112 Yoshimasa, T., Sibley, D. R., Bouvier, M., Lefkowitz, R. J., and Caron, M. G., Cross-talk between cellular signalling pathways suggested by phorbol-ester induced adenylate cyclase phosphorylation. Nature *327* (1987) 67–70.
113 Yu, G., Smitgall, T. E., and Glazer, R. I., K562 cells transfected with the human c-fes gene acquire the ability to undergo myeloid differentiation. J. biol. Chem. *264* (1989) 10276–10281.
114 Zannis, V. I., Cole, F. S., Jackson, C. L., Kurnit, D. M., and Karathanasis, S. K., Distribution of apolipoprotein A-I, C-II, C-III, and E mRNA in human fetal tissues. Time-dependent induction of apolipoprotein E mRNA by cultures of human monocyte-macrophages. Biochemistry *24* (1985) 4450–4455.

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Captivity affects behavioral physiology: Plasticity in signaling sexual identity

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Summary. Little is known about the link between captivity, physiology, and behavior in wild-caught vertebrates. Anecdotal evidence suggests that hormonal changes are responsible for behavioral changes in wild animals brought into captivity. Studying the effects of captivity on reproduction is hampered because wild animals often fail to exhibit sexual behavior under captive conditions. In weakly discharging electric fish, field studies have reported sex differences in electric organ discharges which are rarely seen in the laboratory. I now report the results of a series of laboratory investigations which show that *Gnathonemus petersii* exhibits seasonal, hormone-dependent, phase-specific sex differences in electric organ discharges. Captivity dramatically alters and may even reverse these sex differences as a result of rapid changes in endogenous plasma hormone levels. These findings have broad implications for research on animal physiology and behavior performed in laboratory settings.
Key words. Captivity; electric organ discharge (EOD); sex differences; plasma hormone levels; androgens; estrogen; external morphology; behavioral plasticity.

Introduction

Laboratory research performed on wild animals is typically generalized to naturalistic settings with little regard to the effects of captivity upon the phenomena under investigation. While it is known that captivity has profound effects on both behavior and reproductive physiology in most vertebrates[8, 19, 25–27, 29, 38], the physiological causes underlying the behavioral differences found in field versus laboratory settings have only been surmised. The fact that animals often do not show sexual behavior under captive conditions[19, 25, 29] has made it particularly difficult to study the mechanisms by which reproduction may be inhibited in wild-caught species.

Here I report on a series of laboratory investigations which show robust and replicable captivity effects on the communication of sexual identity in a weakly discharging electric fish, *Gnathonemus petersii.* Newly imported fish exhibit clear hormone-dependent sex differences in the duration of specific phases of their electric organ discharges (EODs). In the laboratory, these sex differences are dramatically altered and may even reverse as a function of profound changes in plasma gonadal steroid hormone levels. Together, these studies provide the first direct evidence of how captivity alters reproductive behavior and its underlying physiology, and explain numerous discrepancies concerning signaling of sex differences in the weakly discharging electric fish.

Laboratory and field studies suggest the EOD of weakly discharging electric fish is used in social communication, species and sexual identification, and possibly as warning signals analogous to the alarm calls of other vertebrates[13–15, 18, 22–24]. Field studies employing relatively small samples have reported largely descriptive, non-statistical accounts of natural sex differences, with considerable variability and overlap between the sexes, in EOD waveform, duration, or pattern of discharge for several species of African mormyrid and South American gymnotiform electric fish species[2, 10, 11]. These field-reported sex differences are rarely observed in the laboratory. Both laboratory and field studies have employed hormone manipulations to induce male- or female-like EODs[3, 6], indicating that these sexual characters are steroid sensitive.

In a previous laboratory study[18], we found a sex difference in EODs of *Gnathonemus petersii,* with males exhibiting shorter EODs and higher peak power spectral frequencies (PPSFs) of the Fourier transformation (fig. 1a). This was surprising because field reports suggested that males of several other mormyrid species have longer-duration EODs and lower PPSFs than females[2, 10] and *G. petersii* administered male hormones exhibited increases in EOD duration and decreases in PPSF[17]. The present studies were designed to further

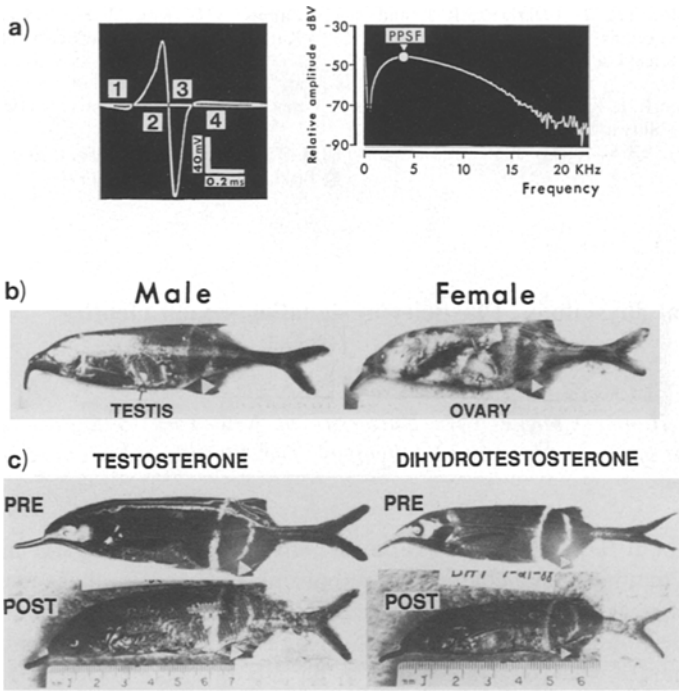


Figure 1. The EOD and Fourier transformation obtained from oscilloscope and spectrum analyzer, respectively, and external sexual character in the mormyrid *G. petersii*. *a* Left: The four phases of the EOD. (1) Phase 1: a slightly negative initial phase, (2) phase 2: a relatively long duration high amplitude positive phase, (3) phase 3: a long duration high negative amplitude phase, (4) phase 4: a slightly positive amplitude phase of highly variable duration. Right: Fourier transformation. Arrow points to peak power spectral frequency (PPSF). *b* External morphology of adult male and female fish. Arrowheads point to sexually dimorphic indentation in the dorsal margin of the anal fin. *c* Androgen-dependence of external sexual dimorphism. Note dorsal margin of the anal fin in juvenile fish (arrowheads) before (top) and 30 days following treatment with implanted T or DHT (bottom). Estradiol did not induce this indentation in juveniles or adult females.

investigate the endocrine basis for the EOD sex difference in *G. petersii* and to experimentally address captivity as a possible confound in laboratory research.

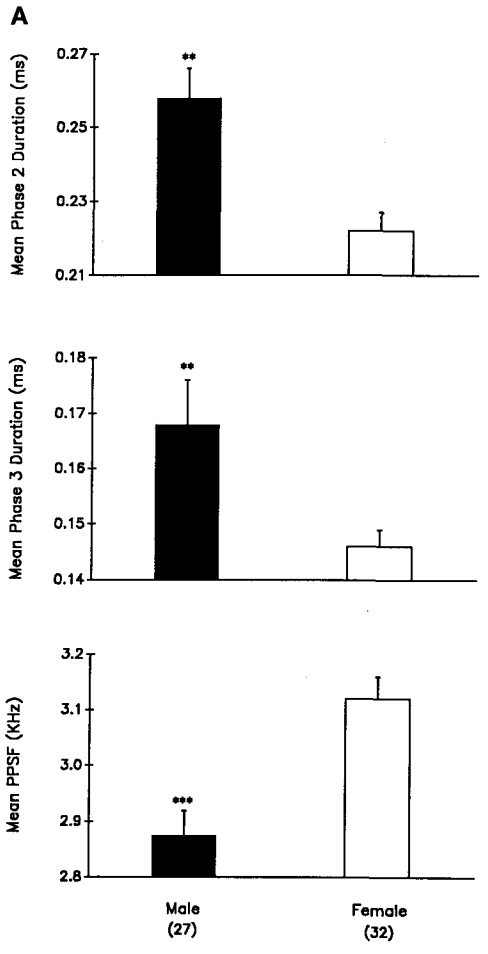
Phase-specific sex differences in EODs

Methods. In an attempt to replicate our previous sex difference findings, I recorded EODs from 27 male and 32 female adult, gonadally ripe (determined by female egg size and color and by testis size and consistency) *G. petersii* on the day they were received from Nigeria during the rainy breeding season. Males were identified by the distinct, hormonally-controlled indentation at the dorsal margin of the anal fin (fig. 1b). EODs were recorded while fish were at rest in a porous ceramic shelter at the bottom of their individual aquaria. The same pair of Ag/AgCl electrodes was used for all subjects to ensure that variability in EODs between subjects and studies was not a function of different input impedances. The stationary electrodes were located approximately 8 cm from head and tail of the fish when at rest in its porous ceramic shelter. EODs were fed directly into an oscilloscope (Tektronix, model 455, input impedance = 1M ohm, high frequency bandpass filter) which triggered a spectrum analyzer (Hewlett-Packard, model 3582A, range: 0–25 kHz, resolution: 100 Hz).

Results and discussion. EODs of *G. petersii* have four phases (fig. 1a). One-way ANOVAs were computed to assess for sex differences in EOD parameters. Large sex differences were obtained only in the durations of phases 2 and 3 (fig. 2A), with males (means ± SEMs: phase 2 = 0.26 ± 0.01 ms, phase 3 = 0.169 ± 0.005 ms) exhibiting longer phases than females (means ± SEMs: phase 2 = 0.22 ± 0.01 ms, phase 3 = 0.146 ± 0.003 ms) [$F(1,57) = 12.24$, $p < 0.001$, and $F(1,57) = 11.91$, $p < 0.001$, respectively]. These sex differences in phase are reflected in the Fourier transformations with males (mean ± SEM = 2.875 ± 0.047 kHz) exhibiting significantly lower PPSFs than females (mean ± SEM = 3.120 ± 0.037 kHz) [$F(1,57) = 16.86$, $p < 0.0001$] (fig. 2A). These findings are opposite those reported in our earlier sex difference study[18], but are in accord with the effects of androgens on the EOD in this species[17].

Hormonal control of EOD sex differences

Methods. To investigate the hormonal control of these sex differences, testosterone (T), dihydrotestosterone (DHT), estradiol 17β (E_2), or DHT + E_2 in silastic capsules were implanted into gonadectomized juvenile *G. petersii*. Following pre-implant data collection, fish were anesthetized (buffered tricaine methane sulfonate,



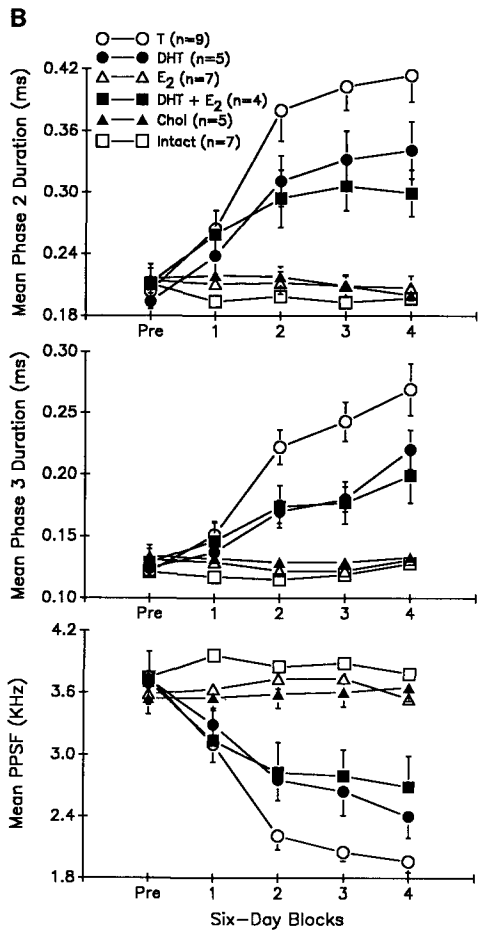


Figure 2. Androgen-dependent phase-specific sex differences in EODs in *G. petersii*. *A* Data were collected on the day fish arrived from Nigeria during the local rainy breeding season. Top: Phase 2; males exhibit significantly longer mean durations than females. Middle: Phase 3; males exhibit significantly longer phase 3 durations than females. Bottom: PPSF; multiple regression analyses [males: $F(4,22) = 11.81$, $p < 0.0001$; females: $F(4,27) = 31.48$, $p < 0.00001$] indicated that only phases 2 and 3 contribute significantly to PPSFs. Both phases are inversely related to PPSF [$r(n = 59) = -0.83$, $p < 0.0005$ and $r(n = 59) = -0.66$, $p < 0.0005$, respectively]. Thus, the mean male PPSF is significantly lower than the females'. $^{**}p < 0.001$ $^{***}p < 0.0001$. *B* Time-course of effects of implanted hormones on phases 2, 3 and on the PPSF in juvenile gonadectomized and non-implanted intact controls over six-day mean blocks. Top: Phase 2; only androgens increased the duration of this phase by the first 6-day post-implant block [F hormone × 6-day blocks $(20,124) = 22.32$, $p < 0.00001$]. Middle: Phase 3; only androgens increased the duration of this phase by the second 6-day post-implant block [$F(20,124) = 20.71$, $p < 0.00001$]. Bottom: PPSF; only androgens decreased the PPSF by the first 6-day post-implant block [$F(20,124) = 32.62$, $p < 0.00001$].

1:20 000) and implanted with one or two silastic capsules (Dow Corning 0.065 inch o.d. and 0.03 inch i.d.) containing a total of 1 mm/1 g b.wt (0.4 mm/1 g b.wt for E_2 because of its greater potency in producing behavioral effects) packed T, DHT, E_2, DHT + E_2, or cholesterol. The gonad was removed and implant(s) inserted into the gut cavity through a 1.5-cm incision on the left ventral surface, approximately 5 mm posterolateral of the ventral fins. The incision was sutured, and each implanted fish was returned to its aquarium and treated with chloramphenicol (Sigma, 1:100 000), an antimicrobial agent. EOD data were recorded and then analyzed with two-way ANOVAs for mixed designs (hormone × 6-day blocks), followed by the Newman Keuls test for multiple comparisons. After 30 days of EOD data collection, blood was collected into heparinized microcapillary tubes from the caudal vein of subjects, centrifuged for 5 min at 2000 rpm, and the plasma stored for future analyses. E_2 and T were measured by radioimmunoassay (RIA) techniques[33] validated for measurement in *G. petersii* plasma. The T antiserum cross-reacted 1.5% with 11-keto T, but not with E_2 (< 0.002%). The E_2 antiserum was highly specific for E_2 showing less than 1% cross-reactivity with estrone, estriol, and 16-ketoestradiol. The estradiol antiserum did not cross-react with T or other androgens. 25–100 µl plasma were extracted with 2 ml hexane/ethyl acetate (70:30). The extracts were dried under nitrogen and reconstituted in 375 µl of assay buffer. Recovery of labeled hormone carried through the extraction procedures ranged from 76% to 95%. 10-, 25-, 50-, and 100-µl aliquots of the reconstituted samples were measured in each RIA to test for parallelism. The

unchromatographed T RIA procedure was validated by chromatography of plasma samples on Sephadex LH-20 columns using iso-octane/toluene/methanol (62:20:15) to elute T. The T values obtained by the unchromatographed and chromatographed procedures were in good agreement (variation 11.2%). The intrassay coefficients of variation were 7.5% for E_2 (N = 13) and 7.5% for T (N = 16).

Results and discussion. Figure 2B shows the time-course of steroid effects. By the end of the first 6-day post-implant block, both T and DHT caused significant increases in the duration of phase 2 (both $p < 0.05$) [fig. 2B]. DHT + E_2 had effects similar to DHT alone, while E_2 implanted fish did not differ from either the cholesterol-implanted or nonhandled controls. Testosterone and DHT also significantly increased phase 3 by the end of the second post-implant block (both $p < 0.05$). Again, the effects of DHT + E_2 were similar to DHT alone, while neither E_2- nor cholesterol-implanted fish differed from the nonhandled controls. The PPSFs for both T- and DHT-implanted fish decreased during the first post-implant block (both $p < 0.05$). DHT + E_2 had similar effects to DHT alone. The PPSFs of E_2- and cholesterol-implanted fish did not differ from the controls'. By the second 6-day post-implant block, androgen-treated fish exhibited phase 2 and 3 durations and PPSFs comparable to adult males (compare figs 2A and 2B). Removal of the implants for only 24 h following 18 days of treatment resulted in reversal of androgen-treated fish' EODs to pre-implant phase durations (phase 2: from means ± SEMs: 0.38 ± 0.01 ms to 0.23 ± 0.02 ms, N = 3; phase 3: from means ± SEMs: 0.25 ± 0.01 ms to 0.14 ± 0.01 ms, N = 3) and PPSFs (from 2.2 ± 0.1 kHz to 3.2 ± 0.2 kHz, N = 3), indicating that the effects of androgens on the EOD in *G. petersii* are rapidly reversible. Finally, only T and DHT induced the adult male-like indentation in the dorsal margin of the anal fin (fig. 1c). In other studies (data not shown), juveniles were implanted with lower hormone doses (1 mm/2.5 g b.wt). The effects of this lower dose treatment were equivalent to the higher dose effects for T shown for phase 2, but slower and less profound for phase 3, suggesting that phase 3 is less sensitive to low androgen doses. Competitive RIAs on pooled plasma samples from high-dose subjects revealed plasma T levels ranging from 5.77 ng/ml, comparable to levels found in sexually mature adult males on the day they were received by the importer (see fig. 4A), to 40.87 ng/ml, both well within the range reported for other fish species during reproductive season[9, 28]. High-dose E_2 implants resulted in pooled plasma estrogen levels ranging from 1.34 ng/ml to 5.55 ng/ml compared to 0.579 ng/ml of estrogen found in a newly imported, sexually mature, adult female.

These results indicate that there are natural sex differences in morphology and in two phases of the EOD in adult *G. petersii* and that exogenous treatment with androgens induces these sexual characters in juveniles. The

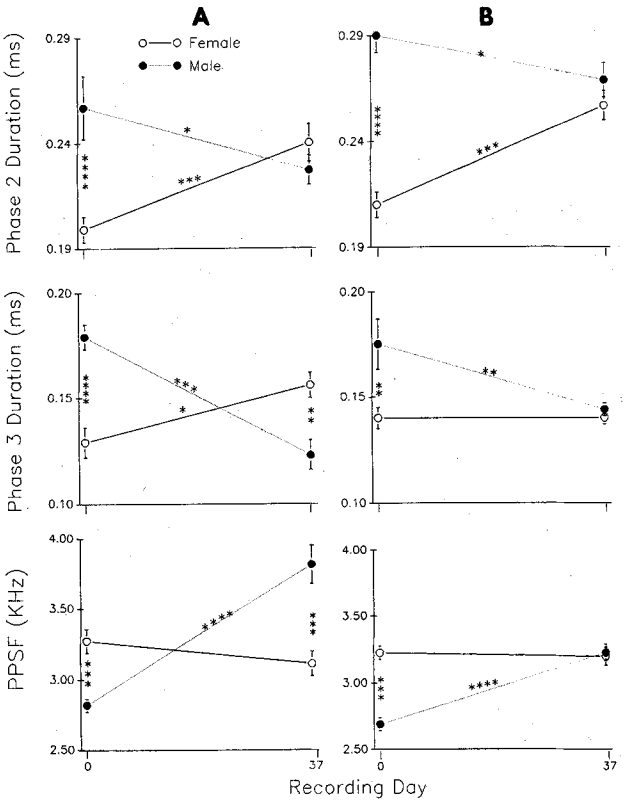


Figure 3. The effects of laboratory maintenance on sex differences in EODs in *G. petersii*. Means ± SEMs for male and female (top) phase 2 and (middle) phase 3 durations, and (bottom) PPSFs for fish maintained in (A) individual or (B) group aquaria. The lines connecting the day-0 and day-37 data points are included only to highlight EOD changes and may not accurately reflect the time-course of change. Stars between male and female symbols at day-0 or day-37 indicate significant sex differences and stars on lines indicate significant differences between the day-0 and day-37 means for each sex. *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; ****$p < 0.0005$.

effects observed were androgen specific and not attributable to aromatization of the hormones, since the non-aromatizable androgen, DHT, caused similar responses to T, while E_2 was without effect. Similar androgen effects were also obtained in gonadectomized adult males and females (data not shown). However, in the adults, E_2 caused a slight, but significant increase in PPSFs. This is the first report of a phase-specific, androgen-dependent sex difference in phase duration in EODs of weakly discharging electric fish.

Effects of laboratory captivity on the EOD

Stress manipulations in vertebrates including fish result in alterations in plasma levels of adrenal and gonadal hormones[1, 21, 25, 26, 30–32, 34]. The data from the fish in the presently reported sex difference study were collected on the day of arrival, whereas the fish in our earlier sex difference publication[18] were maintained in the laboratory in isosexual groups for approximately 3 weeks for adaptation purposes prior to data collection. This sug-

gested that changes over time since the fish were caught, imported, and maintained under laboratory conditions could have altered the EOD sex differences.

Methods. Initial (day-0) EOD data were collected from 17 female and 18 male newly imported adult fish. Day-0 data were collected within 2 h following arrival of the fish at the importers, and fish were then weighed, fin-clipped for later identification, and assigned to either 189-l isosexual (n = 7 for males and females) or heterosexual (n = 4 males and 3 females) group tanks, or to 26.50-l individual aquaria (n = 7 males and 7 females). All group and individual aquaria contained live plants (*Cryptocoryne griffithii* and *Echinodorus intermedius*), gravel, ceramic shelters, and corner filters.

Results and discussion. Figure 3 shows the effects of laboratory maintenance in individual (fig. 3A) and group (fig. 3B) aquaria on sex differences in EODs. At the end of 37 days of captivity, all sex differences were abolished in group-maintained subjects, and the sex differences in phase 3 duration and PPSF were reversed in subjects maintained in individual aquaria. Both sexes exhibited significant changes in phase durations; however, only the mean male PPSFs changed significantly between days 0 and 37, from (means ± SEMs) 2.69 ± 0.05 kHz to 3.22 ± 0.06 kHz [t (df = 10) = −6.67, $p < 0.0005$] (group) and from 2.82 ± 0.04 kHz to 3.81 ± 0.14 kHz [t (df = 6) = −6.89, $p < 0.0005$] (individual), causing the sex difference in PPSF to disappear (fig. 3B) or reverse (fig. 3A). Clearly, captivity has dramatic effects on the phase-specific EOD sex differences in *G. petersii*, either eliminating or reversing them, depending on housing conditions.

Effects of captivity on behavioral physiology

Methods. The profound effects of laboratory captivity on the male EOD along with the sensitivity of the EOD to androgens suggested that changes in endogenous testosterone levels might be responsible for the behavioral ef-

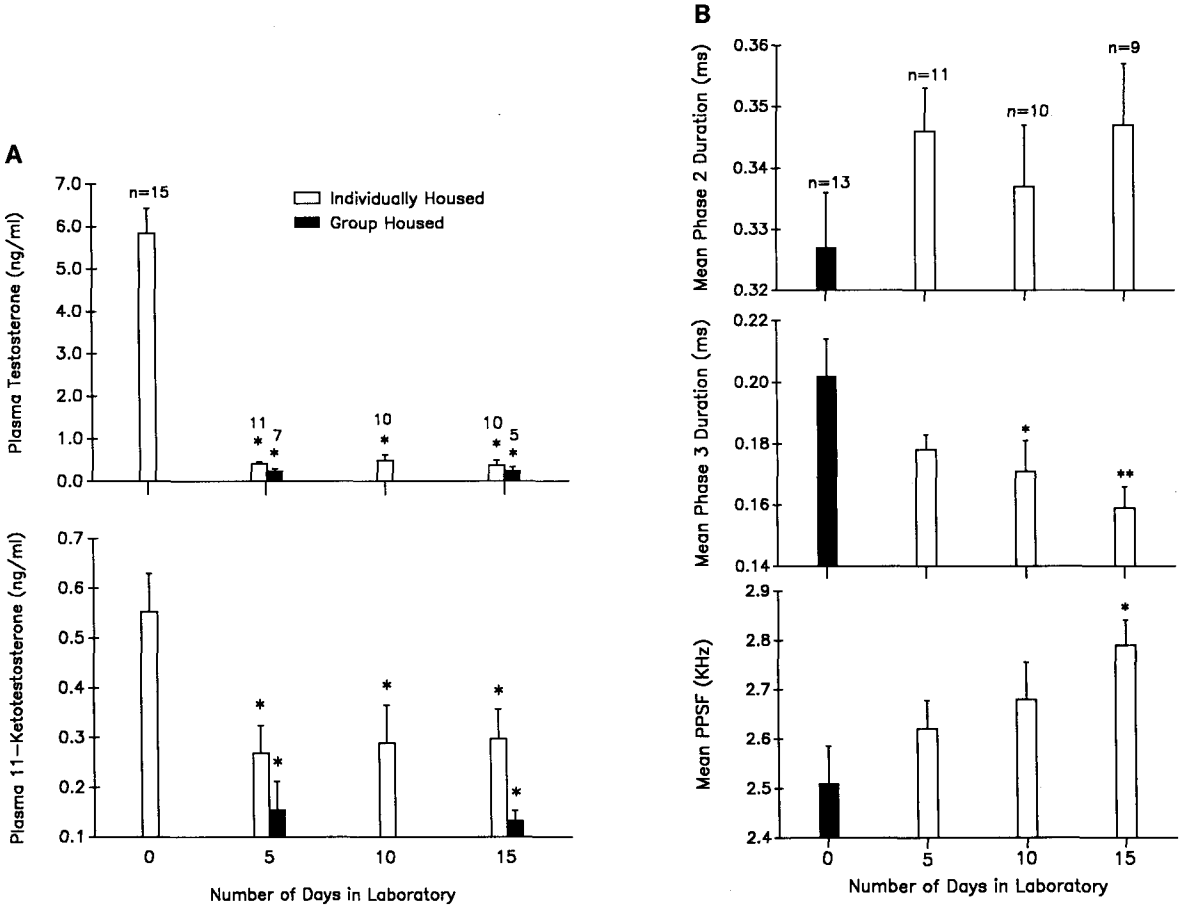


Figure 4. Simultaneous effects of laboratory captivity on plasma androgen levels and EODs. *A* Mean (± SEM) plasma T and 11-keto T levels in sexually mature adult male *G. petersii* obtained from Nigeria during the rainy breeding season. 5-, 10-, and 15-day plasma T (top) and 11-keto T (bottom) levels were lower than the 0-day levels (*$p < 0.001$ and *$p < 0.005$, respectively) regardless of whether maintained in groups with uncontrolled water conductivity or individually with conductivity maintained between 100 and 120 µS/cm. *B* EODs collected from the individually-housed fish. Top: Phase 2 was not significantly affected by the decline in androgen levels over the 15-day study. Middle: Mean Phase 3 durations on day-10 and day-15 were significantly shorter than the mean day-0 duration. Bottom: The mean day-15 PPSF was significantly higher than the day-0 mean PPSF. *$p < 0.05$, **$p = 0.02$.

fects of captivity in *G. petersii*. To investigate this possibility, EOD data were collected and plasma T and 11-keto T (another major androgen in fish) levels determined in breeding season males within 2 h of arrival at the importers, and after 5, 10, and 15 days in the laboratory. EOD behavior was recorded first from all but two fish in the day-0 and one fish in the day-15 groups. Fish were fed tubifex worms ad libitum. Blood was collected into heparinized micro-capillary tubes (Sigma) from the caudal vein, and fish sacrificed on the day they were received (day-0) or following 5, 10, or 15 days in the laboratory. Testosterone and 11-keto T were analyzed in the same extracts in two blocks. Radioimmunoassay (RIA) techniques[33] were validated in *G. petersii* using column chromatography, and 50- and 100-µl aliquots from the reconstituted samples were measured in each RIA to test for parallelism. The T antiserum cross-reacted 1.5% with 11-keto T. The 11-keto T antiserum did not cross-react with testosterone. The detection limits were 1.25 pg per assay tube for both androgens. Recovery of labelled hormone carried throughout the extraction procedures ranged from 76% to 95%. The intrassay coefficients of variation (CVs) were less than 8.0% for both blocks for both the T and 11-keto T assays. The interassay CVs were 7.19% for T and 7.48% for 11-keto T.

Results and discussion. Plasma levels of both androgens dramatically decreased by the fifth day in the laboratory [$F(5,54) = 47.15$, $p < 0.00001$ for T; $F(5,54) = 5.44$, $p < 0.0001$ for 11-keto T], and remained at the new low levels for the subsequent 10 days (fig. 4A). The effects were the same whether fish were maintained in group or individual aquaria (means ± SEMs day-0 vs day-5 plasma T levels: individually-housed: 5.84 ± 0.59 ng/ml vs 0.41 ± 0.05 ng/ml; group housed: 5.84 ± 0.59 ng/ml vs 0.22 ± 0.05 ng/ml); means ± SEMs day-0 vs day-5 plasma 11-keto T levels: individually-housed: 0.55 ± 0.08 ng/ml vs 0.27 ± 0.06 ng/ml; group-housed: 0.55 ± 0.08 ng/ml vs 0.14 ± 0.02 ng/ml). Figure 4B shows the effects of these decreases in plasma androgen levels on the EOD. Although there were no phase 2 effects, the duration of phase 3 decreased from (means ± SEMs) 0.203 ± 0.015 ms to 0.158 ± 0.008 ms [$F(3,39) = 3.63$, $p = 0.02$] over 15 days in the laboratory. The PPSF showed a corresponding increase from (means ± SEMs) 2.50 ± 0.08 kHz on day-0 to 2.79 ± 0.05 kHz [$F(3,39) = 2.88$, $p < 0.05$] by day 15 in the laboratory. These findings explain the loss of EOD sex differences in the laboratory. As androgen levels fall, it is only a matter of time before the hormonally-dependent male EOD phases decrease in duration (fig. 3) and the sex differences disappear (fish recorded on day of import are probably already hormonally-altered because of handling and transport stress[21, 31, 34]). The fact that the duration of phase 2 did not decrease with the decline in androgen levels is not surprising as even low-dose androgen implants caused rapid and profound increases in phase 2 duration equal to those resulting from the high-dose implants (fig. 2B). Thus, low levels of circulating T may be sufficient for maintaining the duration of phase 2.

However, females under laboratory conditions also exhibit statistically significant changes in phases 2 and 3 in the male direction (fig. 3). Plasma T levels were analyzed in 2 males and 2 females following 37 days of laboratory maintenance. Both females exhibited longer phase 2 (0.29, 0.26 ms) and phase 3 (0.15, 0.15 ms) durations, and lower PPSFs (2.8, 2.9 kHz) than the males (both phase 2: 0.24 ms; phase 3: 0.13 ms; and 3.6 and 3.5 kHz, respectively). Plasma T levels were higher in both females (1.66, 1.44 ng/ml) than in the males (0.42, 0.94 ng/ml). These data suggest that females in captivity may actually exhibit increases in androgens affecting EODs. These findings can account for our previously reported laboratory sex difference in *G. petersii* in which males had shorter discharges and higher PPSFs than females[18].

Seasonal sex differences in the EOD of G. petersii

Figure 5 shows seasonal variations in phases 2 and 3 and in the PPSF over the five months in 1988 during which adult fish were available from importers. EOD record-

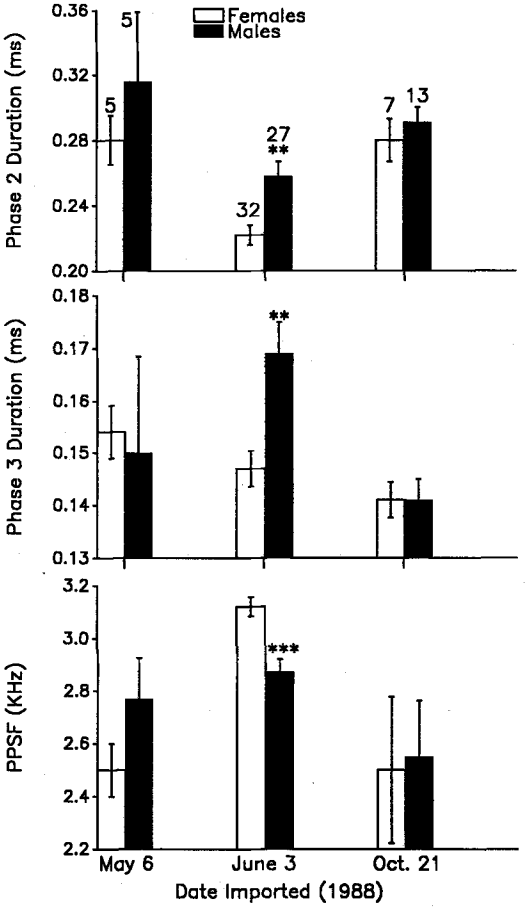


Figure 5. Seasonal sex differences in phase 2 (top), phase 3 (middle) and PPSF (bottom). Numbers (top) indicate sample sizes. **$p < 0.001$; ***$p < 0.0001$.

ings were taken within 2 h of arrival of the fish at the importers. These data imply that there are seasonal phase-specific duration sex differences in EODs of *G. petersii*.

Discussion

Together, the results of these studies can explain large discrepancies in the results of sex-difference studies in weakly discharging electric fish species. Westby and Kramer[14] failed to find a sex difference in the EOD waveform of *G. petersii*, while Landsman, Jou and Moller[18] reported that males of this species exhibited shorter EODs and higher PPSFs than females. These previous findings were incongruent with the present results in which males had longer durations in specific phases of the EOD and lower PPSFs than females. Hopkins[12] reported that mature female *Eigenmannia virescens* (gymnotiform) discharged at higher frequencies than mature males while Westby and Kirschbaum[36] reported a sex difference in the opposite direction. A sex difference in the preceding negative wave of the EOD in the mormyrid *Pollimyrus isidori*[35] could not be replicated[37]. Further, while Lücker and Kramer[20] failed to find any EOD waveform sex difference in *P. isidori*, Westby and Kirschbaum[37] reported a "clear and unambiguous sexual dimorphism within the major phases of the EOD itself" (p. 400). Hormonally-controlled sex differences in EODs are extremely sensitive to environmental perturbations, particularly the stress of captivity. This can account for the failure to find EOD sex differences[16] as well as for the non-reproducibility of sex differences in this and other electric fish species in the laboratory[12, 20, 35–37], and identifies a possible confounding factor in all electro-communication studies performed under captive conditions in field and laboratory.

This is the first report to provide evidence that weakly discharging African mormyrid fish could use phase-duration cues of the EOD, as opposed to the entire duration of the EOD, to communicate sexual identity. We have just found in another mormyrid species, *Brienomyrus brachyistius*, that T and 11-keto T elongate only phases 2 and 3 of the EOD, and have no effect on the duration of phases 1 and 4 (Landsman and Moller, unpublished), suggesting that like *G. petersii*, this species may also exhibit sex differences only in specific EOD phases. Finally, a hormone-dependent sex difference was also reported in EOD symmetry of the two phases of the diphasic EOD in a gymnotiform species[11]. Phase-specific EOD plasticity suggests that individual EODs can communicate much about current motivational and endocrine status of the fish. Steroid hormones appear to exert their effects peripherally by altering membrane properties in the electric organs of mormyrid and gymnotiform species[4, 5, 7]. Communication of sexual identity in these fish appears to be seasonal, abolished by captivity, and based on phase-specific components extremely susceptible to laboratory manipulation and internal hormonal milieu. Therefore, an understanding of electro-communication can only be attempted through interdisciplinary laboratory and field studies examining behavioral processes, physiology, and biochemistry.

Together, these new findings explain why behavioral sex differences in the electro-communication systems of weakly discharging electric fish, as well as hormone-dependent sexual behaviors exhibited by other animals in the wild, are seldom, if ever, observed in captivity[25]. Even institutionalized humans exhibit behaviors that are similar in many respects to the aberrant behaviors exhibited in captive primates in zoos and laboratories[8], suggesting that the effects of captivity cross a wide spectrum of vertebrate species. When wild-caught animals are brought into the laboratory, rapid and profound physiological and behavioral changes occur which may intervene in the processes being studied.

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1 Bardin, C., and Peterson, R., Studies of androgen production by the rat: testosterone and androstenedione content of blood. Endocrinology *80* (1967) 38–44.
2 Bass, A. H., Electric organs revisited: evolution of a vertebrate communication and orientation organ, in: Electroreception, pp. 13–70. Eds T. H. Bullock and W. Heiligenberg. Wiley and Sons Inc., New York 1986.
3 Bass, A. H., A hormone-sensitive communication system in an electric fish. J. Neurobiol. *17* (1986) 131–156.
4 Bass, A. H., Steroid-sensitive neuroeffector pathways for sonic and electric communication in fish. Brain, Behav. Evol. *28* (1986) 7–21.
5 Bass, A. H., Species differences in electric organs of mormyrids: substrates for species-typical electric organ discharge waveforms. J. comp. Neurol. *244* (1986) 313–330.
6 Bass, A. H., and Hopkins, C. D., Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes. J. comp. Physiol. *A 156* (1985) 587–604.
7 Bass, A. H., Denizot, J. P., and Marchaterre, M. A., Ultrastructural features and hormone-dependent sex differences of mormyrid electric organs. J. comp. Neurol. *254* (1986) 511–528.
8 Erwin, J., and Deni, R., Strangers in a strange land: abnormal behaviors or abnormal environments?, in: Captivity and Behavior, pp. 1–28. Eds J. Erwin et al. Van Nostrand Reinhold, New York 1979.
9 Fostier, A., Jalabert, B., Billiard, R., Breton, B., and Zohar, Y., The gonadal steroids, in: Fish Physiology, pp. 277–372. Eds W. S. Hoar et al. Academic Press, New York 1983.
10 Hagedorn, M., The ecology, courtship, and mating of gymnotiform electric fish, in: Electroreception, pp. 497–525. Eds. T. H. Bullock and W. Heiligenberg. Wiley and Sons Inc., New York 1986.
11 Hagedorn, M., and Carr, C., Single electrocytes produce a sexually dimorphic signal in South American electric fish, *Hypopomus occidentalis* (Gymnotiformes, Hypopomidae). J. comp. Physiol. *A 156* (1985) 511–523.
12 Hopkins, C. D., Electric communication: Functions in the social behavior of *Eigenmannia virescens*. Behavior *50* (1974) 270–305.
13 Hopkins, C. D., Evolution of electric communication channels in mormyrids. Behav. Ecol. Sociobiol. *7* (1980) 1–13.
14 Hopkins, C. D., Neuroethology of species recognition in electroreception, in: Advances in Vertebrate Neuroethology, pp. 871–881. Eds J. Ewert et al. Plenum, New York 1983.

15 Kramer, B., Electric and motor responses of the weakly electric fish, *Gnathonemus petersii* (Mormyridae) to play-back of social signals. Behav. Ecol. Sociobiol. *6* (1979) 67–79.
16 Kramer, B., and Westby, G. W. M., No sex difference in the waveform of the pulse type electric fish, *Gnathonemus petersii* (Mormyridae). Experientia *41* (1985) 1530–1531.
17 Landsman, R. E., and Moller, P., Testosterone changes the electric organ discharge and external morphology of the mormyrid fish, *Gnathonemus petersii* (Mormyriformes). Experientia *44* (1988) 900–903.
18 Landsman, R. E., Jou, S. H., and Moller, P., Stress obscures signalling of sexual identity in *Gnathonemus petersii* (Mormyriformes), in: Reproductive Physiology of Fish 1987, p. 307. Eds D. R. Idler et al. Proc. 3rd Int. Symp. Reprod. Physiol. Fish, St. John's, Newfoundland 1987.
19 Leopold, A. S., The nature of heritable wildness in turkeys. Condor *46* (1944) 133–197.
20 Lücker, H., and Kramer, B., Development of a sex difference in the preferred latency response in the weakly electric fish, *Pollimyrus isidori* (Cuvier et Valenciennes) (Mormyridae, Teleostei). Behav. Ecol. Sociobiol. *9* (1981) 103–109.
21 Mazeaud, M. M., and Mazeaud, F., Adrenergic responses to stress in fish, in: Stress and Fish, pp. 49–75. Ed. A. D. Pickering. Academic Press, New York 1981.
22 Moller, P., Electroperception. Oceanus *23* (1980) 44–54.
23 Moller, P., Electroreception and the behaviour of mormyrid fish. Trends Neurosci. *3* (1980) 105–109.
24 Moller, P., Serrier, J., and Bowling, D., Electric organ discharge displays during social encounter in the weakly electric fish *Brienomyrus niger* L. (Mormyridae). Ethology *82* (1989) 177–191.
25 Moore, F. L., and Miller, L. J., Stress-induced inhibition of sexual behavior: corticosterone inhibits courtship behaviors of a male amphibian (*Taricha granulosa*). Horm. Behav. *18* (1984) 400–410.
26 Moore, F. L., and Zoeller, R. T., Stress-induced inhibition of reproduction: evidence of suppressed secretion of LH-RH in an amphibian. Gen. comp. Endocr. *60* (1985) 252–258.
27 Moore, F. L., and Deviche, P., Neuroendocrine processing of environmental information in amphibians, in: Processing of Environmental Information in Vertebrates, pp. 19–45. Ed. M. H. Stetson. Springer-Verlag, New York 1988.
28 Ozon, R., Androgens in fishes, amphibians, reptiles, and birds, in: Steroids in Nonmammalian Vertebrates, pp. 328–389. Ed. D. R. Idler. Academic Press, New York 1972.
29 Richter, C. P., The effects of domestication and selection on the behavior of the Norway rat. J. natl. Cancer Inst. *15* (1954) 727–738.
30 Rivier, C., Rivier, J., and Vale, W., Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. Science *231* (1986) 607–609.
31 Safford, S., and Thomas, P., Effects of capture and handling on circulating levels of gonadal steroids and cortisol in the spotted seatrout, *Cynoscion nebulosus*, in: Reproductive Physiology of Fish 1987, p. 312. Eds D. R. Idler et al. Proc. 3rd Int. Symp. Reprod. Physiol. Fish, St. John's, Newfoundland 1987.
32 Sapolsky, R. M., The endocrine stress-response and social status in the wild baboon. Horm. Behav. *16* (1982) 279–292.
33 Singh, H., Griffith, R. W., Takahashi, A., Kawauchi, H., Thomas, P., and Stegeman, J. J., Regulation of gonadal steroidogenesis in *Fundulus heteroclitus* by recombinant salmon growth hormone and purified salmon prolactin. Gen. comp. Endocr. *72* (1988) 144–153.
34 Sumpter, J. P., Carragher, J., Pottinger, T. G., and Pickering, A. D., The interaction of stress and reproduction in trout, in: Reproductive Physiology of Fish 1987, pp. 299–302. Eds D. R. Idler et al. Proc. 3rd Int. Symp. Reprod. Physiol. Fish, St. John's, Newfoundland 1987.
35 Westby, G. W. M., and Kirschbaum, F., Emergence and development of the electric organ discharge in *Pollimyrus isidori*. I. The larval discharge. J. comp. Physiol. *122* (1977) 251–271.
36 Westby, G. W. M., and Kirschbaum, F., Sex differences in the electric organ discharge of *Eigenmannia virescens* and the effect of gonadal maturation, in: Sensory Physiology of Aquatic Lower Vertebrates, Adv. Physiol. Sci., vol. 31, pp. 179–194. Eds T. Szabo and G. Czeh. Pergamon Press, Budapest 1981.
37 Westby, G. W. M., and Kirschbaum, F., Sex differences in the waveform of the pulse-type electric fish, *Pollimyrus isidori* (Mormyridae). J. comp. Physiol. *145* (1982) 399–403.
38 Wingfield, J. C., Changes in reproductive function of free-living birds in direct response to environmental perturbations, in: Processing of Environmental Information in Vertebrates, pp. 121–148. Ed. M. H. Stetson. Springer-Verlag, New York 1988.

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Research Articles

Multiple prismatic calcium phosphate layers in the jaws of present-day sharks (Chondrichthyes; Selachii)

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Summary. Jaws of large individuals, over 2 m in total length, of the shark species *Carcharodon carcharias* (great white shark) and *Isurus oxyrinchus* (mako shark) of the family Lamnidae, and *Galeocerdo cuvieri* (tiger shark) and *Carcharhinus leucas* (bull shark) of the family Carcharhinidae were found to have multiple, up to five, layers of prismatic calcium phosphate surrounding the cartilages. Smaller individuals of these species and other known species of living chondrichthyans have only one layer of prismatic calcium phosphate surrounding the cartilages, as also do most species of fossil chondrichthyans. Two exceptions are the fossil shark genera *Xenacanthus* and *Tamiobatis*. Where it is found in living forms, this multiple layered calcification does not appear to be phylogenetic, as it appears to be lacking in other lamnid and carcharhinid genera and species. Rather it appears to be functional, only appearing in larger individuals and species of these two groups, and hence may be necessary to strengthen the jaw cartilages of such individuals for biting.
Key words. Chondrichthyes; sharks; jaws; prismatic calcium phosphate.