

Androgen Correlates of Socially Induced Changes in the Electric Organ Discharge Waveform of a Mormyrid Fish

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Weakly electric fish from the family Mormyridae produce pulsatile electric organ discharges (EODs) for use in communication. For many species, male EODs are seasonally longer in duration than those of females, and among males, there are also individual differences in EOD duration. While EOD elongation can be induced by the administration of exogenous androgens, androgen levels have never before been assessed under natural or seminatural conditions. By simulating the conditions occurring during the breeding season in the laboratory, we provide evidence of a sex difference in EOD duration as well as document levels of circulating androgens in males. In this study, we analyzed the nature of social influences on male EOD duration and plasma androgen levels in *Brienomyrus brachyistius*. Individual males, first housed with a single female and then placed into social groups consisting of three males and three females, showed status-dependent changes in EOD duration. Top-ranking males experienced a relatively large increase in EOD duration. Second-ranking males experienced a more modest increase, and low-ranking males experienced a decrease in EOD duration. These changes were paralleled by differences in circulating levels of plasma 11-ketotestosterone (11-KT), but not testosterone, suggesting that the changes in EOD duration may have been mediated by changes in plasma 11-KT levels. Thus, it appears that EOD duration is an accurate indicator of male status, which is under social and hormonal control. © 2000 Academic Press

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African electric fish in the family Mormyridae produce pulsatile electric organ discharges (EODs) that are used for both active electrolocation and communication (Hopkins, 1986). The waveform of the EOD pulse is often a species-typical signature (Hopkins, 1981). For many mormyrids, sex differences in EODs arise during the breeding season (Bass and Hopkins, 1983, 1985; Hopkins, 1980, 1981; Hopkins and Bass, 1981; Kramer, 1997; Landsman, 1993a; Landsman and Moller, 1993). In all known cases, male EODs are longer than those of females. These species and sex differences in EOD waveform play an important role in electric communication (Hopkins and Bass, 1981; Kramer, 1997). Although there is also a high degree of individual variation in EOD duration (Crawford, 1992; Friedman and Hopkins, 1996), no study has yet addressed the social correlates of individual differences in EODs and their relation to communication.

In all known cases of species with sex differences in EOD waveforms, the EOD duration can be increased (masculinized) by administration of exogenous androgens in juveniles, females, and nonreproductive males (Bass, Denizot, and Marchaterre, 1986; Bass and Hopkins, 1983, 1985; Bass and Volman, 1987; Freedman, Olyarchuk, Marchaterre, and Bass, 1989; Herfeld and Moller, 1998; Landsman, Harding, Moller, and Thomas, 1990; Landsman and Moller, 1988). These results suggest that increased gonadal activity occurring at the onset of the breeding season results in elevated androgen levels that cause elongation of the EOD. To our knowledge, this inference has never been tested, nor have circulating androgen levels been reported in breeding animals. However, freshly imported *Gnathonemus petersii*, collected during the breeding season, showed sex differences in the EOD

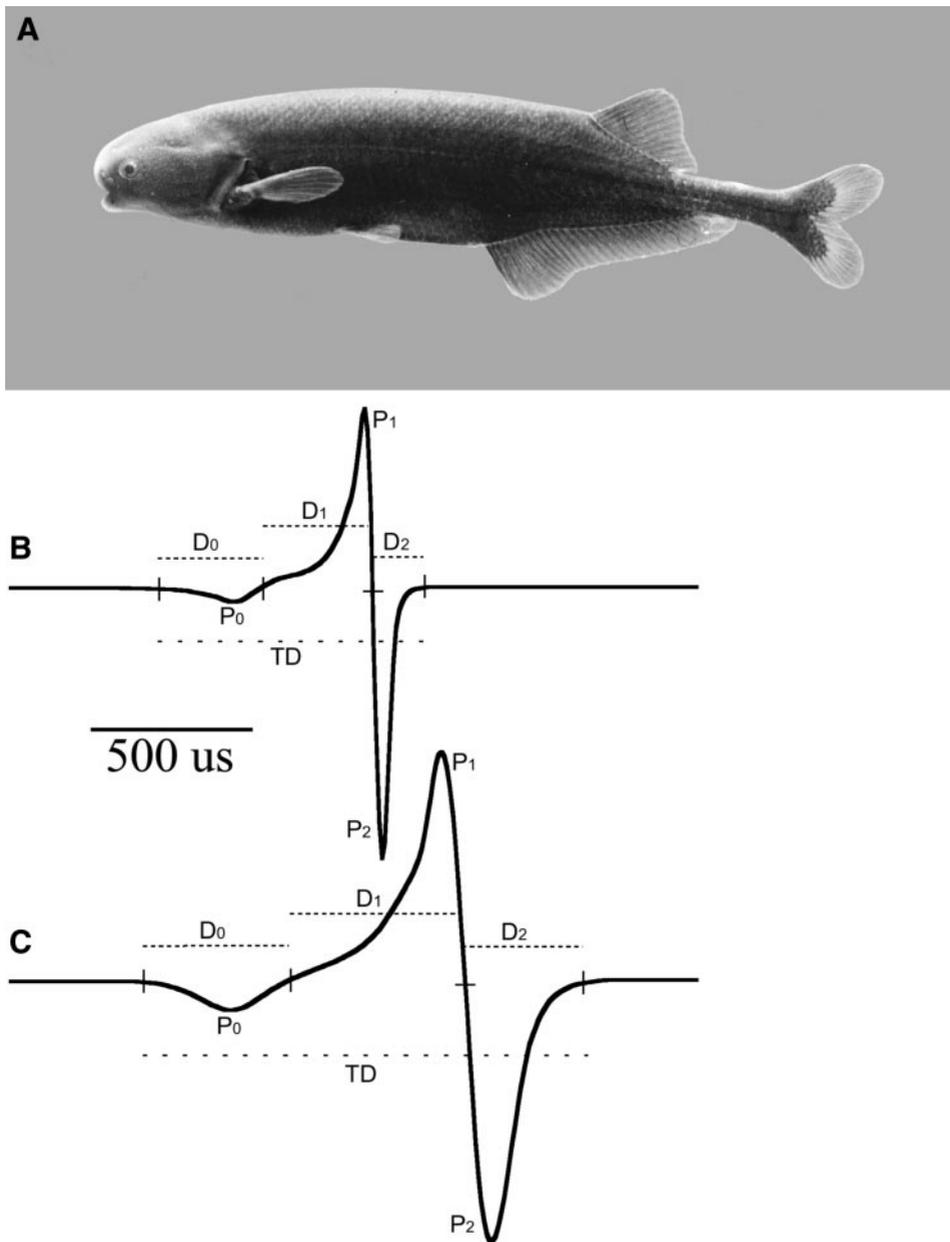


FIG. 1. Photograph of a male *Brienomyrus brachyistius* (A) and EODs of a female (B) and a male (C) *B. brachyistius* in breeding condition. Vertical scale is normalized for both EODs. The three peaks (P_0 , P_1 , and P_2) are noted as are the measurements for total duration (TD) and the duration of each phase (D_0 , D_1 , and D_2). Scale bar = 500 μ s.

which were gradually eliminated in captivity (Landsman, 1993b). In males, these captivity-induced changes in the EOD were correlated with decreases in plasma androgen levels, supporting the hypothesis that sex differences arising in the breeding season are induced by increases in androgen titers (Landsman, 1993b).

Our study species, *Brienomyrus brachyistius* (Gill,

1862; Fig. 1A), is a commonly available aquarium fish. We chose to work with *B. brachyistius* because we have bred this species successfully in the laboratory and it has been the subject of previous work on androgenic regulation of the EOD waveform (Bass and Hopkins, 1983, 1985; Bass and Volman, 1987; Freedman *et al.*, 1989). *Brienomyrus brachyistius* has a widespread distribution from Liberia to the Niger Delta to coastal

drainages in Cameroon and Gabon (Bigorne, 1989; Sullivan, Hopkins, and Arnegard, personal observation). Very little is known about the ecology and breeding behavior of this species or any other mormyrid in the field. However, it is common in slow-moving rocky areas of main river channels of the Ogooué River of Gabon at depths of 3 to 10 m (Sullivan, Hopkins, and Arnegard, personal observation). The EOD duration of captive, breeding males can range from 0.8 to 2.2 ms. Thus, *B. brachyistius* is an ideal species for studying individual variation in the EOD waveform.

If EOD duration is important for electrical communication, this marked variation might have biological relevance for receivers. One goal of the present study was to explore social factors underlying male variation in EOD duration. In distantly related Gymnotiform electric fish, EOD duration in males changes within hours following agonistic encounters, with winners experiencing an increased EOD duration and losers experiencing a decreased EOD duration (Franchina, Stoddard, Volmar, and Salazar, 1998; Hagedorn and Zelick, 1989). We hypothesized that EOD duration might also vary in relation to dominance status in mormyrids.

Since EOD duration is under the influence of androgens, we also hypothesized that the variation among males may be caused by differences in circulating androgen levels. Social interactions among males are known to have influences on androgen levels in a wide range of vertebrate species, including teleost fish, reptiles, birds, and mammals (Greenberg and Crews, 1990; Hannes, 1984; Hannes, Franck, and Lie-mann, 1984; Harding, 1981; Hegner and Wingfield, 1987b; Holberton, Able, and Wingfield, 1989; Mench and Ottinger, 1991; Munro and Pitcher, 1985; Sapol-sky, 1982; Wingfield, 1985; Wingfield and Wada, 1989). Agonistic encounters typically result in asym-metrical changes in circulating androgen levels; win-ners experience increases, while losers experience de-creases (Wingfield, Hegner, Dufty, and Ball, 1990).

Less well known are the relationships among status, androgen levels, and signaling behavior. In many cases, relative status among males is reflected in one or more signals, known as "badges of status" (see Møller, 1988; Rohwer, 1982; Rohwer and Ewald, 1981). In sparrows, plumage coloration is an honest indicator of status that is under androgen control (Rohwer and Ewald, 1981). However, plumage coloration is depen-dent on androgen levels only during molting, so it is a relatively fixed signal over short time spans. The EOD waveform of mormyrids, by contrast, can change in a

matter of days or weeks (Bass *et al.*, 1986; Bass and Hopkins, 1983, 1985; Freedman *et al.*, 1989) and is therefore more amenable to studying short-term ef-fects of status on signaling behavior. Therefore, a ma-jor goal of this study was to analyze the physiological basis for honest signaling in mormyrids in terms of the interplay between social interactions and plasma an-drogen levels. A portion of these results has appeared in abstract form (Carlson and Hopkins, 1998).

MATERIALS AND METHODS

Subjects

Adult mormyrids, identified as *B. brachyistius* were imported from Nigeria. Prior to the experiments, all individuals were housed in large (200- to 400-liter), single-species aquariums in groups of 20–40 individ-uals. Temperature was kept at 23–29°C, and conduc-tivity at 150–300 $\mu\text{S}/\text{cm}$, on a 12:12-h light:dark cycle. We fed fish live black worms daily. A total of 18 males (12.6–17.0 cm total length) and 18 females (7.9–11.0 cm total length) were used in these experiments. All individuals were sexually mature as indicated by their size, anal fin notches in males (Herfeld and Moller, 1998; Landsman, 1993a), and swollen abdomens in females (Crawford, 1992).

Experimental Protocol

Pretreatment period. Eighteen males were each in-dividually paired at random with a single female from the same holding tank and placed into separate 40-liter tanks for a period of 3 weeks. Over a period of 2 weeks, we gradually lowered water conductivity from 150–200 $\mu\text{S}/\text{cm}$ to 10–15 $\mu\text{S}/\text{cm}$ by partial water changes using deionized water. Lowered water con-ductivity is an important factor in stimulating repro-ductive behavior in *Pollimyrus isidori* and *Pollimyrus adspersus* (Crawford, 1992; Kirschbaum, 1995). Pairs were housed for an additional 7 days while maintain-ing lowered conductivity. We then recorded EODs from all individuals and took blood samples from each male. Water temperature was held at $26 \pm 1^\circ\text{C}$ during this 3-week period.

Social groups. Following the 3-week pretreatment period, we established 6 280-liter social tanks, each with 3 males and 3 females from the 18 pretreatment pairs. Male placement was determined by minimizing the size differences between males within each group (mean \pm SEM of maximum difference in total

length = 24.7 ± 1.9 mm). The social tanks were maintained at a conductivity of 10–15 $\mu\text{S}/\text{cm}$ and a temperature of $26 \pm 1^\circ\text{C}$. These conditions simulated natural breeding conditions as courtship and spawning behavior was observed in 3 of the 6 social tanks and fertilized offspring were produced in 2 of these tanks.

Prior to introduction into the social tanks, we determined the dominance relationships among the three males to be placed in each tank by placing two males at a time into a 20-liter tank during the day in a competition trial for a single plastic tube for shelter (see Hagedorn and Zelick, 1989; Hopkins, 1974). Mormyrids immediately seek and defend shelter during the day, so we determined relative dominance by scoring the outcome of an interaction for control of the shelter. A male was designated as dominant if it inhabited the shelter for five consecutive minutes, while its competitor was designated as subordinate. Dominance establishment took between 5 min and 3 h. We tested each male twice, once against each of its tank-mates, for a total of three tests per tank. The order of testing was randomized, with a minimum interval between trials of 2.5 h. Each of the six social groups had a linear dominance hierarchy, with the top-ranking (alpha) male, able to displace the second-ranking (beta) male and third-ranking (omega) male.

After establishing social groups, we recorded EODs for 10 consecutive days (days 0–9) and then intermittently on days 23–24, 32–34, and 39–41. Two recordings were made per day, one in the morning (0900–1100) and one in the evening (2000–2200), to account for possible diel changes in the EOD (Franchina and Stoddard, 1998). There was, however, no evidence of diel changes in EOD duration for any group, as determined by paired *t* tests ($P > 0.05$). Therefore, all EOD data are pooled in subsequent analyses. We took a final EOD measurement 46 days after social group establishment. Immediately after the final EOD recording, blood samples were taken from each male.

EOD Recording and Analysis

EODs were recorded with chlorided silver wire electrodes, digitized at 200 kHz with 16 bits with a Tucker Davis Technology XB2 analog-to-digital board and stored using custom-made software. We recorded six EODs from each fish during each recording session. Total EOD duration (TD) was determined by measuring the first and last points of the waveform that differed from baseline by more than 2% of the peak amplitude (Figs. 1B and 1C). The EOD of *B. brachyistius* consists of three phases, denoted as P_0 , P_1 ,

and P_2 (Figs. 1B and 1C). The start of the EOD to the first zero crossing marked the duration of P_0 (D_0), the first zero crossing to the second zero crossing marked the duration of P_1 (D_1), and the second zero crossing to the end of the EOD marked the duration of P_2 (D_2). We determined the peak power frequency (PPF) from the power spectrum of each EOD using the Fast Fourier Transform. Data from all six waves recorded from a fish in one session were averaged to yield a single data point.

Blood Sampling

All blood samples were taken between 1000 and 1100. Males were lightly anesthetized using tricaine methanesulfonate (MS-222; Sigma, St. Louis, MO), dissolved in aquarium water at a concentration of 100 mg/l. A 28-ga heparinized needle was inserted ventrolaterally, just dorsal to the anal fin and placed in the caudal vein immediately ventral to the spinal cord. Two to three hundred microliters of blood were collected from the vein in a syringe and placed in a microcentrifuge tube. Fish were revived and returned to their home tanks. Blood samples were then centrifuged at 1500 rpm for 7 min. The overlying plasma layers were removed and stored at -10°C for later analysis. These procedures are in accordance with the guidelines established by the National Institute of Health and the Cornell University Institutional Animal Care and Use Committee.

Hormone Analysis

Plasma levels of testosterone (T) and 11-ketotestosterone (11-KT) were determined for each male using radioimmunoassay (RIA; see Singh, Griffith, Takahashi, Kawauchi, Thomas, and Stegeman, 1988). Steroids were extracted from plasma (50 μl per assay) with a 70:30 mixture of hexane:ethyl acetate. The aqueous portion was removed and the solvent evaporated using a stream of N_2 . The steroids were then reconstituted in phosphate buffer and incubated with either T or 11-KT antiserum and the corresponding tritiated steroid tracer. Unbound steroid was removed by charcoal and subsequent centrifugation, and the amount of bound steroid tracer was determined with a scintillation counter. All T levels were determined in a single assay as were all 11-KT levels, so interassay coefficients of variation are not given. The intraassay coefficient of variation for T was 12.36% and for 11-KT was 17.57%. The minimum detectable concentration for the T assay was 44.74 pg/ml and for the 11-KT assay was

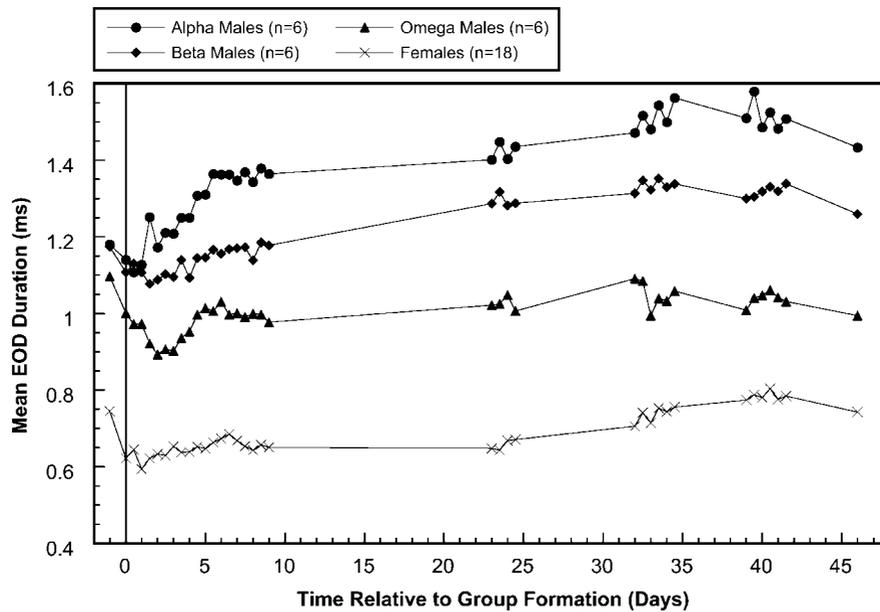


FIG. 2. Changes in mean EOD duration for the three groups of males and females throughout the course of the experiment. Day 0 (solid vertical line) refers to the day of social tank establishment. EODs were recorded starting the day before social tank establishment (Day -1) until 46 days after (Day 46). Error bars are omitted for clarity. A repeated-measures ANOVA demonstrated significant effects of status/sex, time, and a status/sex \times time interaction (see text for detailed statistics).

117.24 pg/ml. All samples yielded detectable levels of steroid.

Statistical Analyses

All statistical measures were computed using Statistica 5.1 (Statsoft, Inc., Tulsa, OK) for Windows. For all tests, α was set at 0.05. We used the *t* test, linear regression techniques, analysis of variance (ANOVA), and Newman-Keuls test for post hoc analyses.

RESULTS

EOD Waveform in the Pretreatment Period

At the end of the pretreatment period, the mean total duration (TD) of male EODs was 1.1507 ± 0.0654 ms ($n = 18$) and the mean TD of female EODs was 0.7443 ± 0.0553 ms ($n = 18$). Typical male and female EODs are shown in Figs. 1b and 1c. This difference is highly significant ($t = 4.7437$; $P < 0.0001$). Thus, the pretreatment condition confirms the existence of a sex difference in waveform.

Before placement into social groups, the males from each tank were scored as alpha (top-ranking), beta (second-ranking), or omega (third-ranking) males

based on relative status. Looking back at the EOD data from each male prior to determining relative status showed that the mean TDs in the pretreatment period were 1.1805 ± 0.1237 ms for alpha males ($n = 6$), 1.1744 ± 0.1058 ms ($n = 6$) for beta males, and 1.0972 ± 0.1279 ms for omega males ($n = 6$). These differences were not significant ($P > 0.05$). Thus, EOD duration did not influence the determination of dominance relationships.

Changes in the EOD Waveform in Social Groups

After introduction into the social tanks, the dominance hierarchy remained stable, as determined by qualitative observations of agonistic behavior in all six groups. Individual males frequently displayed aggressive acts such as head butts and charges toward subordinates, but the reverse was never observed.

In all three groups of males as well as females, TD initially decreased immediately following introduction into the social tanks, likely due to the stress of transfer (Fig. 2). Following this initial drop, the mean TD of females remained stable, while the mean TD of males changed rapidly in a status-dependent manner (Fig. 2). Nine days after social group formation, the mean TD of alpha males increased to 1.4113 ± 0.1407

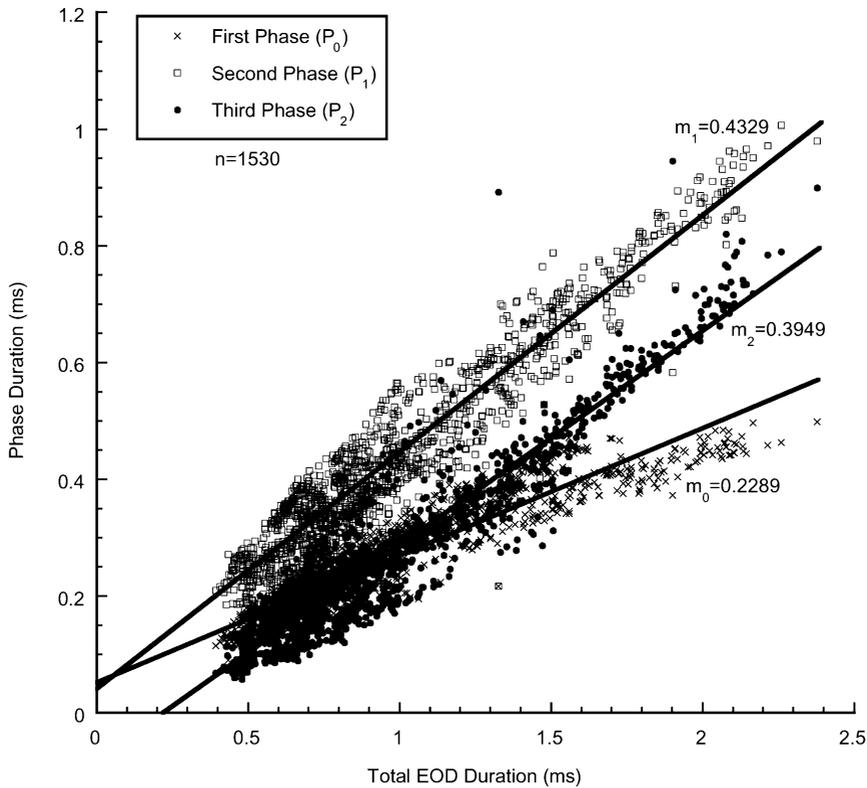


FIG. 3. Correlations between total EOD duration (TD) and the duration of each of the three phases (P_0 , P_1 , and P_2). Multiple-regression analysis showed that the EOD duration of all three phases correlated with total EOD duration ($r^2 > 0.99$; $P < 0.0001$; P_0 : $m_0 = 0.2289$, $P < 0.00001$; P_1 : $m_1 = 0.4329$, $P < 0.00001$; P_2 : $m_2 = 0.3949$, $P < 0.00001$). The slope of each line differs, however, demonstrating a difference in the influence of each phase on TD.

ms (+19.55%), the mean TD of beta males decreased slightly to 1.1611 ± 0.1852 ms (-1.13%), and the mean TD of omega males decreased to 0.9742 ± 0.0695 ms (-11.21%).

Following this initial period of divergence in TD, the EODs of all three groups remained relatively stable (Fig. 2). Forty-six days after social group formation, the mean TD of alpha males was 1.4331 ± 0.1898 ms, that of beta males was 1.2594 ± 0.1717 ms, and that of omega males was 0.9947 ± 0.1059 ms. These changes represent relative changes of +21.40%, +7.24%, and -9.34% , respectively, relative to pretreatment TD. Therefore, the direction and magnitude of changes in EOD duration were dependent on relative status. By contrast, the TD of females was 0.7430 ± 0.0416 ms 46 days after social group formation, a decrease of only -0.1747% . Thus, on average, TD in females was not affected by social group formation.

We used a repeated-measures analysis of variance (ANOVA) to analyze changes in TD using status/sex (alpha, beta, omega, or female), time (days 0–46), and

social tank number (to control for differences in variability within and between tanks) as independent variables and TD as the dependent variable. Highly significant effects were seen for status/sex [$F_{(3,12)} = 94.891$; $P < 0.0001$], time [$F_{(37,444)} = 27.316$; $P < 0.0001$], and the status/sex \times time interaction [$F_{(111,444)} = 2.976$; $P < 0.0001$]. A post hoc analysis on the effects of status/sex indicated significant differences for all comparisons (Newman-Keuls test; $P < 0.05$). Thus, TD was highly dependent on status/sex, TD changed significantly over time, and the direction and degree of change was dependent on status/sex.

Multiple-regression analysis showed that changes in the EOD duration of all three phases (D_0 , D_1 , and D_2) paralleled those in total EOD duration (Fig. 3; $r^2 > 0.99$; $P < 0.0001$; D_0 : $\beta = 0.2289$, $P < 0.00001$; D_1 : $\beta = 0.4329$, $P < 0.00001$; D_2 : $\beta = 0.3949$, $P < 0.00001$). Thus, changes in EOD duration arise from changes in all three phases and not just a single phase. However, the difference in slopes of the three lines (0.2289 for D_0 , 0.4329 for D_1 , and 0.3949 for D_2) indi-

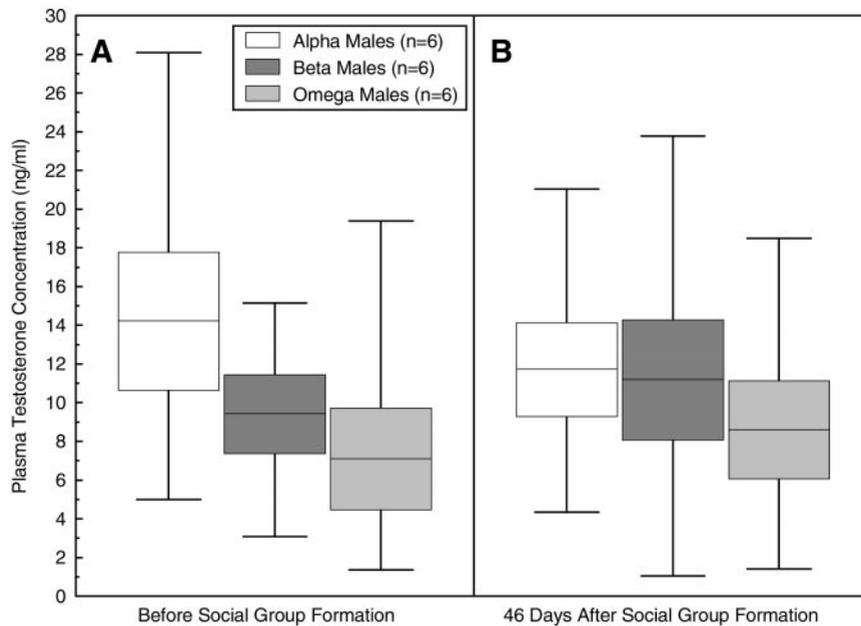


FIG. 4. Box plots of plasma testosterone levels for the three groups of males before (A) and 46 days after (B) social group establishment. Horizontal lines refer to the mean, boxes mark the standard error of the mean, and bars refer to the total range of values. There were no significant differences before or after ($P > 0.05$).

cates that the greatest change resulted from changes in P_1 , less so from P_2 , and minimally from P_0 . Changes in TD were also paralleled by changes in peak power frequency (PPF) of the EODs. PPF correlated negatively with changes in TD ($r^2 = 0.57$; $P < 0.01$).

Changes in Plasma Androgen Levels

Plasma levels of testosterone (T) varied from 1.04 ng/ml to 28.09 ng/ml, measured at the beginning and end of the experiment. Plasma levels of 11-ketotestosterone (11-KT) varied from 0.47 ng/ml to 27.14 ng/ml. There was a significant correlation between individual plasma T levels and 11-KT levels ($r^2 = 0.23$; $P < 0.003$).

Testosterone. Before the establishment of social groups, mean plasma levels of T showed a trend relating to status, with alpha males having the highest levels and omega males having the lowest (Fig. 4A). However, these differences were not significant ($P > 0.05$). At the end of the experiment, there were no significant differences in plasma T levels among the three groups of males ($P > 0.05$; Fig. 4B).

11-Ketotestosterone. At the beginning of the experiment, plasma levels of 11-KT did not significantly differ across the three groups of males ($P > 0.05$; Fig. 5A). By the end of the experiment, average plasma

11-KT levels correlated with status, alpha males having the highest levels, followed by beta males and finally omega males (Fig. 5B). These differences were significant [$F_{(2, 15)} = 5.07$; $P < 0.025$]. A post hoc analysis of the data demonstrated that plasma 11-KT levels of alpha males were significantly greater than plasma 11-KT levels of omega males (Newman-Keuls test; $P < 0.02$). There was no significant difference between alpha and beta males ($P > 0.05$) or beta and omega males ($P > 0.05$).

DISCUSSION

Our results confirm the existence of a sex difference in EODs for *B. brachyistius* and demonstrate that it can arise in captivity when lowering water conductivity, as occurs in *Pollimyrus isidori* (Crawford, 1992). In addition, we have shown that EOD duration correlates with relative status among males. High-ranking males have longer EODs and lower PPFs than low-ranking males, and these differences result from correlated differences among all three phases. Moreover, EOD duration is a plastic feature that changes in response to newfound social status.

Field and laboratory observations of numerous species of mormyrids demonstrate that there is often

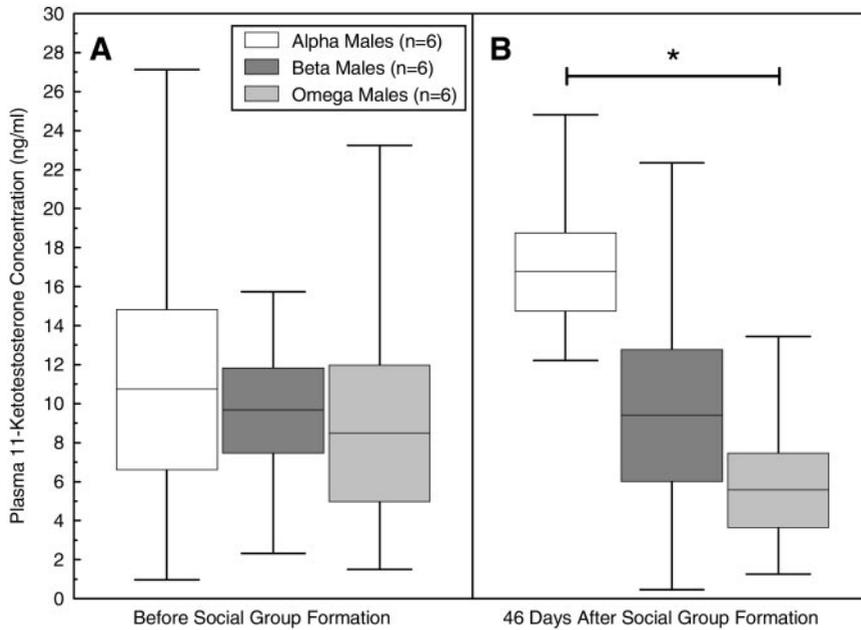


FIG. 5. Box plots of plasma 11-ketotestosterone levels for the three groups of males before (A) and 46 days after (B) social group establishment. For description of plot layout, see legend to Fig. 4. There were no significant differences before establishment ($P > 0.05$), but there were significant differences 46 days after establishment ($F_{(2, 15)} = 5.07$; $P < 0.025$). A post hoc analysis using a Newman-Keul's test demonstrated a significant difference between alpha and omega males ($P < 0.02$), but not between alpha and beta males ($P > 0.05$) or beta and omega males ($P > 0.05$).

marked individual variation (Crawford, 1992; Friedman and Hopkins, 1996), and a study using trained fish demonstrated distinct responses to EODs of different conspecifics (Graff and Kramer, 1989). It is unclear, however, whether these different responses relate to individual recognition or some feature of the signal that relates to another meaningful variable, such as relative status. Additional studies are needed to address the causal factors underlying individual variation across a wide range of species as well as determine the significance of these differences to both male and female conspecifics.

Electrical communication has evolved independently in the South American order Gymnotiformes, and numerous convergent features are seen in relation to mormyrids (Hopkins, 1995). In two species of Gymnotiformes, pairwise encounters between males resulted in rapid (2- to 24-h) changes in EOD amplitude, duration, and spectral features; winners experienced increases in duration and amplitude and decreases in peak power frequencies, while losers experienced opposite effects (Franchina *et al.*, 1998; Hagedorn and Zelick, 1989). This suggests that, as in mormyrids, individual variation in EOD duration may relate to relative status.

The levels of both 11-ketotestosterone (11-KT) and testosterone (T) in *B. brachyistius* males are within the range seen among teleosts in general (Borg, 1994). They are higher than levels seen in the Gymnotiform electric fish *Sternopygus macrurus* (Zakon, Thomas, and Yan, 1991). In the only other mormyrid studied, *Gnathonemus petersii*, the levels of T and 11-KT were lower than those observed here (Landsman, 1993b). This may reflect a species difference in androgen levels. However, the fish used in this study were freshly imported during the breeding season and not engaged in breeding behavior in the laboratory. Therefore, these lower levels may be due to stress from transfer and likely do not reflect the naturally occurring androgen levels of breeding animals. In both mormyrids and gymnotiforms, masculinization of the EOD can be induced by the administration of exogenous androgens (for reviews see Zakon, 1993, 1996). In this study, status-dependent differences in the EOD were paralleled by differences in plasma levels of 11-KT, suggesting that androgens may also be involved in mediating individual variation in EOD waveform.

It is well established that agonistic interactions can have major effects on circulating levels of androgens in a wide range of vertebrates (for review see Wing-

field *et al.*, 1990). As in *B. brachyistius*, relative status generally shows a positive correlation with androgen levels, and differences relating to status are especially pronounced shortly after an aggressive encounter. However, these differences generally persist only through times of social instability (Wingfield *et al.*, 1990). In this study, despite the stability of the dominance hierarchies, we observed disparate levels of plasma 11-KT 46 days after social group formation.

This difference may result from differences in breeding strategies and the period of female receptivity. Most of the species studied are birds in which mating occurs during a relatively fixed, narrow window of time and in which males and females both provide parental care (Holberton *et al.*, 1989; Mench and Ottinger, 1991; Wiley, Piper, Archawaranon, and Thompson, 1993; Wingfield, 1985; Wingfield and Wada, 1989). In the absence of renewed mating opportunities, the benefits to having elevated androgen levels are reduced (Wingfield *et al.*, 1990), and the role of androgens in aggression and mate attraction is incompatible with paternal care (Hegner and Wingfield, 1987a; Wingfield, 1984). In contrast, both field and laboratory observations suggest that many species of mormyrid may breed throughout the rainy season (Crawford, Jacob, and Benech, 1997; Carlson, Krosby, Boone, and Hopkins, personal observation). In addition, we have not observed any form of parental care in *B. brachyistius*; instead, fertilized eggs are left at the bottom of the tank following spawning (Carlson, Krosby, Boone, and Hopkins, personal observation).

Alternatively, in the natural environment of mormyrids, stable hierarchies may not exist. Given the turbulent nature of a riverine environment, especially during the rainy season, there is likely to be high individual turnover in a given location. A given individual may therefore have to establish a pairwise dominance relationship frequently. EOD duration would thereby serve as an androgen-sensitive "badge of status" that correlates with relative quality and is used in intermale assessment (Møller, 1988; Rohwer, 1982; Rohwer and Ewald, 1981). Field studies of the ecology and behavior of mormyrids are needed to clarify these issues and address the adaptive significance and evolutionary basis for androgen-sensitive, status-dependent signaling behavior in weakly electric fish.

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REFERENCES

- Bass, A. H., Denizot, J. P., and Marchaterre, M. A. (1986). Ultrastructural features and hormone-dependent sex differences of mormyrid electric organs. *J. Comp. Neurol.* **254**(4), 511–528.
- Bass, A. H., and Hopkins, C. D. (1983). Hormonal control of sexual differentiation: Changes in electric organ discharge waveform. *Science* **220**(4600), 971–974.
- Bass, A. H., and Hopkins, C. D. (1985). Hormonal control of sex differences in the electric organ discharge of mormyrid fishes. *J. Comp. Physiol. A* **156**(5), 587–604.
- Bass, A. H., and Volman, S. F. (1987). From behavior to membranes: Testosterone-induced changes in action potential duration in electric organs. *Proc. Natl. Acad. Sci.* **84**(24), 9295–9298.
- Bigorne, R. (1989). The genera *Brienomyrus* and *Isichthys* (Pisces; Mormyridae) from west Africa. *Rev. Hydrobiol. Trop.* **22**(4), 317–338.
- Borg, B. (1994). Androgens in teleost fishes. *Comp. Biochem. Physiol. C* **109**, 219–245.
- Carlson, B. A., and Hopkins, C. D. (1998). Socially-mediated changes in the electric organ discharge of a mormyrid fish. In *International Congress for Neuroethology*, p. 328.
- Crawford, J. D. (1992). Individual and sex specificity in the electric organ discharges of breeding mormyrid fish (*Pollimyrus isidori*). *J. Exp. Biol.* **164**, 79–102.
- Crawford, J. D., Jacob, P., and Benech, V. (1997). Sound production and reproductive ecology of strongly acoustic fish in Africa: *Pollimyrus isidori*, Mormyridae. *Behav.* **134**(9–10), 677–725.
- Franchina, C. R., and Stoddard, P. K. (1998). Plasticity of the electric organ discharge waveform of the electric fish *Brachyhyppopomus pinnicaudatus* I. Quantification of day-night changes. *J. Comp. Physiol. A* **183**(6), 759–768.
- Franchina, C. R., Stoddard, P. K., Volmar, C. H., and Salazar, V. (1998). Social stimulation elicits both sudden and gradual increases in duration and amplitude of the electric organ discharge of male gymnotiform electric fish. In *International Congress of Neuroethology*, p. 328.
- Freedman, E. G., Olyarchuk, J., Marchaterre, M. A., and Bass, A. H. (1989). A temporal analysis of testosterone-induced changes in electric organs and electric organ discharges of mormyrid fishes. *J. Neurobiol.* **20**(7), 619–634.
- Friedman, M. A., and Hopkins, C. D. (1996). Tracking individual mormyrid electric fish in the field using electric organ discharge waveforms. *Anim. Behav.* **51**(2), 391–407.
- Gill, T. (1862). On the West African genus *Hemichromis* and description of new species in the museums of the Academy and Smithsonian Institutions. *Proc. Natl. Acad. Sci. Phila.* **14**, 134–139.
- Graff, C., and Kramer, B. (1989). Trained weakly electric fishes *Pollimyrus isidori* and *Gnathonemus petersii* (Mormyridae, Teleostei) discriminate between waveforms of electric pulse discharges. *Ethology* **90**, 279–292.
- Greenberg, N., and Crews, D. (1990). Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. *Gen. Comp. Endocrinol.* **77**, 246–255.

- Hagedorn, M., and Zelick, R. (1989). Relative dominance among males is expressed in the electric organ discharge characteristics of a weakly electric fish. *Anim. Behav.* **38**(3), 520–525.
- Hannes, R. (1984). Androgen and corticoid levels in blood and body extracts of high- and low-ranking swordtail males (*Xiphophorus helleri*) before and after social isolation. *Z. Tierpsychol.* **66**, 70–76.
- Hannes, R., Franck, D., and Liemann, F. (1984). Effects of rank-order fights on whole-body and blood concentrations of androgens and corticosteroids in the male swordtail (*Xiphophorus helleri*). *Z. Tierpsychol.* **65**, 53–65.
- Harding, C. F. (1981). Social modulation of circulating hormone levels in the male. *Am. Zool.* **21**, 223–231.
- Hegner, R. E., and Wingfield, J. C. (1987a). Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* **104**, 462–469.
- Hegner, R. E., and Wingfield, J. C. (1987b). Social status and circulating levels of hormones in flocks of house sparrows, *Passer domesticus*. *Ethology* **76**, 1–14.
- Herfeld, S., and Moller, P. (1998). Effects of 17 α -methyltestosterone on sexually dimorphic characters in the weakly electric fish, *Brienomyrus niger* (Gunther, 1866) (Mormyridae): Electric organ discharge, ventral body wall indentation, and anal-fin ray bone expansion. *Horm. Behav.* **34**(3), 303–319.
- Holberton, R. L., Able, K. P., and Wingfield, J. C. (1989). Status signalling in dark-eyed juncos, *Junco hyemalis*: Plumage manipulations and hormonal correlates of dominance. *Anim. Behav.* **37**, 681–689.
- Hopkins, C. D. (1974). Electric communication: Functions in the social behavior of *Eigenmannia virescens*. *Behaviour* **50**, 270–305.
- Hopkins, C. D. (1980). Evolution of electric communication channels of mormyrids. *Behav. Ecol. Sociobiol.* **7**, 1–13.
- Hopkins, C. D. (1981). On the diversity of electric signals in a community of mormyrid electric fish in west Africa. *Am. Zool.* **21**, 211–222.
- Hopkins, C. D. (1986). Behavior of Mormyridae. In T. H. Bullock and W. Heiligenberg (Eds.), *Electroreception*, pp. 527–576. Wiley, New York.
- Hopkins, C. D. (1995). Convergent designs for electrogenesis and electroreception. *Curr. Opin. Neurobiol.* **5**(6), 769–777.
- Hopkins, C. D., and Bass, A. H. (1981). Temporal coding of species recognition signals in an electric fish. *Science* **212**(4490), 85–87.
- Kirschbaum, F. (1995). Reproduction and development in mormyrid form and gymnotiform fishes. In P. Moller (Ed.), *Electric Fishes—History and Behavior*, pp. 267–301. Chapman and Hall, London.
- Kramer, B. (1997). Electric organ discharges and their relation to sex in mormyrid fishes. *Naturwissenschaften* **84**(3), 119–121.
- Landsman, R. E. (1993a). Sex differences in external morphology and electric organ discharges in imported *Gnathonemus petersii* (Mormyridae). *Anim. Behav.* **46**(3), 417–429.
- Landsman, R. E. (1993b). The effects of captivity on the electric organ discharge and plasma hormone levels in *Gnathonemus petersii* (Mormyridae). *J. Comp. Physiol. A* **172**, 619–631.
- Landsman, R. E., Harding, C. F., Moller, P., and Thomas, P. (1990). The effects of androgens and estrogen on the external morphology and electric organ discharge waveform of *Gnathonemus petersii* (Mormyridae; Teleostei). *Horm. Behav.* **24**(4), 532–553.
- Landsman, R. E., and Moller, P. (1988). Testosterone changes the electric organ discharge and external morphology of the mormyrid fish *Gnathonemus petersii* (Mormyridae). *Experientia* **44**(10), 900–903.
- Landsman, R. E., and Moller, P. (1993). Captivity and signal plasticity in mormyrid fish communication. *J. Comp. Physiol. A* **173**(6), 732–733.
- Mench, J. A., and Ottinger, M. A. (1991). Behavioral and hormonal correlates of social dominance in stable and disrupted groups of male domestic fowl. *Horm. Behav.* **25**, 112–122.
- Møller, A. P. (1988). Variation in badge size in male house sparrows *Passer domesticus*. *Anim. Behav.* **35**, 1637–1644.
- Munro, A. D., and Pitcher, T. J. (1985). Steroid hormones and agonistic behavior in a cichlid teleost, *Aequidens pulcher*. *Horm. Behav.* **19**, 353–371.
- Rohwer, S. A. (1982). The evolution of reliable and unreliable badges of fighting ability. *Am. Zool.* **22**, 531–546.
- Rohwer, S. A., and Ewald, P. W. (1981). The cost of dominance and advantage of subordination in a badge signalling system. *Evolution* **35**, 441–454.
- Sapolsky, R. M. (1982). The endocrine stress response and social status in the wild baboon. *Horm. Behav.* **16**, 279–292.
- Singh, H., Griffith, R. W., Takahashi, A., Kawauchi, H., Thomas, P., and Stegeman, J. J. (1988). Regulation of gonadal steroidogenesis in *Fundulus heteroclitus* by recombinant salmon growth hormone and purified salmon prolactin. *Gen. Comp. Endocrinol.* **72**, 144–153.
- Wiley, R. H., Piper, W. H., Archawaranon, M., and Thompson, E. W. (1993). Singing in relation to social dominance and testosterone in white-throated sparrows. *Behavior* **127**(3–4), 175–190.
- Wingfield, J. C. (1984). Androgens and mating systems: Testosterone-induced polygyny in normally monogamous birds. *Auk* **101**, 665–671.
- Wingfield, J. C. (1985). Short-term changes in plasma levels of hormones during establishment and defense of a breeding territory in male song sparrows, *Melospiza melodia*. *Horm. Behav.* **19**, 174–187.
- Wingfield, J. C., Hegner, R. E., Dufty, A. M., and Ball, G. F. (1990). The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am. Nat.* **136**(6), 829–846.
- Wingfield, J. C., and Wada, M. (1989). Changes in plasma levels of testosterone during male-male interactions in the song sparrow, *Melospiza melodia*: time course and specificity of response. *J. Comp. Physiol. A* **166**, 189–194.
- Zakon, H. H. (1993). Weakly electric fish as model systems for studying long-term steroid action on neural circuits. *Brain Behav. Evol.* **42**(4–5), 242–251.
- Zakon, H. H. (1996). Hormonal modulation of communication signals in electric fish. *Dev. Neurosci.* **18**(1–2), 115–123.
- Zakon, H. H., Thomas, P., and Yan, H. (1991). Electric organ discharge frequency and plasma sex steroid levels during gonadal recrudescence in a natural population of the weakly electric fish *Sternopygus macrurus*. *J. Comp. Physiol. A* **169**, 493–499.