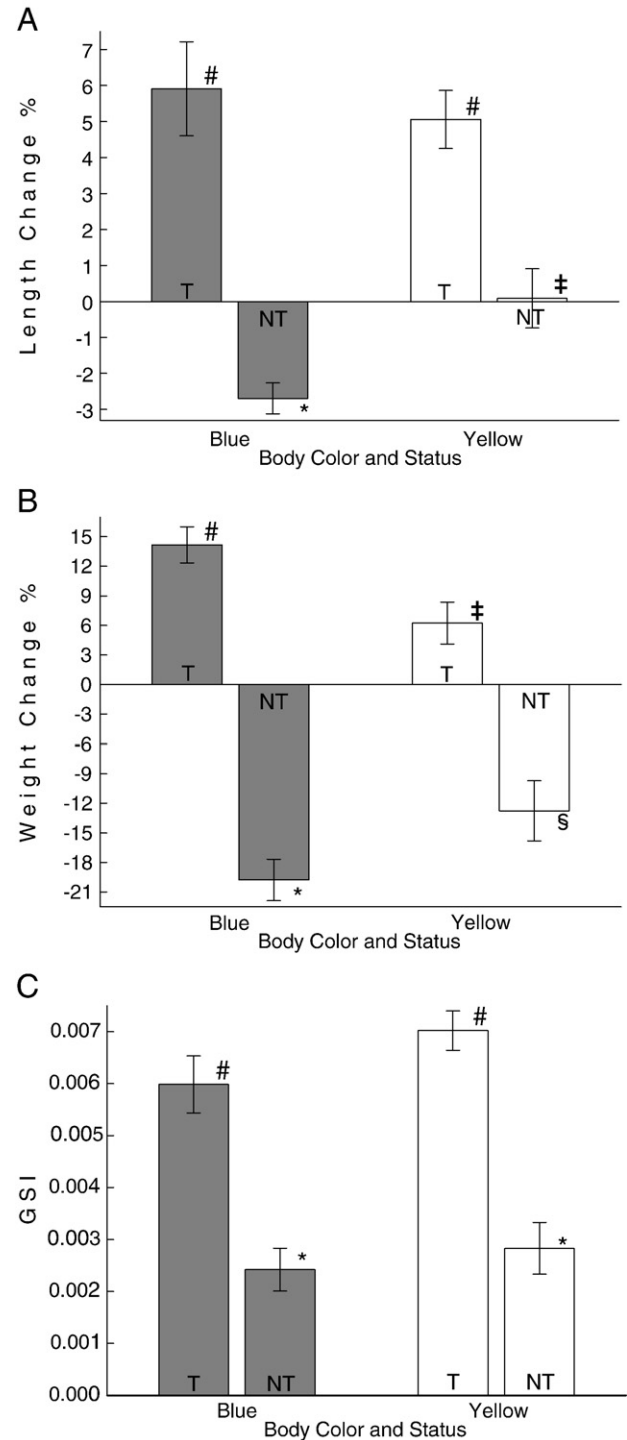

Fig. 2. Growth and reproductive capacity reflect male status and color. The mean (±SEM) percent change of length (A), weight (B) and gonodosomatic index (GSI; C) plotted as a function of body color and status. Both yellow and blue territorial males grow in body length (A) and weight (B). However, blue T males gain significantly more weight (B) than yellow T males. NT males lose weight and length and blue NT males lose more weight (B) and length (A) than do yellow NT males. Gonado-somatic index (GSI) (C) is larger in T as compared with NT males as expected. Means that share or do not have a superscript symbols are not significantly different, and those with no common superscript symbols are significantly different ($p < 0.05$).

Moreover, in mixed pairs, yellow T males became dominant significantly more often (13 of 16 pairings = 77%) ($t_p = 3.10$, $P < 0.007$) (data not shown).

Both yellow and blue T males grew in length (Fig. 2A) and weight (Fig. 2B) significantly more than did NT males during the experiment
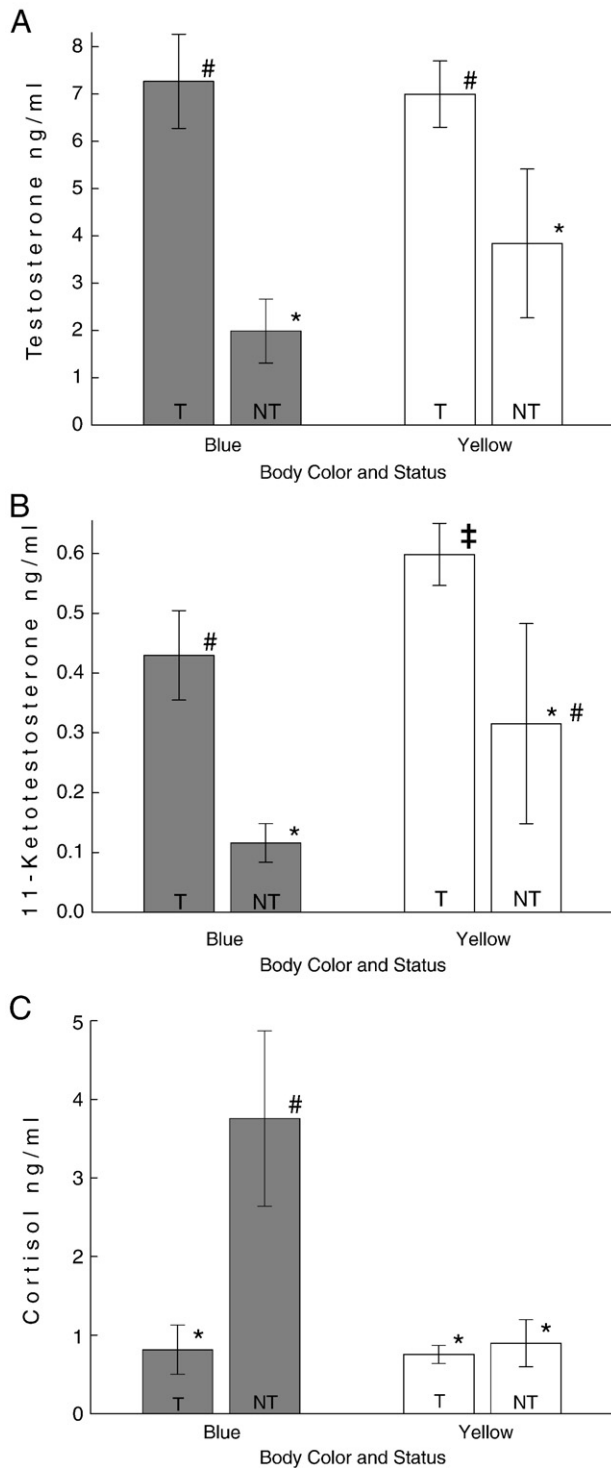
Fig. 3. Circulating levels (mean±SEM) of testosterone (A), 11-ketotestosterone (B) and cortisol (C) plotted as a function of male coloration and status. Testosterone (A) and 11-ketotestosterone (B) levels were higher for T males than NT males of the same color. However, yellow NT 11-ketotestosterone (B) levels were not different from blue T or NT males. Surprisingly blue NT males were the only group with increased cortisol (C) levels. Means that share or do not have a superscript symbols are not significantly different, and those with no common superscript symbols are significantly different ($p < 0.05$).

$[F(3, 36) = 22.98, P < 0.0001]$ but there was no significant difference in length between yellow and blue T males (Fig. 2A). Unexpectedly, the interpretation of *post hoc* analyses revealed that overall length decreased significantly in blue NT males but not in yellow NT males whose length remained unchanged (Fig. 2A [$F(3, 36) = 22.98$, $P < 0.0001$]). Body weight increased significantly for all T males

(Fig. 2B [$F(3, 33) = 40.10$, $P < 0.0001$]) but *post hoc* analyses revealed body weight increased significantly more in blue than in yellow T males. Both yellow and blue NT male body weights decreased significantly (Fig. 2B [$F(3, 33) = 40.10$, $P < 0.0001$]) and *post hoc* analyses revealed that blue NT males lost significantly more weight. Unsurprisingly, gonado-somatic index (GSI) was significantly higher for both yellow and blue T males when compared to NT males, as previously reported (Fig. 2C [$F(3, 45) = 20.58$, $P < 0.0001$]).

Steroid hormone levels

The levels of circulating hormones in blue and yellow males were compared as a function of social status and body color. As expected, circulating testosterone levels were significantly higher for both yellow and blue T males as compared to yellow or blue NT males (Fig. 3A [$F(3, 33) = 10.54$, $P < 0.0001$]). Consistent with our hypothesis, circulating 11-ketotestosterone levels were significantly higher in yellow T males compared to all other groups (Fig. 3B [$F(3, 28) = 16.83$, $P < 0.0001$]). *Post hoc* analysis revealed that, although 11-ketotestosterone levels were significantly higher in blue T males than blue NT's (Fig. 3B [$F(3, 28) = 16.83$, $P < 0.0001$]), they were not different from those of yellow NT's (Fig. 3B). Unexpectedly, circulating cortisol levels were significantly elevated only in blue NT males compared to all other groups (Fig. 3C [$F(3, 34) = 5.30$, $P < 0.004$]).

The testosterone and 11-ketotestosterone levels of yellow NT males were variable and, remarkably, this variance depended on the color of their T opponent (Fig. 4). Yellow NT males paired with a blue T male had significantly higher (T-like) testosterone (Fig. 4A [$u = 1.73$,
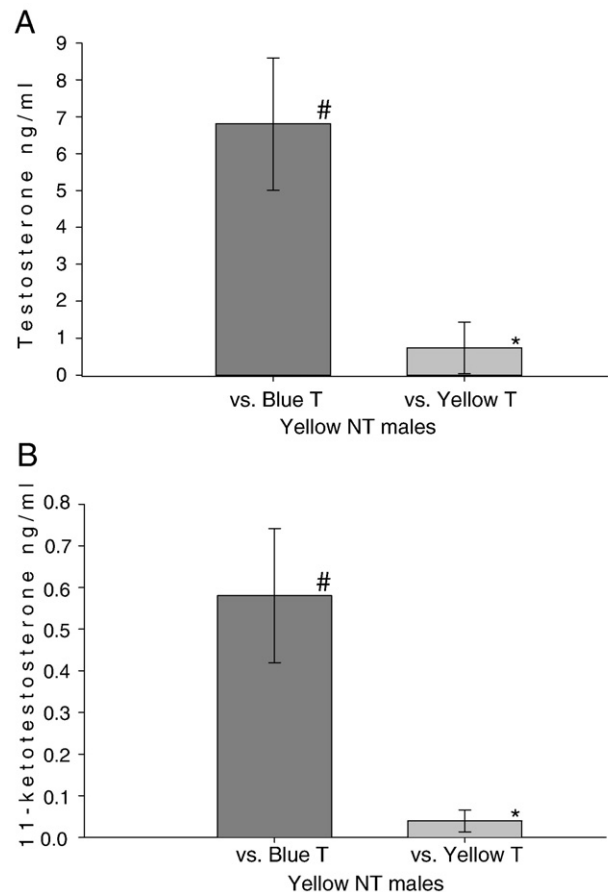

Fig. 4. Androgen levels (testosterone and 11-ketotestosterone, mean±SEM) of yellow NT males depend significantly on the color of the T opponent. Yellow NT males paired with blue T opponents had high levels (~T-like) of both testosterone (A) and 11-ketotestosterone (B) while yellow NT males paired with a yellow T opponent had low (~NT-like) levels of both androgens.

$P<0.0001$]) and 11-ketotestosterone (Fig. 4B [$u=1.73$, $P<0.0001$]) levels while yellow NT males paired with a yellow T male had low (NT-like) levels of both androgens (Fig. 4).

Discussion

Since *A. burtoni* behavior is influenced by body color (Korzan and Fernald, 2007), we asked whether there were corresponding physiological differences. We found that growth rate, reproductive capacity, and steroid hormone levels all vary with status as a function of body color, suggesting that blue and yellow dominant male phenotypes use distinctly different coping strategies.

Hormones vary with body color of male A. burtoni

Aggressive behaviors are typically associated with increased testosterone levels in vertebrates (Lincoln et al., 1972) and increased levels of both testosterone and 11-ketotestosterone in fish (Pottinger and Pickering, 1985). We found that hormone levels reflect color patterns in T males and correlate with the agonistic differences between yellow and blue males. Similar to previous studies of T and NT males (Parikh et al., 2006b), both yellow and blue T males had elevated levels of circulating testosterone (Fig. 3A). This is consistent with the effect of injecting male *A. burtoni* with testosterone, which intensifies body coloration and aggressive behavior (Fernald, 1976) Injections of exogenous testosterone also intensified ventral and facial coloration of males in the color polymorphic lizard *Sceloporus undulates erythrocheilus* (Rand, 1992). Also in the lizard *Uta stansburiana* testosterone implants change some less aggressive yellow males to physiological and color characteristics of the more aggressive blue males (Sinervo et al., 2000) Similarly, we found 11-ketotestosterone levels were significantly higher for yellow T males compared to blue T males (Fig. 3B). This higher level of 11-ketotestosterone may be associated with increased aggression displayed by yellow T males in semi-natural environments. Remarkably, yellow NT males have significantly different levels of circulating testosterone and 11-ketotestosterone depending on the color of their T opponent (Fig. 4). This dependence of androgen levels upon opponent's coloration may suggest a priming affect, if only passively, for future agonistic social interactions.

An unexpected finding was that circulating cortisol levels in yellow NT males were not different from yellow or blue T males. Previously Fox et al. (1997) demonstrated a significant difference in circulating cortisol between paired T and NT males after 2 months in of interaction. Although animal color was not reported, it is possible that the majority of the NT males in that experiment were blue.

Changes in growth rate

Previous studies conducted under more naturalistic conditions showed that male *A. burtoni* changing status from NT to T grew more than either T males or males descending in status (T to NT). Moreover, NT males in both stable and fluctuating environments grew more than descending or T males (Hofmann et al., 1999). Interestingly, in the present study when males were maintained as stable pairs, T males grew in length and weight while NT males shrank or remained the same. Moreover, blue T males grew more and blue NT males shrank more than their yellow counterparts These data show that social setting strongly influences both growth and reproduction.

Growth regulation has been seen in clownfish (*Amphiprion percula*) which live in hierarchical social groups. Subordinate individuals that ascended in rank grew more than subordinates that did not change rank (Buston, 2003). Also, in pairs of Nile tilapia (*Oreochromis niloticus*), growth rate is suppressed in subordinate males compared to dominant males (Vera Cruz and Brown, 2007). The differences in *O. niloticus* growth rate that are dependent on status were attributed to behavioral

changes (i.e. reduced feeding) regulating physiological factors such as lowering hepatic IGF-I mRNA expression in subordinate males (Vera Cruz and Brown, 2007). Clearly growth rate may be fine tuned by social factors in fish species via complex social interactions.

Growth regulation has also been observed in other species, but largely in relationship to food availability as found in the marine iguanas (*Amblyrhynchus cristatus*). Individual iguanas that shrink in length more when food is scarce survive longer than larger iguanas suggesting that smaller iguanas have increased efficiency in foraging due to decreased energy use (Wikelski and Thom, 2000). This effect appears unrelated to social conditions.

Color polymorphism: New dimensions?

Body color in *A. burtoni* appears to have multiple functional consequences for an individual but we do not know how color relates to behavior in naturally occurring habitats. For example, could color affect the foraging or mating success of *A. burtoni* T males? If so, what mechanisms might allow an animal to *know* what color it is, to change color or even how to modulate its aggressive behavior based on its opponent's color?

African cichlid species exist with a wide variety of color patterns. In many species of cichlid, individuals use color as a signal between conspecifics (Hurd, 1997; Korzan and Fernald, 2007; Seehausen and Schluter, 2004). Some of the signals cause immediate effects such as the rapid change in the black bar through the eye in *A. burtoni* which signals a change in aggressive intent (Muske and Fernald, 1987). In other cases, color can change in all or part of the body due to stress (Barlow, 1983; Hulscher-Emeis, 1992) or signal efficiency (Enquist et al., 1990; Hurd, 1997; Leimar and Enquist, 1988) or hormones (Sage, 1970; Fernald, 1976) or pigments (Webber et al., 1973). Color polymorphism is present in some of these species (e.g. *Pelvicachromis pulcher* and *N. omnicaeruleus*) (Barlow, 1973; Barlow, 1983; Maan et al., 2006a,b; Martin and Taborsky, 1997). In cichlid species with color polymorphism, some change their entire body color as reported here. For example, Maan et al. (2006a,b) found that in *N. omnicaeruleus* yellow and blue males occur in the population and many of the yellow males change to blue when they become large enough to defend a territory in the wild. This is not true for *A. burtoni* males, who are cryptically colored and appear similar to females until they reach reproductive maturity when their bodies become either vivid blue or yellow (Fraley and Fernald, 1982).

As noted above, prior work on agonistic preferences in color polymorphic cichlids from Lake Victoria showed that males prefer to interact aggressively with males of the same color (Seehausen and Schluter, 2004) depending on the frequency of each morph in the population (Dijkstra et al., 2007b). Clearly *A. burtoni* prefer to interact with opposite colored males so what might explain this difference? *A. burtoni* with two basic color patterns and two male morphs has the potential for phenotypic change to accompany its phenotype plasticity consistent with cichlid exploitation of a variety of niches in the African lakes. This species must have a neuronal/hormonal regulatory system that regulates body color though nothing is known about how this is achieved.

A. burtoni can change colors from yellow to blue or vice-versa (Robison, 2000) but it is not clear when or why this happens. To document this phenomenon, 64 males were selected at random from a naturally derived population and distributed, along with several females, among six aquaria. 38 (59%) changed color at least once during a 6 month period, 11% were consistently blue, and 30% were always yellow (Robison, 2000). In all fish and all observations, the sample had 50% yellow NT, 12% blue NT, 19% yellow T and 19% blue T males. Territorial and NT males were both seen to change color without a change in status, and to change status without a change in color. In a tank of 12 males observed over 5 months (Robison 2000), nine males changed from yellow to blue and five changed in the

opposite direction. In addition, when males in this sample changed both color and status at the same time (a relatively rare occurrence), the change was typically from a yellow NT to a blue T or vice versa (Robison, 2000). Since blue and yellow fish, and T males in particular, can co-exist in aquaria, we can tentatively rule out diet, depth, lighting, the presence of female and/or male conspecifics, or the presence of other species as proximal causes, though it remains possible that factors such as these are related to the original evolutionary development of color change in this species. Color differences were associated with natural habitat: more blue males were found in murky shore pools and more yellow males were found

in clearer open water (Fernald and Hirata, 1977; Fernald and Liebman, 1980). However, it is not known whether these color patterns were due to predation or other factors associated with specific habitats.

Both behavior and color can serve as external social signals of reproductive and social strategy by males. In *A. burtoni*, our data show that color predicts both behavior and some specific hormone profiles. While the relationship between sex hormones, such as testosterone, and behavior is well known (Lincoln et al., 1972), it is less well known how color relates to hormone profiles.

In Harris's sparrows *Zonotrichia querula* males with darker feathers on their head and breast dominate mating contests and have higher
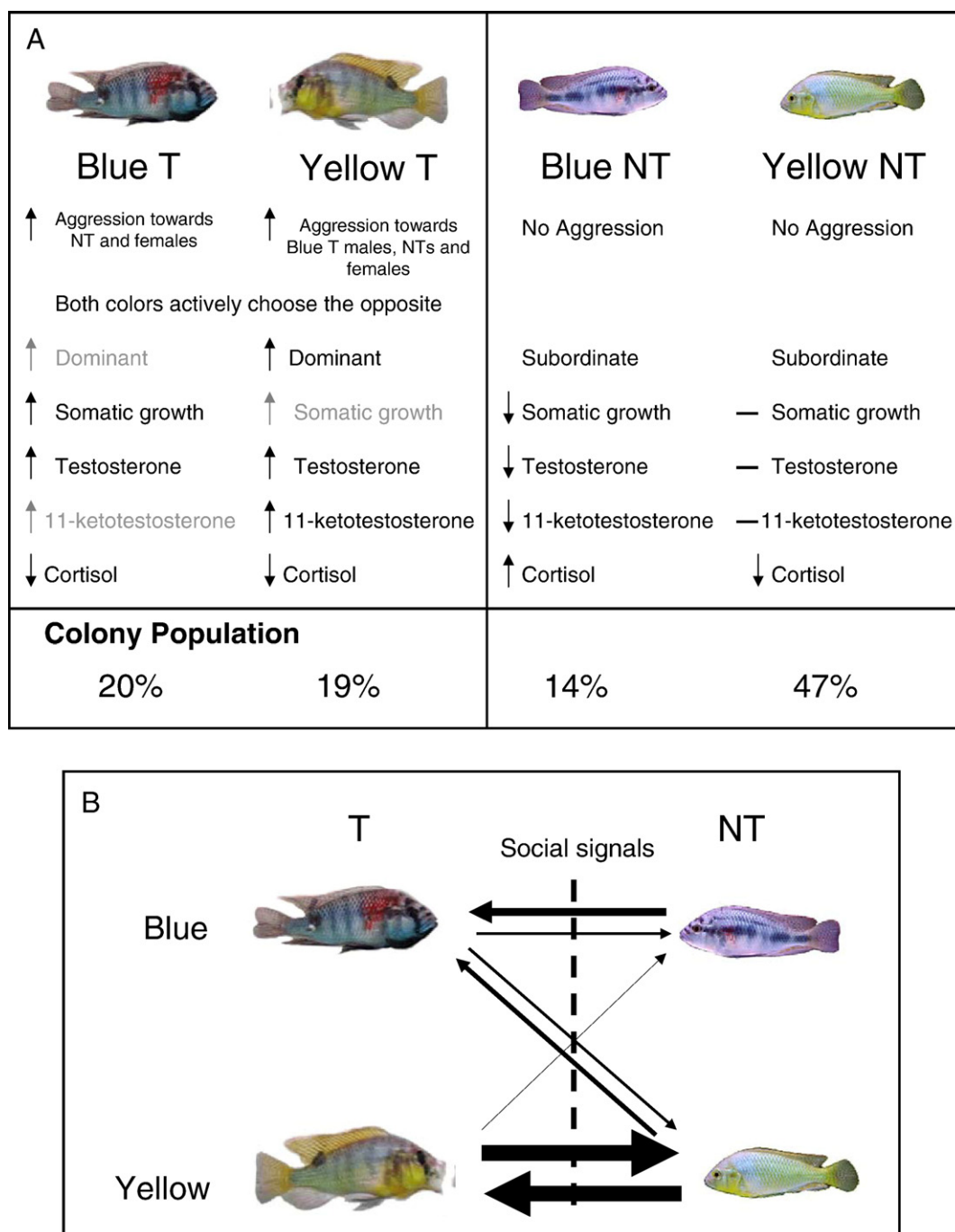

Fig. 5. Synthesis of recent findings. (A) Comparison of behavioral, physiological and population frequency profiles for male *A. burtoni* by color and status. Gray text represents a significantly lesser effect compared to Territorial males of the opposite color. (B) Graphical representation of changes in coloration and status in a semi-natural setting. Arrows indicate direction and arrow thickness is proportional to percentage of changes observed. Changes in color without simultaneous change in status (vertical arrows) are not shown, but do occur. Hashed line represents important social signals such as eyebar and body color expression during status change.

circulating testosterone levels when compared with lighter colored males (Rohwer, 1975; Rohwer and Wingfield, 1981). Experimentally darkening or lightening the feathers alone did not affect social status but when testosterone levels were increased with implants in birds with artificially darkened head feathers, social status dramatically increased (Rohwer and Rohwer, 1978). Recent investigations of body coloration in fish has shown that color can act as an external signal of an individual's potential or established social rank and levels of androgens (e.g., 11-ketotestosterone) needed for these behaviors. Dijkstra et al. (2007a) found that the intensity of red nuptial coloration and social rank were positively correlated in *P. nyererei* and that socially stimulated males expressed more nuptial coloration and elevated 11-ketotestosterone. In *A. burtoni*, aggression and the intensity of body color in both males and females can be increased by testosterone injection (Fernald, 1976; Wapler-Leong and Reinboth, 1974).

Perhaps most surprising, cortisol levels in yellow NT males were not high, as predicted from prior research. This suggests that yellow NT males may have a coping strategy that includes modifying hormone levels. Taken together it may be that both behavioral and hormonal responses play a role in maintaining color polymorphism and ability to change from one color to the other in *A. burtoni*.

Yellow and blue male strategies

Given that *A. burtoni* can change colors, and that each color morph has distinct behavioral, metabolic and hormonal characteristics, color may serve as a signal of an individual's behavioral strategy. Both blue and yellow T male strategies have benefits and costs (Fig. 5A) and this balance may sustain the color polymorphism. Blue T males that actively attack and chase NT males and females may be perceived as more active, which females prefer (Clement et al., 2005). In addition, blue T males gain weight faster than yellow T males, which could be beneficial since larger animals usually become and stay dominant (Bronstein, 1984; Tokarz, 1985). Alternatively, the more aggressive yellow T males that dominate in paired competitions may have an advantage in invading established habitats or maintaining larger territories, which may also appeal to females (Clement et al., 2005). Like T males, the color of NT males may be an external signal of their strategy. Previous studies have shown that, in tanks where territories are frequently shifted, when males change both color and dominance status, NT to T changes were always yellow to blue (Robison, 2000). In the current study, when yellow NT males were paired with a blue T the former showed NT-like behavior but maintained T-like androgen and cortisol levels, suggesting that yellow NT males are not only more likely to become T males but that they also have the necessary physiological substrate and potentially the priming effects of androgens to change status quickly when paired with a blue T male. Together, these studies suggest that yellow males have the physiological substrate necessary for agonistic and social advantage. Nevertheless, the majority of color and status changes appear to be independent (Robison, 2000), so the yellow male advantage seen in this paradigm is likely only part of the explanation. New experiments will examine possible motivation responsible for color changes more carefully.

Close examination of the behavioral and hormonal response associated with each color and status reveals distinct differences between phenotypes (Fig. 5A). Moreover, these differences may translate into advantages or disadvantages depending on the male colony population profile (Fig. 5A). Both blue and yellow T males exhibit behavioral and physiological advantage over the other possible contributing to the equal representation in the population. However, for the NT males the potential advantages associated with physiology exhibited by yellow males may explain their increased representation in the population (Fig. 5A). It is important to note that majority of males changing color and status at the same time is yellow NT male to blue T male, suggesting a clear advantage of yellow NT over blue NT (Fig. 5B).

The modification of basic body color may be a novel mechanism through which animals optimize their reproductive advantage in response to social and environmental circumstances raising several questions: Under what circumstances do males change color and how is this color change regulated physiologically? How are specific physiological states and color linked? Do females prefer yellow or blue males? And finally, how could a male fish know or choose their own color?

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