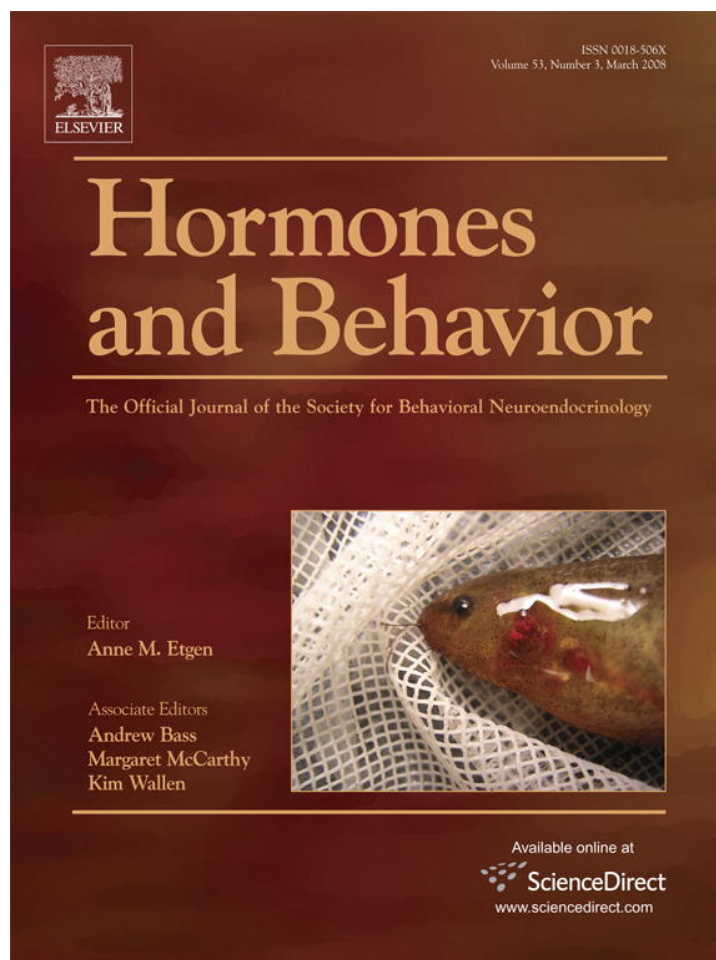


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Reciprocity between endocrine state and contest behavior in the killifish, *Kryptolebias marmoratus*

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Abstract

Given the dramatic behavioral effects of winning and losing contests, and pronounced changes in stress and sex steroid hormones post-fight, it is reasonable to suppose that these hormones also dictate future behavior. We sampled water-borne cortisol, testosterone (T), and 11-ketotestosterone (KT) before and after contests in the mangrove killifish, *Kryptolebias marmoratus*, to determine how endogenous steroid hormone levels might predict and respond to contest dynamics or success. Pre-fight cortisol related negatively, and pre-fight T related positively to contest initiation and winning, particularly in the smaller opponent. In the pairs where a larger fish won the contest, winners with higher pre-fight T and lower pre-fight cortisol delivered more attacks to the losers. Contest duration and escalation influenced post-fight hormone concentrations primarily in losers. Escalation significantly increased post-fight cortisol, T, and KT for losers but not for winners. However, winners that attacked losers at higher rates had higher levels of post-fight cortisol. Losers also demonstrate the most consistent post-fight hormone responses, particularly to contest escalation and duration. Despite the bidirectional relationship between hormones and contest behavior, we found no overall mean differences in pre- or post-fight cortisol, T, or KT between eventual winners and losers. Thus, it is evident that the categorical states of winner and loser cannot alone reveal the complex, reciprocal associations between endocrine systems and social behavior.

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Keywords: Aggression; Dominance; Cortisol; 11-ketotestosterone; Testosterone

Introduction

Winning or losing competitive interactions can impact a host of behavioral traits including courtship, aggression, and success in future dominance encounters (Schuett, 1997; Hsu et al., 2006). Numerous neuroendocrine factors can contribute to such behavioral change, and it is likely that various mechanisms exert independent or interactive influences on post-fight behavior (Clement et al., 2005; Hay and Pankhurst, 2005; discussed by Mikics et al., 2007). Of these mechanisms, steroid hormones – particularly glucocorticoids, androgens, and estrogens – have taken center stage because they appear to contribute in various capacities to the expression of aggressive behavior or the attainment of dominance status (Haller et al., 1998; Summers et al., 2005; Wingfield, 2005; Trainor et al., 2006). Decades of research in this area suggest a two-way relationship between

endocrine state and contest behavior, where contest performance triggers changes in endocrine state that then influence performance during subsequent encounters (Mazur and Booth, 1998). This bidirectional relationship is essential to our understanding of the role of the neuroendocrine system in mediating the behavioral effects of winning and losing (Hsu et al., 2006). Although reciprocity between contest behavior and steroid hormone levels is intuitive, most studies focus their attention either on using endocrine state to predict behavior or on how contest performance influences endocrine profiles but not both.

A great deal is known about how stress and sex hormones can affect contest performance. Short-term pulses of glucocorticoids intensify aggression in rats (Mikics et al., 2004) and predict escalated aggression in convict cichlids (*Archocentrus nigrofasciatus*; Earley et al., 2006). Persistently high glucocorticoid levels, however, tend to have the opposite effect, reducing aggression and predicting subordinate status (Øverli et al., 2002; DiBattista et al., 2005; see also Sloman et al., 2001). Testosterone (T), on the other hand, has long been linked to

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increases in aggressive behavior. This relationship between T and aggression has proven especially complex because it is modulated by season, sex, and mating system (Wingfield, 2005) and can manifest differently across vertebrate taxa (Hirschenhauser and Oliveira, 2006). However, recent studies in California mice (*Peromyscus californicus*) indicate that transient elevations in T are required to reveal winner characteristics such as short attack latencies (Trainor et al., 2004; Oyegbile and Marler, 2005). Another potent androgen, 11-ketotestosterone (KT), prevalent only in fish, can also facilitate aggressive behavior. For example, infusion of KT in gulf toadfishes' food (*Opsanus beta*) dramatically increased the rate of agonistic vocalizations emitted by nesting males (Remage-Healey and Bass, 2006). Importantly, much of what we know regarding how endocrine state might predict contest performance has been revealed through exogenous administration of steroid hormones; much less is known about how endogenous stress and sex steroid concentrations might relate to contest behavior.

In addition to their influence on contest behavior, steroid hormones also are sensitive to social interactions, although the response patterns are far from ubiquitous. Following dyadic conflicts, glucocorticoids are typically more elevated in losers than winners (e.g., Fernandez et al., 1994; Schuett et al., 1996; Winberg and Lepage, 1998). In many cases, however, glucocorticoid concentrations of winners and losers are indistinguishable immediately after a contest (e.g., Knapp and Moore, 1995; Buchner et al., 2004; Earley et al., 2006) although losers may experience delayed latency to return to baseline levels (e.g., Øverli et al., 1999; Summers et al., 2003). Elevated T in response to social confrontation, particularly in monogamous parental species, forms the basis for the 'challenge hypothesis' and has gained considerable support (Wingfield, 2005). In species ranging from fish to humans, dominant animals, winners or territory holders exhibit higher T levels than subordinates, losers, or nonterritorial animals (e.g., Johnsen and Zuk, 1995; Oliveira et al., 2002; Archer, 2006; Parikh et al., 2006), although in some studies of fish KT supplants T as the primary androgenic responder to social challenge (Hirschenhauser et al., 2004; Desjardins et al., 2006). Surges of T or KT subsequent to social interaction have been hypothesized to maintain high aggression levels and/or prepare animals for future contests (Wingfield, 2005; Hirschenhauser and Oliveira, 2006; see references in previous paragraph), thus connecting, conceptually, the 'responsive' and 'predictive' elements of hormone–behavior relationships (Mazur and Booth, 1998).

Although seldom evaluated, it appears that endocrine systems can respond not only to categorical states of dominance (e.g., winner/loser, territorial/nonterritorial) but also to contest dynamics. For instance, in Arctic charr (*Salvelinus alpinus*), post-fight T and KT levels of both winners and losers were positively correlated with the number of aggressive acts performed on the day of hormone collection, while losers' cortisol was positively correlated to the number of acts received on that day (Elofsson et al., 2000; see also Earley et al., 2006). Similar relationships between aggression and endocrine profiles have been uncovered in primates (androgens: Ross et al., 2004; cortisol: Muller and Wrangham, 2004).

Our goal was to investigate reciprocity between hormones and contest behavior in the mangrove killifish, *Kryptolebias marmoratus*. Specifically, the first objective of our study was to explore endogenous pre-fight cortisol, T, and KT concentrations as predictors of contest initiation, escalation probabilities, and outcome. Administering hormones exogenously might allow greater control in evaluating their impact on behavior, but they often are used in extra-physiological doses and, unless carefully controlled (e.g., Trainor et al., 2004), might disguise the role of endogenous steroids. The second objective of our study was to determine how contest participants' cortisol, T, and KT respond to winning or losing and to contest escalation, duration, and dominance reinforcement. Ultimately we hope to identify whether changes in cortisol, T, or KT might govern the pronounced behavioral modifications often witnessed after animals win or lose aggressive contests.

Materials and methods

Study organism

The mangrove killifish, *K. marmoratus*, formerly *Rivulus marmoratus* (Costa, 2004), is an internally self-fertilizing hermaphroditic fish (Taylor et al., 2001). The fish start to lay eggs between 3 and 6 months of age (Harrington, 1975; Grageda et al., 2005). Their total body length (TL, the distance from the tip of the snout to the tip of the caudal fin) could be less than 2 cm when first matured (Sakakura and Noakes, 2000) but can grow to 4–5 cm in the laboratory (personal observation). This species is capable of producing fertilized eggs all year round and does not have obvious oviposition cycles (Harrington, 1963). Most populations of this fish exist in nature as isogenic, homozygous strains, although outcrossing heterozygous populations have been discovered in Twin Cays, Belize (Taylor et al., 2001; Mackiewicz et al., 2006).

This study used individuals of 4 strains of *K. marmoratus* from various geographical areas (HON9: Utila, Honduras; RHL: San Salvador, Bahamas; SLC8E: St. Lucie County, Florida, USA; VOL: Daytona Beach, Florida, USA) which were F2 to F5 generations of fish originally collected from the field by Dr. D. Scott Taylor. Fish were isolated within a week after hatching in a laboratory at National Taiwan Normal University and kept alone in a 10×10×10 cm translucent polypropylene container filled with 400–500 ml of approximately 25 ppt synthetic sea water (Instant Ocean™ powder). Maintenance containers were cleaned and water replaced at least every two weeks. All maintenance containers were labeled with unique codes for individual identification. Fish were kept at 25±2 °C on a 14:10 h photoperiod, and fed newly hatched brine shrimp (*Artemia*) nauplii daily.

Experiments were conducted in accordance with a protocol approved by The Animal Care and Use Committee of National Taiwan Normal University (permit #93008).

Experimental procedures

We determined pre-fight hormone concentrations of cortisol, T, and KT for each individual (see below). Individuals then engaged in a contest against a pre-designated opponent, after which we determined post-fight hormone concentrations. We used fish with TL greater than 25 mm to maximize the likelihood of obtaining detectable amounts of hormone from the water samples (see below). Available fish of each strain were divided into two groups based on the TL: smaller or greater than 26.5 mm. Contest pairs were formed by randomly choosing a fish from each of the two groups such that the smaller opponent of a pair was always smaller than 26.5 mm while the larger opponent was larger than 26.5 mm. All fish used in this study had been used in other studies previously. Prior contest experiences have been shown to influence the contest behavior of the fish (Hsu and Wolf, 1999, 2001) but the effects disappear in 7 days (i.e., after this time the probability of winning and the likelihood of escalating a fight revert to their previous state, unpublished data). As a precaution and not to overuse

fish, only fish that had been re-isolated for at least a month were used in this study and the two contestants of a pair were matched for their last contest outcome. The experiences that these test individuals gained from previous studies were results of 'self-selection' procedures (see Hsu et al., 2006 for a discussion). Bégin and colleagues (1996) concluded that self-selected winners have a 0.67 (as opposed to 0.5) probability of having intrinsically higher fighting ability than a size-matched naïve opponent. Consequently, any differences that might be found in later analyses between prior winners and losers would more likely to result from differences in intrinsic aggressiveness than from so-called winner/loser effects. All contests took place in standard aquaria (12 × 8 × 20 cm) containing water 16 cm deep and gravel 2 cm deep. Twenty pairs from each of the 4 strains were used for the study (Total $N=80$).

Pre-fight hormone collection

On the first day of the experiment (Day 1), a needle was used to break the nonvascularized thin membrane between two soft-rays in the upper or lower margins of the two contestants' caudal fins for individual identification (one upper and one lower margin of the caudal fin, randomly assigned). Immediately after being marked, the fish were fed with small amounts of newly hatched brine shrimp. All fish resumed regular feeding behavior within 5 s. The procedure did not cause any bleeding or observable adverse effects upon the health or behavior of the fish. On Day 2, we transferred the fish from their maintenance containers to 400 ml glass beakers (one fish/beaker) filled with 200 ml clean 25 ppt synthetic seawater and kept for 4 h; from 11 am to 3 pm. The glass beakers were housed inside individual translucent plastic containers such that the experimental fish were visually isolated from the environment to reduce possible disturbance. At 3 pm, we removed the fish from the water and divided the water of each beaker equally into four 50 ml polypropylene tubes. Water samples were stored in a -80°C freezer for hormone extraction.

Staging contests

We divided a standard aquarium into two equal-sized compartments with an opaque partition. After being removed from the pre-fight water collection beakers, the two individuals of a contest were placed in the standard aquarium, one in each randomly assigned compartment and allowed to acclimate until 10 am the next (Day 3) morning. At 10 am on Day 3, the partition was lifted and the interactions of the fish were videotaped for 1 h. At 11 am, we separated the contestants by re-inserting the opaque partition into the aquarium.

Post-fight hormone collection

After the contest, we removed the fish from the standard aquarium by netting, and they were treated the same as was described for pre-fight hormone collection. The fish were returned to their maintenance containers after the experiments.

Contest behaviors

The fighting behavior of *K. marmoratus* has been described in Hsu and Wolf (2001). After the partition was lifted, the fish that first oriented and moved toward its opponent was defined as the initiator of display. The fish that first swam rapidly toward, and pushed against or bit its opponent was the initiator of attack. The fish that first chased and/or attacked its opponent for 5 min without retaliation was the winner of the contest. The time that the loser first retreated was defined as the retreat time. Contest duration was measured as the time period between first display and first retreat. After retreat, the number of attacks that the winner delivered to the loser for the first 5 min was recorded and used to calculate the rate of post-retreat attacks (=number of attacks per minute). A contest was classified as escalated or nonescalated. An escalated contest was resolved after some period of mutual attacks between the two opponents, while a nonescalated contest involved only displays until the winner chased/attacked its opponent.

Hormone extraction and assay

For each fish, hormones were extracted from 100 out of the 200 ml water sample collected using C18 solid phase extraction columns (Lichrolut RP-18,

500 mg, 3.0 ml; Merck) fitted to a 12-port manifold. Columns first were primed using 2 consecutive washes with 2 ml HPLC grade methanol (MeOH) followed by 2 consecutive washes with 2 ml distilled water. Tubing was then fastened securely to the top of each column and was placed into the water sample collected from the fish. The vacuum was engaged and the water sample was passed through the tubing into the column at a rate of approximately 2 ml per minute. Salts were purged from the column with 2 consecutive 2 ml washes of distilled water. The columns were then frozen until further processing. Freeze storage of water samples and columns has been determined not to impact steroid concentrations (Ellis et al., 2004). Columns were thawed and purged with 2 consecutive 2 ml washes of distilled water. Hormone was eluted from the columns into 12 × 75 mm (6 ml) borosilicate vials by 2 consecutive 2 ml washes with HPLC grade MeOH. The 4 ml of eluted solvent was evaporated at 40 °C (water bath) with a gentle stream of nitrogen (~0.7 bar), which was passed over the samples through an evaporating manifold. The resulting hormone pellet was resuspended in 840 µl of enzyme-immunoassay (EIA) buffer supplied with the EIA kits (see below), and the samples were stored at -20°C until assayed.

Cayman Chemicals Inc. EIA kits were used for all hormones (cortisol, KT, and T). Each of the three hormones was assayed for each individual (before and after the contest) in duplicate on five pairs of plates (one standard curve used for two 96-well plates; 30 plates total for all hormones). All procedures recommended by the manufacturer were followed for each assay. Plates were read at 405 nm on a BioMek microplate reader. Two *K. marmoratus* pooled water extracts (see below) run in duplicate were used as controls on each plate. Intra-assay coefficients of variation were (assay pair 1, 2, 3, 4, 5) cortisol (5.8%, 3.6%, 8.0%, 6.6%, 5.2%), T (2.0%, 3.4%, 2.4%, 3.6%, 5.4%), and KT (3.2%, 6.1%, 6.8%, 5.6%, 4.9%). Inter-assay coefficients of variation were cortisol (7.5%), T (5.3%), and KT (10.2%).

The EIA kits were validated for *K. marmoratus* water-extracted hormones by assessing parallelism of a serial dilution curve with the standard curve and by determining quantitative recovery. Hormones were obtained from 50 non-experimental fish (10 per clone VOL, HON9, SLC8E, RHL, and DAN2K) using collection (4 h) and extraction methods similar to those described above. Evaporated samples were then resuspended in 170 µl EIA buffer and combined into a concentrated pool of 8.5 ml. The pool was kept either at 1:1 (for serial dilutions) or diluted 1:5 (for quantitative recovery), and stored at -20°C in aliquots of 200 µl.

One milliliter (1 ml) of the pooled (1:1) control was used for the serial dilutions. Briefly, 500 µl of this sample was transferred to a 1.5 ml microcentrifuge tube and mixed (by vortexing) with 500 µl of EIA buffer to create a 1:2 dilution; 500 µl of 1:2 dilution was mixed with an equal volume of EIA buffer to create a 1:4 dilution, and so on until 1:128. The serial dilutions were run in duplicate using the EIA protocol described above. The log-logit transformed dilution curve was constructed using average % maximum binding and pg/ml concentrations for the eight dilution samples. The dilution curve was parallel to the standard curve for all hormones (comparison of slopes, Zar, 1996, p. 355; cortisol: $t_{12}=-0.504$, $P=0.62$; T: $t_{12}=0.278$, $P=0.79$; KT: $t_{12}=0.00$, $P=1.00$).

For quantitative recovery, 110 µl of the 1:5 diluted *K. marmoratus* pool was pipetted into each of nine 1.5 ml microcentrifuge tubes and mixed with an equal volume of each of the standards provided with the EIA kit; a 220 µl unmanipulated sample also was assayed. Expected recovery concentrations were based on the known amount of cortisol, T, or KT in the *K. marmoratus* control sample (220 µl 1:5 control). Minimum observed recovery for cortisol, T, and KT was 73.2%, 69.5%, and 83.7%, respectively. The slopes of the observed vs. expected curves were 1.19 (cortisol), 0.916 (T), and 0.851 (KT), indicating linear relationships between expected and observed values for all hormones. The sensitivities of the assays (range: plates 1–10) were as follows: cortisol (1.76–10.06 pg/ml); T (3.64–14.50 pg/ml); KT (0.68–2.27 pg/ml). All data are presented as pg/sample.

Statistical analysis

Pearson's correlation coefficients were used to measure relationships between body size (standard length: the distance from the tip of the snout to the beginning of the tail fin) and pre-fight cortisol, T, and KT levels for contestants. Differences in pre-fight cortisol, T, and KT between eventual

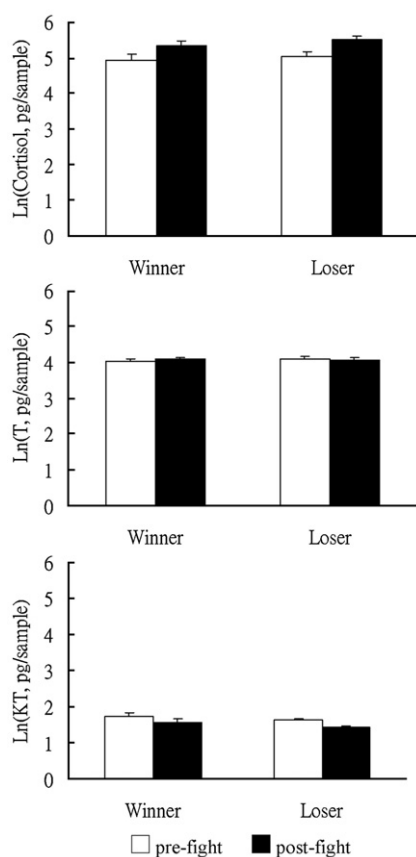


Fig. 1. Pre- and post-fight cortisol, T, and KT levels (ln transformed, mean ± SE) of eventual winners and losers ($n=77$ for all bars).

winners and loser were tested with paired-sample t tests. Changes in these hormones after the contests were also analyzed with paired-sample t tests for winners and losers. F tests were used to analyze strain differences in pre- and post-fight hormones. Multiple logistic regressions (Hosmer and Lemeshow, 2000) were employed to examine relationships between pre-fight hormones of the two contestants and their likelihoods of initiating attacks, and escalating and winning contests while multiple linear regressions were used to examine relationships between pre-fight hormones and contest duration and post-retreat attacks. We controlled for size, last contest outcome, and strain type of the contestants for all regression analyses.

We tested relationships between contest interactions (i.e., whether or not a contest was escalated, which contestant won, contest duration, and post-retreat attacks) and the levels of post-fight hormones using multiple linear regression, controlling for size, last contest outcome, strain type, and level of the corresponding pre-fight hormone of the contestants. Hormone levels, contest duration, and rates of post-retreat attacks were ln transformed to improve distribution. JMP (v. 5.0.1 SAS Institute Inc., Cary, NC, U.S.A.), a commercial statistical package, was used for the statistical analyses in this study.

Results

Of the 80 contests staged, 3 (all between individuals of VOL strain) did not resolve but were terminated after 1 h of interaction and reduced the sample sizes of our statistical analyses to 77. Most (72.7%) of the resolved contests were won by the larger opponents (number contests won, 56:21, $G_1=16.51$, $P<0.001$). Out of the three pre-fight hormones measured, only the levels of KT ($r^2=0.38$, $n=154$, $P<0.001$), but not cortisol

($r^2=0.07$, $n=154$, $P=0.410$) or T ($r^2=-0.01$, $n=154$, $P=0.946$), significantly correlated with the size of the constants. The eventual winners and losers did not differ in their pre-fight cortisol (paired-sample $t_{76}=-0.110$, $P=0.506$), T (paired-sample $t_{76}=-0.046$, $P=0.506$), and KT (paired-sample $t_{76}=0.136$, $P=0.110$) levels (Fig. 1). After contests, cortisol levels increased significantly for both winners (paired-sample $t_{76}=2.8$, $P=0.007$) and losers (paired-sample $t_{76}=3.17$, $P=0.002$), while KT levels decreased for both (winner: paired-sample $t_{76}=-2.34$, $P=0.022$; loser: paired-sample $t_{76}=-2.73$, $P=0.008$) (Fig. 1). Changes in these two hormones after contests were of the same directions and similar magnitudes for winners and losers (cortisol: pair-sample $t_{76}=0.00$, $P=1.000$; KT: pair-sample $t_{76}=0.01$, $P=0.914$). There were no detectable changes in T levels after a contest for winners (pair-sample $t_{76}=0.72$, $P=0.474$) or losers (pair-sample $t_{76}=0.04$, $P=0.970$). There were significant strain effects on pre- and post-fight cortisol (pre-fight: $F_{3,150}=10.22$, $P<0.001$; post-fight: $F_{3,150}=9.15$, $P<0.001$), T (pre-fight: $F_{3,150}=6.43$, $P<0.001$; post-fight: $F_{3,150}=16.72$, $P<0.001$), and KT (pre-fight: $F_{3,150}=6.18$, $P<0.001$; post-fight: $F_{3,150}=10.38$, $P<0.001$) levels (Fig. 2), providing perhaps a physiological basis for individuals of these clones to behave differently.

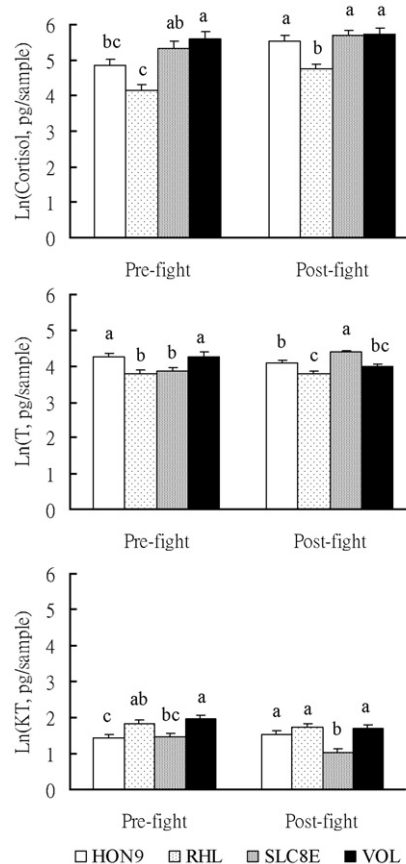


Fig. 2. Pre- and post-fight cortisol, T, and KT levels (ln transformed, mean ± SE) of different strains of individuals ($n=40$ for HON9, RHL, and SLC8E, $n=34$ for VOL). Means labeled with different letters are significantly different at $P<0.05$ (Tukey multiple comparisons).

Table 1
Multiple logistic regressions modeling the relationships between pre-fight hormone levels and contest behaviors (the probability of the larger opponent initiating attacks, the probability of escalation, and the probability of the larger opponent winning the contest), controlling for body size, last contest outcome, and strain type of the contestants (Pre: Pre-fight; Larger: the larger opponent; Smaller: the smaller opponent; *: $P < 0.05$)

Variable	df	Initiating attacks (Larger)			Escalation			Winning (Larger)		
		$(N=76, \chi^2_{12}=30.26, P=0.003)$			$(N=77, \chi^2_{12}=21.03, P=0.050)$			$(N=77, \chi^2_{12}=29.30, P=0.004)$		
		b±SE	χ^2	P	b±SE	χ^2	P	b±SE	χ^2	P
Pre-cortisol (Larger)	1	-0.42±0.29	2.18	0.140	0.09±0.27	0.11	0.742	-0.18±0.31	0.34	0.559
Pre-cortisol (Smaller)	1	1.15±0.45	7.97	0.005*	-0.18±0.37	0.25	0.618	1.30±0.52	8.17	0.004*
Pre-T (Larger)	1	1.84±0.86	5.17	0.023*	-0.67±0.73	0.86	0.354	0.64±0.90	0.51	0.476
Pre-T (Smaller)	1	-1.87±0.89	4.98	0.026*	0.11±0.71	0.02	0.875	-2.67±1.27	5.55	0.019*
Pre-KT (Larger)	1	-0.75±0.55	1.83	0.176	0.37±0.47	0.64	0.423	0.74±0.90	0.73	0.392
Pre-KT (Smaller)	1	0.34±0.88	0.15	0.695	-0.17±0.74	0.05	0.816	2.40±1.51	3.48	0.062
Size (Larger)	1	0.11±0.21	0.29	0.588	-0.40±0.18	5.54	0.019*	0.40±0.23	3.39	0.066
Size (Smaller)	1	-3.79±1.12	17.67	<0.001*	1.66±0.83	4.45	0.035*	-2.47±1.04	6.66	0.010*
Last outcome (W ^a)	1	0.85±0.68	1.63	0.202	1.24±0.57	4.97	0.026*	2.32±0.87	9.50	0.002*
Strain	3		4.51	0.211		2.64	0.451		0.28	0.964

^a An indicator variable, "loser" pairs were used as the baseline group (0 for loser pairs, 1 for winner pairs).

Relationships between the levels of pre-fight hormones and contest behaviors

Behavior 1: probability of the larger opponent initiating attacks

In one of the 77 resolved contests, the loser retreated as soon as the winner displayed and constantly swam away from the winner. No attacks were observed in this contest and thus only data from 76 contests were available for analyzing the likelihood of initiating attacks (Table 1). Multiple logistic regression indicated that the likelihood of the larger opponent initiating attacks was related positively with its own pre-fight T level ($P=0.023$) and the pre-fight cortisol level of its opponent ($P=0.005$), but negatively with the pre-fight T level ($P=0.026$) and the body size of its opponent ($P<0.001$). Overall, an individual was more likely to initiate attacks in a contest when it had higher T levels, and when it fought against a smaller opponent with higher cortisol and lower T levels.

Behavior 2: probability of escalation

None of the hormones had significant relationships with the probability of escalation (Table 1). Instead, the likelihood of escalation related negatively with the size of the larger opponent ($P=0.019$) and positively with the size of the smaller opponent ($P=0.035$). Moreover, escalation likelihood was higher if both contestants were intrinsically aggressive and had won their previous fights ($P=0.026$).

Behavior 3: probability of the larger opponent winning

Probability of winning was correlated mainly with measurements from the smaller opponent (Table 1). The likelihood of the larger opponent winning was related positively with the smaller opponent's pre-fight cortisol levels ($P=0.004$), and negatively with pre-fight T levels ($P=0.019$) and body size ($P=0.001$). In addition, the probability of the larger opponent winning was higher in contests where both contestants were intrinsically aggressive and had won their previous fights ($P=0.002$).

Behavior 4: contest duration

None of the pre-fight hormones had any predictive power ($P>0.30$ for all) on contest duration and the overall model fit was nonsignificant ($F_{12,64}=0.82, P=0.626$).

Behavior 5: post-retreat attacks

Winners delivered attacks to losers even after losers retreated (post-retreat attacks). Whether winners were larger or smaller than the losers might influence the frequency of the attacks; thus, we estimated two separate regression models based on the relative size of the winners. We examined the relationship between post-retreat attacks and the hormones of the winners and the losers (instead of the larger and the smaller opponents as for the other behaviors) (Table 2). When winners were larger than losers, the pre-fight cortisol ($P=0.009$) and T ($P=0.004$) of the winners had important effects on the rate of post-retreat attacks. Winners with lower cortisol and higher T levels

Table 2
Multiple linear regressions modeling the relationships between pre-fight hormone levels and post-retreat attacks; controlling for body size, last contest outcome, and strain type of the contestants (Pre: Pre-fight; Larger: the larger opponent; Smaller: the smaller opponent; *: $P < 0.05$)

Variable	df	Post-retreat attacks: Larger opponent won			Post-retreat attacks: Smaller opponent won		
		$(F_{12,43}=2.53, P=0.013)$			$(F_{12,8}=3.23, P=0.052)$		
		b±SE	F	P	b±SE	F	P
Pre-cortisol (Winner)	1	-0.21±0.08	7.40	0.009*	0.55±0.20	7.38	0.026*
Pre-cortisol (Loser)	1	0.03±0.11	0.08	0.776	0.08±0.12	0.45	0.521
Pre-T (Winner)	1	0.67±0.22	9.37	0.004*	-1.02±0.58	3.09	0.117
Pre-T (Loser)	1	-0.06±0.20	0.10	0.756	0.73±0.37	3.91	0.084
Pre-KT (Winner)	1	0.06±0.13	0.20	0.653	-0.14±0.54	0.07	0.803
Pre-KT (Loser)	1	-0.22±0.23	0.94	0.338	-0.27±0.58	0.22	0.650
Size (Winner)	1	0.00±0.05	0.01	0.922	-0.35±0.43	0.67	0.437
Size (Loser)	1	-0.27±0.22	1.62	0.209	-0.14±0.12	1.53	0.251
Last outcome (W ^a)	1	0.33±0.16	4.30	0.044*	0.18±0.41	0.19	0.675
Strain	3		3.25	0.031*		7.23	0.012*

^a An indicator variable, "loser" pairs were used as the baseline group (0 for loser pairs, 1 for winner pairs).

attacked losers at higher rates. Moreover, larger winners that had won previous contests tended to attack losers at higher rates ($P=0.044$). However, when winners were smaller than losers, the winners with higher pre-fight cortisol tended to attack losers at higher rates ($P=0.026$), while the pre-fight T of the winners no longer had significant relationship with the attack rate ($P=0.117$). Winners of different strains attacked losers at different rates (larger winner: $P=0.031$; smaller winner: $P=0.011$).

Relationships between the levels of post-fight hormones and contest behaviors

Losers' levels of post-fight hormones

Table 3 shows that contest escalation had the greatest impacts on post-fight hormone levels of losers: losers of escalated contests tended to have higher post-fight cortisol ($P=0.046$), T ($P<0.001$), and KT (nonsignificant trend, $P=0.057$) than losers of nonescalated contests. Contest duration correlated negatively with 2 of the 3 post-fight hormones measured: losers of longer contests tended to have lower post-fight T ($P=0.048$) and KT ($P=0.007$). Moreover, losers that received higher rates of post-retreat attacks had lower post-fight T ($P=0.033$). Whether a contest was won by the larger or the smaller opponent did not have any important impact on losers' levels of post-fight cortisol, T, or KT.

Losers' post-fight cortisol, T, and KT levels related positively with the corresponding pre-fight hormone levels ($P=0.007$, 0.013, and 0.013, respectively). Losers of different strains differed significantly in all 3 post-fight hormones (cortisol: $P=0.033$, T: $P<0.001$, KT: $P=0.002$).

Winners' levels of post-fight hormones

Contest interactions had little impact on winners' post-fight cortisol, T, or KT (Table 4). Winners that exhibited more post-retreat attacks toward losers had higher levels of post-fight cortisol ($P=0.006$). Moreover, there was a nonsignificant ten-

dency for winners of longer contests to have higher post-fight T ($P=0.053$).

Winners' post-fight cortisol, T, and KT also tended to relate positively with the corresponding pre-fight hormone levels ($P=0.026$, 0.072, and <0.001 , respectively). Winners' post-fight KT levels related positively with their body size ($P=0.015$). Also, winners of different strains differed significantly in all 3 post-fight hormones (cortisol: $P=0.005$, T: $P<0.001$, KT: $P<0.001$).

Discussion

Our study provides support for reciprocity between hormones and agonistic behavior. Contests alter hormone levels and hormones, in turn, predict behavioral change (see Mazur and Booth, 1998). Our data also show the benefits of correlating hormone levels with contest behavior rather than just outcome. Winning or losing status alone did not correlate significantly with pre- or post-fight hormone levels; just concentrating on status would have hidden how specific contest behaviors could be predicted by pre-fight hormone levels or could have influenced post-fight endocrine profiles. In addition, a finer analysis enabled us to find differences in the relationship between hormones, status and behavior, e.g., only losers mounted significant cortisol, T, and KT responses to contest escalation and duration.

Pre-fight hormones and contest behavior/outcome

Although exogenous administration would allow direct causal links to be made between levels of cortisol, T, or KT and contest behavior, we capitalized on a water-borne hormone collection method (Scott and Ellis, 2007; Sebire et al., 2007) to study the relationship between endogenous baseline endocrine profiles, agonistic responses, and competitive success. Pre-fight hormone concentrations likely reflect the baseline state for *K*.

Table 3

Multiple linear regressions modeling the effects of contest interaction and outcome on loser's post-fight cortisol, T, and KT levels; controlling for body size, last contest outcome, strain type, and the corresponding pre-fight hormone level of the contestants (Loser/Winner: the opponent that lost/won the contest; Pre: pre-fight; *: $P<0.05$; †: $0.05<P<0.1$)

Variable	df	Post-cortisol (Loser) ($F_{11,65}=4.06, P<0.001$)			Post-T (Loser) ($F_{11,65}=5.39, P<0.001$)			Post-KT (Loser) ($F_{11,65}=2.99, P=0.003$)		
		b±SE	F	P	b±SE	F	P	b±SE	t	P
Escalate ^a	1,65	0.51±0.25	4.15	0.046*	0.41±0.10	17.64	<0.001*	0.22±0.11	3.76	0.057 †
Winner (Larger) ^b	1,65	-0.72±0.52	1.92	0.170	-0.15±0.20	0.58	0.447	-0.30±0.24	1.64	0.205
Contest Duration	1,65	-0.12±0.08	2.11	0.152	-0.07±0.03	4.06	0.048*	-0.11±0.04	7.92	0.007*
Post-contest attacks	1,65	0.19±0.21	0.88	0.353	-0.17±0.08	4.73	0.033*	-0.14±0.09	2.43	0.124
Size (Loser)	1,65	0.06±0.14	0.21	0.647	-0.04±0.05	0.44	0.509	-0.03±0.06	0.22	0.640
Size (Winner)	1,65	0.12±0.07	3.38	0.070	0.00±0.02	0.02	0.879	0.01±0.03	0.19	0.664
Last outcome (W ^c)	1,65	-0.28±0.23	1.44	0.235	0.04±0.09	0.18	0.670	0.16±0.11	2.33	0.132
Strain	3,65		3.09	0.033*		10.19	<0.001*		5.73	0.002*
Pre-cortisol (Loser)	1,65	0.28±0.10	7.76	0.007*						
Pre-T (Loser)	1,65				0.17±0.07	6.47	0.013*			
Pre-KT (Loser)	1,65							0.28±0.11	6.55	0.013*

^a An indicator variable, contests that were not escalated were used as the baseline group (0 for nonescalated contests, 1 for escalated contests).

^b An indicator variable, contests that the smaller opponent won were used as the baseline group (0 for smaller winner, 1 for larger winner).

^c An indicator variable, "loser" pairs were used as the baseline group (0 for loser pairs, 1 for winner pairs).

Table 4
Multiple linear regressions modeling the effects of contest interaction and outcome on winner's post-fight cortisol, T, and KT levels; controlling for body size, last contest outcome, strain type, and the corresponding pre-fight hormone level of the contestants (Loser/Winner: the opponent that lost/won the contest; Pre: pre-fight; *: $P < 0.05$; †: $0.05 < P < 0.1$)

Variable	df	Post-cortisol (Winner) ($F_{11,65}=3.00, P=0.003$)			Post-T (Winner) ($F_{11,65}=2.98, P=0.003$)			Post-KT (Winner) ($F_{11,65}=11.57, P<0.001$)		
		b±SE	F	P	b±SE	F	P	b±SE	F	P
Escalate ^a	1,65	0.02±0.25	0.01	0.933	-0.08±0.11	0.52	0.474	0.11±0.15	0.55	0.459
Winner (Larger ^b)	1,65	0.21±0.50	0.18	0.674	-0.03±0.23	0.02	0.902	0.19±0.31	0.39	0.534
Contest duration	1,65	0.12±0.08	2.21	0.142	0.07±0.04	3.88	0.053 †	0.06±0.05	1.45	0.233
Post-contest attacks	1,65	0.57±0.20	8.22	0.006*	0.13±0.09	2.06	0.156	0.09±0.12	0.57	0.451
Size (Loser)	1,65	0.20±0.14	1.91	0.171	0.03±0.06	0.21	0.647	0.12±0.08	2.01	0.161
Size (Winner)	1,65	0.09±0.06	1.76	0.190	0.03±0.03	0.92	0.340	0.10±0.04	6.27	0.015*
Last outcome (W ^c)	1,65	-0.33±0.23	2.01	0.161	0.03±0.10	0.08	0.785	-0.08±0.14	0.29	0.591
Strain	3,65		4.77	0.005*		8.17	<0.001*		7.89	<0.001*
Pre-cortisol (Winner)	1,65	0.17±0.07	5.23	0.026*						
Pre-T (Winner)	1,65				0.14±0.08	3.34	0.072			
Pre-KT (Winner)	1,65							0.629±0.09	52.75	<0.001*

^a An indicator variable, contests that were not escalated were used as the baseline group (0 for nonescalated contests, 1 for escalated contests).

^b An indicator variable, contests that the smaller opponent won were used as the baseline group (0 for smaller winner, 1 for larger winner).

^c An indicator variable, "loser" pairs were used as the baseline group (0 for loser pairs, 1 for winner pairs).

marmoratus because the water-borne collection apparatus approximates both laboratory housing and the crab burrows that the fish occupy in natural conditions (Taylor, 2000).

Our results show that higher pre-fight T correlates positively with aggressive behavior such as attack initiation and that higher pre-fight cortisol correlates with lower aggression. This is consistent with early and more recent models of the impact of cortisol (or corticosterone) and T on agonistic behavior (e.g., Leshner, 1975; Mikics et al., 2004; Summers et al., 2005; Wingfield, 2005). Furthermore, pre-fight cortisol in the smaller opponent was negatively related and pre-fight T positively related to contest success. These trends are similar to those revealed using exogenous hormone implants or other methods for changing endocrine state. For instance, castration and the accompanying reduction in T levels decreased aggression in cichlid fish (*Astatotilapia burtoni*; Francis et al., 1992). Oliveira et al. (2005), however, proposed that contest escalation might be independent of circulating androgens. Our data support this; neither pre-fight T nor KT predicted escalation. DiBattista et al. (2005) showed that exogenously administered cortisol decreases aggression and forecasts subordinate status in rainbow trout (*Oncorhynchus mykiss*). Other studies in rainbow trout (Øverli et al., 2002) and in mammals (e.g., Mikics et al., 2004) agree. A burgeoning literature on stress coping also indicates neuroendocrine stress axis activity as a predictor of aggression and dominance success (Koolhaas et al., 1999; Øverli et al., 2005, 2007).

Pre-fight KT had negligible effects on the behaviors measured in this study. KT is an androgen particular to fish. Its effect on aggressive behavior is unclear, in part due to a paucity of research in this area. Some studies demonstrate that supplementing KT increases aggressive behavior (Ros et al., 2004) or aggressive acoustic emissions (Remage-Healey and Bass, 2006); castration-induced depression in KT also correlates with reduced aggression in cichlids (Francis et al., 1992). Our study, however, showed little correlation between endogenous

pre-fight KT and aggression or contest success, although higher KT levels slightly increased a smaller opponent's chance of winning. Future studies of KT and aggression or dominance should clarify its role as a predictor of contest performance.

In the present study, the relationship between pre-fight hormones and post-retreat attacks (i.e., dominance reinforcement) depended on the relative sizes of winners and losers. When the larger fish won, its post-retreat attacks related positively to pre-fight T levels and negatively to pre-fight cortisol levels. This suggests that these hormones have a similar effect on behavior during both contests and post-fight dominance reinforcement. Interestingly, when the smaller fish won, its attacks related positively to pre-fight cortisol levels. This finding is difficult to understand, but there are a number of possible explanations. Perhaps pre-fight cortisol is not responsible for elevated post-retreat aggression. Rather some other correlated system (e.g., serotonergic) triggers extended bouts of post-retreat attacks when a fish wins against a larger opponent. Alternatively, our finding might reflect behavioral overcompensation by animals that win despite their size and high cortisol level. Perhaps it pays an animal that wins against all odds to maintain high levels of aggression, even after contest settlement. Continuing to attack a subordinated opponent could quickly depress fight-induced cortisol, much as displaced aggression does in rainbow trout (Øverli et al., 2004). This might be especially important for animals that entered the contest with high stress hormone levels.

Contests were influenced more by the body size and endocrine state of the smaller than the larger opponent. Eventual losers dictate the course of a contest, especially escalation and duration (Taylor and Elwood, 2003) so it is not surprising that most predictions about contest dynamics emerged from the smaller animals, which lost a significant majority of the contests. This result is similar to that of Earley et al. (2006) who found that pre-fight cortisol of eventual losers, not winners, predicted contest intensity in convict cichlids (*Cryptoheros nigrofasciatus*).

Contest behavior/outcome and post-fight hormones

Contest had similar effects on the endocrine profiles of winners and losers. In both groups, the fight caused cortisol levels to increase, KT to fall, and had no significant effect on T. A rapid increase in cortisol is a hallmark response to contests, often independent of status achieved (Summers et al., 2005; Summers and Winberg, 2006). But when opponents are kept together after the contest, decay of cortisol back to baseline usually occurs more rapidly in winners than in losers (Øverli et al., 1999; Summers et al., 2003). Given the 4 h hormone collection time employed, we could not directly assess how cortisol secretion changed in the time following the contest. New techniques with water-borne hormone collection times reduced to 30 min will allow finer analyses in future (Sebire et al., 2007). Nevertheless, it is reasonable to suppose that had winners' cortisol secretion fallen off more quickly than losers' then we would have found a difference in accumulated concentrations. Thus, an initial conclusion is that killifish winners' and losers' post-fight cortisol secretion patterns may not differ much over time. Studies similar to ours in which opponents are not kept together after the contest demonstrates a similar failure of winners to reduce cortisol secretion quickly (Øverli et al., 2004).

Perhaps the most surprising finding was that contests did not elicit significant overall changes in T and that KT fell significantly (post- vs. pre-fight) in both winners and losers. Elevations in T occur in many animals as a consequence of territorial intrusion or achievement of dominance in a social hierarchy (Wingfield, 2005; Oliveira et al., 1996). Status differences in T, however, are not always evident following dyadic encounters in which neither animal has a resident-intruder role (e.g., Knapp and Moore, 1995; Neat and Mayer, 1999). Thus, it is possible that social modulation of T in a manner consistent with the "challenge hypothesis" depends not only on social and/or mating systems (and their implications for endocrine maxima) but also on the type of competitive encounter. Recent studies also have shown that KT consistently shows a stronger response than T to social challenges in fish (e.g., Oliveira et al., 1996; Hirschenhauser et al., 2004; Remage-Healey and Bass, 2005; Desjardins et al., 2006; but see Elofsson et al., 2000). KT is usually increased relative to control animals or breeding baselines following exposure to social stimuli. Furthermore, a comparative study using closely related species with contrasting mating systems presented evidence for more pronounced KT responsiveness in monogamous/pair-forming than polygynous/lekking cichlids (Hirschenhauser et al., 2004). *K. marmoratus*, a self-fertilizing clonal hermaphrodite with no parental care, perhaps represents an extreme in terms of mating system with baseline KT near its maximum. If this is true, then social interactions can either keep KT at its maximum level or reduce it as we have documented. The adaptive value, if any, for socially induced KT suppression is unclear but, in this species at least, changes in KT do not appear important for mediating future contest behavior or success (see above).

Despite the lack of status differences in mean post-fight hormone concentrations, there were marked status asymmetries in how contest dynamics predicted endocrine profile. Post-fight T and KT of losers responded to contests in the same way,

increasing with escalation and decreasing with contest duration; winners showed no such trends. Two other studies found that androgens correlate positively with aggressive acts performed in dominant and subordinate (pooled) animals (Arctic charr, T, and KT; Elofsson et al., 2000), and with frequency of aggressive behavior in residents (but not intruders) (marmosets, Ross et al., 2004). Thus, contest behavior can predict changes in androgen levels, and the most obvious link between escalation and hormones occurs in individuals that dictate the course of the contest-residents in Ross et al., 2004; losers in our study. Notably, in killifish, not all escalated contests lasted very long. Individuals that engaged in long contests showed depressed androgens. One explanation for this is that extended periods of dominance uncertainty cause a reduction in androgen production. Oliveira et al. (2005) employed mirror-image stimulation to evoke aggressive responses in cichlid fish, and they showed no significant change in post-aggression T or KT levels relative to controls perhaps because dominance is uncertain in the mirror tests. Their results, however, found a qualitative suppression in androgens at 30 min (T and KT) and 2 h (T) post-test. Lastly, cortisol levels in killifish losers, but not winners, also showed a positive relationship with escalation, which supports the status-dependent stress response shown by Earley et al. (2006) in convict cichlids.

Dominance reinforcement (post-retreat attacks) depressed post-fight T levels of losers and increased post-fight cortisol levels of winners in the killifish. Low T levels are common in subordinate, nonreproductive animals (Creel et al., 1997; Bales et al., 2006; Parikh et al., 2006; but see Carlson et al., 2004). Nothing is known about the social system of *K. marmoratus* in the wild, and we would not predict that social suppression of reproduction would be necessary in a hermaphrodite. However, it is possible that vertebrates' reproductive axes show similar responses to repeated attack or subordination. The positive relationship of cortisol with post-retreat attacks indicates that it can be stressful or energetically costly to maintain dominance aggressively (Goymann and Wingfield, 2004).

Conclusion

Reciprocity between hormones and contest behavior is often proposed but seldom examined in a single study (e.g., Mehta and Josephs, 2006). Although our procedures cannot assign causal links between pre-fight hormones, contest behavior, and post-fight hormones, we provide evidence that endogenous T, KT, and cortisol both predict and respond to behavior during a contest. We showed that the killifish endocrine systems are much more sensitive to contest dynamics (e.g., initiation, escalation, duration) than to winning or losing status, although the contestants' pre-fight endocrine profiles partly predicted both winners and contest dynamics. We were able to determine these relationships despite the absence of status differences in mean pre- or post-fight T, KT, or cortisol concentrations. Our results demonstrate that simply evaluating differences in mean hormone levels between statuses or social roles may underestimate or even mask the potent influence of hormones on behavior, and vice versa.

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References

- Archer, J., 2006. Testosterone and human aggression: an evaluation of the challenge hypothesis. *Neurosci. Biobehav. Rev.* 30, 319–345.
- Bales, K.L., French, J.A., McWilliams, J., Lake, R.A., Dietz, J.M., 2006. Effects of social status, age, and season on androgen and cortisol levels in wild male golden lion tamarins (*Leontopithecus rosalia*). *Horm. Behav.* 49, 88–95.
- Bégin, J., Beaugrand, J.P., Zay, R., 1996. Selecting dominants and subordinates at conflict outcome can confound the effects of prior dominance or subordination experience. *Behav. Process.* 36, 219–226.
- Buchner, A., Sloman, K., Balshine, S., 2004. The physiological effects of social status in the cooperatively breeding cichlid *Neolamprologus pulcher*. *J. Fish Biol.* 65, 1080–1095.
- Carlson, A.A., Young, A.J., Russell, A.F., Bennett, N.C., McNeilly, A.S., Clutton-Brock, T., 2004. Hormonal correlates of dominance in meerkats (*Suricata suricatta*). *Horm. Behav.* 46, 141–150.
- Clement, T.S., Parikh, V., Schrupf, M., Fernald, R.D., 2005. Behavioral coping strategies in a cichlid fish: the role of social status and acute stress response in direct and displaced aggression. *Horm. Behav.* 47, 336–342.
- Creel, S., Creel, N.M., Mills, M.G.L., Monfort, S.L., 1997. Rank and reproduction in cooperatively breeding African wild dogs: behavioral and endocrine correlates. *Behav. Ecol.* 8, 298–306.
- Desjardins, J.K., Hazelden, M.R., Van der Kraak, G.J., Balshine, S., 2006. Male and female cooperatively breeding fish provide support for the “Challenge Hypothesis”. *Behav. Ecol.* 17, 149–154.
- DiBattista, J.D., Anisman, H., Whitehead, M., Gilmour, K.M., 2005. The effects of cortisol administration on social status and brain monoaminergic activity in rainbow trout *Oncorhynchus mykiss*. *J. Exp. Biol.* 208, 2707–2718.
- Earley, R.L., Edwards, J.T., Aseem, O., Felton, K., Blumer, L.S., Karom, M., Grober, M.S., 2006. Social interactions tune aggression and stress responsiveness in a territorial cichlid fish (*Archocentrus nigrofasciatus*). *Physiol. Behav.* 88, 353–363.
- Ellis, T., James, J.D., Stewart, C., Scott, A.P., 2004. A non-invasive stress assay based upon measurement of free cortisol into the water by rainbow trout. *J. Fish Biol.* 65, 1233–1252.
- Elofsson, U.O.E., Mayer, I., Damsgård, B., Winberg, S., 2000. Internale competition in sexually mature arctic charr: effects on brain monoamines, endocrine stress responses, sex hormone levels, and behavior. *Gen. Comp. Endocrinol.* 118, 450–460.
- Fernandez, Z., Meunier-Salaun, M., Mormede, P., 1994. Agonistic behavior, plasma stress hormones, and metabolites in response to dyadic encounters in domestic pigs: interrelationships and effects of dominance status. *Physiol. Behav.* 56, 841–847.
- Francis, R.C., Jacobson, B., Wingfield, J.C., Fernald, R.D., 1992. Castration lowers aggression but not social dominance in male *Haplochromis burtoni* (Cichlidae). *Ethology* 90, 247–255.
- Grageda, M.V.C., Sakakura, Y., Minamimoto, M., Hagiwara, A., 2005. Differences in life-history traits in two clonal strains of the self-fertilizing fish, *Rivulus marmoratus*. *Environ. Biol. Fish.* 73, 427–436.
- Goymann, W., Wingfield, J.C., 2004. Allostatic load, social status, and stress hormones: the costs of social status matter. *Anim. Behav.* 67, 591–602.
- Haller, J., Halasz, J., Makara, G.B., Kruk, M.R., 1998. Acute effects of glucocorticoids: behavioral and pharmacological perspectives. *Neurosci. Biobehav. Rev.* 23, 337–344.
- Harrington Jr., R.W., 1963. Twenty-four-hour rhythms of internal self-fertilization and of oviposition by hermaphrodites of *Rivulus marmoratus*. *Physiol. Zool.* 36, 325–341.
- Harrington Jr., R.W., 1975. Sex determination and differentiation among uniparental homozygotes of the hermaphroditic fish *Rivulus marmoratus* (Cyprinodontidae: Antheriformes). In: Reinboth, R. (Ed.), *Intersexuality in the Animal Kingdom*. Springer-Verlag, New York, pp. 249–262.
- Hay, A.C., Pankhurst, N.W., 2005. Effects of paired encounters on plasma androgens and behaviour in males and females of the spiny damselfish *Acanthochromis polyacanthus*. *Mar. Freshw. Behav. Physiol.* 38, 127–138.
- Hirschenhauser, K., Oliveira, R.F., 2006. Social modulation of androgens in male vertebrates: meta-analyses of the challenge hypothesis. *Anim. Behav.* 71, 265–277.
- Hirschenhauser, K., Taborsky, M., Oliveira, T., Canario, A.V.M., Oliveira, R.F., 2004. A test of the ‘challenge hypothesis’ in cichlid fish: simulated partner and territory intruder experiments. *Anim. Behav.* 68, 741–750.
- Hosmer, D.W., Lemeshow, S., 2000. *Applied Logistic Regression*, 2nd edn. John Wiley & Sons, Inc., New York.
- Hsu, Y., Wolf, L.L., 1999. The winner and loser effect: integrating multiple experiences. *Anim. Behav.* 57, 903–910.
- Hsu, Y., Wolf, L.L., 2001. The winner and loser effect: what fighting behaviours are influenced? *Anim. Behav.* 61, 777–786.
- Hsu, Y., Earley, R.L., Wolf, L.L., 2006. Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. *Biol. Rev.* 81, 33–74.
- Johnsen, T., Zuk, M., 1995. Testosterone and aggression in male red jungle fowl. *Horm. Behav.* 29, 593–598.
- Knapp, R., Moore, M., 1995. Hormonal responses to aggression vary in different types of agonistic encounters in male tree lizards, *Urosaurus ornatus*. *Horm. Behav.* 29, 85–105.
- Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress physiology. *Neurosci. Biobehav. Rev.* 23, 925–933.
- Leshner, A.A., 1975. A model of hormones and agonistic behavior. *Physiol. Behav.* 15, 225–235.
- Mackiewicz, M., Tatarenkov, A., Taylor, D.S., Turner, B.J., Avise, J.C., 2006. Extensive outcrossing and androdioecy in a vertebrate species that otherwise reproduces as a self-fertilizing hermaphrodite. *Proc. Natl. Acad. Sci. U. S. A.* 103, 9924–9928.
- Mazur, A., Booth, A., 1998. Testosterone and dominance in men. *Behav. Brain Sci.* 21, 353–397.
- Mehta, P.H., Josephs, R.A., 2006. Testosterone change after losing predicts the decision to compete again. *Horm. Behav.* 50, 684–692.
- Mikics, E., Kruk, M.R., Haller, J., 2004. Genomic and non-genomic effects of glucocorticoids on aggressive behavior in male rats. *Psychoneuroendocrinology* 29, 618–635.
- Mikics, E., Barys, B., Haller, J., 2007. The effect glucocorticoids on aggressiveness in established colonies of rats. *Psychoneuroendocrinology* 32, 160–170.
- Muller, M., Wrangham, R.W., 2004. Dominance, cortisol and stress in wild chimpanzees (*Pan troglodytes schweinfurthii*). *Behav. Ecol. Sociobiol.* 55, 332–340.
- Neat, F.C., Mayer, I., 1999. Plasma concentrations of sex steroids and fighting in male *Tilapia zillii*. *J. Fish Biol.* 54, 695–697.
- Oliveira, R.F., Almada, V.C., Canário, A.V.M., 1996. Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*. *Horm. Behav.* 30, 2–12.
- Oliveira, R.F., Hirschenhauser, K., Carneiro, L.A., Canário, A.V.M., 2002. Social modulation of androgen levels in male teleost fish. *Comp. Biochem. Physiol. B* 132, 203–215.
- Oliveira, R.F., Carneiro, L.A., Canário, A.V.M., 2005. No hormonal response in tied fights. *Nature* 437, 207.
- Øverli, Ø., Harris, C.A., Winberg, S., 1999. Short-term effects of fights for social dominance and the establishment of dominant-subordinate relationships on brain monoamines and cortisol in rainbow trout. *Brain Behav. Evol.* 54, 263–275.
- Øverli, Ø., Kotzian, S., Winberg, S., 2002. Effects of cortisol on aggression and locomotor activity in rainbow trout. *Horm. Behav.* 42, 53–61.
- Øverli, Ø., Korzan, W.J., Larson, E.T., Winberg, S., Lepage, O., Pottinger, T.G., Renner, K.J., Summers, C.H., 2004. Behavioral and neuroendocrine correlates of displaced aggression in trout. *Horm. Behav.* 45, 324–329.

- Øverli, Ø., Winberg, S., Pottinger, T.G., 2005. Behavioral and neuroendocrine correlates of selection for stress responsiveness in rainbow trout—a review. *Integr. Comp. Biol.* 45, 463–474.
- Øverli, Ø., Sørensen, C., Pulman, K.G.T., Pottinger, T.G., Korzan, W., Summers, C.H., Nilsson, G.E., 2007. Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neurosci. Biobehav. Rev.* 31, 396–412.
- Oyegbile, T.O., Marler, C.A., 2005. Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. *Horm. Behav.* 48, 259–267.
- Parikh, V.N., Clement, T.S., Fernald, R.D., 2006. Androgen level and male social status in the African cichlid, *Astatotilapia burtoni*. *Behav. Brain Res.* 166, 291–295.
- Remage-Healey, L., Bass, A.H., 2005. Rapid elevations in both steroid hormones and vocal signaling during playback challenge: a field experiment in Gulf toadfish. *Horm. Behav.* 47, 297–305.
- Remage-Healey, L., Bass, A.H., 2006. From social behavior to neural circuitry: steroid hormones rapidly modulate advertisement calling via a vocal pattern generator. *Horm. Behav.* 50, 432–441.
- Ros, A.F.H., Bruinijes, R., Santos, R.S., Canario, A.V.M., Oliveira, R.F., 2004. The role of androgens in the trade-off between territorial and parental behavior in the Azorean rock-pool blenny, *Parablennius parvicornis*. *Horm. Behav.* 46, 491–497.
- Ross, C.N., French, J.A., Patera, K.J., 2004. Intensity of aggressive interactions modulates testosterone in male marmosets. *Physiol. Behav.* 83, 437–445.
- Sakakura, Y., Noakes, D.L.G., 2000. Age, growth, and sexual development in the self-fertilizing hermaphroditic fish *Rivulus marmoratus*. *Environ. Biol. Fish* 59, 309–317.
- Schuett, G.W., 1997. Body size and agonistic experience affect dominance and mating success in male copperheads. *Anim. Behav.* 54, 213–224.
- Schuett, G.W., Harlow, H.J., Rose, J.D., VanKirk, E.A., Murdoch, W.J., 1996. Levels of plasma corticosterone and testosterone in male copperheads (*Agkistrodon contortrix*) following staged fights. *Horm. Behav.* 30, 60–68.
- Scott, A.P., Ellis, T., 2007. Measurement of fish steroids in water—a review. *Gen. Comp. Endocrinol.* 153, 392–400.
- Sebire, M., Katsiadaki, I., Scott, A.P., 2007. Non-invasive measurement of 11-ketotestosterone, cortisol and androstenedione in male three-spined stickleback (*Gasterosteus aculeatus*). *Gen. Comp. Endocrinol.* 152, 30–38.
- Sloman, K.A., Metcalfe, N.E., Taylor, A.C., Gilmour, K.M., 2001. Plasma cortisol concentrations before and after social stress in rainbow and brown trout. *Physiol. Biochem. Zool.* 74, 383–389.
- Summers, C.H., Winberg, S., 2006. Interactions between the neural regulation of stress and aggression. *J. Exper. Biol.* 209, 4581–4589.
- Summers, C.H., Summers, T.R., Moore, M.C., Korzan, W.J., Woodley, S.K., Ronan, P.J., Höglund, E., Watt, M.J., Greenberg, N., 2003. Temporal patterns of limbic monoamine and plasma corticosterone response during social stress. *Neuroscience* 116, 553–563.
- Summers, C.H., Watt, M.J., Ling, T.L., Forster, G.L., Carpenter, R.E., Korzan, W.J., Lukkes, J.L., Øverli, Ø., 2005. Glucocorticoid interaction with aggression in non-mammalian vertebrates: reciprocal action. *Eur. J. Pharmacol.* 526, 21–35.
- Taylor, D.S., 2000. Biology and ecology of *Rivulus marmoratus*: new insights and a review. *Fla. Sci.* 63, 242–255.
- Taylor, D.S., Fisher, M.T., Turner, B.J., 2001. Homozygosity and heterozygosity in three populations of *Rivulus marmoratus*. *Environ. Biol. Fishes* 61, 455–459.
- Taylor, P.W., Elwood, R.W., 2003. The mismeasure of animal contests. *Anim. Behav.* 65, 1195–1202.
- Trainor, B.C., Bird, I.M., Marler, C.A., 2004. Opposing hormonal mechanisms of aggression revealed through short-lived testosterone manipulations and multiple winning experiences. *Horm. Behav.* 45, 115–121.
- Trainor, B.C., Kyomen, H.H., Marler, C.A., 2006. Estrogenic encounters: how interactions between aromatase and the environment modulate aggression. *Front. Neuroendocrinol.* 27, 170–179.
- Winberg, S., Lepage, O., 1998. Elevation of brain 5-HT activity, POMC expression, and plasma cortisol in socially subordinate rainbow trout. *Am. J. Physiol.* 274, R645–R654.
- Wingfield, J.C., 2005. A continuing saga: the role of testosterone in aggression. *Horm. Behav.* 48, 253–255.
- Zar, J.H., 1996. *Biostatistical Analysis*, 3rd Edition. Prentice-Hall, Upper Saddle River, NJ, p. 355.