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Seasonal, hormonally controlled, phase-specific sex differences in electric organ discharges of the weakly electric fish, *Gnathonemus petersii* (Mormyridae), and the effects of captivity on these discharges

Landsman, Robert Eric, Ph.D.

City University of New York, 1990

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SEASONAL, HORMONALLY CONTROLLED, PHASE-SPECIFIC SEX DIFFERENCES IN
ELECTRIC ORGAN DISCHARGES OF THE WEAKLY ELECTRIC FISH,
GNATHONEMUS PETERSII (MORMYRIDAE), AND THE EFFECTS
OF CAPTIVITY ON THESE DISCHARGES

by

ROBERT ERIC LANDSMAN

A dissertation submitted to the Graduate Faculty in Psychology in
partial fulfillment of the requirements for the degree of Doctor of
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1990

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This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

SEASONAL, HORMONALLY CONTROLLED, PHASE-SPECIFIC SEX DIFFERENCES IN
ELECTRIC ORGAN DISCHARGES OF THE WEAKLY ELECTRIC FISH, GNATHONEMUS
PETERSII (MORMYRIDAE), AND THE EFFECTS OF CAPTIVITY ON THESE
DISCHARGES

by

Robert Eric Landsman

Advisers: Professors Cheryl F. Harding and Peter Moller

These studies were designed to examine plasticity in the signaling of sexual identity in the adult weakly discharging electric fish, Gnathonemus petersii (Mormyriformes, Teleostei). In the initial study, a sex difference in the peak power spectral frequency (PPSF) of the Fourier transformation of the electric organ discharge (EOD) was found, with males exhibiting higher PPSFs than females. The PPSF was found to be inversely related to the duration of phase 3 of the EOD, indicating a trend for males to have shorter phase 3 durations than females. Confinement or restraint caused dramatic increases in individual variation in EODs so that the sex difference in PPSF was abolished. Experiments 2 and 3 investigated the effects of low and high doses of steroid hormones on EODs and the external morphology of

juvenile and adult fish. Testosterone (T) and 17α -methyltestosterone (17α -MT) both caused decreases in the PPSF as well as increases in the durations of phases 2 and 3 of the EOD. The effects of T on phases 1 and 4 of the EOD were highly dose-dependent with low doses resulting in decreases and high doses resulting in increases in the durations of both phases. High-dose dihydrotestosterone (DHT) had similar, but less dramatic effects than T, while low-dose DHT appeared to have no effects. Estradiol (E_2) affected neither PPSFs nor phase durations of the EOD in juveniles, but caused a slight but significant increase in adult PPSFs. Only androgens induced an indentation in the dorsal margin of the anal fin typical of other adult male mormyrid species. In Experiment 4, when data were collected from adults within hours of arrival from Africa during the pre-rainy, rainy breeding, and post-rainy seasons, non-overlapping sex differences were found only in PPSFs and specific phases of the EOD in fish imported during the rainy breeding season. These seasonal, phase-specific sex differences were characterized by males having longer durations in only phases 2 and 3, and lower PPSFs than females. Clear indentations were observed in the dorsal margin of the anal fin only in males for all 3 seasons, indicating that this external morphological character is sexually dimorphic. The final Experiment investigated the effects of captivity on the sex differences in the EOD of newly imported fish. Whether maintained in groups or individually, the PPSF and phase-specific sex differences were abolished by 14 days in captivity and in some cases reversed by the end of 37 days in captivity. Only male PPSFs increased, while both sexes showed changes in the durations of phases 2 and 3, but in opposite directions. Plasma T and 11-keto T levels in

males dramatically decreased by the 5th day of captivity and remained at these levels for the remaining days in captivity. These endocrine changes were accompanied by a significant gradual decrease in the duration of phase 3 by Day 3, and a significant gradual increase in PPSF by Day 15. Females appeared to have higher T levels than males following 37 days of captivity. Electrocommunication signals appear to exhibit much more plasticity than previously thought. Specific phases of the EOD rather than the entire duration or PPSF may signal information about current motivational states. Together, these studies demonstrate the link between captivity, hormones, and reproductive behavior.

PREFACE

To me, a scientist is one who searches for truth. Searching for truth, however, is not the easiest of tasks. The scientist must employ rigorous research design, observation, measurement, experimentation, statistical evaluation of data, good thinking, and replication in his research. Perhaps the most important skill to acquire as a student of science is to learn how to learn. To this end, many individuals contributed to my development as scientist both as teachers, critical reviewers, and confidence builders.

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GENERAL INTRODUCTION

Stress is deleterious to mental health in humans, and to physical health, social and reproductive behavior across species. Many commonalities exist in hormonal and behavioral stress responses across vertebrates, and so, a suitable animal model for the study of stress is one in which behavior is observable, hormones are measurable, and with which laboratory and field studies are practicable. Weakly electric fish may serve as excellent subjects in which to study stress as they are readily obtainable from fish importers at reasonable prices, emit a unique behavior--the EOD--which is readily observable, recordable, and quantifiable using oscilloscope, FM tape recorder, and spectrum analyzer. Many species exhibit sexually dimorphic EODs which are modulated by gonadal hormones. These fish have been shown to alter their EODs in response to manipulation of various environmental factors, a response which may be a function of endocrine responses. This makes the weakly electric fish potentially suitable for studying the effects of hormones on behavior, behavior on hormones, and hormonal interactions. The study of stress and reproduction in fish is important for commercial fish culturing and is also of comparative value in understanding the development and evolution of steroid sensitive pathways controlling behavior.

STRESS IN FISH

The hormonal stress response in fish is similar to that in

mammals. In the latter, a releasing factor from the hypothalamus stimulates release of adrenocorticotropin (ACTH) from the anterior pituitary which in turn stimulates release of glucocorticoids from the adrenal cortex, epinephrine from the adrenal medulla, and norepinephrine from the sympathetic nerves. Exposure to stress can affect reproduction by blocking ovulation (Britton, Koob, Rivier, & Vale, 1982), causing dysmenorrhea in women (Baker, 1981), or by altering androgen secretion in males (Cumming, Vickovic, Wall, & Fluker, 1985). Stress in fish is typically assessed by measuring blood corticosteroid levels. Exposure to stress results in an increase in plasma cortisol secreted from the interrenal tissue (i.e., in most fish, cortisol is secreted from dispersed interrenal tissue rather than adrenal glands). Handling and confinement of fish results in increases in circulating corticosteroids (Hane, Robertson, Wexler & Krupp, 1966; Donaldson & McBride, 1967; Fagerlund & Donaldson, 1970; Grant & Mehrle, 1973; Nemeth & Jurani, 1974; Fuller, Scott, & Fraser, 1974; Spieler, 1974; Mazeaud, Mazeaud, & Donaldson, 1977; Strange, Schreck, & Golden, 1977; Strange & Schreck, 1978; Strange, Schreck, & Ewing, 1978; Pickering, Pottinger, Carragher, & Sumpter, 1987). Exposure to pollutants such as copper (Donaldson & Dye, 1975), chromium (Hill & Fromm, 1968), pesticides (Grant & Mehrle, 1973; Yaron & Ilan, 1974; Ilan & Yaron, 1983), a component of kraft mill effluent (Dye & Donaldson, 1974), or to electroshock (Schreck, Whaley, Bass, Maughan, & Solazzi, 1976), antibiotic injection (McBride, Strasdine, & Fagerlund, 1975), unbuffered anesthetic (Wedemeyer, 1969), formalin (Wedemeyer & Yasutake, 1974), saline (Chavin, 1973; Singley & Chavin, 1975; Assem & Hanke, 1981; Hegab & Hanke, 1986), or oxygen depletion

(Tomasso, Davis & Parker, 1981), all lead to increases in blood cortisol levels in all fish species investigated.

Stress also causes changes in behavior in fish. Such behavioral changes may be related to the hormonal changes which typically accompany stress manipulations. Newton (1982) reared guppies (Poecilia reticulata) in low and high density groups subjected to handling or handling plus cold stress. Her results indicated that female control fish displayed significantly more aggression than either the handled or cold stressed fish. Further, both socially isolated and overcrowded rearing conditions resulted in adult fish exhibiting less aggression than control fish. Thines and Heuts (1968) determined dominance in pairs of young swordtail (Xiphophorus helleri) X platyfish (Xiphophorus maculatus) and then subjected the alpha member of each pair to either large dominating or smaller non-dominating fish for one day. When the alpha fish were replaced in their original aquaria with their original omega partners, the omega fish attacked the dominated alpha fish significantly more than the non-dominated alpha fish.

When pumpkin-seed sunfish (Lepomis gibbosus) were confined in small quarters, they established and defended territories and a negative relationship was found between aggressive behavior and interrenal volume (Erickson, 1967). The highest ranking swordtails (Xiphophorus helleri Heckel) in groups of three showed the least adrenocortical activity as measured by mean nuclear diameter of 25 adrenocortical cells, while the remaining two fish in each group showed no significant differences from each other, regardless of distinguishable behavioral differences in rank (Scott & Currie, 1980).

However, it has been shown that mean nuclear diameter provides only an approximate indication of plasma cortisol level in Coregonus lavaretus (Scott & Rennie, 1980). Dominant immature Salmo gairdneri also exhibited lower interrenal activity than subordinates, though top ranking fish had higher activity than expected (Noakes & Leatherland, 1977). Most subordinate Anguilla anguilla L. exhibited enlarged interrenal cells and increased plasma cortisol concentrations, while dominant eels did not differ from controls on either measure (Peters, Delventhal & Klinger, 1980). The above findings suggest that aggressive territorial behavior is related to corticoid levels in such a way that the loser or subordinate fish exhibits an increase in cortisol, indicative of exposure to stress.

Steroid hormones other than corticosteroids have also been implicated in the hormonal stress response in fish. Transfer from a "home" container to crowded conditions resulted in large increases in dihydrotestosterone (DHT), estradiol (E_2), and estrone (E_3) one to three hours later in the sea lamprey (Petromyzon marinus) and yellow eel (Anguilla rostrata) (Epple, Katz & Dashow, 1982). Progesterone (P) and testosterone (T) levels dropped within the first hour and then rose sharply, while androstenedione was not detectable. In prespawning adult lampreys of both sexes, androstenedione titers increased in response to surgery, agitation, decapitation, and anesthesia followed by decapitation. T levels increased only after surgery, and cortisol increased only after agitation. E_2 titers fell after surgery, while E_3 did not change. The authors suggest that in vertebrates many steroid hormones are "stress hormones" (Epple, Katz, & Dashow, 1982). However, the data could also be interpreted as

indicating that only agitation results in a stress response in these species. When spotted seatrout were maintained in captivity for 21 days, after a one day post-capture decrease in plasma levels of T and E_2 , T levels and gonadosomal index (GSI) for males did not change, while GSI values for females decreased over the 21 days in captivity (Safford & Thomas, 1987). In the same study, cortisol levels in females increased by day 1 post-capture, but returned to initial levels by day 21.

Exposure of fish to pollutants has resulted in incongruencies regarding their effects on gonadal steroid hormones. When rainbow trout were exposed to low pH, no suppression of gonadal steroids was observed (Weiner, Schreck & Li, 1986), while plasma levels of T and 11-keto T in Atlantic salmon were significantly lower in water with pH 4.7 than water with pH 5.6 (Freeman, Sangalang, Burns & McMenemy, 1983). Chronic confinement of sexually mature male brown trout for 1 month as well as acute handling stress both resulted in suppression of plasma levels of both T and 11-keto T (Pickering et al., 1987). Exposure of brook trout to cadmium resulted in elevated plasma androgen levels, whereas crude petroleum caused suppression of androgens in salmon and flounder (Truscott, Walsh, Burton, Payne & Idler, 1983).

Hannes and Franck (1983) measured blood androgen and glucocorticoid levels in socially isolated and non-isolated male Haplochromis burtoni and Xiphophorus helleri. Social living fish of both species exhibited significantly higher mean concentrations of both androgens and corticoids, with no relationship between the levels of the two hormones. Thus, social isolation reduced circulating

androgens, but not as a result of isolation stress because corticoid levels also fell. Social isolation of Sarotherodon mossambicus resulted in reduced gonad weight, spermatocyte/spermatogonial ratio, and size of interstitial cell nuclei (Silverman, 1978), all of which are regulated by hypothalamic and pituitary activity. Hannes, Franck, and Liemann (1984) paired male X. helleri for up to 14 days in order to study the time courses of androgen and glucocorticoid changes in whole body and blood samples. Corticoids dramatically increased in both fish during the initial rank-order fight, however this increase was more pronounced in losers than in winners following a fight. Androgen levels decreased in losers and less markedly in winners following a fight. One-hour after the fight, androgen levels of the winners rose above controls' and remained there throughout the 14 day experiment, while loser values returned to control levels within 7 days. Finally, Schwanck (1980) allowed pairs of young male Tilapia mariae to establish dominance in an aquarium large enough for only one territory. The fish with larger genital papilla, indicative of androgenic hormone state, won most of the encounters.

The above studies indicate that cortisol is not the only steroid hormone affected by stressful environmental conditions. In general, it appears that cortisol levels rise and androgen levels fall in response to aversive manipulations. Further, there may be sex differences in endocrine response to such stressors as handling and captivity.

SEXUALLY DIMORPHIC ELECTRIC ORGAN DISCHARGES AND STEROID
HORMONE EFFECTS IN WEAKLY ELECTRIC FISH

Sex differences in EODs have been reported for both South American gymnotoid and African mormyriiform weakly discharging electric fish. These sex differences are typically reversible by gonadal manipulation and steroid hormone administration and are highly correlated with gonadal maturity and reproductive state. The peripherally located electric organs in weakly discharging electric fishes are excited by a central motor pathway to produce an electric discharge. The electric organ consists of electrocytes embryonically derived from myotomes, except for one family of South American electric fish, Aptereronotidae, whose electric organ is derived from nerves (for reviews, see Bennett, 1971 and Bass, 1986a; Moller, 1980; Bass, 1986b).

Mature male Hypopomus occidentalis (gymnotoid) are larger with broader tails containing larger electrocytes and exhibit EODs with lower peak power frequencies in the Fourier spectrum of the discharge than females (Hagedorn & Carr, 1985). When females of this species were injected with 5µg/g of either DHT or E₂, DHT-treated fish developed larger, male-like electrocytes along with male-like EODs, while E₂-treated fish showed no change in electrocytes or discharges (Hagedorn & Carr, 1985). In the gymnotoid Sternopygus macrurus, mature females discharge at higher frequencies than mature males, while juveniles discharge at frequencies intermediate between the two (Hopkins, 1972, 1974a). Males exposed to female-typical emissions increased their electrical courting signals (Hopkins, 1972). Males

did not increase discharge rates when exposed to other frequencies, suggesting that the difference in discharge rates is used as a means of sex discrimination in this species. Female Sternopygus dariensis, a closely related species, also emit EODs at higher frequencies than males (Meyer, 1983). Meyer (1983) injected male and female S. dariensis with T, DHT, or E_2 in various doses ranging from 2.5 to 20.0 $\mu\text{g/g}$ body-weight. Following the T injections, both sexes exhibited decreased discharge frequencies. Fish with higher pretreatment discharge frequencies showed larger responses to the hormone treatment than fish discharging at lower frequencies. Fish treated with E_2 increased their discharge frequencies, with lower-frequency fish exhibiting larger responses than higher-frequency fish. In the same study, gonadectomized males showed an 11% increase in discharge frequency, while ovariectomized females showed an 18% decrease in discharge frequency. Hormone replacement reversed the effects of the surgery, while administering T or DHT to females and E_2 to males increased the effects of gonadectomy. These results suggest that hormonal effects on the EOD of this species are not permanent, but are activational in nature. This means that the electric organ may be bipotential in its ability to emit male- or female-like discharges, and it is likely that the presence or absence of gonadal hormones in adulthood determines the sexual characteristics of the discharge. Hopkins (1974b) reported that mature Eigenmannia virescens (gymnotoid) exhibited a significant sex difference in frequency of discharge in the same direction as S. macrurus but with much overlap between the sexes. In contrast, Westby and Kirschbaum (1981) reported that sexually mature but unripe males of this species showed a

significantly higher EOD frequency than females, but with considerable overlap, while immatures fell within a wide range of intermediate frequencies. As the fish became ripe, the females shifted frequency in the upward direction and in many cases surpassed the males in frequency. These incongruent findings suggest that a) the fish used by Hopkins (1974b) may have been gonadally ripe, b) the EOD is sensitive to seasonal changes in reproductive state, and/or c) the EOD is sensitive to captivity.

Many mormyrids share the same rivers and lakes in Africa where the natural L:D cycle is 12:12, water conductivity ranges from 60-120 $\mu\text{S}/\text{cm}$, and water temperature ranges from 21-30 $^{\circ}\text{C}$ (For a complete review of the mormyrid biogeography, see Hopkins, 1986). They are mostly nocturnal and found in turbid water. All mormyrids breed during rainy seasons. Several mormyrid species have been shown to have sex differences either in the sequence of pulse intervals or in the waveform of the EOD, and these have been shown to be reversed by steroid hormones in all species investigated (See Bass, 1986b, for a review). Brienomyrus brachyistius (long biphasic), Brienomyrus brachyistius (triphasic), Stomatorhinus corneti, Stomatorhinus walkeri, and possibly Hippopotamyrus batesii (triphasic) have sexually dimorphic pulse waveforms. Males exhibit pulses 2 to 3 times longer in duration than females (Moller, 1980; Hopkins, 1980, 1981; Hopkins & Bass, 1981; Bass & Hopkins, 1983, 1985). 17α -methyl T added to the water of juvenile and adult female B. brachyistius (long biphasic) induced male EODs, and following gonadectomy, the discharges of these androgen-treated females reverted to the female type 24 days after treatment ended (Bass & Hopkins, 1982, 1983). Gonadectomized,

DHT-treated females also exhibited male-like EODs, while E_2 had minimal effects on immature males and females (Bass & Hopkins, 1983). These results suggest that the presence of androgen is necessary for fish of this species to exhibit male discharges, but that female discharges are not dependent upon the presence of estrogen, at least in terms of activational effects. Androgen-specific receptors have been found in the electric organs of adult male B. brachyistius (long biphasic), and a possible sex difference has been suggested by preliminary data (see Bass, Segil, & Kelley, 1984). Further, Bass et.al. (1984), using autoradiography, found ^3H -DHT binding cells in the brain adjacent to "relay" cells of the medullary command nucleus. These cells project to spinal motor neurons that innervate the electrocytes of the electric organ. When B. brachyistius (long biphasic) were administered 17α -methyl T or DHT, female and immature fish increased their discharge durations in the male direction and exhibited male-like discharge waveforms with downward shifts in peak power frequency of the Fourier spectra (Bass & Hopkins, 1985). Surprisingly, E_2 treatment also resulted in a downshift in peak power frequency and an increase in discharge duration, however these changes were not as dramatic as those resulting from androgen treatment. In a closely related species, B. brachyistius biphasic, reported to have no EOD sex difference, 17α -methyl T had no effects on EODs (Bass & Hopkins, 1983). However, Landsman & Moller (unpublished data) found that androgens and estrogen both significantly influenced the durations of specific phases of the EOD in that species.

Juvenile S. corneti undergo a transitional stage in the development of the adult EOD (Bass & Hopkins, 1985). When immature

and female fish were treated with 17α -methyl T or T propionate, they showed dramatic downshifts in peak power spectral frequency and increases in discharge duration, both characteristic of male discharges (Bass & Hopkins, 1985).

A sex difference in the waveform of the mormyrid Gnathonemus petersii has recently been discovered (Landsman, Jou, & Moller, 1986; Landsman, 1989). While Kramer and Westby (1985) failed to find a sex difference when comparing individual male and female discharges on an oscilloscope, Landsman et. al. (1986; Landsman, 1989) found a sex difference in the Fourier spectrum of the discharge only under certain recording conditions. When fish were permitted to freely enter ceramic shelters containing electrodes placed at either end, males displayed higher average peak power spectral frequencies than females. However, when fish were confined in gauze envelopes or restrained by tying them in the envelope against a vertical support, the sex difference was eliminated. These restraints were initially introduced to see if they might reduce individual variation in EOD due to movement. In these latter two conditions, fish of both sexes emitted highly variable EODs which deviated from the normal peak power spectral frequency range for either sex. The results of this study indicated that EOD characteristics of the individual fish are much more labile than previously thought (see Moller, 1980).

Unfortunately, the majority of studies involving sex differences in EODs in weakly electric fish are largely descriptive and employed small samples. These studies typically report some to great amounts of overlap between the sexes. Further, the studies involving hormone manipulation lack many important control groups and in many cases used

only methylated androgen or DHT, neither of which are naturally occurring in fish.

ENVIRONMENTALLY-INDUCED CHANGES IN THE ELECTRIC ORGAN
DISCHARGE OF WEAKLY ELECTRIC FISH

When two control female S. corneti were held captive for 6 to 14 days, their average peak power Fourier spectral frequencies decreased in the direction of males or androgen-treated females. However, these changes in frequency were not statistically significant and were well within the range of normal female EODs (Bass & Hopkins, 1985). The EODs of mature males maintained in captivity for periods of 3 to 6 months did not revert to a female-type waveform; in fact, after 12 days in captivity, one male with a "transitional waveform" exhibited a more male-like peak power spectral frequency. These authors suggested that the discharges of adult males are permanent, but have a modifiable appearance based on an individual's overall physiological state. Bass (1986b) reported a similar effect in one captive B. brachyistius (long biphasic) that exhibited a reversal to the natural female EOD waveform over time.

Female H. occidentalis injected with saline as a control for hormone administration increased their peak power spectral frequencies (Hagedorn & Carr, 1985), while S. dariensis of both sexes exhibited increases in EOD frequencies following handling compared to a nonhandled control group (Meyer, 1983). The sexually dimorphic waveform of Pollimyrus isidori may be eliminated by exposure of fish to low or high water conductivities. Male EOD waveforms resembled

those of females at low conductivity, while female EOD waveforms resembled males' at higher conductivity (Bratton & Kramer, 1986). The sexually dimorphic waveform of G. petersii was eliminated when males and females were either confined or restrained (Landsman et. al., 1986; Landsman, 1989).

As reviewed above, manipulations such as handling, confinement, and restraint typically result in hormonal changes, such as increases in cortisol levels, as well as changes in behavior. This, in addition to the above findings, suggests that the EOD of weakly electric fish may reflect the physiological state of the animal.

EFFECTS OF STRESS ON REPRODUCTIVE PHYSIOLOGY IN FISH

Stress-responses in fish are characterized by an increase in cortisol, possible changes in other steroid hormones, and by changes in behavior. All of these effects may have deleterious influences on reproduction. There is some evidence that cortisol may affect reproduction in fish by inhibiting gonadal steroids directly at the level of the gonads or indirectly by inhibiting gonadotropin secretion. The same environmental stimuli which cause increases in cortisol also result in decreases in reproductive success. Extreme variations in temperature; water quality including such aspects as acidity, dissolved oxygen content, salinity, and pollutants; food availability; and social environment may all have detrimental effects on reproduction characterized by inhibition of gonadotropin release, gonad development, gametogenesis, ovulation, pheromone production, spawning, egg fertility, and embryogenesis (for a review, see Billard,

Bry, & Gillet, 1981).

In rats, increased corticotropin-releasing factor (CRF) resulting from stress manipulations has been directly implicated in the inhibition of luteinizing hormone secretion (Rivier, Rivier, & Vale, 1986). A few studies have shown that adverse environmental conditions or aggression may interfere with reproduction in fish via decreases in plasma gonadotropic hormone(s) (GTH, many fish species secrete only one gonadotropin). The endocrine mechanisms controlling reproduction in fish are similar to those in higher vertebrates. The hypothalamus secretes gonadotropin-releasing hormone (GnRH) which stimulates the anterior pituitary gland to secrete GTH (Billard et al., 1981). This hormone stimulates gametogenesis and induces the gonads to produce sex steroids which exert a negative feedback on the hypothalamo-pituitary axis. Both plasma and pituitary GTH declined in starved goldfish (Carassius auratus) (Gillet, Billard, & Breton, 1981). A decrease of plasma GTH and gonadosomatic index (GSI) was observed in male goldfish maintained at 30 °C under hypoxic conditions. Bry and Zohar (1980) and Zohar (1980) reported that the catheterization of the dorsal aorta in one rainbow trout resulted in hypophagia and failure to acclimate following the surgical procedure. This fish exhibited a prolonged elevation of plasma glucocorticoids, evidence of a stress response, and a marked decline in plasma gonadotropin. Finally, Sangalang and Freeman (1974) have shown that androgen metabolism is decreased in brook trout exposed to sublethal levels of cadmium, while Freeman, Sangalang, and Uthe (1981) reported that both gonadal and interrenal steroid hormone synthesis is adversely affected by cadmium and PCB (Aroclor 1254) in the common brook trout (Salvelinus fontinalis) and

Atlantic cod (Gadus morhua L.), respectively. Electric shock has been shown to have adverse effects on the fertilizability of the ova of adult female salmon (Marriott, 1973) and adult females shocked as embryos (Godfrey, 1957; Marriott, 1973).

Only a few studies have investigated the effects of handling and confinement on reproduction in fish. Inhibition of the ovarian response to exogenous gonadotropin and follicular atresia were observed in pike (Esox lucius) following handling and captivity (de Montalambert, Bry, & Billard, 1978). Abraham and Blanc (1966) reported that in the grey mullet, Mugil cephalus, oogenesis fails to occur in freshwater ponds (due to confinement?), but it is completed in lakes. Further, when they were maintained in fresh water, ovulation did not occur; while in sea water, oogenesis and ovulation did occur, except when fish were maintained in ponds. When female minnows, Phoxinus phoxinus, were transferred from the natural environment to an aquarium tank, massive atresia of vitellogenic oocytes occurred within a few days (Scott, 1979). Changes in EOD, such as elimination of the sex differences in waveform, following exposure to aversive environmental stimuli have been discussed in a previous section. As detailed earlier, gonadal steroid levels have effects on EODs. Environmental influences upon the EOD may be exerted through changes in plasma levels of gonadal or interrenal hormones. Cortisol may affect the EOD either by directly influencing the electric organ electrocytes or by influencing gonadal steroid hormone levels. In freshwater Tilapia (Sarotherodon mossambicus) and rainbow trout (Salmo gairdneri), increased cortisol has been shown to augment the entrance of sodium, calcium, and chloride within muscle cells and

support the retention of water within cells and the accumulation of a larger amount of water in the extracellular space (Assem & Hanke, 1981). Thus, cortisol has been implicated in the regulation of cellular ion channels. A similar type of regulation may occur at the electrocyte level in the electric fish.

EXPERIMENTAL PROTOCOL

The studies presented here were designed to examine the EOD in Gnathonemus petersii with special emphasis on plasticity in the waveform of the EOD as a signal in electrocommunication. In addition, sex differences in external morphology were examined. Each study is presented in manuscript form with references following the specific format for the publication for which it was intended. A general bibliography can be found at the end of this volume.

The weakly discharging electric fish, G. petersii, was chosen as subject for a number of reasons based on the findings in the first study, Aversive Manipulation Obscures Signalling of Sexual identity in the Weakly Electric Fish, Gnathonemus petersii. This experiment was designed to assess the effects of confinement- and restraint-stress on the waveform of the EOD. Adult males and females were first confined, then restrained or permitted to rest freely in their shelters in a counterbalanced design. A sex difference with some overlap in PPSF was found, with males exhibiting higher PPSFs than females only when fish were at rest. PPSFs were inversely related to the duration of phase 3 of the EOD. When subjects were either confined or restrained, the sex difference was eliminated due to substantial increases in individual variability in PPSF for both sexes. The sex difference obtained in this study was surprising for three reasons. First, it was contrary to the results of Kramer and Westby (1985) who failed to find a sex difference in the EOD waveform in G. petersii. Second, studies of sex differences in other mormyrid species typically

reported males to have lower PPSFs and EODs of longer duration than females, results which were opposite to those found for male and female G. petersii. Finally, the EOD was previously thought to be stable enough to serve as a "fingerprint" for species, sexual, and individual identity. The results of my first study suggested that the EOD is malleable and may even serve as an alarm signal similar to that of other vertebrate species. The remaining manuscripts address these issues.

The second study, Testosterone Changes the Electric Organ Discharge and External Morphology of the Mormyrid Fish, Gnathonemus Petersii (Mormyriformes), was designed to determine whether the EOD and/or external morphology in this species might be sensitive to gonadal hormones. Juveniles and one adult subject implanted with pellets or silastic capsules containing 17α -methyl T showed dramatic decreases in PPSF and increases in EOD duration, as well as development of adult male-like indentations in the dorsal margin of the anal fin. Although these effects of androgen on the EOD were similar to those reported for other species, they were inconsistent with the sex difference reported in study one in which males exhibited higher PPSFs indicative of shorter duration EODs than females. The third study, The Effects of Steroid Hormones on the External Morphology and Electric Organ Discharge Waveform of Gnathonemus Petersii (Mormyriformes), further investigated the effects of hormonal control of sexual characteristics in this species. Male and female hormones were administered in low- and high-dose silastic capsules to juveniles and high-dose to adults. Only androgens exerted hormone- and dose-dependent effects on specific phases of the EOD in both

juveniles and adults. Although E_2 exerted no effects on the EOD in juveniles, it caused a slight increase in adult PPSFs. Finally, only androgens caused the male-like indentation in the dorsal margin of the anal fin typical of adult mormyrids. Together, the previously mentioned findings regarding the sex differences in EOD and the effects of male hormones were inconsistent. If androgens, but not estrogens, increase the durations of specific phases of the EOD resulting in a lower PPSF, then, assuming males have higher endogenous androgen levels than females at least during breeding season, the male EODs should have longer phases and lower PPSFs than the females'.

The fourth study, Sexual Dimorphisms in Morphology and Electric Organ Discharge in Gnathonemus Petersii (Mormyriformes): Seasonal Phase-Specific Signalling of Sexual Identity, reexamined the EOD for sex differences in just-imported adult fish during the African pre-rainy, rainy breeding, and post-rainy seasons. Regardless of season, all males but no females exhibited the indentation in the dorsal margin of the anal fin. Only rainy season fish exhibited non-overlapping sex differences in phases 2 and 3 of the EOD and in PPSF, with males exhibiting longer durations in both phases and lower PPSFs.

The fifth study, The Effects of Captivity on the Electric Organ Discharge and Plasma Hormone Levels in Gnathonemus Petersii (Mormyriformes), was designed to address the discrepancies between the sex differences in studies one and four. In Experiment 1, the newly imported adult fish from study four were maintained in captivity in groups or individually for up to 37 days during which EOD data were collected periodically. After 37 days of captivity, all EOD sex

differences in group-maintained subjects were gone, while subjects maintained in individual aquaria exhibited sex-reversed EODs such that females exhibited significantly longer phase 3 durations and lower PPSFs than males. Only males showed changes in PPSFs, while both sexes showed phase duration changes in opposite directions. Females appeared to have higher endogenous plasma androgen levels than males following 37 days of captivity. Experiment 2 assessed the effects of up to 15 days of captivity on EOD behavior and endogenous androgen levels in adult males. Captivity caused dramatic decreases in plasma androgen levels by day 5 through day 15. The drop in hormone titers was followed by a decrease in the duration of phase 2 of the EOD and an increase in PPSF.

The final manuscript, Captivity Affects Behavioral Physiology: Plasticity in Signalling Sexual Identity, summarizes the most important findings from the five studies with emphasis on general contributions to the study of electrocommunication as well as captivity effects in vertebrate species.

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PART 1: AVERSIVE MANIPULATION OBSCURES SIGNALLING OF SEXUAL
IDENTITY IN THE WEAKLY ELECTRIC FISH, GNATHONEMUS PETERSII

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Abstract

Several species of weakly electric fish exhibit sexually dimorphic electric organ discharges (EODs) used in social communication. Such a dimorphism was not known to exist in the African mormyrid fish, Gnathonemus petersii. EODs and associated Fourier spectra were obtained for this species under three recording conditions, varying in degree of mobility allowed the fish: 'restrained', 'confined', and 'free'. An EOD-related sexual dimorphism appeared in the 'free' condition, but was not discernible in the other two: the fish's EOD is much more malleable than was previously thought. Stress-producing conditions may affect the EOD waveform changing its signal content from sexual identity to alarm or fear.

The electric organ discharges (EODs) emitted by freshwater African mormyriiform and neotropical gymnotoid fishes serve as signals in social communication (1). Several species exhibit a sex difference in the pulse-like wave of their EOD and in the sequence of pulse intervals (2,3). These differences communicate sexual identity (4). Several mormyrid species, such as Brienomyrus brachyistius (biphasic) and Gnathonemus petersii reportedly exhibit no sex differences in EOD under laboratory recording conditions (5). I now report a sex difference in the EOD of G. petersii. This difference disappeared under recording procedures which produce stress in fish (6). Thus, individual EODs may signal information about the fish's current physiological state, and may not be as invariant as was previously thought.

The fish used in my study were five male and five female sexually mature (gonadally ripe, determined by testis weight and egg size) G. petersii (7). Male fish ranged in standard length from 15.5 to 20.5 cm (SD = 2.02 cm) and females from 15.6 to 18.3 cm (SD = 0.92 cm). The fish were clipped on dorsal and anal fins for identification, and assigned to two 200 l isosexual holding tanks. The light-dark cycle was 12:12 with light onset at 0900 h. Fish were fed tubifex worms twice a week.

For recording, the fish were individually placed into a 30x60x45 cm all-glass aquarium maintained at 22.5 °C and conductivity at 150 μ S/cm. EODs between the fish's head and tail were monitored with a pair of Ag/AgCl electrodes, and fed directly into a Hewlett-Packard Spectrum Analyzer (HP 3582A, range: 0-25 KHz, resolution: 100 Hz), to measure the peak power frequency of the Fourier spectrum. Initially,

fish were confined in a gauze envelope suspended into the water from an overhead support system, allowing the fish some degree of lateral mobility. Following a 15-min habituation period in the test tank, six Fourier power spectra were collected for each subject. Testing was done between 1400 h and 1800 h. The peak power spectral frequencies (PPSFs) were tabulated and a mean PPSF was computed for each fish. Absolute deviation scores were obtained by subtracting each subject's mean PPSF from the group mean for that sex. Individual absolute deviation scores and sex-group absolute deviation means were plotted as a function of mean PPSFs (Fig.1). In general, 'confined' males showed average peak power spectra at higher frequencies than females (means $\pm$ SE = 3102.4 ± 110.4 Hz and 2739.8 ± 117.8 Hz, respectively; Fig. 1a); however, this difference was not statistically significant, $t(8) = 2.25$, $p < 0.06$. The great variability between fish (illustrated by the vertical spread of the male and female distributions in Fig. 1a) suggested that the recording technique which allowed the fish some degree of mobility might have been the source of the observed variability obscuring any sex difference. Therefore, two additional recording conditions were introduced. Following a five-day resting period, the fish were again individually placed into the test tank, this time either 'restrained' within the gauze envelope tied to a vertical support (8) within the tank or 'free' to leave or return to a porous porcelain shelter tube. The 'restrained' condition deprived the fish of all locomotor activity; in the 'free' condition, the fish were permitted to move about freely and EOD recordings were taken only when a fish remained in its shelter.

Since the 'restrained' and 'free' conditions were introduced

following analysis of data from the initial 'confined' condition, fish were assigned to the two treatments in a counterbalanced manner so that all subjects received both conditions separated by a five-day rest period. Restraining the fish did not result in the expected sex difference in average PPSFs, $t(8) = 0.54$, $p = 0.60$; there was great overlap in individual male and female means (Fig. 1b). Surprisingly, in the 'free' condition (Fig. 1c), the male PPSF was located at a significantly higher frequency (mean $\pm$ SE = 2902.6 ± 73.1 Hz) than the female PPSF (mean $\pm$ SE = 2691.5 ± 60.6 Hz), $t(7) = 2.14$, $p < 0.05$, one-tailed, $r_{pb} = 0.63$ (9). The PPSF was not related to the standard length of the fish, $r(N = 9) = -0.49$, $p > 0.05$. There was also a dramatic decrease in variability between individual PPSFs for both males and females in the 'free' condition compared to the relatively large variability between individuals in the 'restrained' condition (Figs. 1b, c). Levene's test (10) was used to assess differences in variability between the sexes and across the 'free' and 'restrained' conditions by subjecting the absolute deviation scores to a split-plot ANOVA. A significant treatment effect, $F(1, 7) = 6.15$, $p < 0.05$, indicated greater variability between the individual means in the 'restrained' condition (mean absolute deviations were 492.8 for males and 324.5 for females) compared to the 'free' condition (male mean = 104.2, female mean = 93.5). No sex or interaction effects were statistically significant. Thus, it appears that a sex difference in mean PPSFs was eliminated by the high variability in the 'restrained' condition, which resulted in greater overlap among individual male and female means. Although the 'confined' condition was not compared with the other two conditions (11) it is interesting to note the trend

toward the expression of a sex difference and the intermediate variability of individual means (Figs. 1a, b, c) (12).

The mean PPSF was plotted for each fish, and the 'free' condition data were then compared with the 'restrained' condition data (Fig. 2). Under the 'free' condition, both males and females exhibited PPSFs between 2540 and 3100 Hz (males: 2660 to 3100 Hz, females: 2540 to 2820 Hz) (13). Under the 'restrained' condition, however, individuals shifted their PPSFs either to the low end of the spectrum (males: 2230 to 2450 Hz, females: 2370 to 2412 Hz) or to the higher end (males: 2875 to 3950 Hz, females: 2867 to 3140 Hz). The PPSF sex difference is probably reflected in the duration of the EOD (Fig. 3). Only the duration of the negative phase of the waveform was inversely related to PPSF of both sexes, $r(N=9) = -0.67$, $p < 0.05$, $r^2 = 0.45$. There is evidence that mormyrids discriminate the EOD waveform on the basis of the duration of its phases (2). I am currently collecting additional data to determine whether a statistically significant sex difference exists in this characteristic.

My results suggest that the EOD is more malleable than was previously thought. Stress may affect reproduction in the electric fish through its effects on communication. As sex differences in EODs of other weakly electric fish species have been successfully altered by gonadal and hormonal manipulations (3), it is likely that the EODs of G. petersii might also be affected by hormones (14). Changes in environmental factors have previously been reported to alter the EODs of other electric fish species and influence the sexually dimorphic characteristics of their electrical signals (15). Stressors such as

capture and confinement increase circulating catecholamines and corticosteroids in many fish species (6). The confinement procedures in the present study may have constituted stressful events for the subjects and resulted in a hormonal stress-response which, in turn, altered the EODs emitted by these fish. Stress may affect all levels of the hypothalamic-pituitary-gonadal axis and is deleterious to reproduction in many species. Corticotropin-releasing-factor has been shown to mediate the inhibition of luteinizing hormone resulting from stressful manipulation (16). Somatic stressors have also been shown to result in decreased plasma testosterone levels, and changes in social dominance status have resulted in changes in plasma testosterone and plasma cortisol concentrations in a variety of species (17). Similar hormonal changes in electric fish may influence the EOD behavior and thus the message broadcast by these signals. As dominant and subordinate individuals exhibit differential hormonal responses to stress (17), this may explain the fact that some fish exhibited increased PPSFs while others exhibited decreases in response to the stress-producing environment (Fig. 2). The EOD waveform of G. petersii could thus serve to signal information about the individual's current physiological status, be it exposure to stressors or readiness to mate. Because of its susceptibility to changing environmental conditions, the EOD of certain weakly electric fish may not be a thumbprint for individual identity (18) but rather involved in broadcasting motivational characteristics. EODs produced in stressful situations may be analogous to the alarm calls of other vertebrates.

Figure 1. EODs recorded from the same fish under a) 'confined', b) 'restrained', and c) 'free' conditions. Absolute deviations, indicating the variability of individual average PPSFs (Y-axis), are plotted as a function of the individual average PPSFs (X-axis). Also, each sex group mean absolute deviation is plotted as a function of its group mean PPSF. Black circles (●) represent female individual means; open circles (○) represent female group means; black triangles (▲) represent male individual means; open triangles (△) represent male group means. The enclosure surrounding each sex group distribution illustrates the amount of variability (vertical spread) and range (horizontal spread) of individual mean PPSFs. The 'confined' condition was completed prior to the introduction of the 'restrained' and 'free' manipulations and thus was not included in the counterbalancing of conditions. The vertical support used for restraining the fish was a styrofoam board. I have also tied fish against a porous porcelain shelter tube and obtained the same EOD alterations. This along with the fact that there was no relationship between the 'free' and 'restrained' PPSFs ($r = 0.20$) indicates the styrofoam support did not systematically affect the EOD as there was no consistent alteration in waveform among fish. The degree of mobility of the fish in the 'free' condition was much larger than in the 'confined' condition. The decrease in variability of the peak power spectra of the Fourier transformations in the 'free' condition indicates that the vicinity of the electrodes to the fish did not affect the waveform. The sample size for the 'free' condition was 9 as one female died prior to testing.

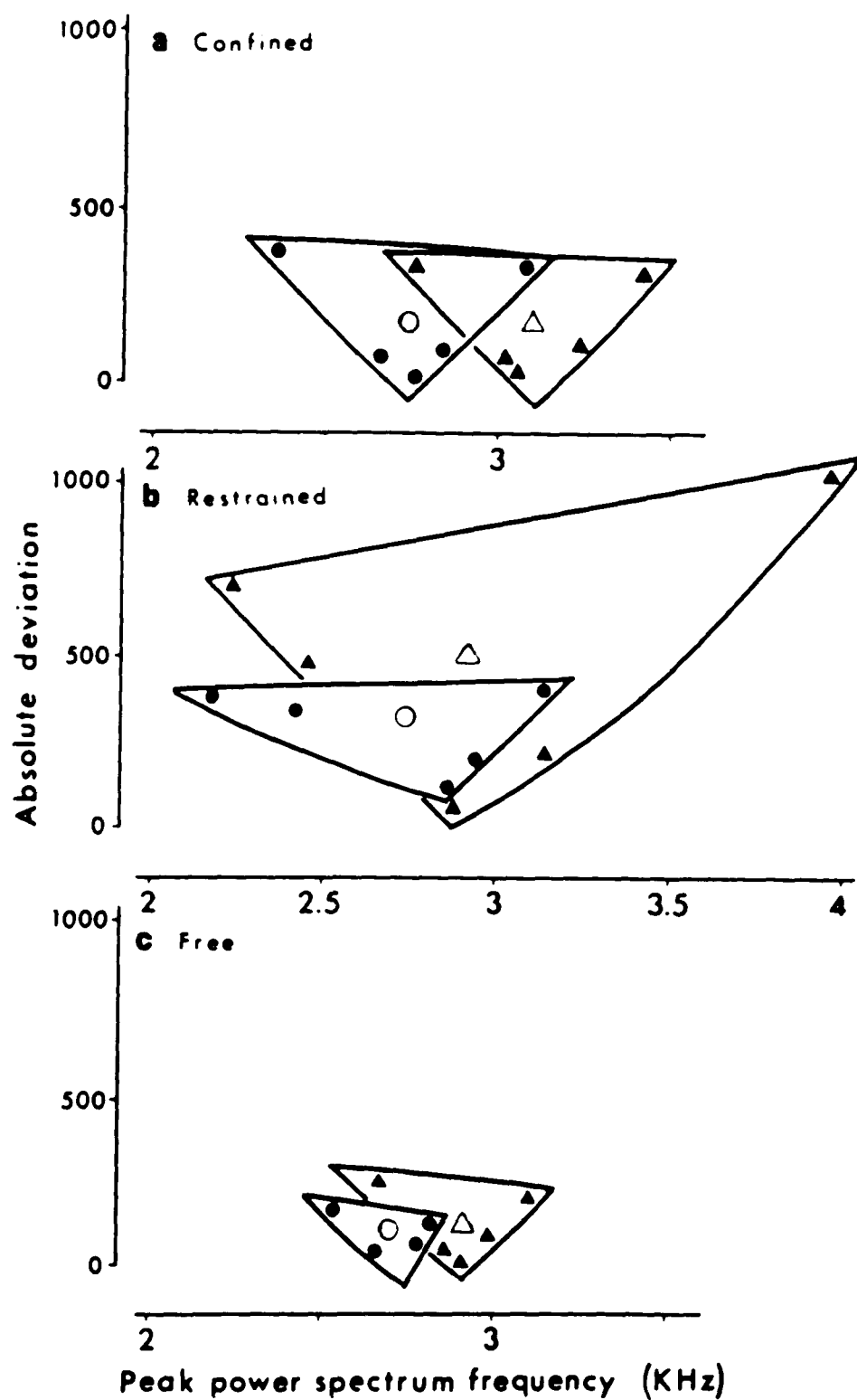


Figure 2. The fate of individual mean PPSFs for males and females under 'free' and 'restrained' conditions. Under the 'free' condition, PPSFs are clustered, and under 'restrained' conditions are dispersed to either end of the spectrum. The numerals identify individual fish. Some overlap in the PPSF between the sexes can be seen in Fig. 1c and the present figure ('free' condition). Investigators have reported overlap in sexually dimorphic EOD characteristics of other species of weakly electric fish^{7,12,14,15}.

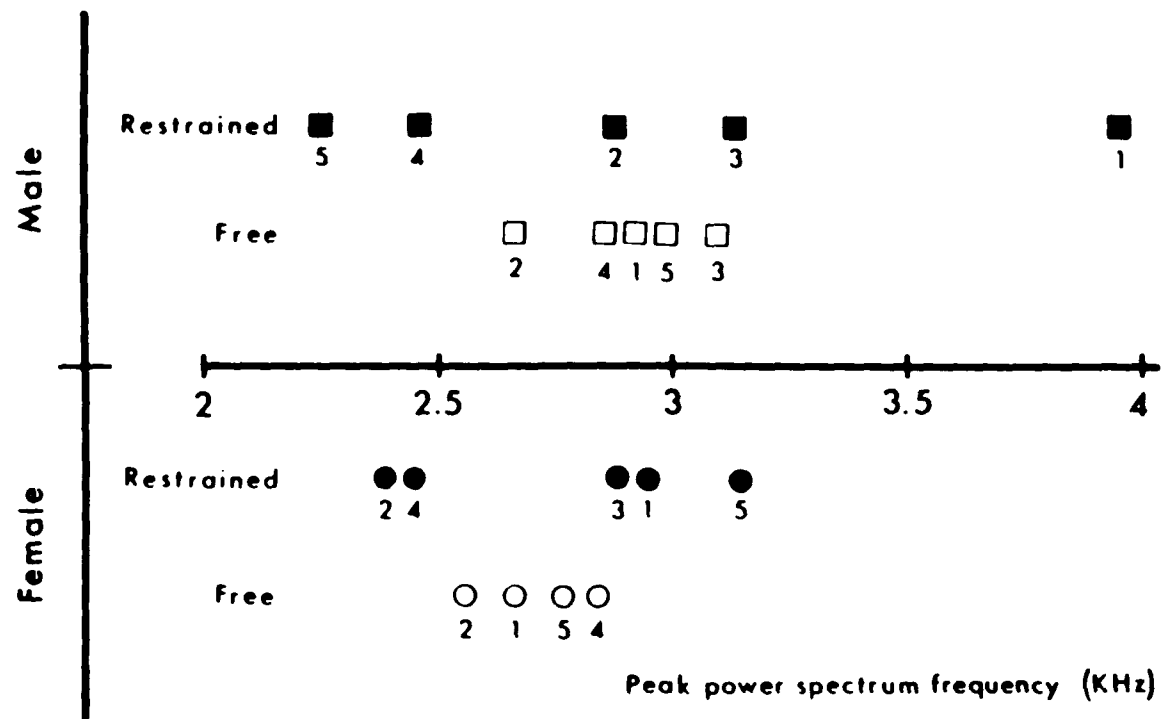
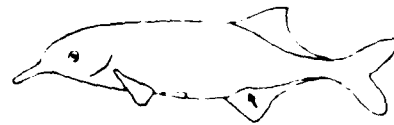
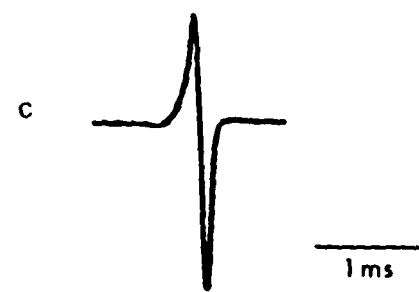
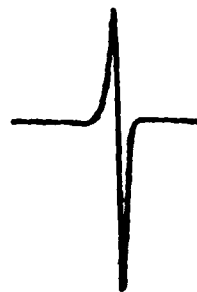
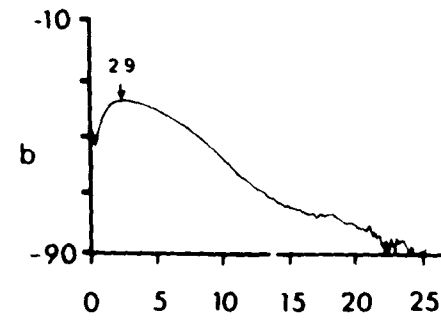
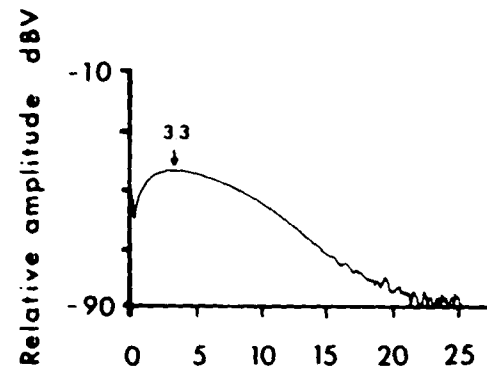
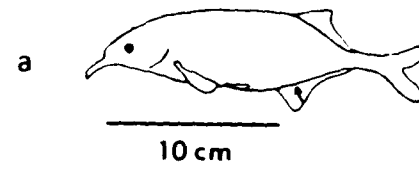


Figure 3. Sexual dimorphisms in the mormyrid G. petersii. (a) external morphology: arrows point to dorsal margin of the anal fin. Notice the indentation displayed by the male. In selecting the subjects for my study, I chose the largest males and females in order to ensure sexual maturity. (b) Fast Fourier Power Spectrum: note difference in peak power spectrum (arrows); (c) duration of EOD waveform of males is shorter than females.

Male



Female



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7. Fish were obtained from African Fish Importers, Hackensack, N. J. two days following import from Nigeria. In selecting subjects, I chose the largest males and females by external sexual dimorphism (Fig. 3a) in order to ensure sexual maturity (B. F. Blake, J. Fish. Biol. 11, 437 (1977)).
8. The support was a styrofoam board. I have also tied fish against a porous porcelain shelter tube and obtained the same EOD alterations. This along with the fact that there was no relationship between the 'free' and 'restrained' PPSFs ($r = 0.20$) indicates the styrofoam support did not systematically affect the EOD as there was no consistent alteration in waveform among fish.
9. A one-tailed test was used because of the trend for males to exhibit higher PPSFs than females in the initial manipulation. The sample size for this comparison was 9 as one female died prior to testing in the 'free' condition.
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11. The 'confined' condition was completed prior to the introduction of the 'restrained' and 'free' manipulations and thus was not included in the counterbalancing of conditions.
12. The degree of mobility of the fish in the 'free' condition was much larger than in the 'confined' condition. The decrease in variability of the peak power spectra of the Fourier

transformations in the 'free' condition indicate that the vicinity of the electrodes to the fish did not affect the waveform.

13. Overlap in sexually dimorphic EOD characteristics of other species of weakly electric fish was also reported by C. D. Hopkins, Behaviour 50, 270 (1974); G. M. W. Westby, F. Kirschbaum, in Sensory Physiology of Aquatic Lower Vertebrates, T. Szabo, G. Czeh, Eds. (Pergamon Press, Budapest, 1981) pp. 179-194; J. H. Meyer, J. Comp. Physiol. 153, 29 (1983); A. H. Bass, C. D. Hopkins, J. Comp. Physiol. 156, 587 (1985).
14. I report here Fourier spectra of EODs from G. petersii that differ from those obtained by M. Boudinot (thesis, Mem. C.N.A.M., Paris, 1972) and B. Kramer, G. M. W. Westby (Experientia 41, 530, 1985). This difference could be due to the respective reproductive states of the fish. My fish were caught during the local rainy (breeding) season and tested 14 days after import to the U.S., while Kramer's fish were tested after a longer stay in the laboratory (Kramer, personal communication, 1986). It is also possible that there are subspecies of G. petersii.
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19. I thank C. F. Harding and B. Kramer for critical reading of the manuscript. This research was supported in part by a grant from the City University of New York PSC-CUNY Research Award Program.

PART II: TESTOSTERONE CHANGES THE ELECTRIC ORGAN DISCHARGE AND
EXTERNAL MORPHOLOGY OF THE MORMYRID FISH, GNATHONEMUS
PETERSII (MORMYRIFORMES)^{1,2}

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Abstract

Effects of silastic and pellet methyltestosterone implants on the waveform of the electric organ discharge of the weakly electric African mormyrid, Gnathonemus petersii, were investigated. Within seven days of implantation, the duration of the discharge increased dramatically while the associated peak power frequency of the Fourier spectrum decreased in all treated fish. By day 35, hormone-treated fish exhibited up to five-fold increases in EOD duration, as well as multiple discharges and variations in the shape of the positive phase of the discharge. Testosterone treatment also changed body morphology, making immature and adult female fish resemble adult males.

Several species of mormyrid fish exhibit sex differences in the characteristics of their electric organ discharge. Such differences appear important for sexual identification³. However, the majority of mormyrids investigated failed to exhibit any sexual dimorphism in waveform or discharge pattern under laboratory conditions⁴. Male hormones administered to juvenile or adult females typically result in fish exhibiting male-like discharges⁵. However, only when a natural sex difference is evident under laboratory or field conditions, is it affected by exogenous gonadal hormone manipulation⁵. Thus, it is believed that treatment with gonadal steroids may predict naturally occurring sexually dimorphic electric-organ-discharge (EOD) waveforms⁵.

Kramer & Westby⁶ did not find a sex difference in the waveform of the EOD of Gnathonemus petersii. Landsman, Jou, and Moller⁷, however, reported a sex difference in the average peak power spectral frequency (PPSF) for this species. Only when the fish were recorded at rest, unrestrained in their shelters, did males exhibit peak power spectra of higher frequencies than females, with some overlap between the sexes. This sex difference was inversely related to the duration of the EOD, with male EODs shorter than those of females.

The present study investigated whether the EOD waveform of G. petersii can be influenced by testosterone, to substantiate a natural EOD sex difference in this species.

Materials and methods. Animals. Fourteen juvenile G. petersii (standard length: 10.2 to 12.0 cm; weight: 10.0 to 19.0 g) and one adult female (S.L.: 16.1 cm, 42 g) were randomly selected from large stock tanks (Lombardos African Fish Imports, Newark, N.J.). Fish were

maintained individually in 20-l aquaria on a 12:12 L:D cycle, with lights on at 0900 h. Water conductivity was kept at $200 \pm 40 \mu\text{S/cm}$ and temperature at $23 \pm 0.5^\circ\text{C}$.

EOD Recordings. A pair of Ag/AgCl electrodes, extending from Plexiglass tubes were fitted to the far ends of the aquarium, 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) which triggered a spectrum analyzer (Hewlett-Packard, model 3582A, range: 0-25 KHz, resolution: 100 Hz). Both EODs and Fourier transformations were plotted with an X-Y plotter (Hewlett-Packard) and photographed from the screens of the oscilloscope and spectrum analyzer, respectively. All recordings were made between 1500h and 1700h.

Gonadal manipulation and hormone implants. The duration of the EOD was tested in each fish. Then, three of the juveniles were anesthetized (tricaine methane sulfonate, 1:20,000) and either (1) implanted with a silastic capsule (Dow Corning 0.065 inch o.d. and 0.03 inch i.d.) containing 1 mm/2.5 g body weight packed 17α - methyltestosterone (17α -T)(Sigma), (2) gonadectomized and implanted with a similarly sized 17α -T silastic capsule, or (3) gonadectomized and implanted with a 1 mg 17α -T pellet. The adult female fish was anesthetized, gonadectomized, and implanted with a 17α -T silastic capsule. The gonad was removed and/or implant inserted into the gut cavity through a 1.5 cm incision on the left ventral surface, approximately 5 mm posterolateral of the ventral fins. Five other juvenile fish were anesthetized, gonadectomized, and implanted with a silastic tube containing cholesterol to control for the effects of

non-gonadal steroid hormones. The animals were returned to their aquaria, and all fish, experimental and cholesterol control, were treated with chloramphenicol (Sigma), an antimicrobial agent. Six of the juvenile fish were not subjected to any anesthetization or surgery and served as nonhandled controls. All operated fish, testosterone- and cholesterol-implanted controls, showed no visible deficiency in their motor behavior and fed normally.

Results and discussion. Figure 1a shows a typical EOD of a juvenile G. petersii along with its Fourier transformation. Before treatment, we had verified that all fish exhibited EODs of 0.6 to 0.8 ms in duration with peak power spectrum frequencies (PPSFs) ranging between 2900 Hz and 4400 Hz (fig. 1a). The EOD duration of the adult female was 0.8 ms with PPSF at 2900 Hz. The EOD duration for all control fish, nonhandled and cholesterol, remained between 0.6 and 0.8 ms duration throughout the study.

Methyltestosterone exerts profound effects on the EOD of G. petersii as evidenced by changes in duration, the associated PPSF, and pulseform. Within the first 7 days post-implant, the 17α -T implanted fish exhibited increased EOD durations ranging from 1.2 to 2.0 ms. These 'day 7' EOD durations (1.26 ± 0.32 ms) were significantly longer than the 'day 0' pre-implant durations (0.67 ± 0.06 ms), $t(3) = -3.67$, $p < 0.05$.

Figure 1b illustrates changes in duration and Fourier transformations over the length of the study for a gonadectomized, 17α -T pellet-treated fish. By day 4 post-implant, the EOD length had increased from 0.6 ms to approximately 1.0 ms, while the associated PPSFs decreased from 4100 Hz to 2100 Hz. By day 35, the EOD lasted

3.4 ms, a more than 5-fold increase in duration, while the PPSF had decreased to 400 Hz. This increase in the duration of the pulse was clearly reflected in the first three phases of the discharge; the duration of the EOD was inversely related to its PPSF, $r(N=8) = -.861$, $p < 0.01$, $r^2 = 0.74$.

All control fish exhibited PPSFs ranging from 2900 to 4400 Hz throughout the study. By post-implant day 7, Fish #1 (silastic 17α -T implant, intact), Fish #4 (silastic 17α -T implant, gonadectomized), Fish #5 (17α -T pellet implant, gonadectomized), and Fish #23 (adult female, silastic 17α -T implant, gonadectomized) exhibited decreases in the PPSF of the Fourier transformations of their EODs (see insert, fig. 2).

Figure 2 shows the mean PPSFs for all control and experimental fish. An analysis of variance revealed a significant treatment effect by post-implant day 7, $F(2,12) = 49.50$, $p < 0.0001$, with the Tukey HSD indicating that the 17α -T mean PPSF (1700 ± 440 Hz) was significantly lower than the mean PPSFs for both the nonhandled (3725 ± 264 Hz, $p < 0.01$) and cholesterol (3560 ± 329 Hz, $p < 0.01$) control groups. There was no statistical difference between the two control mean PPSFs.

By day 15, the EOD duration for Fish #4 reached its maximum length (2.2 ms) with PPSF of 800 Hz, after which it began to decrease in duration resulting in higher PPSFs (see insert, fig. 2). EOD durations increased with sharply decreasing PPSFs for all implanted fish until day 12, and then began to level off. Fish #5 exhibited the EOD of longest duration (3.4 ms, PPSF = 400 Hz), followed by #1 (2.8 ms, PPSF = 600 Hz), #4 (2.5 ms, PPSF = 850 Hz), and Fish #23

(1.9 ms, 1300 Hz). The time-course of changes in PPSFs was identical in all fish with the exception of #4 (see insert, fig. 2).

Changes in EOD duration have been reported in other mormyrids treated with testosterone, but only in species that showed a sex difference under natural conditions⁴. Testosterone-treated immature fish and treated adult females typically exhibit a male-like EOD of longer duration⁴, as was found for all of the treated G. petersii in the present study. This was unexpected, in that results from previous studies^{7,8} found that female pulses were longer than those of males. It has been suggested that hormone treatment may reliably induce naturally occurring EOD sex differences in mormyrids⁴. My results appear to be incongruent with the previously reported sex difference in G. petersii, suggesting that at least for this species, testosterone treatment may not reliably indicate a naturally occurring EOD sex difference. It is also possible, however, that the physiological stress of laboratory housing may alter male EODs in the female direction. EOD duration and waveform obtained from mormyrids in captivity were reported to have undergone a natural sex reversal from maleness to femaleness, and such changes may be a function of rapidly fluctuating steroid levels⁴. Fish exposed to acute or chronic stress from confinement, capture, and handling exhibited stimulation of the hypothalamo-pituitary-interrenal axis, marked by increased ACTH and cortisol levels, and suppression of the hypothalamo-pituitary-gonadal axis, marked by suppression of plasma androgens^{9,10}. Such environmentally induced hormone responses could explain the previously reported EOD sex difference in G. petersii. These differences were: (i) accentuated by atypically high aquatic

conductivity levels (above $700 \mu\text{S}/\text{cm}$)⁸, (ii) immediately abolished following physical restraint⁷, and (iii) eliminated within 48 hrs following sudden, large conductivity changes⁸. Comparable sex-related alterations in EOD as a function of water conductivity were reported in Pollimyrus isidori¹¹.

Testosterone treatment effected other changes in the fish's EOD activity (fig. 3). (i) Frequently, the fish emitted trains of two to six multiple discharges that were identical in form and duration and were separated by 6.1 ms intervals (fig. 3a), suggesting autostimulation of the electric organ. Control fish never exhibited such EOD activity. (ii) Treated fish exhibited characteristic variations in the ascending portion of the initial positive phase (fig. 3b). (iii) On day 60, Fish #5 switched between two pulseforms, the elongated EOD (as shown in fig. 1b on day 35) and a discharge of the same duration, best described as a succession of two biphasic pulses with the second lasting twice as long as, and exhibiting about 4 times the peak-to-peak amplitude of the first (fig. 3c). These changes in the generation of EODs could be related to a testosterone-induced increase in electrocyte membrane surface⁴.

Testosterone treatment also resulted in external morphological changes. Figure 4 shows the appearance of an indentation in the dorsal margin of the anal fin in Fish #5¹². The other three 17α -T implanted fish also exhibited this change, whereas none of the control subjects did. In several species of mormyrid fish, such indentation is characteristic of large, mature males.

Although the extent of the hormone effects could, in part, be due

to pharmacological doses, it is evident that gonadal hormones do influence the electro-communication system in G. petersii. Further studies will be required to resolve the issue of a hormone-dependent natural sex difference in the EOD of this species.

Figure 1. Electric organ discharge waveforms and associated Fourier transformations recorded from a juvenile G. petersii, Fish #5, (a) before and (b) following gonadectomy and 17α -methyltestosterone implantation for a period of 35 days. Note increase in EOD duration and corresponding decrease in peak power spectrum frequency. Fourier spectra (right panel) correspond in successive order to EODs (left panel) recorded on days marked with black dot.

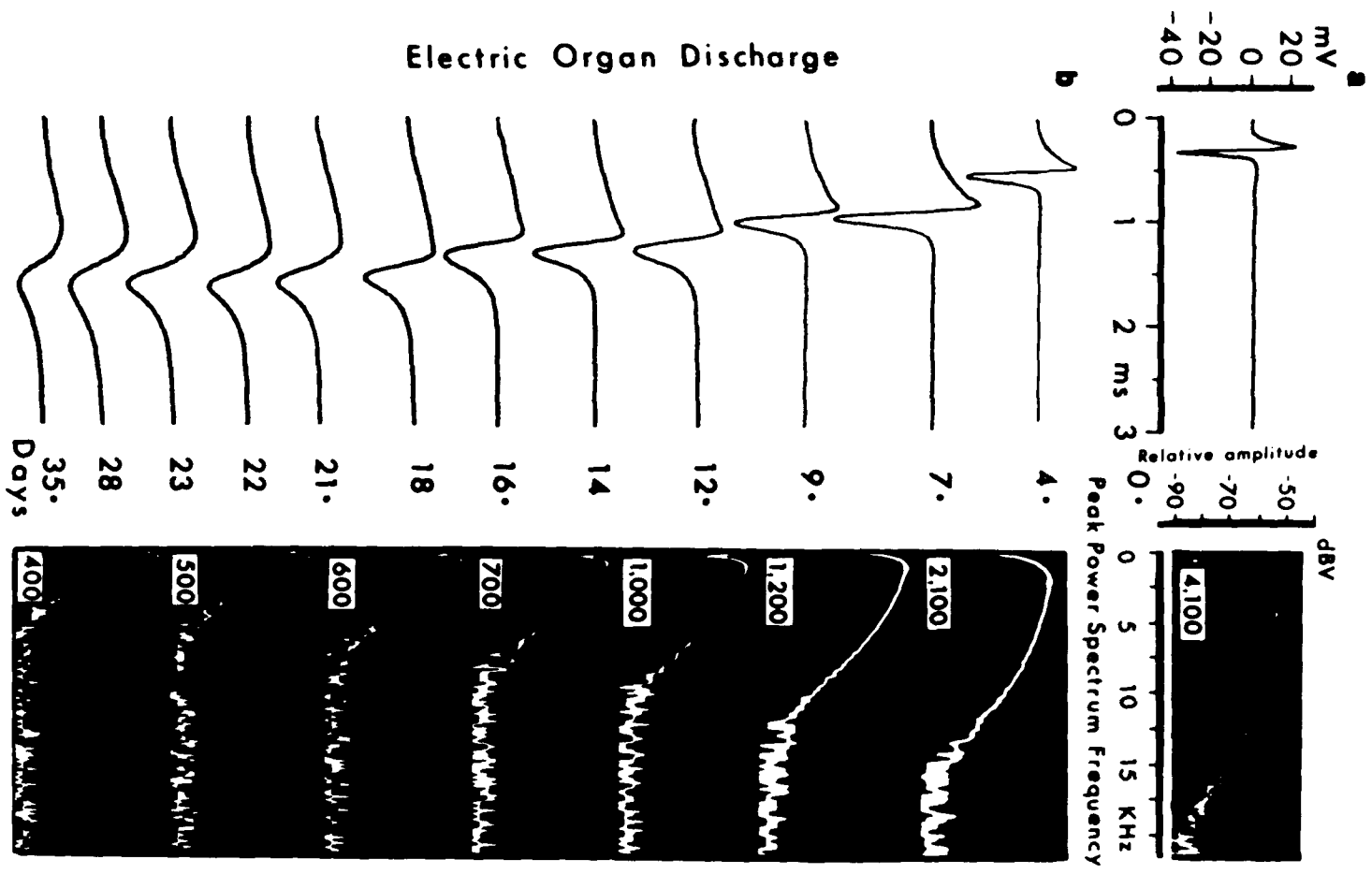


Figure 2. Time-course of hormone treatment on electric organ discharge waveform in Gnathonemus petersii. Mean peak power spectrum frequencies (PPSFs) and SEM for controls (open circles-nonhandled fish, n=6; solid circles-gonadectomized, cholesterol-implanted fish, n=5) and 17α -methyltestosterone-implanted fish, n=4 (solid triangles). Mean pre-implant PPSF ('Day 0', open triangle) was estimated on the basis of pulse duration data. Day 4 solid triangle represents the PPSF from Fish #5 only. Insert (left bottom corner) shows time-course over days of 17α -T treatment on PPSF for the individual fish.

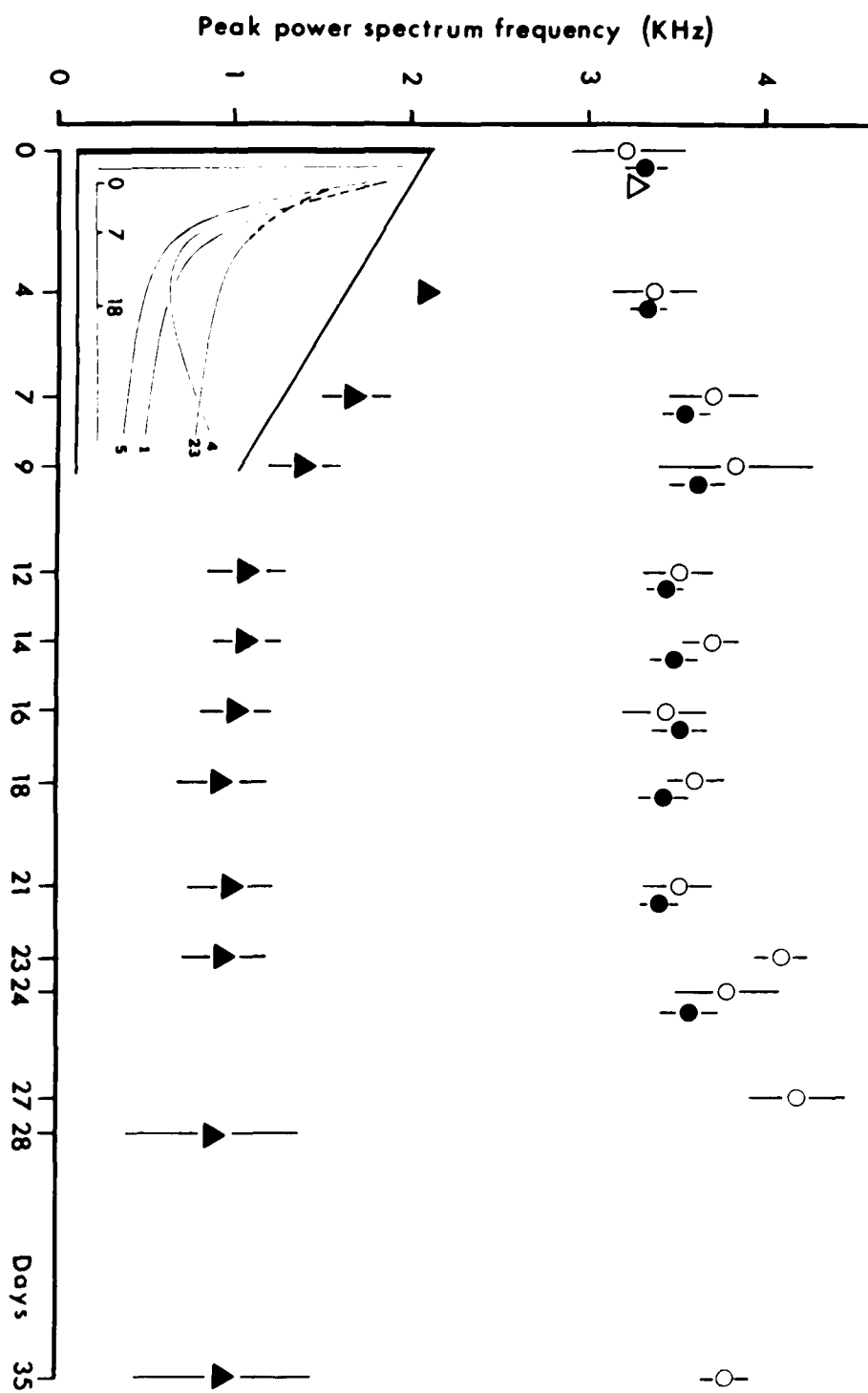


Figure 3. Effects of 17α -methyltestosterone treatment on EOD activity. (a) multiple discharges, (b) EOD of an untreated fish and EODs of 17α -T implanted fish; note variations in ascending positive phase (arrowheads), and (c) EOD pulseform in Fish #5 on day 60 post-implant.

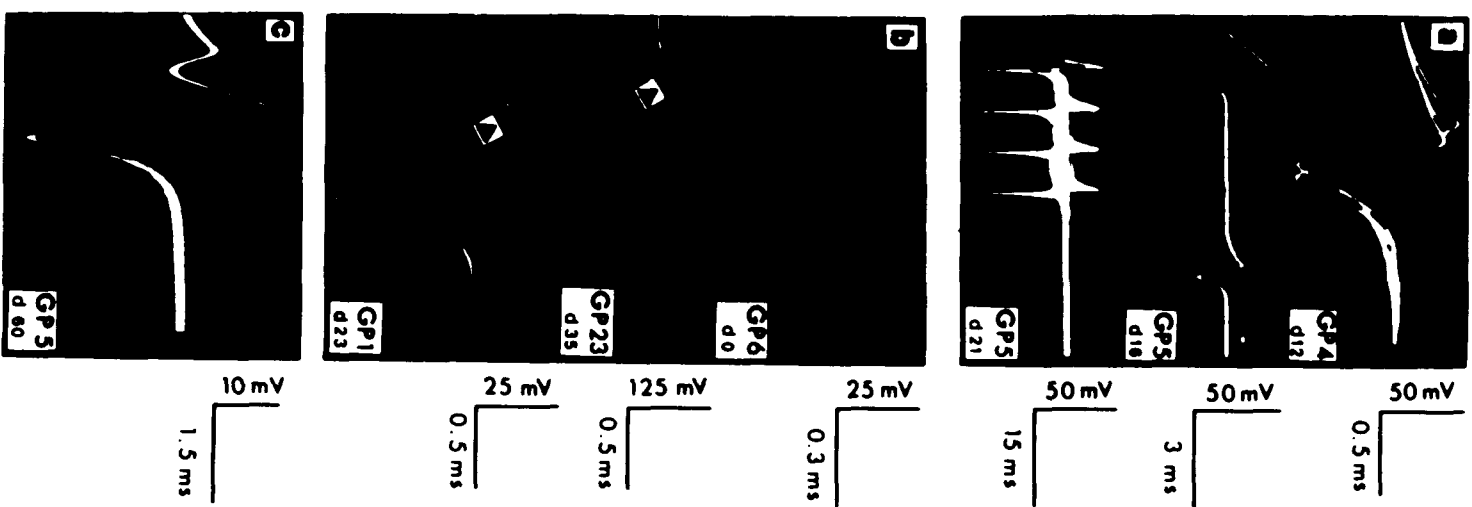


Figure 4. Change in external sexual characteristic in G. petersii #5.
(a) day 1 post-implant and (b) day 60 post-implant. Note indentation
in base of anal fin by day 60 (arrowhead). Length of black bar: 10 cm.
Emaciated appearance of fish is probably due to prolonged hormone
treatment¹³.

a



b



References and Notes

- 1 This study was supported by grants from NIMH (NRSA 1F31MH09664), Sigma Xi, and PSC-CUNY Research Award Program (RF 665405).
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PART III: THE EFFECTS OF STEROID HORMONES ON THE EXTERNAL
MORPHOLOGY AND ELECTRIC ORGAN DISCHARGE WAVEFORM OF
GNATHONEMUS PETERSII (MORMYRIFORMES)

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Abstract

The effects of male and female hormones on the sexually dimorphic external morphology and electric organ discharge waveform in Gnathonemus petersii, a weakly discharging electric fish, were investigated. Following pre-implant data collection, juvenile and adult fish were gonadectomized and implanted with silastic capsules containing testosterone (T), dihydrotestosterone (DHT), estradiol (E_2), or cholesterol (CH) in low-dose (juveniles only) or high-dose blocks. A DHT + E_2 group was included in the high-dose blocks. At either dose, both androgens induced male-like indentations in the dorsal margin of the anal fin of juveniles and adult females. Both low and high doses of T decreased the peak power spectrum frequency (PPSF) of Fourier transformations, and increased the durations of Phases 2 and 3 in juveniles and adults, with the high-dose effects being quicker and more profound. Dramatic dose-dependent T effects were obtained for phases 1 and 4 in juveniles. Low dose T decreased the durations of Phases 1 and 4, while high dose T increased these durations in juveniles. In adults, high dose T increased the duration of Phase 1, but had inconsistent effects on the duration of Phase 4. Total EOD durations were increased by low- and high-dose T in juveniles, while adults showed inconsistent effects due to individual variability in hormone sensitivity. Compared to T, DHT exerted similar, but less dramatic effects on all measures, but only at high doses. E_2 increased adult PPSFs slightly, but had no effects on juvenile PPSFs, or adult or juvenile EOD phases. The effects of DHT +

E_2 on PPSF and Phases 2 and 3 equalled those of DHT alone. These findings demonstrate the steroid sensitivity and plasticity of EODs in G. petersii. The results are discussed with regard to methodological considerations and hormone studies involving sex differences in EODs reported for this and other species.

The electric organ discharges (EODs) of weakly discharging electric fish are used in social communication (Kramer, 1979; Moller, 1980; Hopkins, 1983), species and sexual identification (see Hopkins, 1986, for a review), and possibly for alarm calling (Landsman, Jou, & Moller, 1987). Steroid hormones have been shown to alter both EODs and the physiology of the electric organ in some species which appear to exhibit natural sex differences in the field (Bass & Hopkins, 1985; Bass, 1986a). While the effects of exogenous hormone treatment appear to be good predictors of natural sex differences reported in the field (Bass & Hopkins, 1985), such endocrinological studies have typically used small samples and compared the gross effects of methylated testosterone with non-methylated DHT, estrogen, or cholesterol (CH) [Bass & Hopkins, 1983, 1984; Bass, Denizot, & Marchaterre, 1986; Bass & Volman, 1987; Landsman & Moller, 1988]. Hormone doses may be administered through pellet implants or directly into the water (Bass & Hopkins, 1983, 1984; Bass, Denizot, & Marchaterre, 1986; Bass & Volman, 1987). Although DHT has never been isolated as a naturally occurring hormone in fish, many studies employed this hormone rather than the naturally occurring T or 11-keto T to describe the effects of androgens on the electrocommunication system of weakly discharging electric fish (see Meyer & Zakon, 1982; Bass & Hopkins, 1983, 1984; Hagedorn & Carr, 1985; Mills & Zakon, 1987; Meyer, Leong, & Keller, 1987). Little is known about either the specific effects of hormones on specific EOD phases or the time-course of steroid action on EODs.

The EOD and external morphology of G. petersii can be altered by pellets or silastic implants of 17 α -methyl T (Landsman & Moller, 1988). However, laboratory investigations of sex differences in the

electric organ discharge of this species have produced incongruent findings including no sex differences (Kramer & Westby, 1985), males exhibiting shorter EODs and higher peak power spectral frequencies (PPSFs) of their Fourier transformations than females under non-restrained recording conditions (Landsman, Jou, & Moller, 1987), and males exhibiting longer durations in specific phases of the EOD and lower PPSFs than females in newly imported fish (Landsman, 1989a). These sex differences were abolished when fish were restrained (Landsman et al., 1987) and abolished or even reversed by simply maintaining the fish in the laboratory for 30 days (Landsman, 1989b). Although high doses of male hormones elongated the EOD and lowered the PPSF in juveniles and an adult female (Landsman & Moller, 1988), it is unclear whether female hormones have any effect on the EOD in this species.

To further investigate hormonal control of EODs and external morphology in this species, two doses of T, DHT, and E_2 , and one of DHT + E_2 were administered through silastic capsules. Changes in external morphology as well as the time-course of treatment effects on individual phases of the EOD and its spectral components were assessed.

T, DHT, and E_2 were used to determine whether the EOD and external morphology are sensitive to male and/or female hormones. DHT was used to assess whether the effects of testosterone are androgenic. DHT + E_2 were administered together to one group in order to determine possible potentiating effects of E_2 . CH, a steroid hormone precursor, was used to control for the effects of the steroid hormones, and the non-handled fish served as controls for all surgical

and implant procedures.

Methods

Subjects.

Fifty-six juvenile (standard length = 70-110 mm and weight = 6-10 g) G. petersii and 18 adult (9 males and 9 females, standard length = 152-202 mm and weight = 32-71 g) G. petersii were imported from Nigeria (Lombardo's African Fish Importers, Newark, N.J.) and maintained in 20-l individual aquaria for 14 days prior to pre-implant data collection. Juvenile subjects could not be sexed by gonadal examination or the external sexual character found in adult males of this species. Fish were fed tubifex worms twice per week in amounts so that some food always remained available at the bottom of the aquaria. Fish were not fed for 6 days following surgery to minimize infection from surgical procedures. Fish were maintained on L:D = 12:12, with lights on at 10:00 h (EST), water conductivity at 150 ± 20 μ S/cm, pH = 6.8 ± 0.2 , and water temperature at $24 \pm 1^{\circ}\text{C}$. One-third of the water was changed for each subject every third day to maintain pH and conductivity levels over the course of the study.

Materials and Procedures.

Design and Analyses. The experiments were performed in one low-dose and one high-dose block for juveniles, and one high-dose block for the adults. Subjects for each block were numbered consecutively and the numbers then randomly assigned to treatment

Table 1. Treatment groups and sample sizes for adults and juveniles implanted with low- (1mm/2.5g b. wt.) or high-dose (1mm/1g b. wt.) silastic hormone implants.

Treatment	<u>Subjects and Dose</u>		
	Juveniles		Adults
	Low	High	High
Testosterone (T)	3	9	3
Dihydrotestosterone (DHT)	4	5	3
Estradiol (E ₂)	4	7	3
DHT + E ₂	-	4	4
Cholesterol (CH)	4	5	5
Nonhandled Controls (NHC)	4	7	-

groups. The various treatments and sample sizes for each block are shown in Table I.

The 14 day habituation period prior to pre-implant EOD data collection was based on data which indicated that EOD waveforms in this species are profoundly altered during the first two weeks of captivity (Landsman, 1989b). EOD data were collected daily from each subject over a six-day pre-implant block and for four consecutive six-day post-implant blocks. Individual EOD phase lengths were recorded directly from an oscilloscope while subjects were at rest in their porous ceramic shelters. Fourier transformations from three EODs were collected daily from each subject and individual daily medians were then calculated. Six-day median total EOD and individual phase durations and PPSFs were computed for each subject and data were then averaged for all subjects in each group comprising mixed factorial designs for the three experimental blocks. Two-way ANOVAs with repeated measures were used to assess between-group as well as six-day block within-group treatment effects. Significant interactions were further analyzed with tests for simple main effects, which, when significant ($\alpha = 0.05$), were followed by the Neuman Keuls test for multiple comparisons ($\alpha = 0.05$).

The dependent t-test was used to compare the adult pre- and post-implant block means. By the beginning of the second 6-day post-implant block, some of the adults appeared to be experiencing adverse reactions to the steroid implants and were eliminated from the study at the first appearance of distress or illness. Removal of these fish prevented statistical analyses of the adult data beyond the

first 6-day post implant block, although the data for the remaining subjects are still presented in the figures.

Surgical Procedures. Fish were first anesthetized with tricaine methane sulfonate (Sigma) buffered with sodium bicarbonate (1:20,000). Gonadectomy and insertion of the silastic implant(s) were performed through a 1.5-cm incision on the left ventral surface, approximately 5 mm posterolateral of the ventral fins. Both ends of the gonads were tied with surgical thread before gonad removal in the adults. After the fish were gonadectomized and then implanted with silastic capsules under a dissecting microscope, the incision was sutured (6.0 silk, Beckton Dickson) and fish revived by flowing conditioned aquaria water through their mouths and gills. Fish were returned to their individual aquaria and treated with chloramphenicol (Sigma), an antimicrobial agent. Non-handled controls were not subjected to any pre- or post-surgical treatments. After being sacrificed at the end of the study, subjects were checked for completeness of gonadectomy, and implants recovered.

Data Collection. Fish were weighed and measured prior to assigning them to individual aquaria on the day they were received from the importer. Implanted fish were photographed, initially while anesthetized prior to surgery, and after being sacrificed following blood collection. EODs and Fourier spectra were collected from fish in their individual aquaria through a pair of Ag/AgCl electrodes, extending from Plexiglass tubes fitted to the far ends of the aquarium, approximately 8 cm from head and tail of the fish when at rest in its porous ceramic shelter. The same pair of electrodes was used for all subjects to avoid the possible confound of different

electrode impedances which could affect the EOD waveform. EODs were fed directly into an oscilloscope (Tektronix, model 455, input impedance = 1.0 M Ohm, high bandpass filter) which triggered a spectrum analyzer (Hewlett-Packard, model 3582A, range: 0-25 KHz, resolution: 100 Hz). Both EODs and Fourier transformations were plotted with an X-Y plotter (Hewlett-Packard) and photographed from the screens of the oscilloscope and spectrum analyzer, respectively. Because of the large number of subjects and the time-consuming data collection procedures, order of data collection was randomized on a daily basis. All EOD recordings were made between 12:00 and 20:00 h (EST). The waveform of the EOD in G. petersii does not exhibit a circadian rhythm (Landsman, unpublished data).

Silastic Implants. Low dose androgen implants were 1mm packed hormone in silastic tubing (Dow-Corning 0.065 inch o.d. and 0.03 inch i.d.)/2.5g body weight and high dose were 1mm packed hormone/1g body weight. E₂ implants were 40% of the androgen doses because this hormone has been shown to be more potent in its behavioral effects. Because of the prohibitive length of a single implant in the high dose study, up to 3 implants were used to achieve the required dose. CH controls received matching numbers of implants. Because cholesterol-implanted juveniles never differed from non-handled controls, only cholesterol controls were used for the adult blocks to avoid unnecessary use of animals.

Radioimmunoassay. After 30 days of data collection, blood was collected into heparinized microcapillary tubes from the caudal vein of high-dose subjects, centrifuged for 5 min. at 2000 rpm, and stored frozen for future analyses.

RIA techniques (see H. Singh, Griffith, Takahashi, Kawauchi, Thomas, & Stegeman, 1988) were validated in G. petersii using column chromatography, and 10-, 25-, 50-, and 100- μ l aliquots of reconstituted samples were measured in the RIA to test for parallelism. A combined testosterone-estrogen RIA assay was performed and validated by adding either T, E_2 , or T and E_2 spikes for plasma samples from both E_2 - and T-implanted fish. All samples were run in one assay. Where possible, I analyzed blood from individuals, but where not, blood samples were pooled. The T antiserum cross-reacted 11% with 11-keto T, another major androgen found in fish, but not with E_2 . There was no significant cross-reactivity between estrogen antiserum and T. The detection limits were 1.25 pg per assay tube for both T and estrogen. Recovery of labelled hormone carried through the extraction procedures ranged from 76% to 95%. The intrassay coefficients of variation were 7.54% for E_2 (N=13) and 7.53% for T (N=16).

Results

External Morphology.

Adult males exhibit an indentation in the dorsal margin of the anal fin never seen in adult females (Landsman, 1989a). Figure 1 shows the external morphology of juvenile fish before and 30 days after receiving either T, DHT, or E_2 . Juveniles treated with low- or high-doses of either T or DHT developed the adult male-like indentation, while E_2 -treated juveniles did not develop this

characteristic (Fig. 1). The same male-like indentation was also induced in adult females treated with T and DHT.

Qualitative Effects on EODs and Fourier Transformations.

Figure 2 shows the effects of the various high-dose treatments on the EODs and their Fourier transformations for individual subjects. Only androgen treatment caused EOD duration changes as well as changes in the shape of the Fourier transformation and PPSF. When sound amplified (Archer Mini-amplifier with speaker), EODs of androgen-treated subjects were deeper thumps compared to the high-pitched clicks in E_2 -treated and control subjects.

Fourier Spectrums.

By the end of the study, higher frequency sine waves appeared to decrease in abundance only in androgen-treated fish (Fig. 2).

Low Dose. A significant hormone X block interaction effect, [$F(16,56) = 9.07, p < 0.0001$], revealed that by the end of the first 6-day post-implant block, there was a significant decrease in mean PPSF for only T-implanted fish, which differed significantly from E_2 , CH, the NHC, but not from the DHT-implanted fish (Fig. 3, top). By the end of the second 6-day post-implant block to the end of the third, the mean T PPSF was also significantly lower than the PPSFs for either DHT or DHT + E_2 .

High Dose. The hormone X block interaction effect was again significant [$F(20,124) = 32.63, p < 0.000001$], and multiple comparisons indicated that T-, DHT-, and DHT + E_2 -implanted fish

exhibited lower mean PPSFs than their pre-implant PPSFs by the end of the first 6-day post-implant block (Fig. 3, bottom). E_2 -implanted fish did not change and did not differ from the CH or NHC throughout the study. T decreased PPSFs significantly more than DHT or DHT + E_2 by the end of the second six-day post-implant block.

Adults. Figure 4 shows the effects of the various hormone treatments on PPSFs in individual adult males and females. Androgens had effects similar to those shown for the juveniles. Dependent t-tests indicated significant decreases in PPSFs by the first 6-day post-implant block for groups treated with T [means $\pm$ SEMs = 3.85 ± 0.218 KHz to 2.39 ± 0.215 KHz, $t(2)=5.33$, $p<0.04$], DHT [3.37 ± 0.076 KHz to 2.10 ± 0.093 KHz, $t(2)=14.19$, $p<0.005$], and DHT + E_2 [3.71 ± 0.082 KHz to 2.71 ± 0.133 KHz, $t(3)=7.15$, $p<0.006$].

Surprisingly, the E_2 -implanted adults showed a relatively small, significant increase in PPSF from a pre-implant mean of $3.23 (\pm 0.196)$ KHz to $3.55 (\pm 0.163)$ KHz by the end of the first 6 days of treatment [$t(2)=6.77$, $p<0.03$]. CH-implanted controls did not show any change in PPSF over the course of the study.

Total EOD Duration.

Total EOD duration is the sum of the durations of the 4 phases. At low doses, only T significantly increased total duration by post-implant block 3 [$F(16,56) = 3.54$, $p < 0.001$] (Figure 5, top). The total duration means for post-implant blocks 3 and 4 for T-treated fish were significantly longer than those for DHT, E_2 , or the controls. Total EOD duration for low-dose DHT- or E_2 -implanted fish did not differ from the controls.

High doses of both androgens increased total EOD duration [$F(20,124) = 6.57$, $p < 0.00001$, Fig. 5, bottom]. By the first post-implant block, T increased the mean duration and T-induced increases began to level off by the fourth post-implant block. The high-dose effects of DHT and DHT + E_2 were less dramatic (Fig. 5, bottom). By post-implant block 2, DHT- and DHT + E_2 -treated fish showed slight, but significant increases in mean EOD duration. Mean total durations for both DHT groups leveled off for the remaining two blocks. Again E_2 did not affect total EOD duration over the course of the study.

Adults. Figure 6 shows the effects of hormone treatment on the total duration of individuals' EODs. All but CH-treated subjects appeared to exhibit slight increases in total EOD duration. However, only the subjects which received DHT + E_2 exhibited a change in duration which reached statistical significance [means $\pm$ SEMs = 1.59 ± 0.073 ms to 1.99 ± 0.110 ms, $t(3) = -6.01$, $p < 0.01$]. It is interesting to note that male #101 implanted with T and male #115 implanted with E_2 failed to show any change from their pre-implant durations (Fig. 6), indicating much individual variation in the responsiveness of total EOD duration to steroid hormones.

Phase 2.

Figure 7 shows the effects of both hormone doses on phase 2 of the EOD. At low doses, only T caused a significant increase in the duration of this phase [$F(16,56) = 4.05$, $p < 0.001$, Fig. 7, top]. By the end of the first six-day post-implant block, T-implanted fish increased their mean phase 2 duration and reached their maximum

phase 2 duration by the end of the second post-implant block, leveling off throughout the remainder of the study. None of the other hormones affected the duration of phase 2 when administered at the low dose.

High-dose treatments resulted in significant phase 2 increases for the T, DHT, and DHT + E₂ groups throughout the four treatment blocks compared to pre-implant durations (Fig 7, bottom) [$F(20,124) = 22.32$, $p < 0.00001$]. T treatment, however, resulted in a significantly longer phase 2 mean duration than DHT by the end of the second post-implant block, and throughout the remainder of the study, with both hormones exerting their maximum increase in duration by the end of the last post-implant block (Fig. 7, bottom). The effects of DHT + E₂ did not differ from the effects of DHT alone over the course of the study.

Adults. Figure 8 shows the effects of hormone treatment on phase 2 in individual adults. T and DHT increased the duration of phase 2 by the end of the first 6 days of treatment [means $\pm$ SEMs = 0.20 ± 0.019 ms to 0.33 ± 0.035 ms, $t(2) = -4.31$, $p < 0.05$; 0.26 ± 0.013 ms to 0.36 ± 0.033 ms, $t(2) = -5.30$, $p < 0.04$, respectively]. Neither E₂ nor CH treatment influenced the duration of phase 2, nor did E₂ appear to influence the effects of DHT on phase 2 duration as fish implanted with both hormones showed a significant increase in the duration of this phase [means $\pm$ SEMs = 0.24 ± 0.014 ms to 0.31 ± 0.007 ms, $t(3) = -5.07$, $p < 0.02$] by the end of 6 days of treatment.

Phase 3.

Significant hormone X block effects on the duration of phase 3

were obtained for both low dose [$F(16,56) = 3.25, p < 0.0001$] and high dose [$F(20,124) = 20.72, p < 0.00001$] hormone treatments (Fig. 9). By the fourth post-implant block, low-dose T significantly increased the phase 3 duration from its pre-implant duration (Fig. 9, top). No other hormones exhibited effects on the duration of this phase when given at low dose.

High-dose effects on phase 3 were not significant until the second post-implant block (Fig. 9, bottom). T, DHT, and DHT + E_2 significantly increased phase 3 durations, and these durations continued to increase over the remaining 12 days of the study. By the second post-implant block, phase 3 was significantly longer in the T group than in the DHT and DHT + E_2 groups.

Adults. Figure 10 shows the effects of the various hormones on the duration of phase 3 in individual adults. Again, both T and DHT caused significant increases in the duration of this phase by the end of the first 6-day post-implant block [means $\pm$ SEMs = 0.11 ± 0.012 ms to 0.20 ± 0.028 ms, $t(2) = -4.40, p < 0.05$; 0.14 ± 0.006 ms to 0.24 ± 0.012 ms, $t(2) = -8.02, p < 0.02$, respectively]. E_2 had no effect on phase 3 durations and did not influence the effects of DHT as DHT together with E_2 resulted in the same effects as DHT alone [means $\pm$ SEMs = 0.12 ± 0.005 ms to 0.19 ± 0.006 ms, $t(3) = -7.48, p < 0.005$]. Finally, the CH implanted-fish showed no changes in phase 3 duration.

Phases 1 and 4.

The effects of low- and high-dose hormone treatments resulted in similar effects for both phases 1 and 4 (Figs. 11 and 12, respectively). However, these effects were very different for the two

doses. Low dose T caused significant decreases in phase 1 [$F(16,56) = 2.03$, $p < 0.05$] and in phase 4 [$F(16,56) = 3.06$, $p < 0.001$] (Figs. 11, top and 12, top). By the end of the third post-implant block, the mean durations of phase 1 in T-treated fish decreased significantly, while the mean phase 4 duration decreased significantly by the end of the second post-implant block. T was the only hormone that resulted in durations which differed from the controls.

High-dose T, in contrast to the low dose effects, caused significant increases in phase 1 duration by the end of the second post-implant block [$F(20,124) = 1.82$, $p < 0.05$, Fig. 11, bottom], and in phase 4 duration [$F(20,124) = 2.58$, $p < 0.001$, Fig. 12, bottom] by the end of the first post-implant block. The increase in duration of phase 1 appeared to be only temporary as this phase decreased again by the end of the fourth post-implant block. No other hormone treatments resulted in durations which differed from the controls.

Adults. Figures 13 and 14 show the effects of hormone treatments on the durations of phases 1 and 4 in individual adults. Both T and DHT increased the duration of phase 1 by the end of 6 days of treatment [means $\pm$ SEMs = 0.15 ± 0.003 ms to 0.19 ± 0.007 ms, $t(2) = -12.25$, $p < 0.007$; 0.12 ± 0.006 ms to 0.17 ± 0.010 ms, $t(2) = -4.90$, $p < 0.04$, respectively], while neither E_2 nor CH had an effect. Again, the effects of DHT were not influenced by E_2 as fish implanted with both hormones also showed increases in the duration of phase 1 [means $\pm$ SEMs = 0.12 ± 0.001 ms to 0.16 ± 0.004 ms, $t(3) = -11.31$, $p < 0.002$] comparable to fish implanted with DHT alone (Fig. 13).

Although Figure 14 shows slight increases in phase 4 duration for individual fish following treatment with both androgens and E_2 , the

only significant increase was that exhibited by fish implanted with both DHT and E_2 [means $\pm$ SEMs = 1.13 ± 0.064 ms to 1.30 ± 0.108 ms, $t(3)=-3.77$, $p<0.04$]. These results parallel those found for total EOD duration. Similar to total duration effects, male #101, female #104, and male #115, implanted with T, DHT, and E_2 , respectively, were not responsive to their respective hormone implants by the end of the first 6 days of treatment (Fig. 14).

Plasma Hormone Levels.

Blood collected from fish implanted with either T or DHT was of very thick consistency and deep red in color, whereas blood collected from E_2 - and CH-implanted, as well as NHC subjects was thin and watery in appearance and light-red in color. RIA analyses indicated that plasma samples from high-dose E_2 -implanted fish ranged from 1.34 ng/ml for pooled juveniles ($n=5$) to 5.55 ng/ml for an adult male. Plasma E_2 levels in adult and juvenile NHCs were less than 1.95 ng/ml, compared to 0.833 ng/ml found in pooled plasma from CH-implanted controls (Fig. 15).

Plasma T levels (Fig. 16) from T-implanted fish ranged from 5.77 ng/ml for a low-dose juvenile to up to 40.87 ng/ml in pooled or individual plasma samples from high-dose subjects. In contrast, plasma T levels in NHCs ranged from around 1.00 ng/ml or less in adults to 3.25 ng/ml in pooled juveniles. Plasma T levels in CH-implanted subjects ranged from 0.86 to 1.43 ng/ml.

Discussion

Both the external morphology and EOD waveform in G. petersii are hormone sensitive. Androgens induce the adult male-like indentation in the dorsal margin of the anal fin in juveniles and in adult female fish. Androgens also dramatically increase the durations of phases 2 and 3 of the juvenile and adult EOD, and consequently decrease the PPSF of the Fourier transformation.

The PPSF of the Fourier transformation indicates the characteristic frequency of the most predominant sine wave occurring in the complex waveform of the EOD. Although it has never been established whether weakly electric fish can perform even a rudimentary form of spectral analysis, this measure is typically used as an indicator of sex differences and gross changes in EOD waveform (eg., Westby & Kirschbaum, 1982; Bass & Hopkins, 1985; Hagedorn & Carr, 1985; Bass, 1986a; Bratton & Kramer, 1988). Androgens decreased the PPSFs in both juvenile and adult G. petersii. DHT had no effect at low dose and was less potent than T at the high dose. Surprisingly, E_2 caused a slight, but significant, decrease in PPSFs in adults, but not in juveniles. This is interesting for a number of reasons. First, if the weakly electric fish can in fact use Fourier spectral cues for sexual identification, then this subtle effect of E_2 could accentuate the difference between the PPSF in adult males and females while allowing males to discriminate between adult females and juveniles during periods when both androgens and estrogen levels are relatively high. Second, that E_2 had no effect on PPSF in juveniles

suggests the possibility that receptors for this hormone are not yet developed or functional in juveniles. This is the first reported estradiol effect on the EOD in mormyrid fish that works in the opposite direction of androgens. For example, a male-like EOD was induced in B. brachyistius (triphasic) by treatment with E_2 as well as with T or DHT (Bass, 1986a) and E_2 had a slight effect on the EOD in the male direction in B. brachyistius (long biphasic) [Bass & Hopkins, 1983].

The most dramatic and consistent hormone effects on the EOD were those exhibited in phases 2 and 3 by fish treated with androgens. In juveniles, the durations of phases 2 and 3 are androgen-dependent, with T at low dose being more potent than low dose DHT and at high dose exerting more profound effects than high dose DHT. Androgen-treated adults also showed increases in the durations of phases 2 and 3, while fish treated with E_2 or CH exhibited no changes in the duration of this phase. The effects of the androgens on phase 2 occurred much sooner than those on phase 3 indicating that the duration of phase 3 is less sensitive to androgen at least in juveniles. This can account for the finding that males in captivity showed decreases in endogenous androgen levels accompanied by decreases in the duration of phase 3, but not phase 2 (Landsman, 1989b). As T levels decrease, phase 3 is probably affected before phase 2, because the latter phase appears to be extremely responsive to even low doses of androgen.

Phases 1 and 4 of the EOD in G. petersii are extremely variable and are influenced dramatically by variations in water conductivity and temperature changes (Landsman & Bowling, unpublished data). Both

phases have been shown to decrease to non-detectable amplitudes over long distances within the electrocommunication range (Squire & Moller, 1982). Phases 1 and 4 appear to be affected differently by different doses of male hormones. The finding that low-dose treatment decreased the durations of these phases suggests that during non-breeding seasons when steroid levels are low, slight increases and decreases in hormone titers may induce subtle changes in various phases of the EOD. This phase-specific EOD flexibility enables the signalling of motivational states accompanied by underlying changes in hormone levels. High-doses of both androgens and estrogen appear to cause slight nonsignificant increases in the durations of phases 1 and 4 in adults, while only high doses of androgens cause these effects in juveniles. Steroid hormones appear to exert their effects on the EOD by altering electrocyte membrane characteristics (Bass, 1986a, b; Bass, Denizot & Marchaterre, 1986; Mills & Zakon, 1987). These studies found that androgens cause a thickening of the membrane and possibly changes in the electrocyte membrane ion channels. The dose-dependent hormone effects on specific phases in G. petersii suggest a more complex explanation of how hormones exert their effects on the EOD.

The duration of phase 4 as well as the total duration of the EOD in juveniles was increased by T, but not DHT. It is not surprising that the hormone effects on phase 4 are similar to those found for total EOD duration as this phase comprises most of the duration of the EOD.

E₂ appeared to potentiate the effects of DHT on the duration of phase 4 and on the total EOD duration in adults. The fact that

DHT + E₂, but no other androgen treatments, resulted in significant increases in these durations was probably a function of larger sample size as well as lower individual variability in subject responsiveness to this combined hormone treatment.

The plasma T levels induced by the high-dose implants were comparable to those found in males just imported during the rainy breeding season (Landsman, 1989b). Males imported during the breeding season exhibited longer durations in phases 2 and 3 and lower PPSFs than females imported at the same time. Newly imported males exhibit T levels around 6.0 ng/ml, while adult males held captive in the laboratory for 5 days exhibit near non-detectable levels (Landsman, 1989b) similar to those found for the control fish.

Endocrine studies on weakly electric fish species commonly employ a methylated androgen (Bass & Hopkins, 1983, 1984; Bass, 1986a; Landsman & Moller, 1988), methyl T, which is more potent than T. Although methyl T may be used to induce male-typical behavior in many fish species, the effects of this hormone are exaggerated and may be pharmacological in nature, especially when compared to the effects of T. Landsman & Moller (1988) implanted methyl T into juvenile and adult G. petersii and found up to a five-fold increase in total EOD duration accompanied by decreases in PPSF to 400 Hz! In contrast, the present study which employed T implants resulting in T levels in the range found in breeding males (Landsman, 1989b), resulted in total EOD duration increases of up to 0.33% with decreases in PPSF to above 1 KHz. Problems of hormone dose are compounded when the effects of methyl T are compared to the effects of non-methylated DHT or E₂ (e.g., Bass & Hopkins, 1983, 1984; Bass, 1986a).

To my knowledge, DHT has never been isolated as a circulating hormone in fish. If this is the case, then one must be careful in the interpretation of results obtained with DHT unless substantiated with T or 11-keto T, the two predominant androgens found in fish. In the present study, DHT was found to be less potent than T in producing behavioral effects on the EOD, and did not have any effects on phases 1 and 4 in juveniles. However, the differences in DHT and T may be a dose effect since DHT has been shown to clear more rapidly than an equivalent dose of T in other species (For a discussion, see Harding, 1986). The difference in plasma E_2 levels found in the adult and juveniles implanted with E_2 may have been a function of age-related differences in hormone metabolism rates (Harding, 1986).

Although hormonal sensitivity of EOD duration and PPSFs have been reported for other species of weakly discharging electric fish, it appears that PPSF and overall duration of EODs may in fact have little to do with communication of sex differences or other information regarding motivational states (see Landsman, 1989a). The phase-specific results presented here suggest the likelihood that communication in the weakly discharging electric fish involves the processing of either individual EOD phases or PPSFs rather than total EOD duration.

The major hormone effects found in the present experiment were that male hormones lengthen the durations of phases 2 and 3 and decrease the PPSF. These results are congruent with the seasonal sex differences in PPSF and the durations of phases 2 and 3 of the EOD found in just imported G. petersii (Landsman, 1989a). Landsman (1989a) reported that males exhibited significantly longer phases

2 and 3 and a lower PPSF than females imported during the rainy breeding season. However, these sex differences in EOD were not found in fish imported outside the breeding season of the same year. The results of the present study suggest that these seasonal sex differences in the durations of specific phases of the EOD are probably a function of steroid hormone levels. Whether these sex differences in behavior and morphology are used in sexual identification has not yet been determined for this species.

Figure 1. The external morphology of juvenile G. petersii before and 30 days after being implanted with high doses of either a) T, b) DHT, or c) E_2 . Note development of indentation in dorsal margin of the anal fin (arrowheads) occurred only following T or DHT treatment. The same results were obtained in subjects treated with low doses of each hormone.

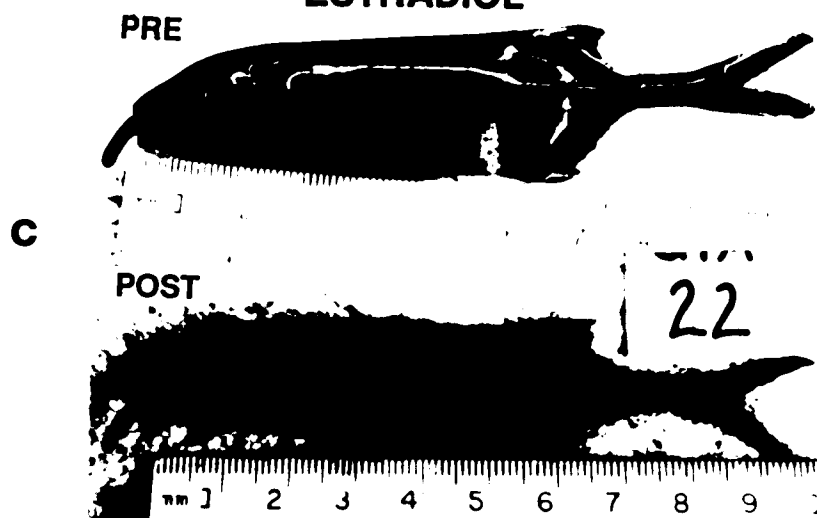
TESTOSTERONE**DIHYDROTESTOSTERONE****ESTRADIOL**

Figure 2. The juvenile EOD (left) and its Fourier transformation (right) before and 24 days following gonadectomy and implantation with high dose a) T, b) DHT, c) E_2 , d) DHT + E_2 , e) CH, and in f) NHC. Arrowheads (1 and 2) point to phases 2 and 3, respectively, and PPSFs are indicated below Fourier transformations. Note clear increases in the durations of phases 2 and 3 and decreases in PPSFs only for subjects treated with T, DHT, or DHT + E_2 . White marker between T pre- and post-implant = 3.0 ms.

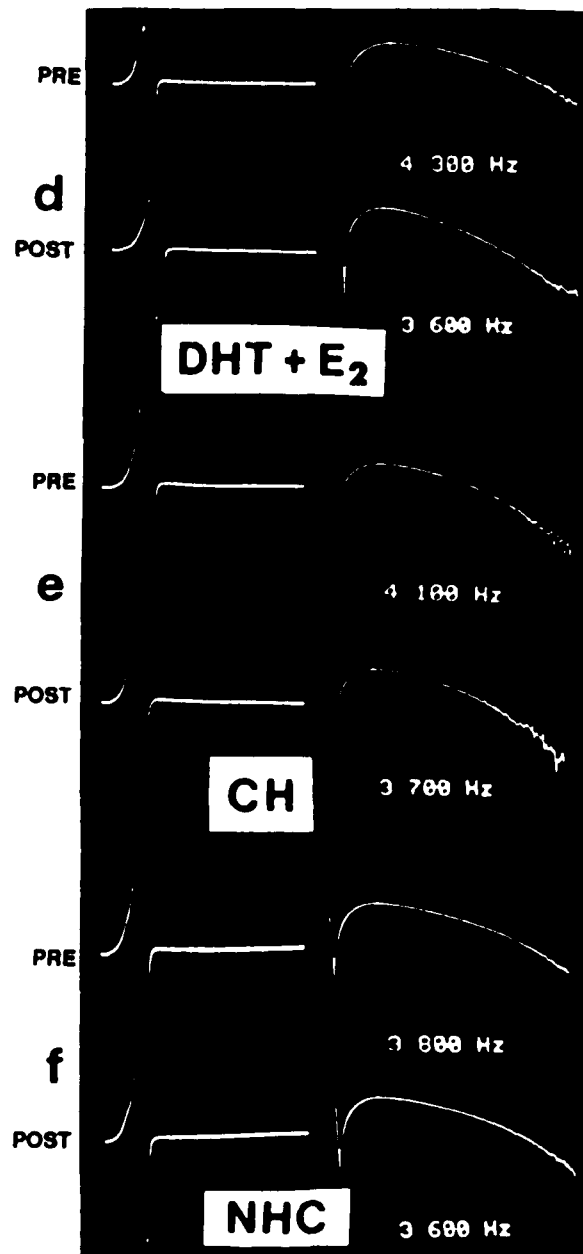
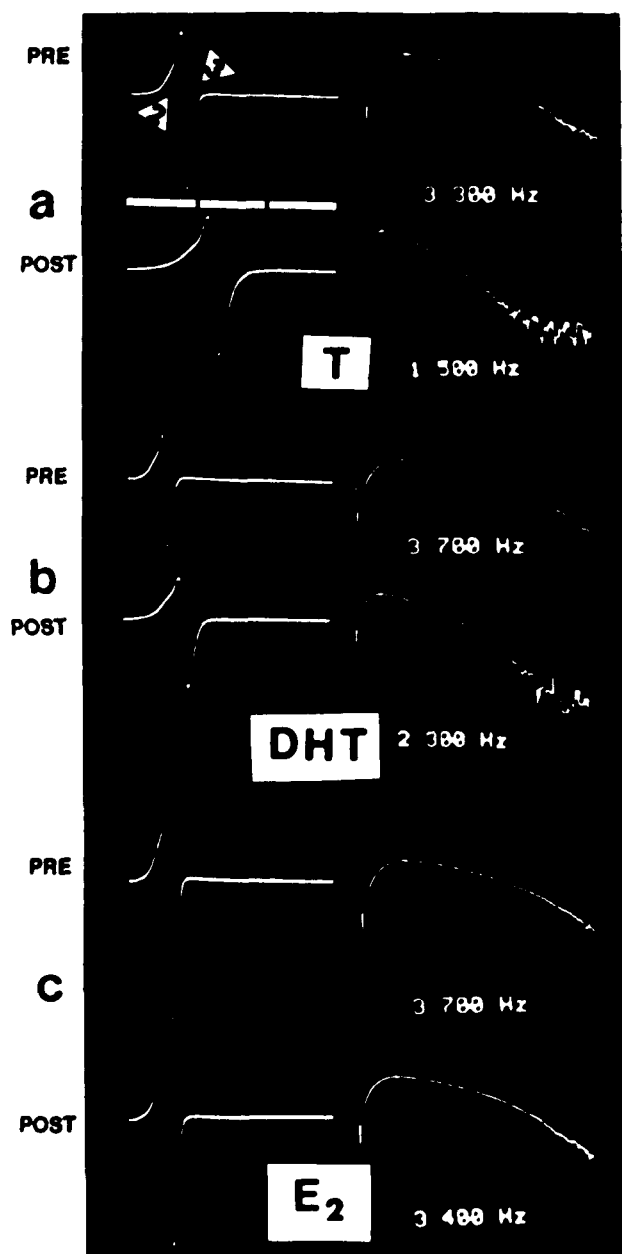


Figure 3. Juveniles. Mean-Median PPSFs for 6-day pre-implant and subsequent 6-day post-implant blocks. (top) Low-dose hormone effects. (bottom) High-dose hormone effects.

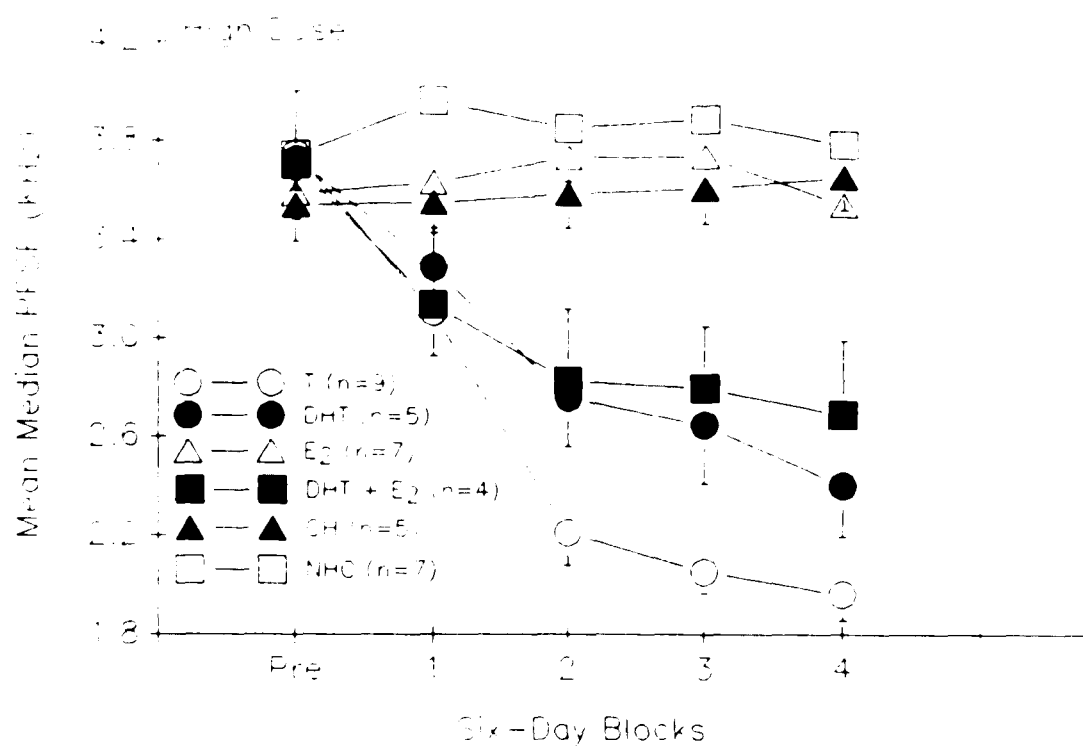
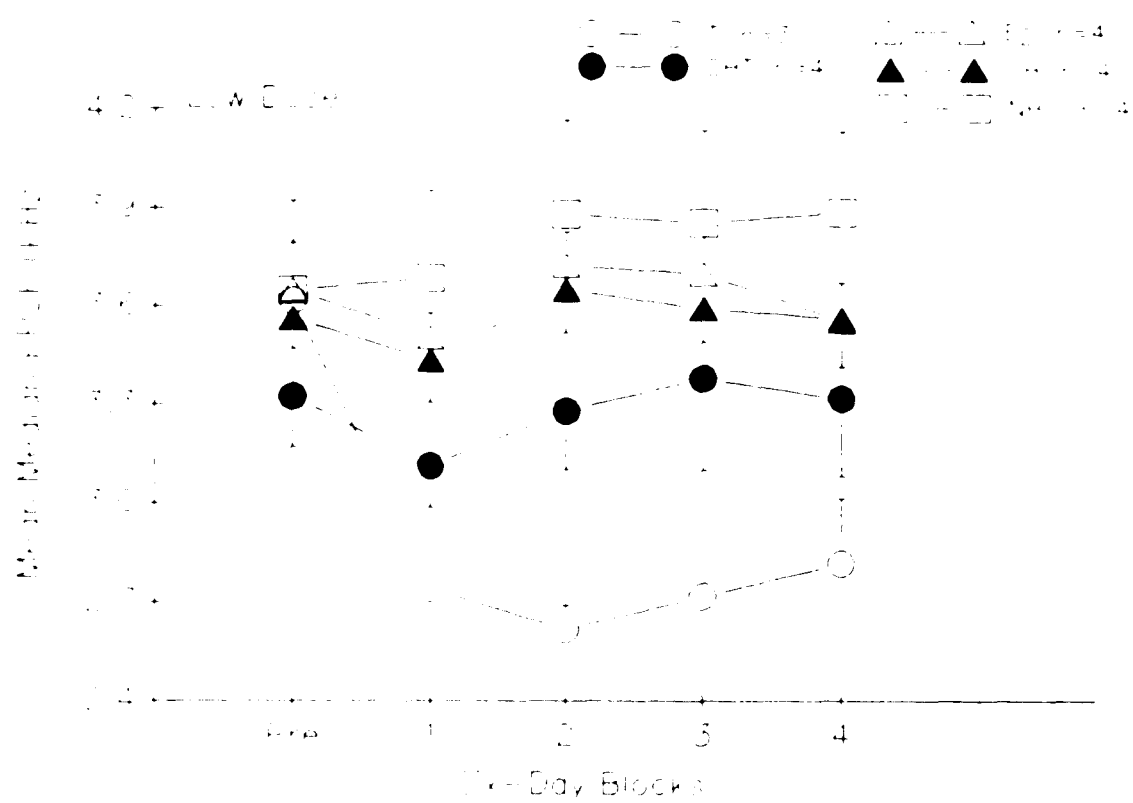


Figure 4. Adults. Time-course for hormone effects on PPSF for individual subjects across 6 pre-implant and 24 post-implant days. Legend refers to individual subject numbers and sex.

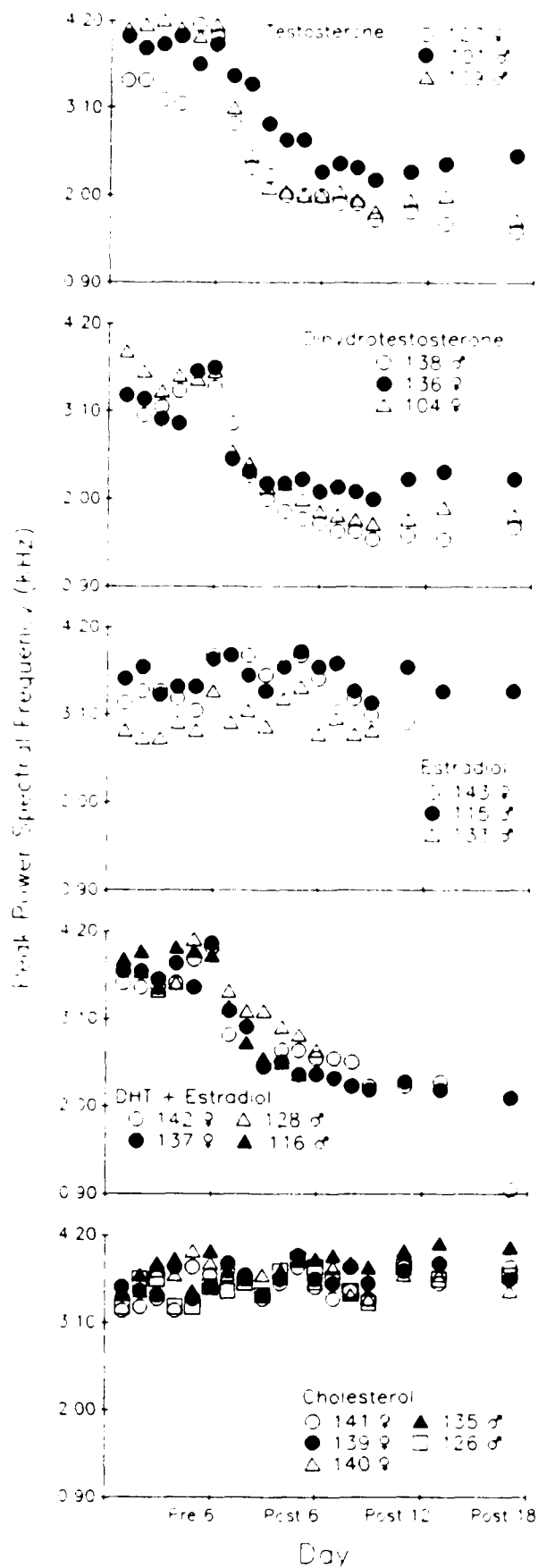


Figure 5. Juveniles. Mean-median total EOD durations for 6-day pre-implant and subsequent 6-day post-implant blocks. (top) Low-dose hormone effects. (bottom) High-dose hormone effects.

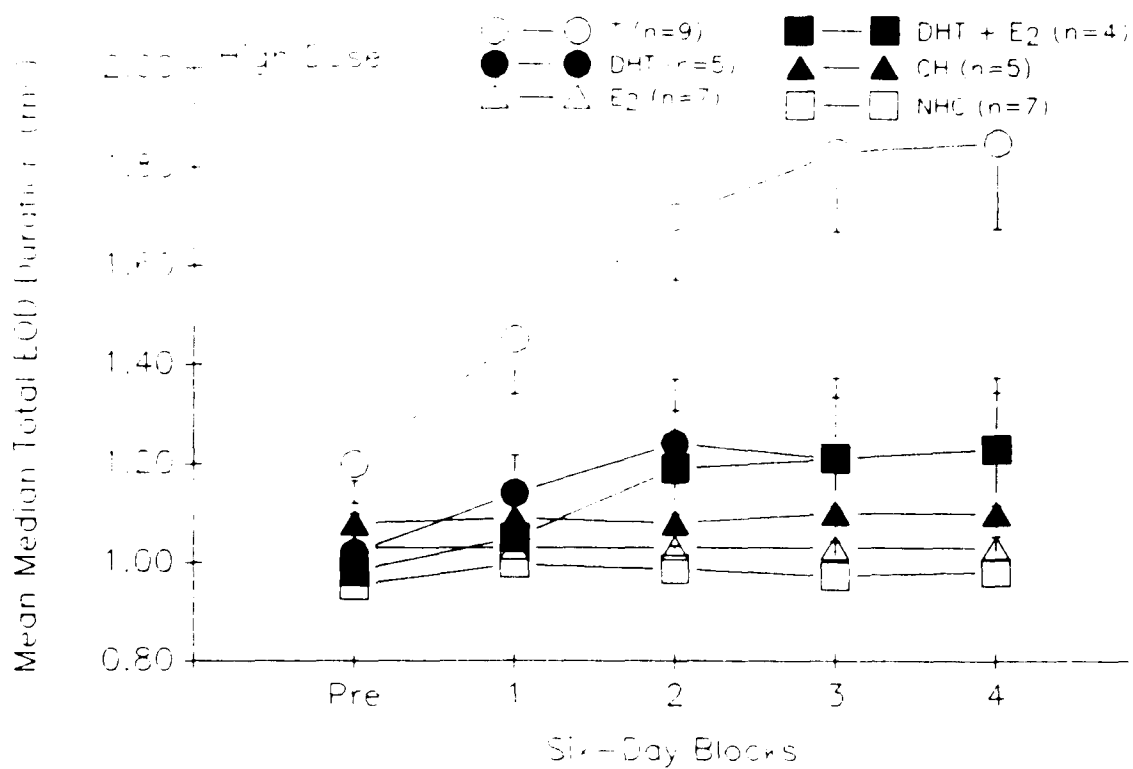
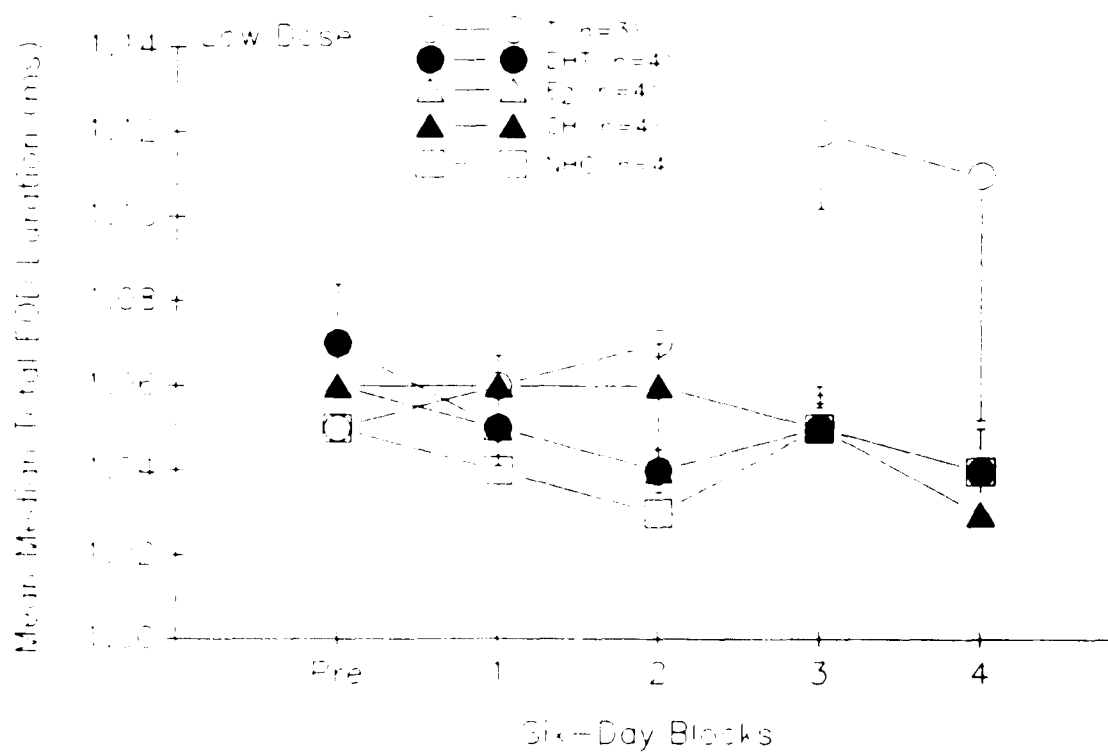


Figure 6. Adults. Time-course for hormone effects on total EOD duration for individual subjects across 6 pre-implant and 24 post-implant days. Legend refers to individual subject numbers and sex.

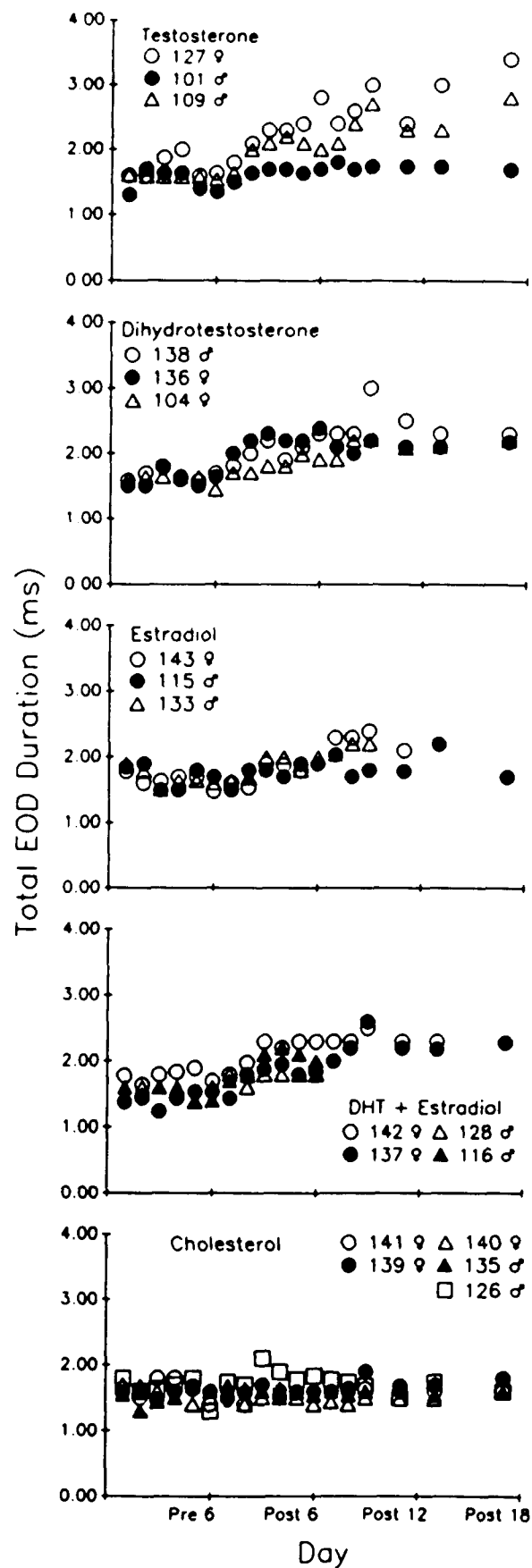


Figure 7. Juveniles. Mean-median phase 2 durations for 6-day pre-implant and subsequent 6-day post-implant blocks. (top) Low-dose hormone effects. (bottom) High-dose hormone effects.

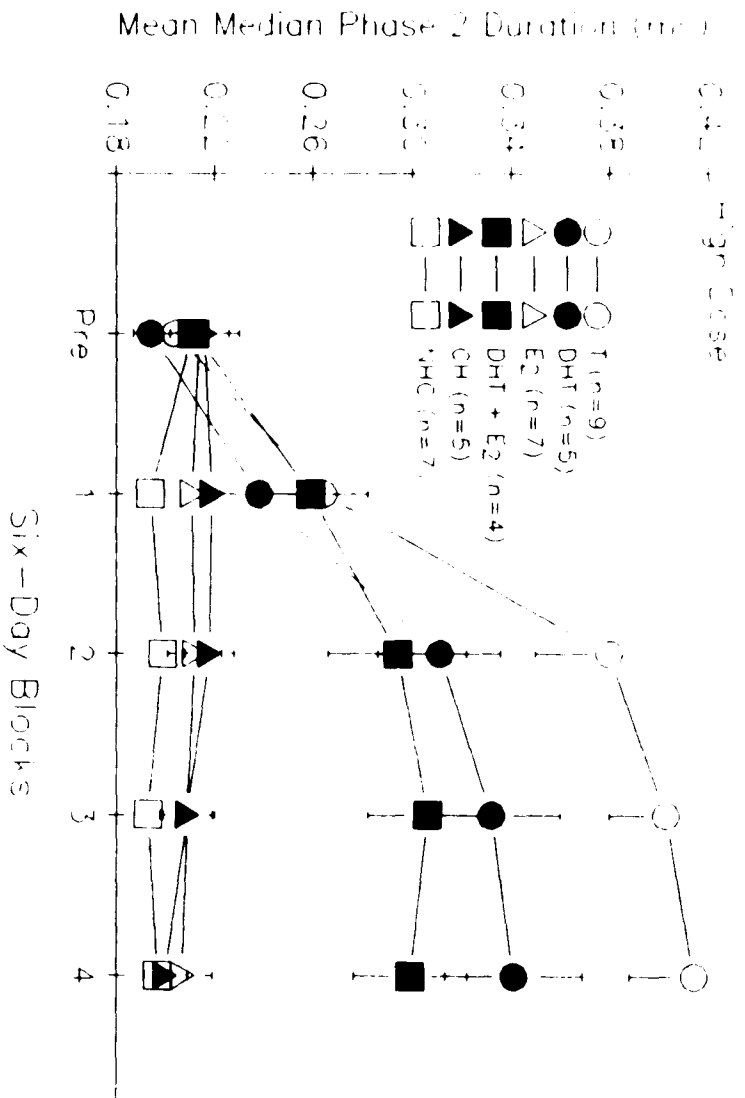
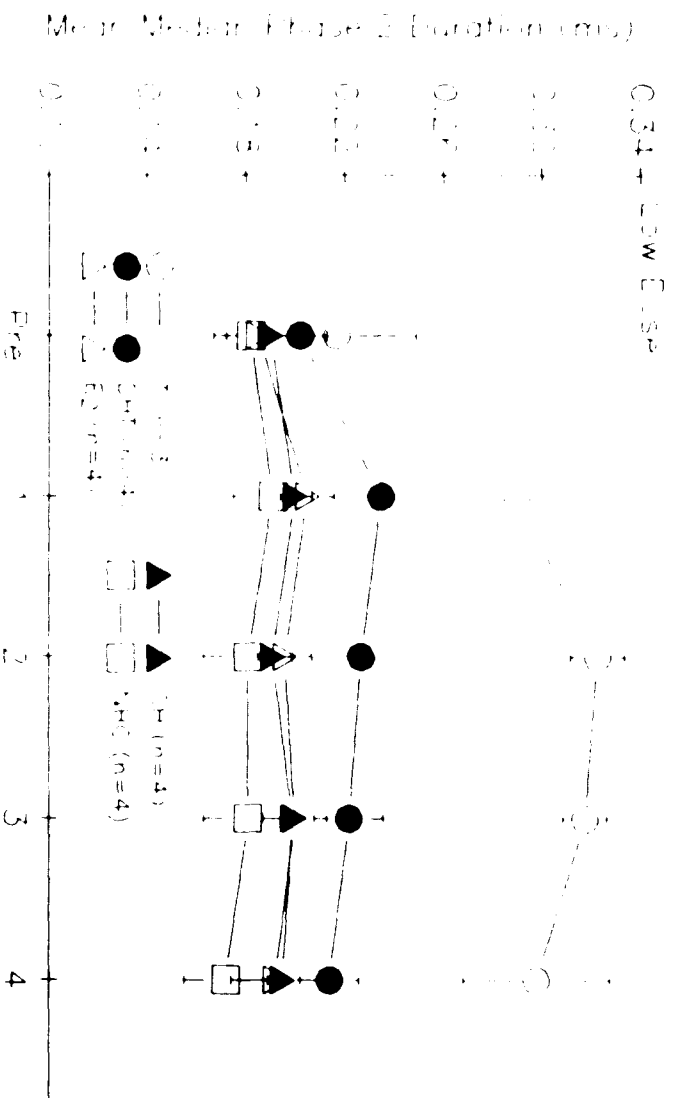


Figure 8. Adults. Time-course for hormone effects on phase 2 duration for individual subjects across 6 pre-implant and 24 post-implant days. Legend refers to individual subject numbers and sex.

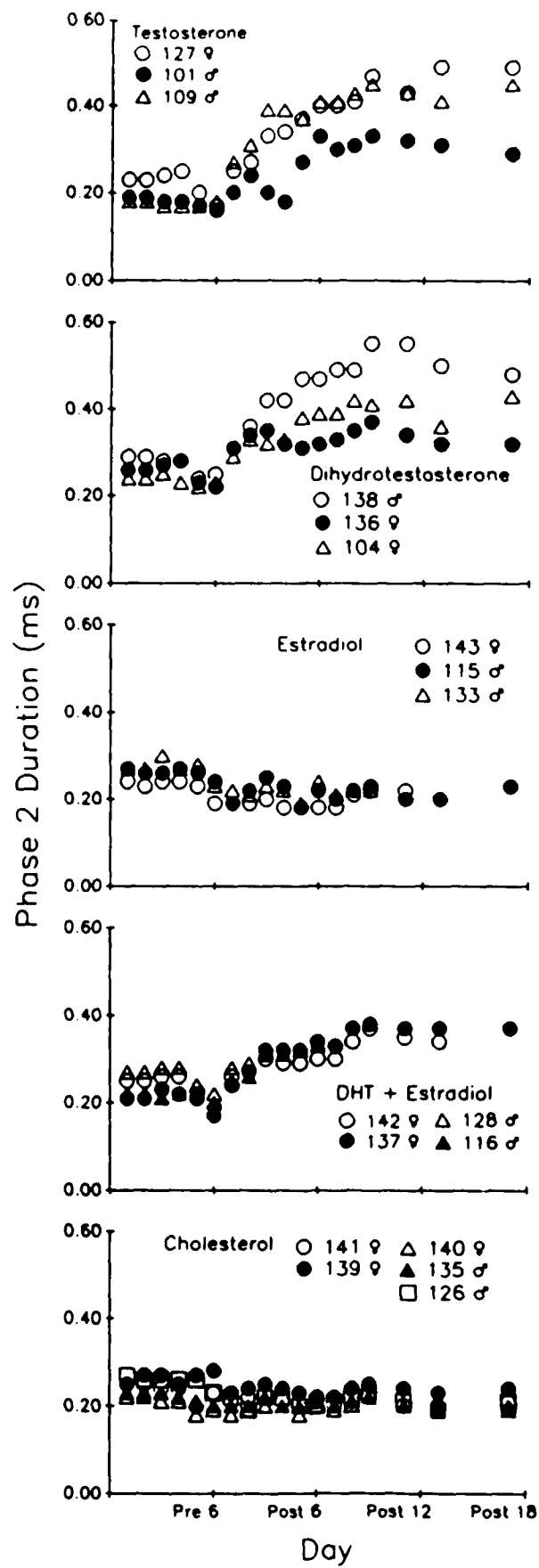


Figure 9. Juveniles. Mean-median phase 3 durations for 6-day pre-implant and subsequent 6-day post-implant blocks. (top) Low-dose hormone effects. (bottom) High-dose hormone effects.

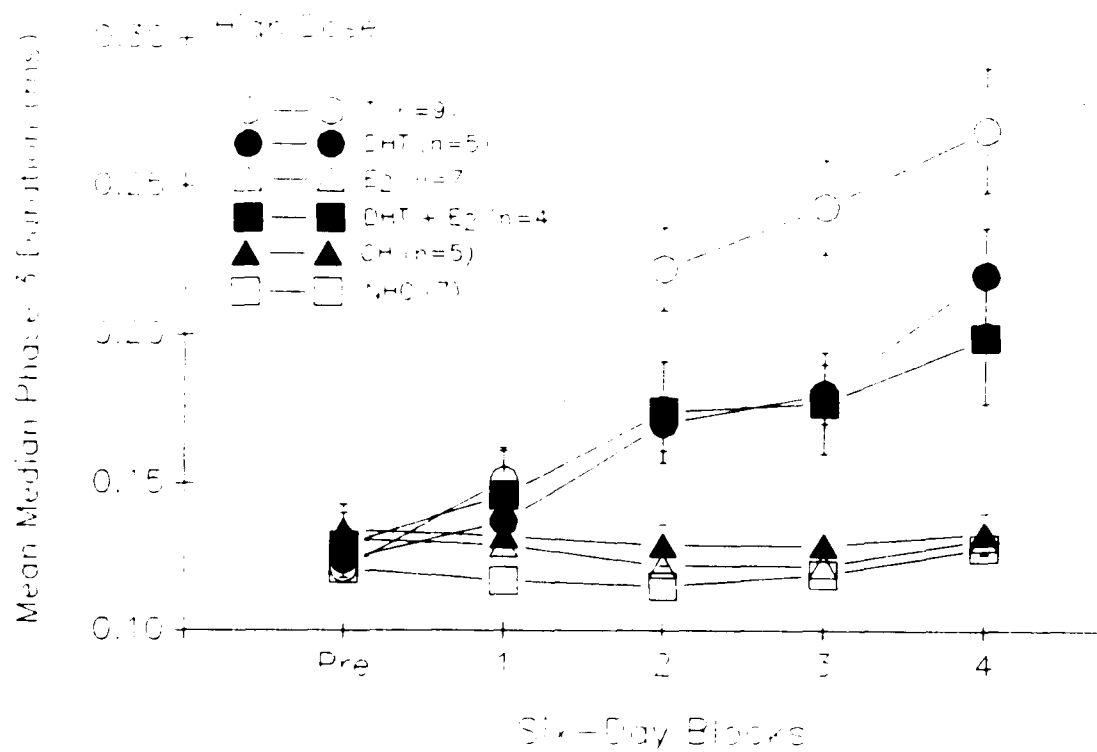
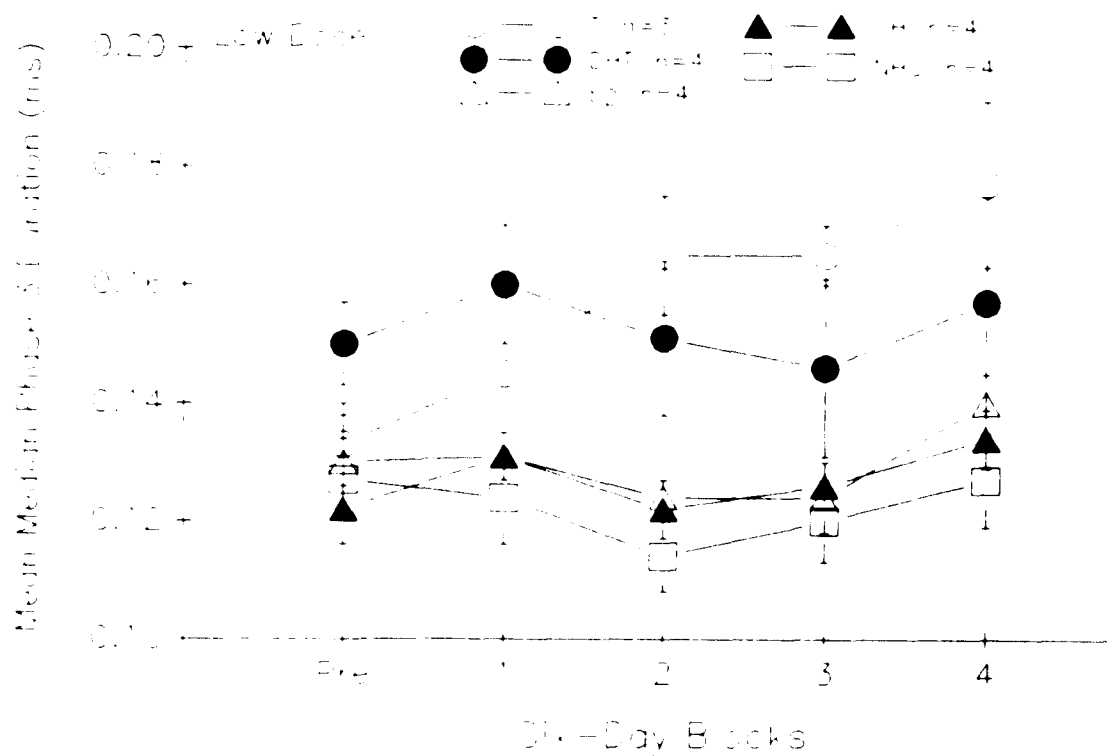


Figure 10. Adults. Time-course for hormone effects on phase 3 duration for individual subjects across 6 pre-implant and 24 post-implant days. Legend refers to individual subject numbers and sex.

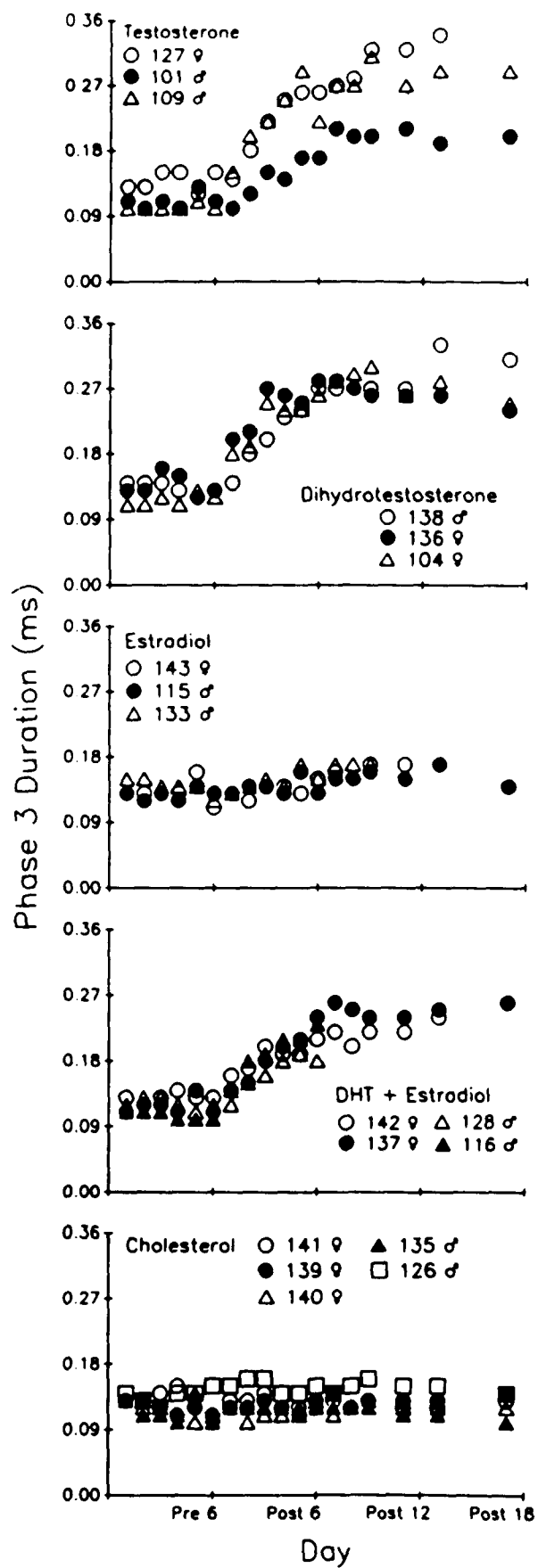


Figure 11. Juveniles. Mean-median phase 1 durations for 6-day pre-implant and subsequent 6-day post-implant blocks. (top) Low-dose hormone effects. (bottom) High-dose hormone effects.

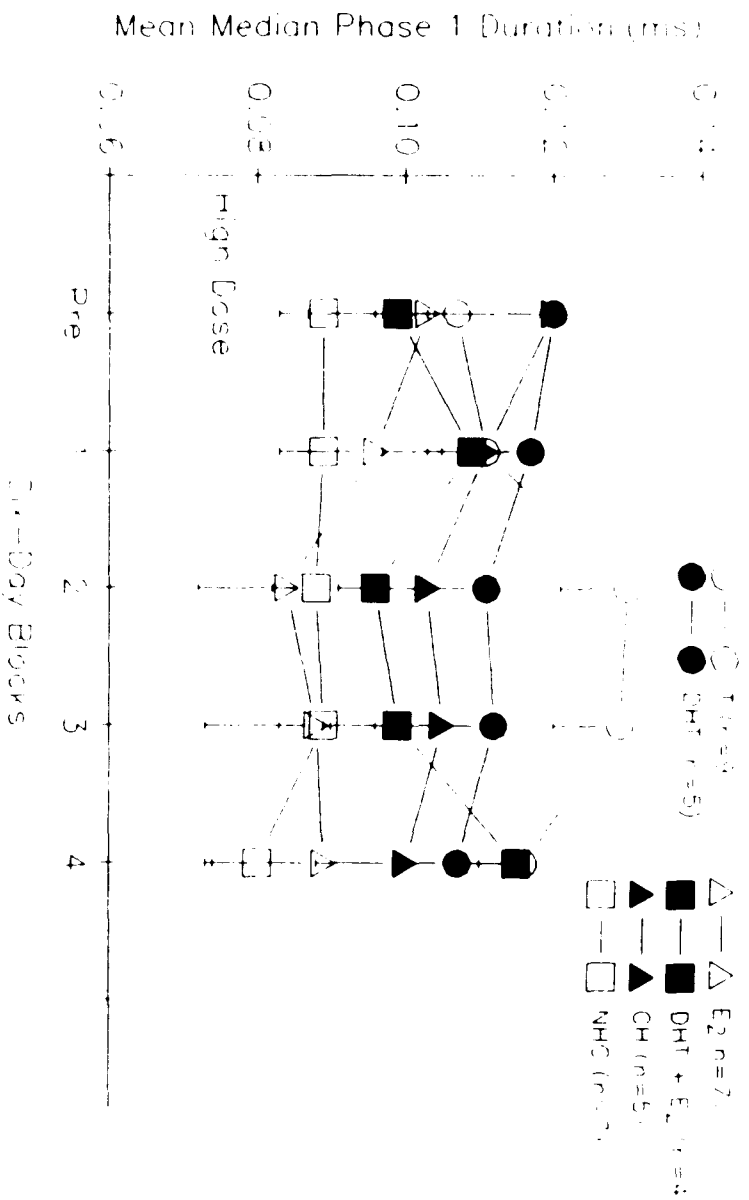
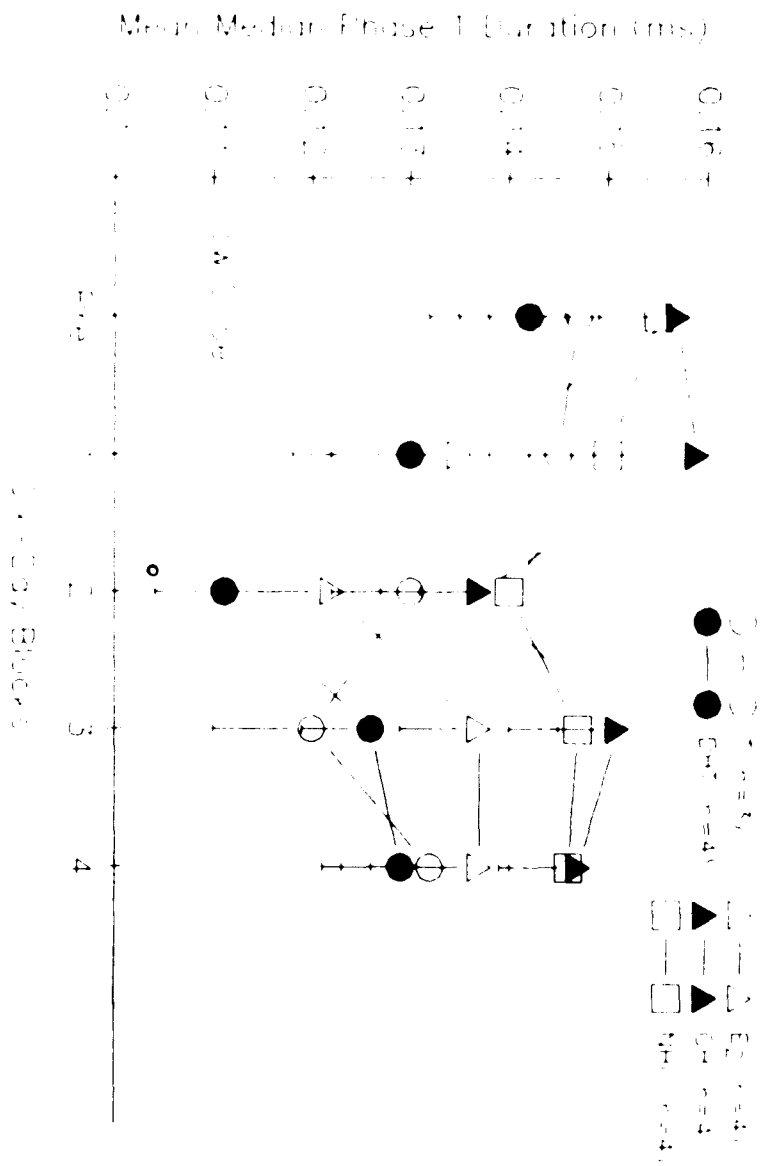


Figure 12. Juveniles. Mean-median phase 4 durations for 6-day pre-implant and subsequent 6-day post-implant blocks. (top) Low-dose hormone effects. (bottom) High-dose hormone effects.

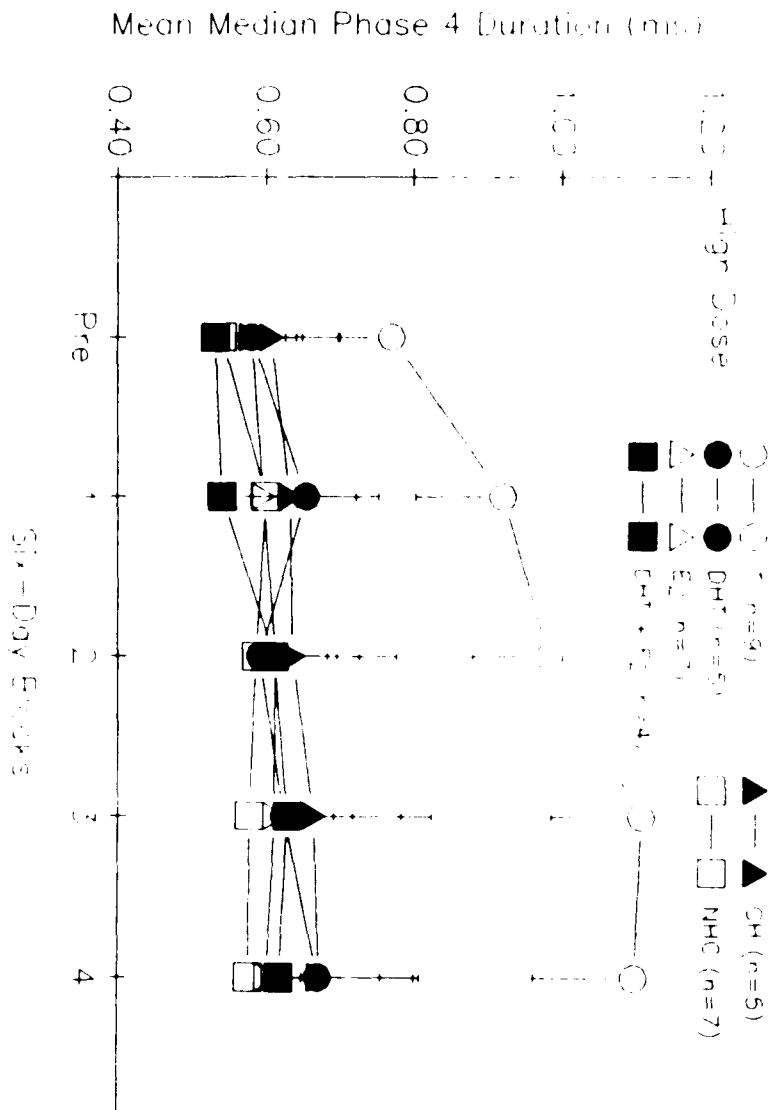
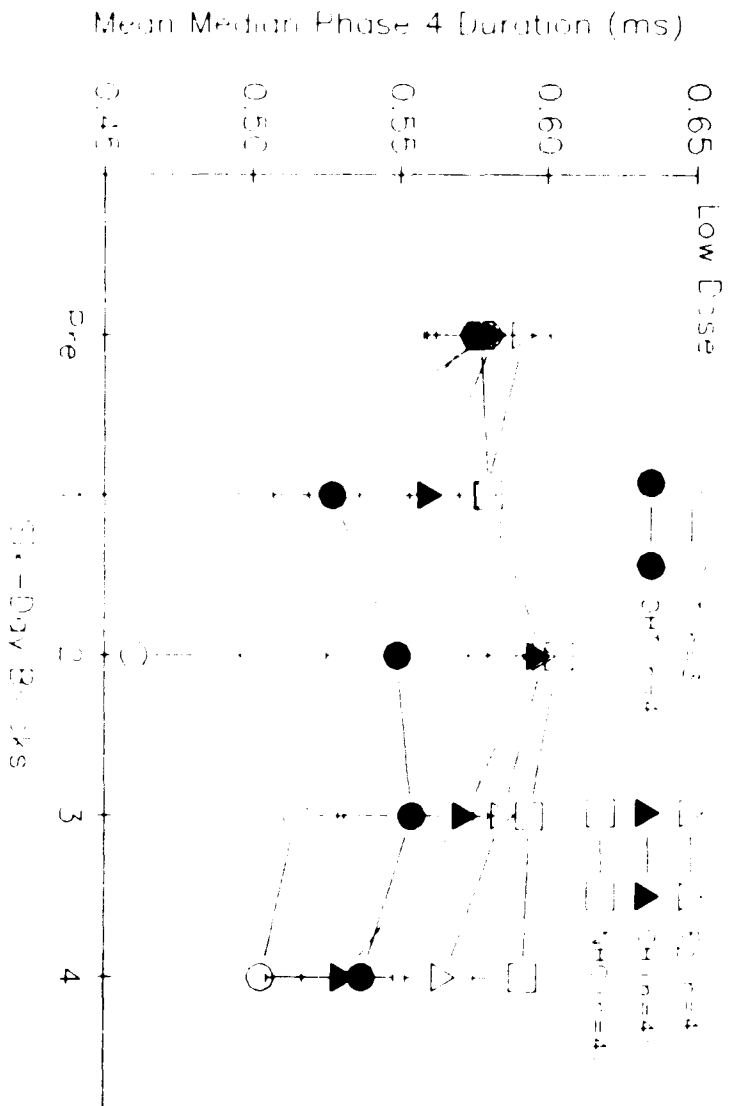


Figure 13. Adults. Time-course for hormone effects on phase 1 duration for individual subjects across 6 pre-implant and 24 post-implant days. Legend refers to individual subject numbers and sex.

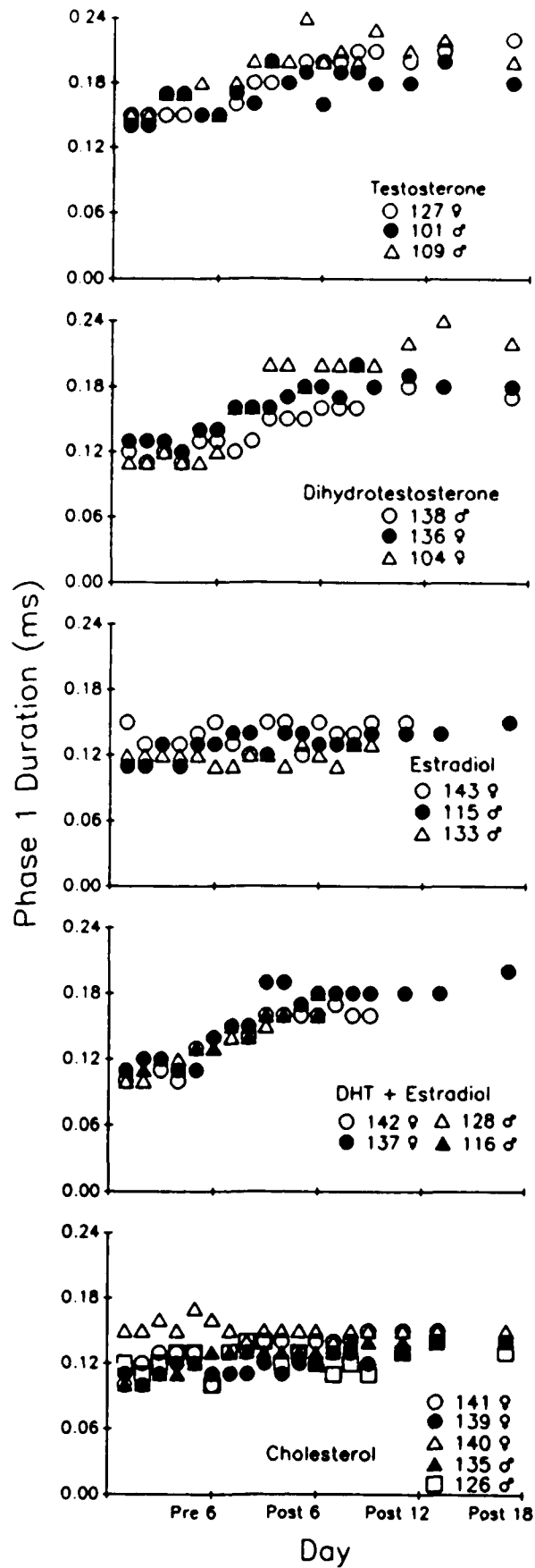


Figure 14. Adults. Time-course for hormone effects on phase 4 duration for individual subjects across 6 pre-implant and 24 post-implant days. Legend refers to individual subject numbers and sex.

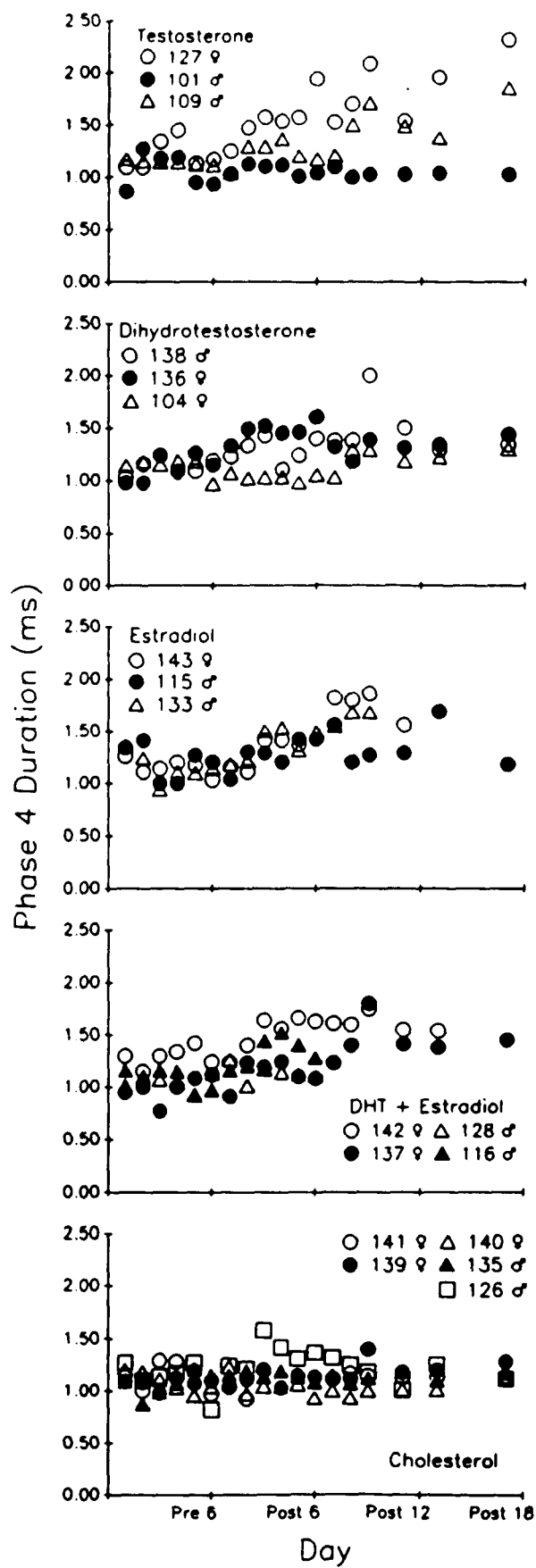


Figure 15. Pooled and individual plasma estradiol (E_2) levels in E_2 - and cholesterol (CH)-implanted subjects and in non-handled controls (NHC).

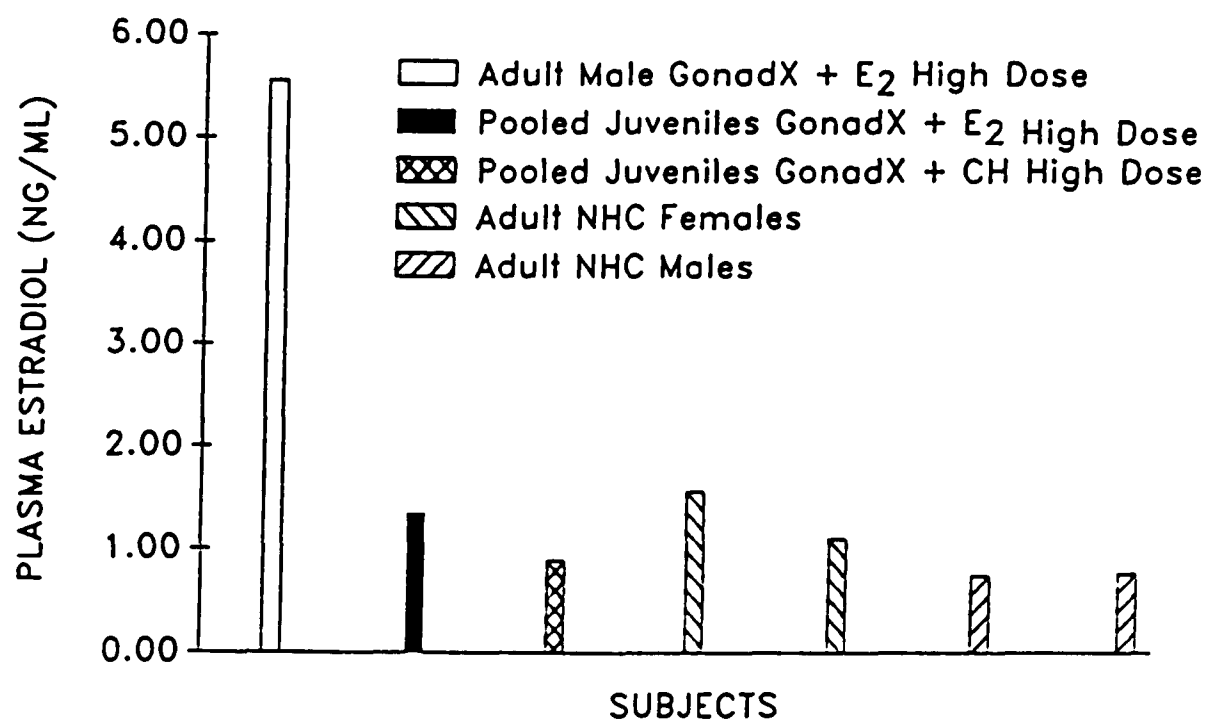
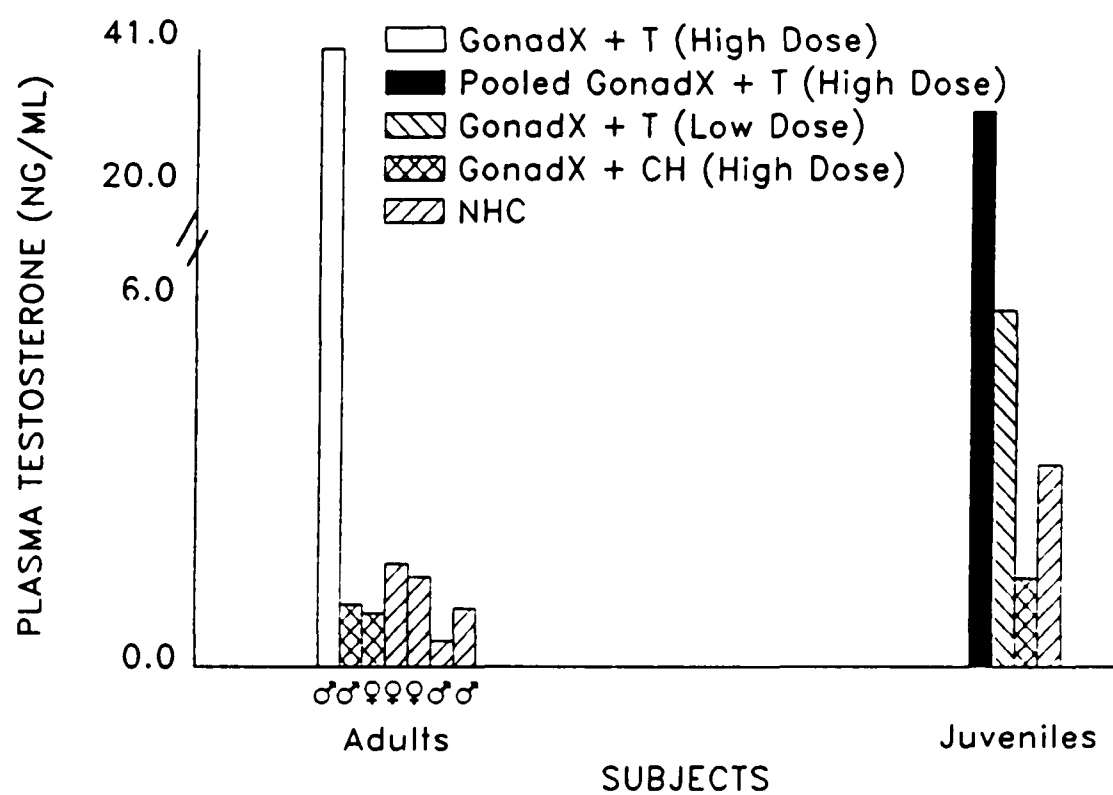


Figure 16. Pooled and individual plasma testosterone (T) levels in T- and cholesterol (CH)-implanted subjects and in non-handled controls (NHC).



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PART IV: SEXUAL DIMORPHISMS IN MORPHOLOGY AND ELECTRIC ORGAN
DISCHARGE IN GNATHONEMUS PETERSII (MORMYRIFORMES): SEASONAL,
PHASE-SPECIFIC SIGNALLING OF SEXUAL IDENTITY

Abstract

The electric organ discharge (EOD) waveform and external morphology of gonadally-ripe, newly imported, adult weakly-discharging electric fish, Gnathonemus petersii, were quantitatively described and examined for sex differences at three times corresponding to the Nigerian pre-rainy, rainy breeding, and post-rainy season in 1988. A sexual dimorphism was found in external morphology with males displaying a clear indentation at the dorsal margin of the anal fin regardless of season. I found pronounced sex differences in EODs only in fish imported during the Nigerian rainy season. These differences were found only in two phases of the tetraphasic discharge, with males exhibiting longer durations of phases 2 and 3. These sex differences were reflected in the Fourier transformation, with male discharges exhibiting lower peak power spectral frequencies than those of females. No sex differences were found in total EOD duration. Correlations between the EOD measures and fish length as well as between the EOD measures themselves revealed relationships which varied in both direction and degree as a function of sex and/or season. Previous work has shown that phase durations of the EOD are steroid dependent and therefore indicative of physiological states of the animal. Thus, the kinds of information conveyed during breeding and non-breeding seasons may be quite different, given great differences in gonadal steroid levels.

Weakly-discharging electric fish use their electric organ discharges (EODs) in social communication including sexual identification (Hopkins 1972; Kramer & Otto 1988), species recognition (Hopkins 1981; Hopkins & Bass 1981; Kramer & Zupanc 1986; Moller & Serrier 1986), schooling (Moller 1976) and possibly alarm calling (Landsman et al. 1987). Field studies have reported sex differences in the EODs of a few waveform gymnotoid and pulseform mormyrid species (Hopkins 1972, 1974; Hopkins & Bass 1981; Bass & Hopkins 1983; Hagedorn & Carr 1985, while sex differences were seldom reported in laboratory studies. Some male mormyrids are reported to have longer EODs than females (Moller 1980; Hopkins 1980, 1981; Hopkins & Bass 1981; Bass & Hopkins 1983). The results of studies employing exogenous hormone manipulations support these sex difference findings (See Bass & Hopkins 1985; Bass 1986, for reviews). However, in Gnathonemus petersii, males were reported to have shorter EODs than females (Landsman et al. 1987). Surprisingly, exogenous androgen treatment increased the durations of two EOD phases, significantly increasing total EOD duration (Landsman, 1989a; Landsman & Moller 1988; Landsman et al. 1988).

The present study was designed to re-address the issue of sex differences in EOD in G. petersii and to better describe the EOD of this species. This study differs from previous studies of sex differences in electric fish in a number of ways. To minimize the effects of transportation and captivity on EODs (Landsman 1989b), data were collected on the day fish were imported into the USA from Nigeria, as opposed to maintaining the fish in the laboratory to allow habituation. Fish were imported during the beginning, middle, and at

the end of the 1988 Nigerian rainy season to assess possible seasonal differences in EODs. Finally, quantitative analyses of individual phases of the EOD were performed and the fishes' external morphology examined for sex differences.

METHODS

Subjects

Adult G. petersii were obtained from a local fish importer (Lombardos African Fish Importers, Newark, NJ) on the day of arrival into the USA and transported directly to the laboratory. Males were initially identified by their sexually-dimorphic, androgen-dependent (Landsman, 1989a) distinct indentation at the dorsal margin of the anal fin. Sex was later corroborated by gonadal inspection. During 1988, subjects were received by the importer on May 6 (n=5 males and 5 females), June 3 (n=27 males and 32 females), and October 21 (n=13 males and 7 females). These were the only three dates when adult G. petersii were available to the importer during 1988, and coincided with the Nigerian pre-rainy season, early rainy season, and post-rainy season, respectively (B. Rosler, Lombardos African Fish Importers, personal communication, 1988). Subjects were selected by size, beginning with the largest males and females available. All available specimens with standard length of 140 mm or greater were included in the studies because gonadal examination indicated that this length insured large eggs or testes which secreted milt when pressed with forceps.

Apparatus

EODs were recorded from each fish in a 20-l aquarium with water temperature at 24°C, pH at 6.7, and conductivity at 120 μ S/cm. The aquarium was equipped with a corner filter for aeration and a porous ceramic shelter to provide a resting place for the fish. The same pair of Ag/AgCl electrodes was used for all three studies to ensure that variability in EODs between subjects and studies was not confounded by different input impedances. The electrodes were fitted into plexiglas tubes so that the exposed leads extended 2 mm beyond the tubes. The tubes were then fitted into larger plexiglas tubes connected to the far ends of the aquarium. This arrangement ensured that the electrodes were stationary and 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).

The dual beam of the oscilloscope was used so that the EOD could be triggered with 5.0 mV/division amplification, while the EOD phases and total durations were read and recorded directly off the oscilloscope using 10.0 mV/division amplification (Fig. 1a, left panel). The peak power spectral frequencies (PPSFs) of the Fourier transformations were read and recorded directly from the spectrum analyzer (Fig. 1a, right panel). Both EODs and Fourier transformations were plotted on an X-Y plotter (Hewlett-Packard).

The fish were sacrificed if not used in subsequent hormone

studies, or anesthetized (tricaine methane sulfonate, 1:20,000) and the gonads removed, photographed, and then preserved (10% formaldehyde) and stored in 70% alcohol.

Procedure

Subjects were netted and placed into individual plastic bags containing oxygenated water at the importers. Transportation to the laboratory was accomplished as carefully and quickly as possible to minimize handling and transportation effects. Upon arrival at the laboratory (within one hour), all subjects were first introduced into a large 1200 l all-glass tank (water temperature 24°C, conductivity 120 μ S/cm, pH 6.7).

Subjects were randomly netted by an assistant and placed into the recording aquarium individually to collect EODs. This allowed me to collect the duration data from the oscilloscope without knowing the sex of the subject. Data read directly from the oscilloscope were independently verified by the assistant. Following EOD data collection, each fish was sexed by external morphology and measured (standard length).

All behavioral data were collected between 12:00 and 18:00 hr. In a previous study on G. petersii, I found that the EOD waveform in this species does not show a circadian rhythm when data are recorded continuously over a 24-hour period. The EOD behavioral data collected included the duration of each of the four phases, total EOD duration, amplitudes of Phases 2 and 3 (measured from the X-Y plots), and the PPSF of the Fourier transformation.

RESULTS

External Morphology and Gonadal Sex

The morphology of the dorsal margin of the anal fin was found to be an excellent indicator of gonadal sex for all three groups of fish. Only fish exhibiting the indentation contained a testis, while all fish lacking this characteristic contained large ovaries with eggs (Fig. 1b). There were no observable differences in testis size across the three imported groups. However, ovaries from May and June subjects filled the gut cavity and contained large eggs, compared to smaller ovaries containing much smaller eggs in the October fish.

Standard Length

Figure 2 shows the mean standard length for males and females selected from the importers for the three samples. There was little within sex variability in the size of the largest fish available from the importers across the May, June, and October import dates. However, males were significantly longer than females for each sample [May: 171.8 ± 3.3 mm, 158.6 ± 0.9 mm ($\bar{X} \pm SE$), respectively, $F=14.64$, $df=1,8$, $P<0.005$; June: 175.3 ± 2.5 mm, 156.8 ± 1.6 mm ($\bar{X} \pm SE$), $F=40.66$, $df=1,57$, $P<0.00001$; October: 177.3 ± 2.8 mm, 154.4 ± 3.1 mm ($\bar{X} \pm SE$), $F=26.20$, $df=1,18$, $P<0.0001$].

Peak Power Spectrum Frequency (PPSF)

PPSFs (Fig. 1a, right panel) from the Fourier transformations of three EODs were collected for each subject and a median was computed for each fish. Mean-medians were then computed for each of the three

imported groups. The overall May, June, and October mean PPSFs were 2.635 (SE= 0.098 KHz, n=10), 3.008 (SE=0.033 KHz, n=59), 2.532 (SE=0.165 KHz, n=20), respectively. Figure 3 shows the mean PPSFs for males and females for each import date. One-way ANOVAs indicated that only the June fish exhibited a statistically significant sex difference in PPSF ($F=16.86$, $df=1,57$, $P<0.0005$) with males exhibiting a lower PPSF ($\bar{X}=2.875$, $SE= 0.047$ KHz) than females ($\bar{X}=3.120$, $SE= 0.037$ KHz). Males tended to exhibit higher PPSFs than females during May 6 [2.770 ± 0.155 KHz, 2.500 ± 0.101 KHz ($\bar{X} \pm SE$), respectively], although the difference was not statistically significant ($F=2.13$, $df=1,8$, $P>0.18$).

The October male and female PPSFs were virtually identical [2.550 ± 0.213 KHz, 2.500 ± 0.277 KHz ($\bar{X} \pm SE$), respectively]. Finally, compared to May and October, females exhibit their highest PPSFs in June.

Total EOD Duration

The mean total EOD durations for each sex are illustrated in Fig. 4. Total EOD durations for both sexes in June were almost twice as long as the May durations. By October 21, the end of the rainy breeding season, total durations appeared to be decreasing.

There were no significant differences between the sexes for any of the three sampling periods (May: $F=1.20$, $df=1,8$, $P>0.30$; June: $F=1.56$, $df=1,57$, $P>0.21$; October: $F=0.12$, $df=1,18$, $P>0.74$). These findings were surprising and suggested a detailed analysis of specific phases of the EOD.

Individual Phases of the EOD

The EOD of G. petersii consists of 4 phases (Fig. 1a, left panel).

Phase 1

Phase 1 is of relatively short duration and low amplitude. It is negative and appears to be highly variable in duration under changing water and temperature conditions (Landsman & Bowling, unpublished data). Inspection of Fig. 5 indicates that the duration of Phase 1 varied dramatically between the three import dates. The mean durations of this phase were approximately one-third longer in June than in May, with the October durations falling in between. No sex differences were observed in the duration of this phase for any of the three imported groups (Fig. 5).

Phase 2

Phase 2 is the major positive phase of the EOD (Fig. 1a). Although the May and October samples revealed no Phase 2 sex differences ($F=0.63$, $df=1,8$, $P>0.45$; $F=0.47$, $df=1,18$, $P>0.50$, respectively), June males ($\bar{X}=0.26$, $SE=0.01$ ms) exhibited significantly longer mean Phase 2 durations than June females ($\bar{X}=0.22$, $SE=0.01$ ms) [$F=12.24$, $df=1,57$, $P<0.001$, see Fig. 6].

Phase 3

Phase 3 is the major negative phase in the EOD (Fig. 1a). Despite the high variability for the May male sample, males appeared to exhibit their longest Phase 3 durations in the June sample, while

females appeared to exhibit a general decrease in duration of this phase over the three import dates (Fig. 7). This may be indicative of high androgen levels in males during the rainy breeding season, and decreasing androgen levels in females (Landsman et al., 1989). A significant sex difference was found in the June sample with males ($\bar{X}=0.169$, $SE=0.005$ ms) exhibiting longer Phase 3 durations than females ($\bar{X}=0.146$, $SE=0.003$ ms) [$F=11.68$, $df=1,57$, $P<0.002$] (Fig. 7). No sex differences in Phase 3 duration were obtained for the May ($F=0.04$, $df=1,8$, $P>0.84$) or the October samples ($F=0.01$, $df=1,18$, $P>0.91$).

Phase 4

As can be seen in Fig. 1a, Phase 4 is a low amplitude positive phase. Similar to Phase 1, the duration of this phase appears to be extremely variable. No sex differences were found in this phase for any of the samples (May: $F=1.32$, $df=1,8$, $P>0.28$; June: $F=0.90$, $df=1,57$, $P>0.55$; October: $F=0.23$, $df=1,18$, $P>0.64$, see Fig. 8).

It is interesting to note the Phase 4 duration differences between the three import dates. The Phase 4 duration for all May subjects ($\bar{X}=0.514$ ms, $SE=0.025$, $N=10$) was less than half the durations for either the June ($\bar{X}=1.365$ ms, $SE=0.063$, $N=59$) or October ($\bar{X}=1.182$ ms, $SE=0.055$, $N=20$) subjects.

P-N Duration Ratio

The ratio of the durations of the major positive Phase 2 to the major negative Phase 3 was computed for all subjects. Similar ratios

have been used in the attempt to identify or describe sex differences in EODs in G. petersii and other electric fish species (Westby & Kirschbaum 1982; Kramer & Westby 1985; Kramer & Otto 1988).

Figure 9 shows the mean P-N duration ratios for both sexes for the three import dates. Analyses of variance indicated that there were no significant P-N ratio sex differences (May: $F=0.68$, $df=1,8$, $P<0.43$; June: $F=0.40$, $df=1,57$, $P<0.50$; October: $F=0.82$, $df=1,18$, $P<0.82$).

P-N Amplitude Ratio

Possible sex differences have been explored in the ratio of the amplitudes of various positive and negative phases of the EOD of mormyrids (Westby & Kirschbaum 1982; Kramer & Westby 1985; Bratton & Kramer 1988). The amplitudes of both Phase 2 and Phase 3 were measured from the X-Y plots of each subject's EOD. A ratio of the positive Phase 2 to the negative Phase 3 amplitudes was computed for each subject.

Figure 10 shows the mean P-N amplitude ratios for both sexes for the three import dates. There were no sex differences for this measure (May: $F=0.16$, $df=1,8$, $P=0.70$; June: $F=0.96$, $df=1,57$, $P>0.30$; October: $F=1.24$, $df=1,18$, $P>0.28$).

Qualitative Sex Difference in EODs

Auditory amplification (Archer Mini-amplifier with speaker) of EODs revealed that males could be identified by their deep, thump-like sounds compared to the higher-pitched click-like sounds of females.

Relationships Between EOD Variables

Relationships between EOD measures and PPSF as well as standard length were computed to see if the same relationships hold in spite of seasonal variability in male and female EODs. In addition, I hoped to be able to characterize the relationship between PPSF and individual EOD phases. Tables 1-3 show the results of Pearson correlations between standard length and EOD measures for both males and females for May, June, and October, respectively.

Standard length

PPSF was significantly related to standard length for all June fish ($R=-0.59$, $df=57$, $P<0.001$, $R^2=0.35$) [Fig. 11a]. PPSFs of males were significantly inversely related to standard lengths in May (Table 1) and June (Table 2) [$R^2=0.77$ and 0.17 , respectively], while the relationship between PPSF and standard length of females only reached statistical significance in June ($R^2=0.18$). PPSF was not related to standard length for or all fish or either sex in the October sample (Table 3).

Phase 1 duration was never statistically related to standard length. Phase 2 duration was positively related to standard length for the June subjects only ($R=0.48$, $df=57$, $P<0.001$, $R^2=0.23$), but these variables were not related separately for males or females. Phase 3 duration was positively related to standard length for females in June ($R^2=0.18$) and males in October ($R^2=0.40$).

It is interesting to note the trends toward inverse relationships

Table 1. Pearson correlation coefficients for May males and females.

<u>Males (N=5)</u>					
	PPSF	Phase 1	Phase 2	Phase 3	Phase 4
St. Length	-0.88 0.047*	-0.33 0.59	0.64 0.248	0.19 0.761	-0.30 0.62
PPSF		0.20 0.752	-0.72 0.172	-0.27 0.658	0.54 0.35
Phase 1			-0.77 0.124	0.87 0.058	0.31 0.608
Phase 2				-0.46 0.438	-0.54 0.348
Phase 3					0.16 0.798
<u>Females (N=5)</u>					
	PPSF	Phase 1	Phase 2	Phase 3	Phase 4
St. Length	-0.68 0.206	0.46 0.44	-0.23 0.709	0.08 0.898	-0.81 0.094
PPSF		0.29 0.641	-0.48 0.415	-0.58 0.304	0.38 0.532
Phase 1			-0.91 0.033*	-0.47 0.424	-0.69 0.197
Phase 2				0.31 0.604	0.35 0.566
Phase 3					0.30 0.625

* Significant probabilities ($P < 0.05$)

Table 2. Pearson correlation coefficients for June males and females.

<u>Males (N=27)</u>					
	PPSF	Phase 1	Phase 2	Phase 3	Phase 4
St. Length	-0.41 0.033*	0.10 0.623	0.31 0.112	0.07 0.732	0.67 0.001*
PPSF		0.32 0.099	-0.78 0.001*	-0.41 0.034*	-0.46 0.016*
Phase 1			-0.62 0.001*	-0.20 0.312	0.04 0.850
Phase 2				0.35 0.074	0.43 0.026*
Phase 3					0.10 0.622

<u>Females (N=32)</u>					
	PPSF	Phase 1	Phase 2	Phase 3	Phase 4
St. Length	-0.42 0.017*	0.01 0.978	0.28 0.115	0.43 0.014*	0.57 0.001*
PPSF		0.24 0.179	-0.81 0.001*	-0.85 0.001*	-0.27 0.136
Phase 1			-0.50 0.004*	-0.16 0.379	-0.24 0.190
Phase 2				0.68 0.001*	0.28 0.123
Phase 3					0.31 0.085

* Significant probabilities ($P < 0.05$)

Table 3. Pearson correlation coefficients for October males and females.

<u>Males (N=13)</u>					
	PPSF	Phase 1	Phase 2	Phase 3	Phase 4
St. Length	0.51 0.073	0.36 0.224	0.01 0.964	0.63 0.022*	0.08 0.789
PPSF		-0.17 0.586	0.05 0.861	0.18 0.546	0.08 0.793
Phase 1			-0.53 0.061	-0.09 0.778	0.44 0.131
Phase 2				0.58 0.038*	-0.01 0.960
Phase 3					0.13 0.678
<u>Females (N=7)</u>					
	PPSF	Phase 1	Phase 2	Phase 3	Phase 4
St. Length	-0.40 0.375	-0.29 0.531	0.22 0.635	0.36 0.433	0.63 0.130
PPSF		0.44 0.317	0.10 0.829	0.32 0.490	-0.80 0.029*
Phase 1			-0.64 0.121	0.13 0.787	-0.46 0.303
Phase 2				0.38 0.394	0.18 0.698
Phase 3					0.30 0.514

* Significant probabilities ($P < 0.05$)

between Phase 4 and standard length in May fish, especially for females (Table 1). In contrast, Phase 4 was positively related to standard length in June (Table 2) for all fish ($R=0.55$, $df=57$, $P<0.001$, $R^2=0.30$), males ($R^2=0.45$), and females ($R^2=0.32$). There were no significant relationships between these variables in the October subjects (Table 3).

Total EOD duration and standard length were related only in the June sample for all subjects ($R=0.59$, $df=57$, $P<0.001$, $R^2=0.35$), males ($R^2=0.46$), and females ($R^2=0.35$).

P-N duration ratio was inversely related to standard length only for October males ($R^2=0.35$). Finally, the P-N amplitude ratio was positively related to standard length for all June fish ($R=0.35$, $df=57$, $P<0.007$, $R^2=0.12$) and for June males ($R^2=0.18$).

PPSF and EOD

The durations of Phases 2, 3, and 4 of the EOD for all June 3 subjects were inversely related to the PPSF (Phase 2: $R=-0.83$, $df=57$, $P<0.001$, $R^2=0.69$; Phase 3: $R=-0.66$, $df=57$, $P<0.001$, $R^2=0.44$; and Phase 4: $R=-0.38$, $df=57$, $P<0.003$, $R^2=0.14$), while Phase 1 was positively related to PPSF ($R=0.34$, $df=57$, $P<0.008$, $R^2=0.12$) [Figs. 11 c,d,e,b, respectively].

Multiple regression analyses were run to assess the relative contribution of specific EOD phase durations to the PPSF for the June subjects. Regression analysis using the four EOD phases as predictors for the PPSF indicated that 77% ($R^2=0.7698$) of the variance in PPSF for both sexes together could be predicted on the basis of the durations of the four phases of the EOD at better than chance levels ($F=45.14$, $df=4,54$, $P<0.00001$). Partial correlation coefficients

indicated that only the Phase 2 duration, when considered without the influence of the three other phases, accounted for a significant amount of the PPSF variability ($R_{\text{partial}}=0.50$, $df=57$, $P<0.01$). The relative importance in descending order of the individual EOD phases as predictors using the standardized regression coefficients was Phase 2 ($\beta=-0.76$), Phase 3 ($\beta=-0.27$), Phase 1 ($\beta=-0.17$), and Phase 4 ($\beta=-0.06$).

Relationships between the PPSF and the EOD phases as well as the entire EOD duration were examined for each sex for the three import dates (Tables 1-3). In June, the Phase 2 duration was significantly inversely related to PPSF for both males and females ($R^2=0.61$ and $R^2=0.66$, respectively). Both male and female Phase 3 durations were significantly inversely related to PPSF ($R^2=0.17$ and $R^2=0.72$, respectively), however, the relationship between these two variables was stronger for females than males ($R'=1.256$ and 0.436 , respectively, $Z=2.97$, $P<0.01$). Phase 4 duration was significantly inversely related to PPSF in males ($R^2=0.21$), but not in females, respectively).

For fish imported in October, only the female Phase 4 duration was significantly inversely related to PPSF ($R^2=0.64$).

Total EOD duration was negatively related to PPSF for all June subjects ($R=-0.45$, $df=57$, $P<0.001$, $R^2=0.20$) [Fig. 11f], and for males ($R^2=0.26$), but not for females. These variables were not related in May or October subjects.

Phases 1-4

Moderate to high negative correlation coefficients were obtained between Phases 1 and 2 for all subjects (May: $R=-0.81$, $df=8$, $P<0.005$, $R^2=0.66$; June: $R=-0.58$, $df=57$, $P<0.001$, $R^2=0.34$; October: $R=-0.58$,

df=18, $P<0.007$, $R^2=0.34$) and for each sex across the three import groups (Tables 1-3). Only the May male and October male and female relationships did not reach statistical significance. The duration of Phase 1 was significantly positively related to the duration of Phase 3 for all May fish ($R=0.70$, df=8, $P<0.03$, $R^2=0.49$), however, only the males showed a trend towards a positive relationship (Table 1). In contrast, there was a trend towards a weak negative relationship between these two phases for all June subjects ($R=-0.25$, df=57, $P=0.059$). Phase 4 duration was not related to Phase 1 duration for either sex across the three groups.

Phase 2 duration was positively related to the duration of Phase 3 for all June and October subjects ($R=0.56$, df=57, $P<0.001$, $R^2=0.31$; $R=0.51$, df=18, $P<0.03$, $R^2=0.26$, respectively), but not for May subjects. Only June females and October males exhibited significant relationships between these two phases ($R^2=0.46$ and $R^2=0.34$, respectively, see Tables 2 and 3).

Positive relationships were obtained between the durations of Phases 2 and 4 only for all June subjects ($R=0.38$, df=57, $P<0.003$, $R^2=0.14$) and June males ($R^2=0.18$).

The durations of Phases 3 and 4 were not related for any of the groups.

DISCUSSION

The mormyrid, G. petersii exhibits a seemingly year-round external morphological sexual dimorphism and a seasonal EOD sexual dimorphism. Males show an indentation in the dorsal margin of the anal fin that does not exist in females. Clear sex differences in the durations of Phases 2 and 3, the two major phases of the EOD in G. petersii, were obtained only in fish imported in June during the Nigerian rainy breeding season. Males exhibited longer durations for both phases than females. There were no sex differences in Phases 1 and 4, total EOD duration, P-N duration or amplitude ratios, or H-T ratio for any import date. Sex differences in phase were reflected in Fourier transformations of the EODs with males exhibiting lower PPSFs than females.

These results agree with the effects of treatment with exogenous hormones (Landsman & Moller 1988; Landsman 1989a). Landsman (1989a) reported that both low and high doses of androgens increased the durations of Phases 2 and 3 in G. petersii. These hormone effects were accompanied by a decrease in PPSF, as well as development of the male-like indentation at the dorsal margin of the anal fin in both juveniles and adult females.

That Kramer & Westby (1985) reported no sex difference in the waveform of this species is not surprising for a number of reasons. First, they only studied one sample of imported fish and did not report the time of year or season when the fish were imported from Africa. The present study clearly shows that sex differences in the

EOD of G. petersii are season-dependent and may easily be missed if subjects are not obtained during the breeding season. In addition, these researchers did not record behavior from their subjects until approximately 30 days following import to the US (Kramer, personal communication, 1986). Landsman (1989b) has shown that G. petersii maintained in the laboratory exhibit profound hormone changes which may abolish or even reverse the sex differences in EODs. Finally, Kramer & Westby (1985) did not compare data for individual phase durations nor PPSFs, but rather reported comparisons between various amplitude or duration ratios as well as between durations of combinations of phases.

We (Landsman et al. 1987) previously reported a sex difference in G. petersii in which males exhibited higher PPSFs than females, with PPSF inversely related to the duration of Phase 3. These findings were similar to the general trends exhibited by the May fish in the present study. The fish in this previous study were obtained during the rainy breeding season in June 1986, but were maintained in the laboratory for approximately 15 days prior to data collection. Further research (Landsman 1989b) demonstrated that prolonged captivity can cause a reversal in the direction of the sex difference.

Estradiol has been shown to cause a slight increase in PPSF in adult G. petersii (Landsman 1989a). In June, females exhibited their highest PPSFs, which may be indicative of higher estrogen levels during breeding. This could serve to accentuate the sex difference and function in sex recognition.

Numerous laboratory investigations designed to examine mormyrid EODs for sex differences have reported negative findings,

non-reproducible sex differences, or sex differences which vary from study to study (Hopkins 1974; Westby & Kirschbaum 1977, 1981, 1982; Lückner & Kramer 1981; Kramer & Westby 1985; Landsman et al. 1987; Bratton & Kramer 1988). All of these studies used fish obtained from importers, pet stores, or local aquariums. In most cases the authors do not specify the season during which the subjects were imported or the length of time they were maintained in the laboratory prior to data collection. Bratton & Kramer (1988) held Petrocephalus bovei in captivity for three to six weeks prior to data collection and did not find any EOD-related sex differences. They did not mention the period of time elapsed between importing Pollimyrus isidori, a sympatric mormyrid species, in which an EOD sex difference was found. It is not surprising that laboratory findings disagree with themselves as well as with field studies. When gonadal hormones are involved in a sexual behavior of seasonal breeders, it is advisable to study the sexual behavior of the species over a range of time periods in order to draw accurate conclusions about the existence or nature of sex differences.

These are the first reported phase-specific sex differences in EOD duration in a mormyrid species. Phase specificity suggests that the EOD is a more complex signal than previously thought. Individual phases may code specific information. Further, it appears that the information communicated may change from season to season. Thus, a lack of sex difference in phase during May and October could simply mean that EODs signal information other than sexual identity. Such an interpretation is supported by the profound effects of hormone treatment (Landsman & Moller 1988; Landsman 1989a) and endogenous hormone changes (Landsman 1989b) on EOD waveform. Clearly, during the

breeding season when gonadal hormone levels are high, EOD characteristics reflect this, whereas when these levels are low, subtle changes in hormonal milieu due to factors other than reproduction may affect duration of EOD phases. Indeed, Landsman (1989a) has also shown that low dose androgen treatment resulted in decreases in the durations of both Phases 1 and 4, while high doses increased the durations of both phases, suggesting a dose specific response for these two phases. This could account for the fact that the durations of these two phases as well as total EOD duration in males were shortest in May, prior to the rainy breeding season. Low androgen levels are likely to be present during the non-breeding season, and, based on results from hormone studies (Landsman, 1989a), would be expected to decrease the durations of Phases 1 and 4 well as the total EOD duration. Because of the extreme variability in both of these phases as a function of changing environmental factors such as water conductivity and temperature (Landsman & Bowling, unpublished data), it is not surprising that sex differences were not found in either phase. Surely, it would be difficult for these phases to serve as unambiguous signals of physiological state for this species.

The PPSF is commonly examined for sex differences in weakly discharging electric fish species (Westby & Kirschbaum 1982; Bass & Hopkins 1983, 1985; Hagedorn & Carr 1985; Bass 1986; Bratton & Kramer 1988). However, it has not been established whether weakly discharging electric fish actually use this or other Fourier characteristics in species or sexual identification. For the researcher, the PPSF is useful as a gross indicator of (sex or otherwise) differences in some aspects of the EOD waveform.

In G. petersii and other weakly discharging electric fish, it is largely determined by the individual phase durations of the EOD.

Previous research on other mormyrid species has demonstrated that sex differences in PPSF accompany sex differences in the overall duration of the EOD (eg. Bass & Hopkins 1985; Bass 1986). However, the total duration of the EOD may not necessarily reflect existing sex differences in the individual phases, as was seen in the current study. This is a case where the sum of the parts may be greater than the whole, and suggests that studies investigating sex differences in EOD should include quantitative analyses of the individual phases. Further, the fact that no sex differences were found in the P-N duration ratio indicates that sexual identity is not signalled by the combined duration of the two phases in which sex differences were found. Kramer and Westby (1985) also failed to find a sex difference in this ratio.

The unambiguous sex differences in duration of specific EOD phases reported here would require only that the receiving fish has the ability to detect differences in phase length. This would be a more parsimonious assertion than to suggest that this species is capable of performing a Fourier analysis or detailed examination of the power spectrum of the EOD. One study (Hopkins & Bass 1981) found that three male Brienomyrus brachyistius triphasic (mormyrid) responded with more rasps to the playback of a female EOD than to computer phase-altered or time reversed EODs, with power spectral and sequences of pulse intervals held constant. Further, males were able to discriminate between a 0.1 ms and 0.4 ms computer generated square wave. These findings suggest that weakly discharging electric fish

have the ability to use phase spectral cues alone (Hopkins & Bass 1981). However, the ability of G. petersii to discriminate between individual phase durations is yet to be investigated.

Although males were always larger than females, the phase-specific sex differences cannot be attributed to sex differences in size. Phase 3 duration was related to standard length for females, but not for males. Also, sex differences were obtained only for June fish while standard length did not vary within sex across the three import dates. However, while fish length might not dictate sex differences, it might provide constraints on how large the differences could be.

The degree and direction of relationships between the various EOD parameters are particularly interesting in that they depended upon both sex and season. Although the relationship of the PPSF to individual phases of the EOD has not been evaluated for any weakly electric fish species, it is commonly used as an indicator for EOD-related species (Hopkins 1981), sex and individual differences (Westby & Kirschbaum 1982; Hagedorn & Carr 1985; Kramer & Westby 1985; Bass & Hopkins 1985; Bass 1986; Kramer & Otto 1988). The fact that only June fish exhibit relationships between PPSF and the durations of phases 2 and 3 indicates a seasonal difference in contribution of these phases to the Fourier transformation. Further, PPSF was more strongly related to phase 3 duration for June females than males. Thus, extreme caution must be taken in the interpretation of sex differences in PPSF. Apparently, the PPSF may reflect different EOD characteristics in the two sexes during different seasons. These changes in relationship between phase durations and PPSF can be

explained by the sum of the relative contributions of the various phases to the PPSF. At certain times, a given phase may change in duration without having a major impact on the PPSF, while at other times slight changes in phase duration may be accompanied by large changes in PPSF, both situations causing the overall relationship between these variables to be altered. Further, Landsman (1989b) demonstrated that the sex difference in Phase 2 of the EOD in newly imported G. petersii disappeared following 7 days of captivity in the laboratory, while the sex difference in PPSF was still evident. Thus, phase-specific changes in the EOD may not be unambiguously reflected in the PPSF.

It is also interesting that PPSF and Phase 2 were significantly inversely related to the duration of Phase 1, with Phase 3 also exhibiting a trend towards a negative relationship with Phase 1. This further illustrates the flexibility in EOD phases: as Phases 2 and 3 increase, Phase 1 duration decreases. The negative relationship and trend for Phases 2 and 1 and 3 and 1, respectively, could, in part, account for the fact that total EOD duration is similar for June males and females in spite of the sex differences in phase durations. This is supported by the findings that low-dose testosterone implants increased the durations of Phases 2 and 3, but decreased the duration of Phase 1 (Landsman 1989a).

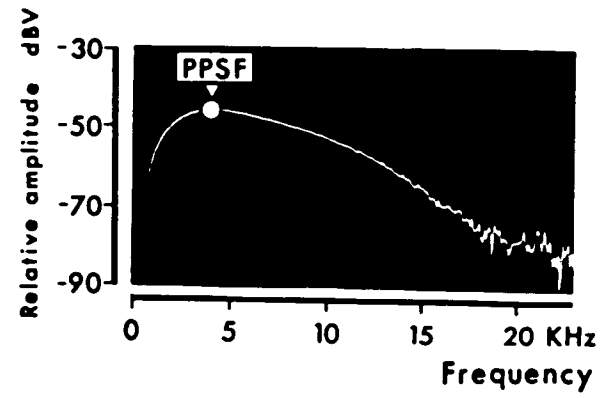
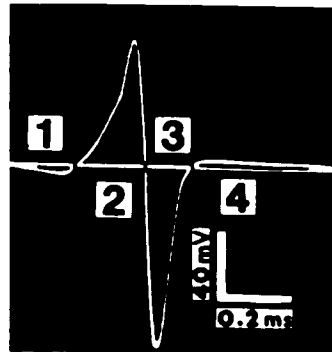
Previous work has shown that phase durations of the EOD are steroid dependent and therefore indicative of physiological states of the animal. Thus, the kinds of information conveyed during breeding and non-breeding seasons may be quite different, given great differences in gonadal steroid levels. During the Nigerian rainy

breeding season, when hormone levels are high, both the duration of specific phases of the electric organ discharge as well as external morphology are likely to carry information regarding sexual identity in G. petersii. During the non-breeding season, it is likely that the various EOD phases convey motivational information based on rapid fluctuations in plasma hormone levels.

Figure 1. a) The G. petersii EOD (left) and its Fourier transformation (right). Note the four phases of the EOD and the peak power spectral frequency (PPSF) of the Fourier transformation.

b) Adult gonadally ripe male and female G. petersii. Note male testis and female ovary with eggs (arrows), indentation at the dorsal margin of the anal fin in the male is absent in the female (arrowheads).

a



b



Figure 2. Mean ($\pm$ SE) standard lengths of imported males and females during 1988. * $P < 0.005$ ** $P < 0.0001$ *** $P < 0.00001$ that the differences between male and female standard length were due to chance.

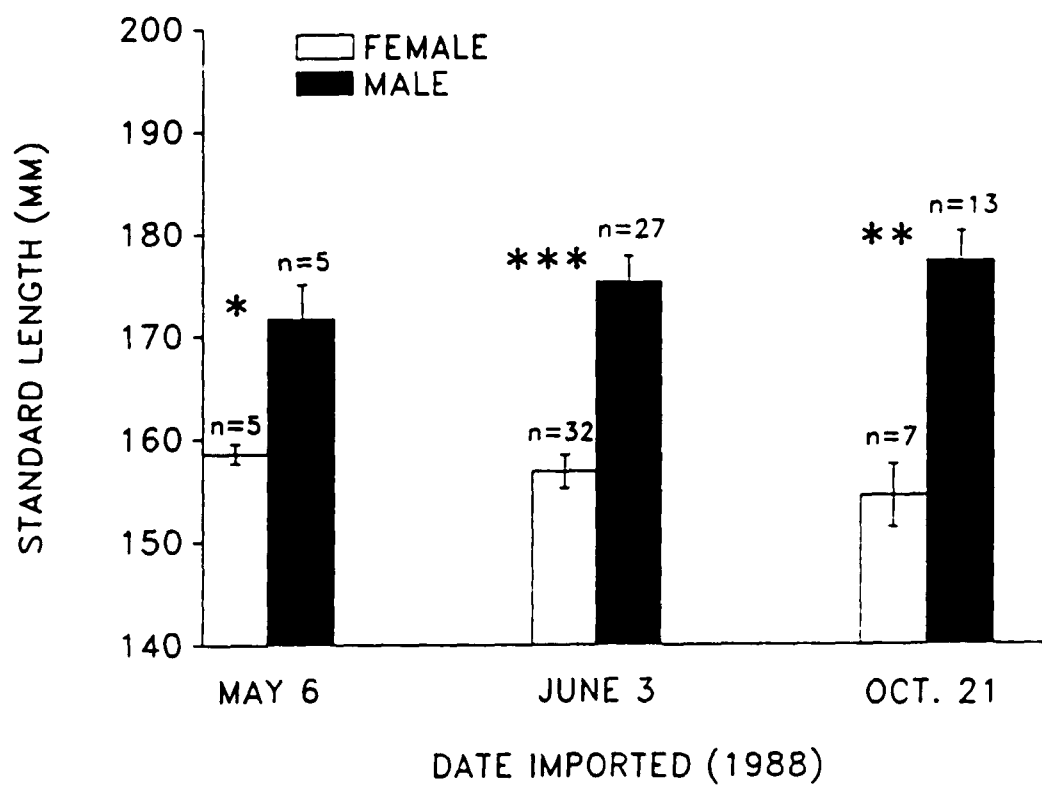


Figure 3. Mean ($\pm$ SE) peak power spectral frequencies (PPSFs) for males and females imported during 1988. * $P < 0.0005$ that the difference in PPSFs for June males and females was due to chance.

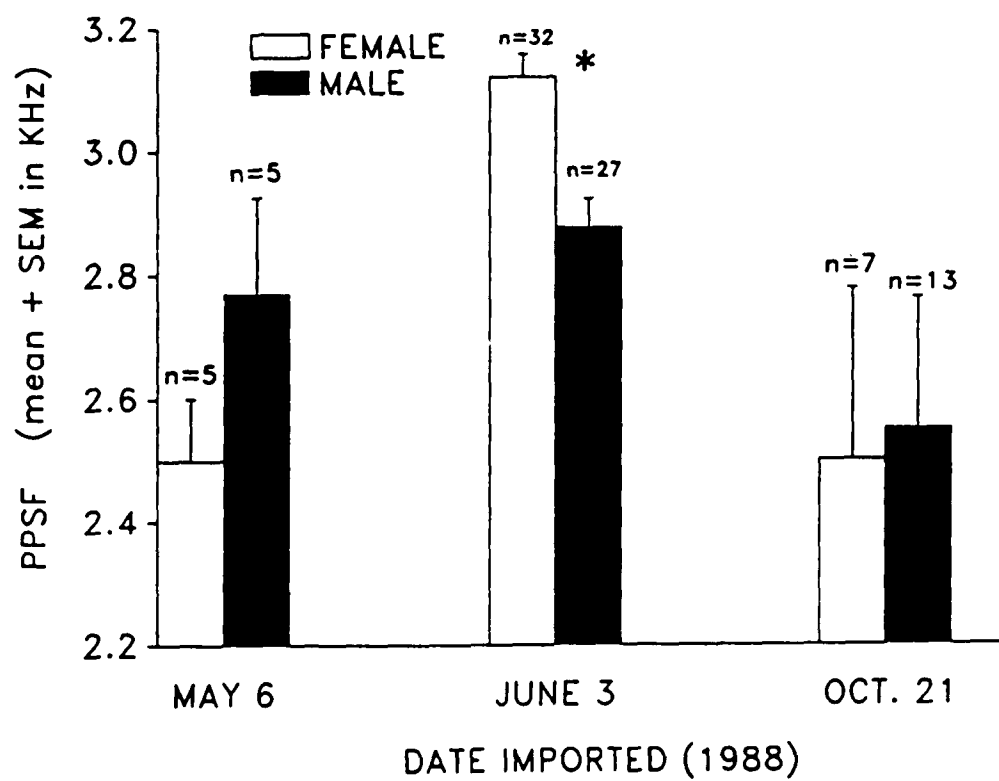


Figure 4. Mean ($\pm$ SE) total EOD durations for males and females imported during 1988.

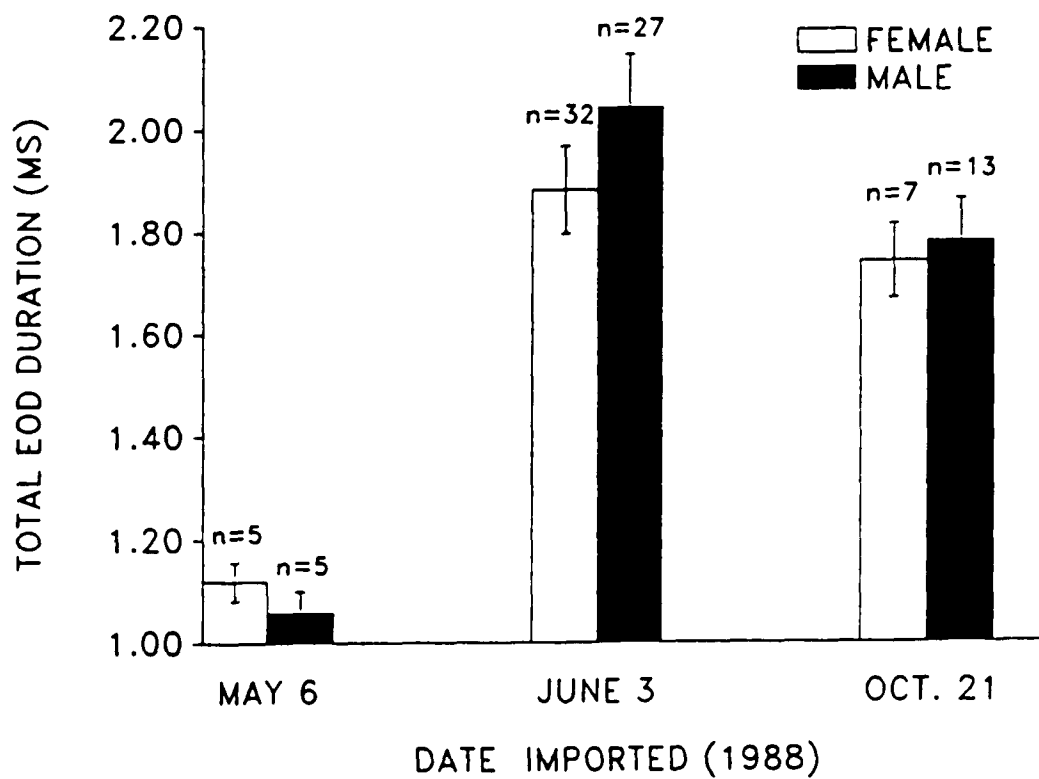


Figure 5. Mean ($\pm$ SE) Phase 1 durations for males and females imported during 1988.

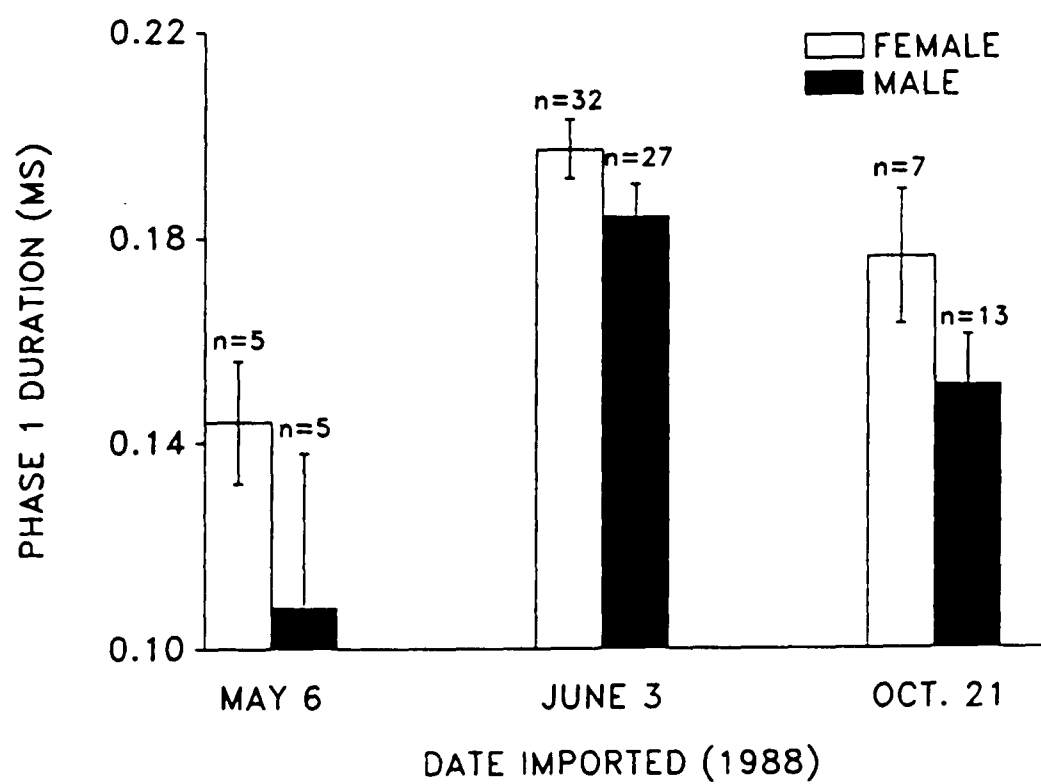


Figure 6. Mean ($\pm$ SE) Phase 2 durations for males and females imported during 1988. * $P < 0.001$ that the Phase 2 duration difference between June males and females was due to chance.

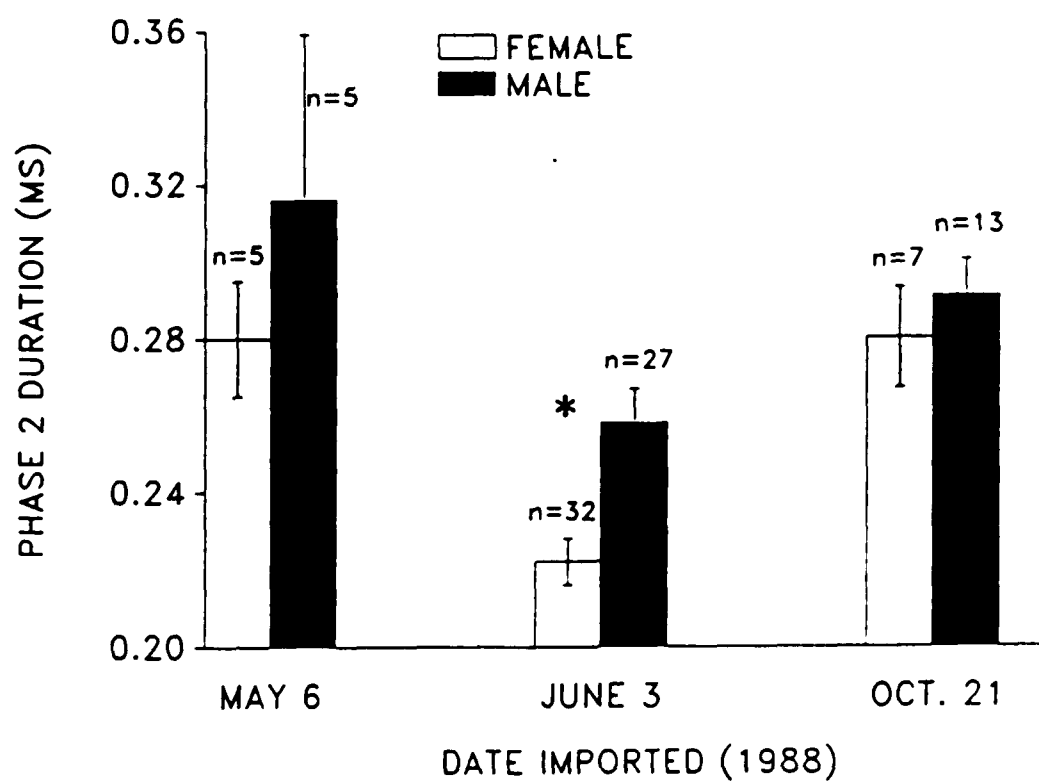


Figure 7. Mean ($\pm$ SE) Phase 3 durations for males and females imported during 1988. * $P < 0.002$ that the Phase 3 duration difference between June males and females was due to chance.

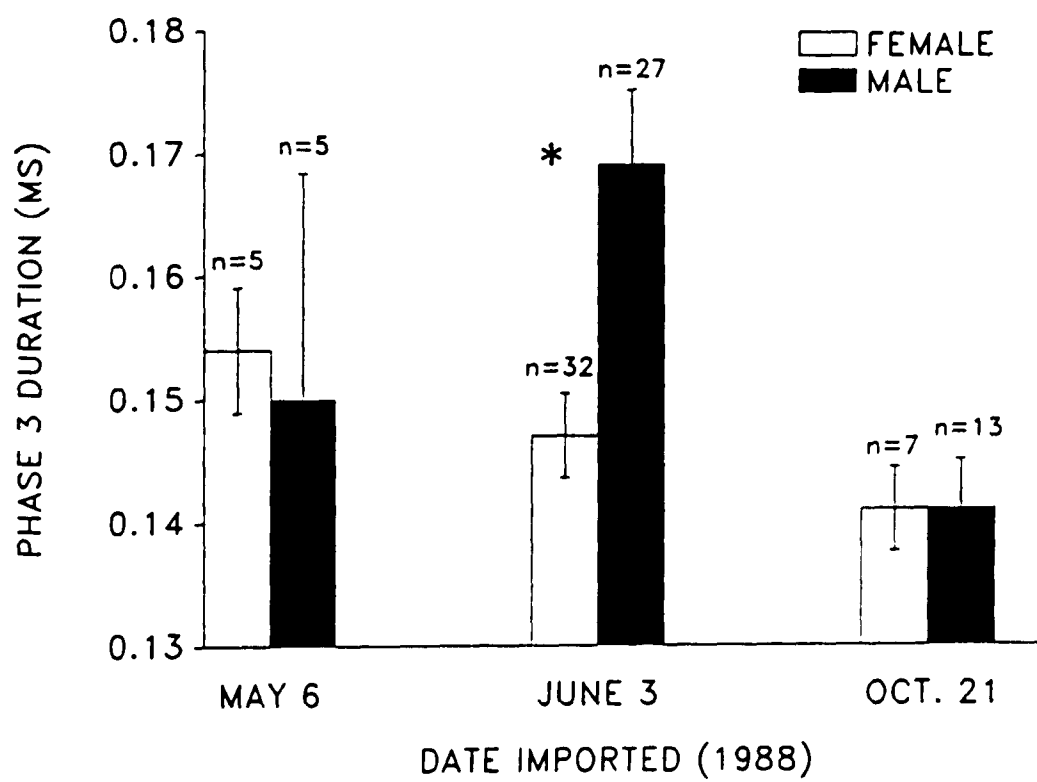


Figure 8. Mean ($\pm$ SE) Phase 4 durations for males and females imported during 1988.

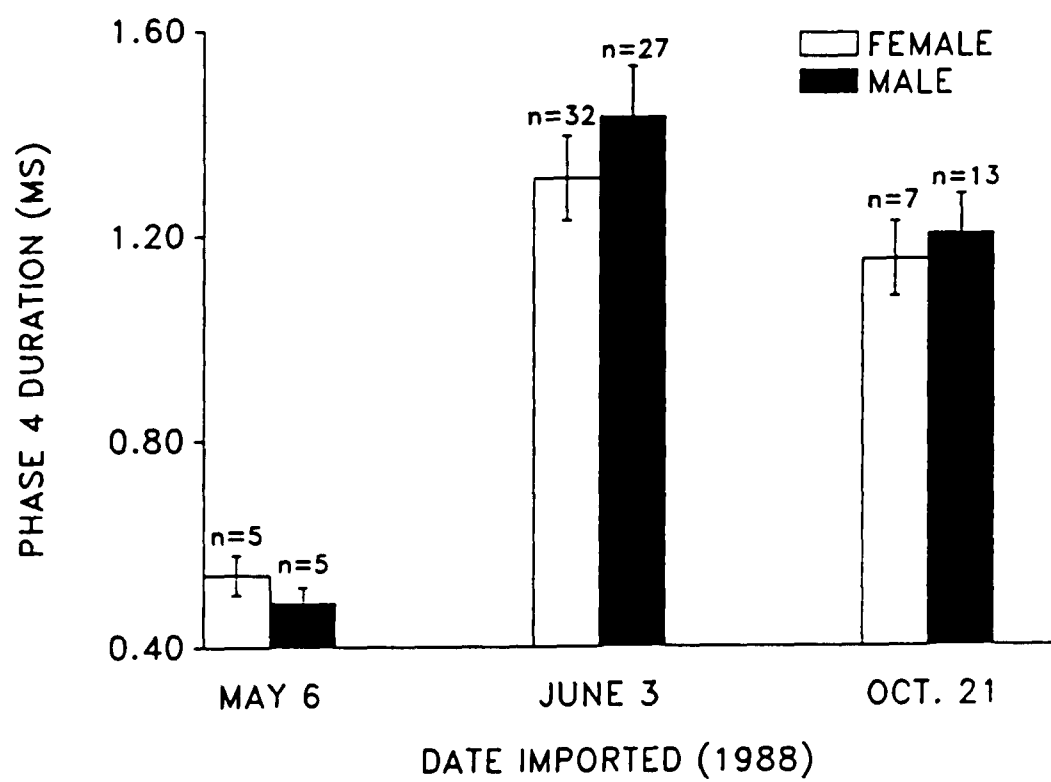


Figure 9. Mean ($\pm$ SE) P-N duration ratios for males and females imported during 1988.

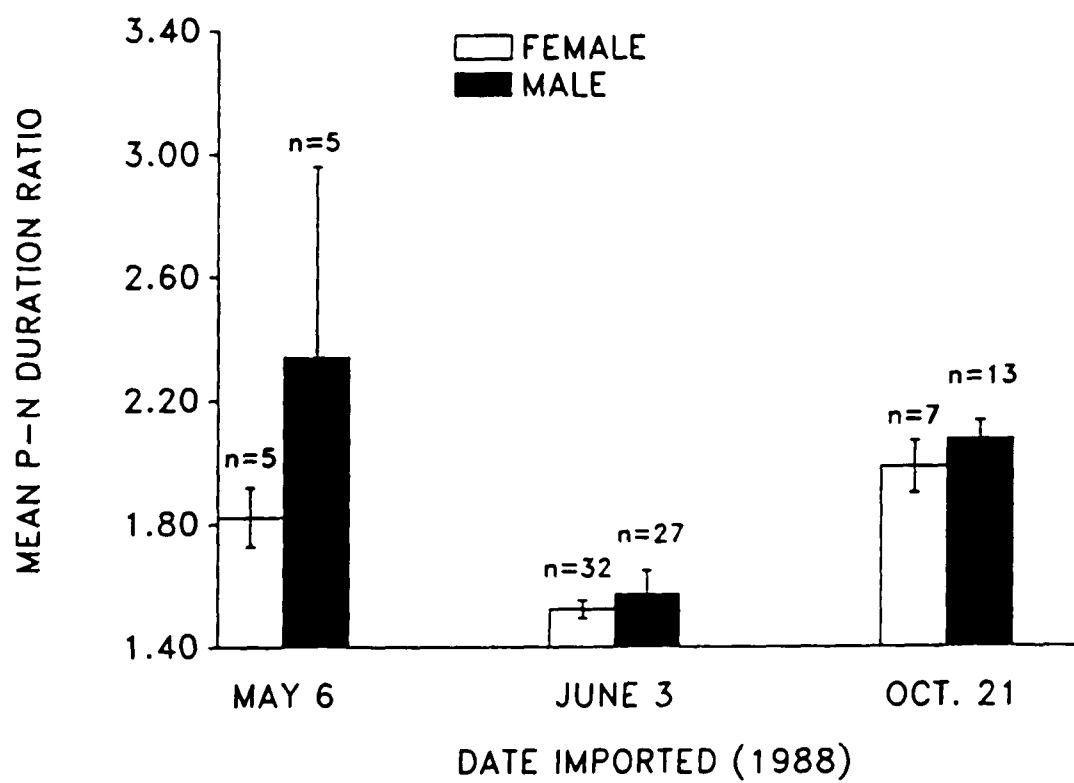


Figure 10. Mean ($\pm$ SE) P-N amplitude ratios for males and females imported during 1988.

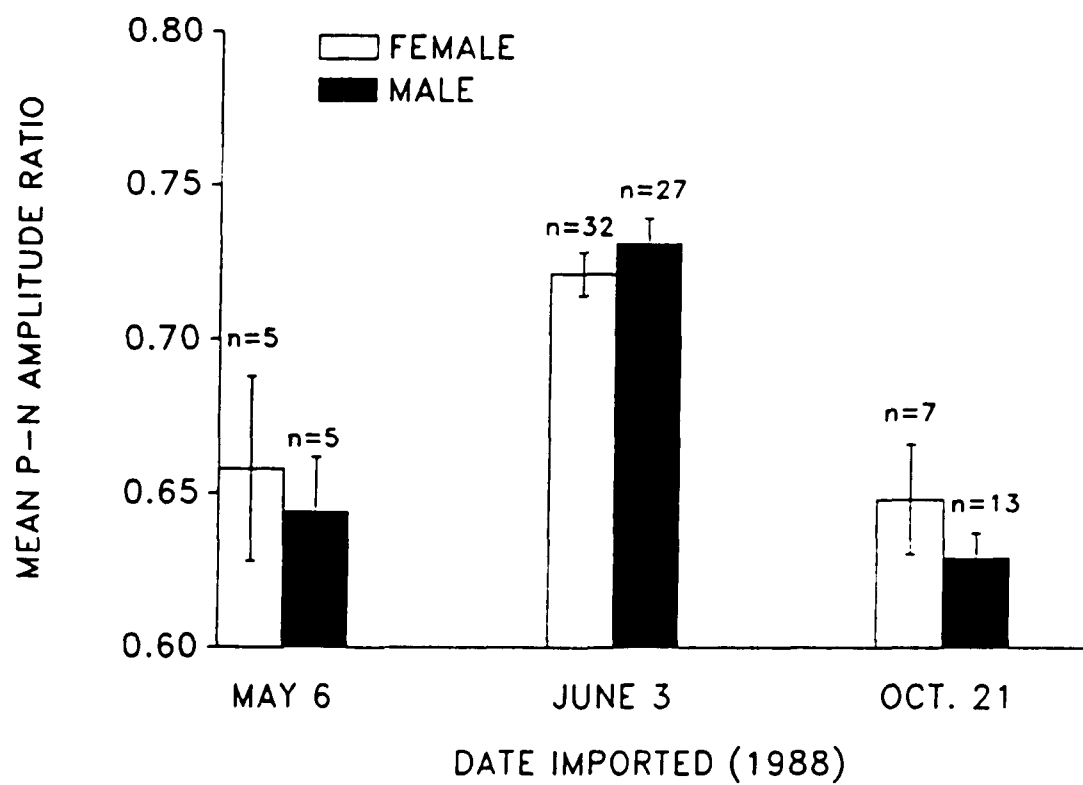
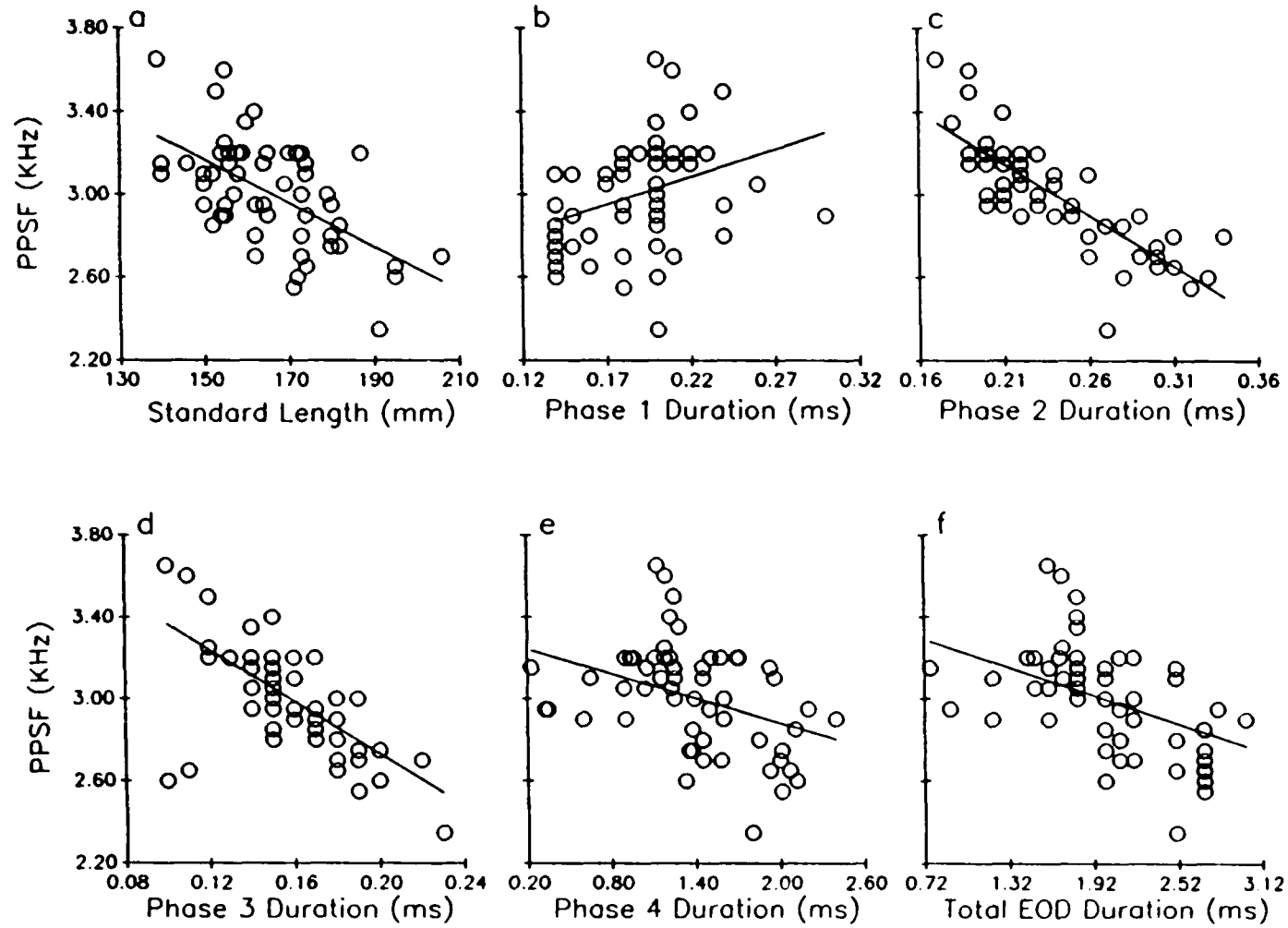


Figure 11. Scatter plots and regression lines depicting the relationship between PPSF and a) standard length, b) Phase 1 duration, c) Phase 2 duration, d) Phase 3 duration, e) Phase 4 duration, and f) total EOD duration for all subjects imported in June (N=59). See text for statistics and explanations.



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PART V: THE EFFECTS OF CAPTIVITY ON THE ELECTRIC ORGAN DISCHARGE AND
PLASMA HORMONE LEVELS IN GNATHONEMUS PETERSII (MORMYRIFORMES)

Abstract

Experiment 1 employed a repeated measures design to examine the effects of 37 days of captivity on sex differences in the electric organ discharge (EOD) of 18 male and 17 female adult Gnathonemus petersii newly imported from Africa. On the day subjects were imported, males exhibited longer durations of phases 2 and 3 of the EOD and lower peak power spectral frequencies (PPSFs) than females. Following 7 days of initial group maintenance, 11 males and 10 females were placed in hetero- or isosexual group tanks where they were maintained for 30 days. Following 14 days in the initial group tank, the remaining 7 males and 7 females were placed in individual aquaria for 23 days. After 7 days in captivity, the sex difference in the duration of phase 2 was abolished, and by Day 14 the sex differences in phase 3 and the PPSF were eliminated. After the 37 days of captivity, the durations of phases 2 and 3 and the PPSFs were dramatically altered so that all sex differences were abolished or even reversed depending on housing conditions. Males appeared to exhibit the most dramatic changes in behavior. Females appeared to have higher testosterone levels than males after 37 days in captivity. Experiment 2 was designed to investigate the effects of captivity on both behavior and endocrine status in 58 newly imported males. In this independent group design, EOD data and blood were collected from subjects on Day 0, and on Days 5, 10, and 15 for individually-housed subjects, and blood collected on Days 5 and 15 for group-housed subjects. Decreases in phase 3 of the EOD and increases

in PPSFs progressed over the 15 day experimental period, becoming statistically significant by days 10 and 15, respectively. Regardless of whether housed individually or in groups, both testosterone and 11-ketotestosterone dramatically decreased to near non-detectable levels by Day 5 in the laboratory. These findings emphasize the plasticity of the EOD as a communication signal. Together with previous research indicating the sex differences in this species are steroid dependent, these studies can explain major discrepancies in electric fish research by showing that captivity causes rapid and profound changes in the endocrine system which result in dramatic changes in steroid-sensitive EODs. These findings are the first to directly link captivity, hormones, and behavior, and show why feral animals brought into captivity usually do not exhibit sexual behavior.

It is well established that stress disrupts reproduction across a wide spectrum of species. Exposure to stress can affect reproduction by blocking ovulation, causing dysmenorrhea in women, or by causing dysfunction in sexual behavior (Baker, 1981; Britton, Koob, Rivier, & Vale, 1982; see reviews by Christian, Lloyd, & Davis, 1965; Kime, Vinson, Major, & Kilpatrick, 1980; Nowell, 1980; Wingfield, 1988). Stress manipulations typically result in measurable hormone changes including alterations in pituitary, adrenal, and gonadal hormone levels (Bambino & Hsueh, 1981; Bardin & Peterson, 1967; Cumming, Vickovic, Wall, & Fluker, 1985; Dessypris, Kuoppasalmi, & Adlercreutz, 1976; Deviche, 1983; Francis, 1981; Gray, Smith, Damassa, Ehrenkranz, & Davidson, 1978; Kawakami & Higuchi, 1981; Kreuz, Rose, & Jennings, 1972; Krulich, Hefco, Illner, & Read, 1974; Mazeaud & Mazeaud, 1981; Mazeaud, Mazeaud, and Donaldson, 1977; Moore & Miller, 1984; Moore & Zoeller, 1985; Pickering, Pottinger, Carragher & Sumpter, 1987; Rasmussen & Malven, 1983; Repceková & Mikulaj, 1977; Rivier, Rivier, & Vale, 1986; Saez, Morera, Haour, & Evain, 1977; Safford & Thomas, 1987; Sapolsky, 1982; Silver & Buntin, 1973; Sirinathsinghji, Rees, Rivier, & Vale, 1983; Tache, Du Ruisseau, Ducharme, & Collu, 1978; Welsh, Bambino, & Hsueh, 1982; Welsh & Johnson, 1981; Wilson & Follett, 1975; Wingfield, 1988, for a review). Further, laboratory manipulations (such as food deprivation, overcrowding, extensive handling, isolation, extreme temperatures, surgical procedures, injections, etc.) on animals which will breed in the laboratory have demonstrated that severe stressors inhibit reproductive behaviors (Miller & Moore, 1983; Moore, 1978; Moore & Miller, 1983, 1984; Moore, Miller, Spielvogel, Kubiak, & Folkers, 1982; Moore & Muller, 1977;

Moore & Zoeller, 1979; Zoeller & Moore, 1982). To the extent that such reproductive behaviors are hormone-dependent, it has generally been assumed that the endogenous hormone changes resulting from such stress manipulations cause the behavioral changes. However, these laboratory experiments employ severe manipulations and leave the animal no recourse other than a severe stress response (Wingfield, 1988).

Feral animals subjected to capture and maintained in captivity also exhibit changes in reproductive physiology (e.g., Licht, McCreery, Barnes, & Pang, 1983; Wingfield, Smith, & Farner, 1982; Mazeaud & Mazeaud, 1981; Mazeaud et al., 1977) and in reproductive behaviors (Elton, 1979; Erwin & Deni, 1979, for a review). Unfortunately, little is known regarding the link between captivity, endogenous hormone milieu, and reproductive behavior in wild-caught animals because, in most cases, such species fail to exhibit any sexual behavior in the laboratory (Moore & Miller, 1984).

In fish, experimental manipulations thought to be stressful usually result in increases in plasma corticosteroid levels (e.g., for a review, see Mazeaud & Mazeaud, 1981; Pickering, et al., 1987). Very little is known about the effects of stress on reproductive endocrinology in fish (for a review, see Billard, Bry, & Gillet, 1981). A few recent studies with incongruent findings have shown that stress alters plasma gonadal hormone levels in fish (Freeman, Sangalang, Burns, & McMenemy, 1983; Morrison, Leatherland, & Sonstegard, 1985; Pickering et al., 1987; Safford & Thomas, 1987; Sangalang & Freeman, 1974; Truscott, Walsh, Burton, Payne, & Idler, 1983). For example, pollutants have been shown to cause increases

(Sangalang & Freeman, 1974) and decreases (Truscott et al., 1983) in plasma androgen levels in fish. One study examined the effects of 21 days of laboratory captivity on endocrine state in feral Cynoscion nebulosus (Safford & Thomas, 1987). Cortisol levels were reported to increase by the end of the first day, but returned to initial post-capture levels by the third week in captivity. Following initial post-capture day 1 decreases in both testosterone (T) and estradiol (E_2) levels, T levels and gonadosomal index (GSI) for males did not change, while GSI values for females decreased over the 21 day captivity period, suggesting a sex difference in response to captivity.

Little is known about the effects of captivity or other stressors on reproductive behavior in fish. However, one would expect that steroid hormone responses to stressors should be accompanied by changes in steroid-sensitive behaviors.

Some species of weakly discharging electric fish exhibit hormone-dependent sex differences in their electric organ discharges (EODs) [For reviews, Bass, 1986 and Bass & Hopkins, 1985; Landsman, 1989a, b; Landsman & Moller, 1988]. Such sex differences appear to function in the communication of sexual identity during the breeding season (Hopkins, 1972; Kramer & Otto, 1988). Although instances of these sex differences have been observed in field studies (Bass & Hopkins, 1983; Hagedorn & Carr, 1985; Hopkins, 1972, 1974; Hopkins & Bass, 1981), they have rarely been reported under laboratory conditions. In fact, the majority of laboratory studies performed on imported fish have reported major discrepancies regarding sex differences in EODs (Bratton & Kramer, 1988; Hopkins, 1974; Kramer &

Westby, 1985; Landsman, Jou, & Moller, 1987; Landsman, 1989a; Lückner & Kramer, 1981; Westby & Kirschbaum, 1977, 1981, 1982). Nothing is known about the levels of endogenous hormones or their effects on the EOD in weakly electric fish.

Recently, Landsman et al. (1987) reported a sex difference in the EODs of Gnathonemus petersii held in the laboratory, with males exhibiting shorter EODs and higher peak power spectral frequencies (PPSFs) than females. Kramer & Westby (1985) did not find a sex difference in the EOD of this species. In a subsequent laboratory study, a seasonal sex difference in specific phases of the EOD was reported in G. petersii when EOD data were collected on the day of arrival from Africa (Landsman, 1989a). This sex difference in EOD was in the opposite direction to that shown by Landsman et al. (1987), in that males exhibited longer phase 2 and 3 durations and lower PPSFs than females. These sex differences were found to be steroid dependent; male hormones increased the duration of phases 2 and 3 and decreased the PPSF (Landsman, 1989b; Landsman & Moller, 1988; Landsman, Harding & Moller, 1989).

Because of its sensitivity to hormones and because newly imported fish do exhibit sex differences in EOD behavior in the laboratory, I thought that the EOD behavior of G. petersii might provide an excellent opportunity to demonstrate the link between captivity, hormones and behavior. The present experiments were designed to investigate the effects of laboratory captivity on the sex differences in EOD and endogenous hormone levels in newly imported G. petersii. Experiment 1 was designed to examine the effects of 37 days of captivity upon the sex differences in the EOD of G. petersii.

Experiment 2 was designed to investigate the effects of laboratory captivity on both EOD behavior and plasma androgen levels in adult males over their first fifteen days in the laboratory.

Experiment 1

The purpose of this experiment was to determine the effects of captivity on phase-specific sex differences in EODs in newly imported G. petersii. Specifically, it was designed to investigate whether maintaining subjects individually or in groups would influence the durations of phases 2 and 3 and PPSFs, and to assess changes in these EOD measures over time.

Method

Subjects.

The subjects were 17 female [standard length = (mean $\pm$ SD) = 157.35 ± 7.45 mm, range = 149-172 mm; weight = 43.41 ± 7.03 g, range = 36-57 g] and 18 male [standard length = (mean $\pm$ SD) = 173.28 ± 16.68 mm, range = 145-204 mm; weight = 53.44 ± 12.41 g, range = 29-76 g] adult G. petersii. The subjects for this study were randomly selected from a group of 59 fish imported on June 3, 1988 from Nigeria during the local rainy breeding season. Sex was initially determined by the sexually dimorphic external morphology, and confirmed by gonadal examination at the end of the study.

Fish were maintained on L:D = 12:12 with lights on at 10.00 EST throughout the study. Fish were fed tubifex worms twice per week in amounts so that some food was always present in the aquaria.

Materials.

Test tank. The test tank was a 18.93 l aquarium equipped with

aeration through a corner filter. Testing water was maintained at $24 \pm 1^{\circ}\text{C}$ and $130 \mu\text{S/cm}$. Plexiglas tubes were permanently installed at the far ends of the aquarium and a porous ceramic shelter was permanently placed in the center of the aquarium with open ends facing the electrode tubes.

Group Tank. A large Plexiglass tank (150 x 150 cm, water level at 25 cm) equipped with filter system (Eheim model 2013) and aeration, thermostatically controlled heaters (Supreme), tropical plants, and porous ceramic shelters of various sizes was used to house the fish over the first 7-14 days of the study. Water temperature was maintained at $24 \pm 1^{\circ}\text{C}$ and conductivity remained at $130 \pm 5 \mu\text{S/cm}$ during this 14 day period.

Group aquaria. Three 189.27 l aquaria, each equipped with under gravel filters, corner filters, aeration, gravel, plants, heater, and porous ceramic shelters, were used to house fish in groups. Water temperature was maintained at $24 \pm 1^{\circ}\text{C}$. Conductivity was not adjusted to prevent possible confounding effects due to disruption caused by water changes. The outsides of these aquaria were covered with dark construction paper to ensure that fish were undisturbed by the researcher's activities. Fluorescent aquarium lights (Perfecto 20 Watt) were used for illumination.

Individual aquaria. Fourteen 18.93 l aquaria were used for maintaining individual fish in isolation. These aquaria were located on wall racks elevated above human height so that it was unnecessary to cover the plexiglas front. Each aquarium was equipped with permanent Plexiglass tubes for electrode insertion, a porous ceramic shelter, corner filter with aeration and were identical to the 18.93 l

test tank. Water temperature was maintained at $24 \pm 1^{\circ}\text{C}$ and conductivity at $130 \pm 10 \mu\text{S/cm}$. Conductivity adjustments were accomplished every third day by siphoning out approximately 3.78 l of aquarium water through the Plexiglass tubes using standard air tubing, and replaced in the same manner with conditioned water.

EOD recording. The same pair of Ag/AgCl electrodes were used throughout the experiment. The electrodes were contained in Plexiglass tubes with 2mm of the tips extending beyond the tube so that when placed within the aquarium tubes, the exposed electrode tips were in direct contact with the water. EODs were fed to a dual beam oscilloscope (Tektronix, model 455; input impedance 1M Ohm) which triggered a spectrum analyzer (Hewlett-Packard, model 3582A, range: 0-25 KHz, resolution: 100 Hz). The dual beam of the oscilloscope was used so that the EOD could be triggered with 5.0 mV/division amplification, while the EOD phases and total durations were read and recorded directly off the oscilloscope using 10.0 mV/division amplification. Data were collected only when fish were at rest in the porous ceramic shelter. Fourier transformations were photographed and peak power spectral frequencies read directly from the spectrum analyzer. All EODs and Fourier transformations were plotted on a Hewlett-Packard X-Y plotter.

Procedure.

Day 0. Subjects were placed in individual oxygenated bags with water conductivity of $380 \mu\text{S/cm}$ at the importers and transported to the laboratory within 30 minutes. Prior to data collection EODs were recorded from one male and one female first in the $380 \mu\text{S/cm}$ water and

then in the 130 $\mu\text{S}/\text{cm}$ water to ensure that the conductivity change from transportation water to test tank water would not effect EOD waveform or Fourier transformation.

EOD data were recorded from each subject in the test tank immediately upon arrival from the importer. Following data collection, each subject was sexed, measured, weighed, and placed in the group tank.

Day 7. Subjects were maintained in the large group tank for either 7 or 14 days. On day 7, 21 subjects (10 females and 11 males) were individually netted from the group tank, EOD data were collected from each fish in the test tank, and each animal was again identified by sex, standard length, and weight. Each subject was then assigned to either an iso- (n=7 females or 7 males) or a hetero-sexual (n=3 females and 4 males) 30 day group aquarium (189.27 l). All but the shortest and longest males and females assigned to the 30 day group aquaria were fin-clipped for identification purposes. To control for possible effects of fin-clipping, the same fins were clipped for the isosexual-grouped males and females, and for 3 males and 2 females in the heterosexual group.

Day 14. The remaining 7 females and 7 males were individually netted from the group tank and placed into the test tank for EOD data collection. These subjects were then identified and placed into the 18.93 l individual maintenance aquaria.

Day 22. EOD data were collected from the individually- housed subjects 8 days following isolation by introducing the recording electrodes into the Plexiglass tubes attached to each aquarium. Data collection from the group-housed subjects was not done because this

would have necessitated disrupting the group aquaria and moving the subjects to the test tank.

Day 37. Following the 30 days of undisturbed group and 23 days of individual maintenance, all subjects were again netted and placed into the test tank for the final EOD data collection. Each subject was then weighed, identified (the 30 day grouped subjects were identified by sex, fin-clip, and standard length if not fin-clipped).

Two males and two females from the iso-sexual group aquaria were sacrificed following Day 37 data collection, and blood was collected for radioimmunoassay analyses performed in Experiment 2. All other subjects were then anesthetized and gonadectomized for use in a subsequent hormone implant study. The gonads were examined to confirm sex.

Data and Analyses. The duration of phases 2 and 3 of the EOD were read directly from the oscilloscope and the EOD was photographed. The peak power spectral frequency (PPSF) of the Fourier transformation was read directly from the spectrum analyzer and the Fourier transformation and EOD plotted.

The PPSFs from three EODs were collected from each subject on each recording day. Although these did not vary by more than 100 Hz, the resolution of the spectrum analyzer, mean PPSFs were computed for each subject. Mean PPSFs were then computed for each sex in each group for each data collection day. Mean durations for EOD phases 2 and 3 were also computed for each sex in each group over the course of the study.

Neither the 4 males nor 3 females maintained in the heterosexual group differed from their isosexual group counterparts on any EOD

measures on Day 37. Thus data for the group maintained subjects were pooled for analyses.

Two-way ANOVAs with repeated measures on one variable were used to test for sex differences across the data collection days for EOD phases, PPSF, and weight. Significant interactions were followed by tests for simple main effects, and the Neuman-Keuls test was used for multiple comparisons ($\alpha = 0.05$). Neuman-Keuls tests were also used to test for differences between means following significant main effects. One-Way ANOVAs were performed to assess sex differences in standard length and weight for all subjects on Day 0.

Results

Physical Characters.

Standard Length. Males were significantly longer than females [means $\pm$ SDs = 173.28 ± 16.21 mm and 157.35 ± 7.23 mm, respectively; $F(1,34) = 13.02$, $p < 0.005$]. Males maintained both in group and individual aquaria were significantly longer than females [male means $\pm$ SEMs = 175.91 ± 5.31 mm and 170.14 ± 3.51 mm, females = 158.7 ± 2.21 mm and 155.86 ± 3.47 mm; $F(1,20) = 7.56$, $p < 0.025$ and $F(1,13) = 7.18$, $p < 0.025$, respectively].

Weight. On Day 0, males ($N = 18$) weighed more than females ($N = 17$) [means $\pm$ SDs = 53.44 ± 12.06 g and 43.41 ± 6.82 g, respectively; $F(1,34) = 8.52$, $p < 0.01$].

Figure 1 shows the mean weights for males and females assigned to group and individual aquaria. Males weighed more than females over

the 37 day study regardless of whether maintained in groups (Fig. 1a) or individually (Fig. 1b) [$F(1,19) = 7.96$, $p < 0.025$ and $F(1,12) = 7.57$, $p < 0.025$, respectively]. However, both sexes lost weight by Day 37 regardless of whether maintained in groups [females, $F(2,38) = 7.93$, $p < 0.005$; males, $F(2,38) = 19.97$, $p < 0.001$] or individually [females, $F(2,24) = 26.64$, $p < 0.001$; males, $F(2,24) = 52.13$, $p < 0.001$].

Gonads. Gonadal examination revealed that all subjects were accurately sexed by external morphology. Males have a clear indentation at the dorsal margin of the anal fin, while females do not. Although a gonadasomal index was not calculated, there were no obvious differences in gonad size following the 37 days of captivity between any of the groups for either sex. In addition, there were no obvious gonad size differences after 37 days of captivity compared to 15 females and 9 males imported at the same time which were sacrificed on Day 0.

EODs.

Auditory amplification (Archer Mini-amplifier with speaker) revealed that male Day 0 EODs were characterized by low thump-like sounds compared to the higher clicking sounds of female EODs. On Day 37, however, all male EODs clicked, while females either clicked or thumped.

PPSF. Mean PPSFs for males and females maintained in the group aquaria after 7 days are shown in Figure 2a. Analysis of variance revealed a significant interaction effect indicating that the differences between males and females maintained in groups varied over the course of the study [$F(2,38) = 12.66$, $p < 0.0001$]. Males had

lower PPSFs than females on Day 0 [$F(1,19) = 27.29, p < 0.0001$]. By Day 7 in the laboratory, males still exhibited lower PPSFs than females, although the difference was not as dramatic as Day 0's [$F(1,19) = 9.41, p < 0.01$]. However, by Day 37 of laboratory maintenance in groups, there was no difference between male and female PPSFs [$F(1,19) = 0.07, p > 0.05$].

Males, but not females, maintained in groups exhibited significant changes in PPSF over the course of the study [$F(2,38) = 24.02, p < 0.0001$; $F(2,38) = 0.08, p > 0.05$, respectively]. The mean male PPSF increased significantly between days 0 and 7 and between days 7 and 37 (Fig. 2a).

Figure 2b shows the mean PPSFs for both sexes maintained first in the group tank and then in individual aquaria. A significant interaction effect between sex and recording day was obtained and is clearly depicted in Figure 2b [$F(3,36) = 21.68, p < 0.0001$]. Again, Day 0 male PPSFs were lower than females' [$F(1,12) = 6.14, p < 0.05$]. By Day 14 in the group tank, male PPSFs did not differ from those of females [$F(1,12) = 0.1, p > 0.05$]. On Day 22, 8 days after being placed in individual aquaria, male PPSFs again did not differ from females', [$F(1,12) = 0.45, p > 0.05$]. Surprisingly, by Day 37, individually maintained fish exhibited a significant PPSF sex difference opposite the Day 0 sex difference (Fig. 2b). Female PPSFs were significantly lower than males' [$F(1,12) = 14.86, p < 0.001$].

Similar to group-housed subjects, individual males, but not females, exhibited significant changes in PPSF over the course of the study [$F(3,36) = 33.73, p < 0.0001$; $F(3,36) = 2.26, p > 0.05$, respectively]. These males exhibited significant increases in PPSFs

between Days 0 and 14 in the group tank, and between Days 14 and 37 in individual aquaria (Fig. 2b).

Phase 2. Figure 3a shows the mean male and female phase 2 durations for fish maintained in groups. Analysis of variance revealed a significant interaction effect between sex and recording day [$F(2,38) = 7.37$, $p < 0.001$]. Males exhibited longer phase 2 durations than females only on Day 0 [$F(1,19) = 22.48$, $p < 0.0001$]. By Day 7, and at Day 37, the sex difference in phase 2 duration was absent (Fig. 3a).

Females, but not males, exhibited significant changes in phase 2 duration over the course of the study [$F(2,38) = 5.50$, $p < 0.01$; $F(2,38) = 2.19$, $p > 0.05$, respectively]. Mean phase 2 durations for females increased significantly from Day 0 to Day 7, with no significant change between Days 7 and 37 (Fig. 3a). Although not statistically significant, the mean male phase 2 duration decreased from Day 0 to Day 37.

Analysis of variance also revealed a significant interaction between sex and recording days for phase 2 duration of subjects maintained in individual aquaria [$F(3,36) = 10.07$, $p < 0.0001$] (Fig. 3b). Males exhibited longer phase 2 durations than females only for Day 0 [$F(1,12) = 12.35$, $p < 0.005$]. By Day 14 in the laboratory group tank, the sex difference in phase 2 duration was abolished, and by Day 37, following the 23 days of individual maintenance, females tended to have longer durations than males (Fig. 3b), although the difference was not significant.

Both males and females maintained in individual aquaria exhibited changes in phase 2 duration over the course of the study [$F(3,36) =$

7.15, $p < 0.001$; $F(3,36) = 11.69$, $p < 0.0001$, respectively]. The phase 2 duration of males decreased significantly between Days 14 and 37. In contrast, females in the group tank exhibited a significant increase in the duration of this phase by Day 14, which they maintained while in individual aquaria over the remaining 23 days (Fig. 3b).

Phase 3. Figure 4a shows the mean phase 3 durations for males and females maintained in group aquaria. Analysis of variance revealed significant sex and recording day effects [$F(1,19) = 25.76$, $p < 0.0001$; $F(2,38) = 3.89$, $p < 0.03$, respectively], but no significant interaction. Phase 3 durations were longer in males than females on Day 0 and on Day 7. However, by Day 37, there was no phase 3 duration sex difference (Fig. 4a). The lack of a significant interaction between sex and recording day together with inspection of Figure 4a suggests that a slight decrease in male and increase in female phase 3 durations between Days 7 and 37 accounted for the loss of the sex difference. Multiple comparisons revealed that the mean phase 3 duration for all subjects decreased significantly from Day 0 to Day 7, and remained near this duration on Day 37.

Figure 4b presents mean phase 3 durations for males and females maintained first in the group tank and then in individual aquaria. Analysis of variance revealed a significant interaction between sex and recording day [$F(3,36) = 20.94$, $p < 0.0001$]. The duration of phase 3 was longer in males than females on Day 0 [$F(1,12) = 26.27$, $p < 0.0001$]. By Day 14 there was no difference between the male and female mean phase 3 durations [$F(1,12) = 0.09$, $p > 0.05$]. The Day 22 phase 3 durations, after subjects were maintained for 8 days in

individual aquaria, also revealed no sex difference (Fig. 4b). However, by Day 37, following 23 days in individual aquaria, a significant sex reversal was obtained with females exhibiting significantly longer phase 3 durations than males [$F(1,12) = 12.02$, $p < 0.01$].

Both males and females exhibited significant changes in phase 3 duration [$F(3,36) = 24.74$, $p < 0.0001$; $F(3,36) = 5.43$, $p < 0.005$, respectively]. The male mean phase 3 duration decreased significantly from Day 0 to Day 14. By Day 37, males exhibited their shortest phase 3 durations. Female phase 3 durations remained relatively unchanged through the first 22 days, but increased significantly between Days 22 and 37.

Discussion

The overall results are quite clear. The phase-specific and associated PPSF sex-differences in the EOD of G. petersii are extremely susceptible to captivity. When fish are imported during their native breeding season, males exhibit significantly longer durations in phases 2 and 3 of their EOD and lower PPSFs in the associated Fourier transformations. Over 37 days of laboratory maintenance, these phases decrease in duration in males, and may increase in duration in females causing the sex differences to disappear or reverse. The durations of phases 2 and 3 of the EOD in G. petersii account for approximately 80% of the variability in PPSF (Landsman, 1989a). The dramatic effects of captivity on the PPSF appear to be most evident in males. PPSFs increase in males and

remain relatively constant in females over the 37 days of laboratory maintenance.

These findings can account for incongruent results reported in previous work on sex differences in EOD in G. petersii (Kramer & Westby, 1985; Landsman et al., 1987). Kramer & Westby (1985) were unable to find any sex difference in the EOD of this species, while Landsman et al. (1987) reported that males exhibited higher PPSFs and shorter EODs than females. The subjects used by Kramer & Westby were maintained in the laboratory for approximately 6 weeks prior to data collection (Kramer, personal communication, 1986), while the subjects in the Landsman et al., (1987) study were maintained in isosexual groups for approximately 3 weeks prior to data collection.

The present results also suggest that subjects maintained in individual aquaria show more dramatic effects of captivity on the EOD than those shown by subjects maintained in groups. Subjects maintained in groups in this and in previous studies establish dominance hierarchies (Bell, Myers, & Russell, 1974; Crockett, 1986; Kramer, 1976) which involve much aggression. The subjects maintained in group aquaria in the present experiment displayed much biting and chasing over the entire experimental period. In contrast, subjects maintained in individual aquaria appeared calm and, when not scavenging for food, were at rest in their shelters. It would appear that the subjects maintained in groups were more stressed than those maintained in the individual aquaria. If so, then the captivity effects exhibited on EODs in G. petersii were not due to "stress". To further address the stress interpretation for the present results, future research could measure cortisol levels to determine the

relative stress levels in fish maintained in groups or individually. It is also possible that the amount of time the subjects were maintained in the initial group tank was responsible for the more pronounced EOD changes in the individually-housed subjects. It is also possible that the effects of the initial group tank maintenance for all fish was responsible for the changes which progressed over the subsequent course of the study.

In G. petersii, phases 2 and 3 of the EOD as well as the corresponding PPSF are androgen sensitive (Landsman & Moller, 1988; Landsman, 1989b). Male hormones increase the durations of these two phases and decrease the PPSF. Silastic implants containing dihydrotestosterone or T result in lower-pitched, thump-like EODs compared to higher click-like EODs of estradiol-treated and control subjects (Landsman, 1989b). Male EODs reverted from thump- to click-like sounds, and females from click- to thump-like sounds over the 37 days of captivity. Together, these findings suggest that endogenous changes in plasma androgen levels are the physiological mechanism responsible for captivity-induced inhibition of sex differences in EOD behavior.

Experiment 2

This experiment was designed to investigate the endocrine basis for captivity-induced changes in the steroid-dependent EOD of newly imported adult male G. petersii maintained individually or in groups. I used an independent groups design to collect EOD behavior and to determine plasma levels of both testosterone (T) and 11-ketotestosterone (11-keto T) from males maintained in the laboratory for different lengths of time. Plasma T levels were also analyzed for the 2 males and 2 females from Experiment 1. In addition, to determine whether the initial 7-14 day group-maintenance was responsible for the EOD changes in males in Experiment 1, the individually-housed subjects in this experiment were not initially maintained in a group tank.

Method

Subjects.

Fifty-eight adult male G. petersii (standard length = 169.1 ± 12.4 mm, range = 146-183 mm; weight = 50.29 ± 9.6 g, range = 28-75 g) were obtained from the importer on the day on which they were received from Nigeria during the rainy breeding season. Subjects were individually placed into plastic bags with oxygenated water and transported to the laboratory within 30 minutes.

Subjects were maintained on the same feeding and lighting schedule and with the same water conditions as subjects maintained in individual aquaria in Experiment 1.

Materials.

The same maintenance aquaria were used as in Experiment 1 to house subjects individually or in groups. The same EOD recording apparatus and test tank were used as well.

Plasma Hormone analyses. Blood was collected from the caudal vein into heparinized micro-capillary tubes (Sigma) and immediately centrifuged for 5 min at 2000 rpm. The plasma was removed and stored at -70°C until analyzed.

Radioimmunoassays (RIA) for both T and 11-keto T were performed on the same extracts in two blocks. The RIA procedures were previously validated for channel catfish plasma as described by Singh et al. (1988). The T antiserum cross-reacted 11% with 11-keto T, while there was no cross-reaction of the 11-keto T antiserum with T.

Seventy-five microliters of plasma from each sample was extracted with 1.5 ml hexane/ethyl acetate (70:30) in 12 x 75 mm borosilicate tubes. Extraction efficiency was determined by adding tritiated T (1000 cpm) to each sample prior to extraction. The extracts were dried under a gentle stream of nitrogen and reconstituted in 375 μl of assay buffer (51.2 mM phosphate buffer, pH 7.6). Fifty and 100 μl aliquots of the reconstituted sample were measured in each RIA to test for parallelism. Recovery of labelled hormone carried throughout the extraction procedures ranged from 76% to 95%.

In the T assay, 25 μl of tritiated steroid tracer (1,2,6,7 ^3H -T, 3600 cpm, Research Products International) and 25 μl of antiserum (1 in 9000 dilution) were added to the samples and standards. In the 11-keto T assay, 25 μl of steroid tracer (1,2,6,7 ^3H -11=O-T, 4000 cpm,

Amersham) and 25 μ l of antiserum (1 in 10,000 dilution) were added to the samples and standards. The assay mixtures were incubated overnight at 4 °C and bound steroid was separated from free steroid with dextran-coated charcoal. The detection limits were 1.25 pg per assay tube for both androgens.

Column chromatography [Sephadex LH-20 (7.9 x 0.8 cm) using iso-octane:toluene:methanol (62:20:15)] was used to validate the one-step extraction procedures. Values obtained by the one-step T assay were approximately 17% higher [mean coefficient of variation (CV) = 9.26%], probably a function of the 11% cross-reactivity of the antiserum with 11-keto T. The one-step extraction 11-keto T assay resulted in sample values approximately 11% higher (mean CV = 11.3%). The intra-assay CVs were less than 8.0% for both blocks for both the T (N = 23 and 24, respectively) and 11-keto T (N = 14 and 10, respectively) assays. The inter-assay CV was 7.19% (N=8) for T and 7.48% (N=6) for 11-keto T.

Procedure.

Upon arrival at the laboratory, 13 subjects were individually placed into the test tank and EOD behavior collected. Blood was collected from these 13 subjects immediately following data collection, and from 2 additional Day 0 subjects from which EOD data were not collected. The remaining subjects were randomly placed into individual (n=30) or group (n=12) aquaria.

On Day 5, 11 individually-housed subjects were placed into the test tank and EOD data were recorded. EOD data from these subjects were recorded both while the subject was still in its individual

aquaria and again after the subject was placed into the test tank to ensure that the process of netting and transporting did not affect the EOD phases or PPSF. Blood was collected from both the individually-housed Day 5 subjects and one group of 7 subjects maintained for 5 days in a group aquarium.

Day 10 EOD behavior was collected in the test tank from 10 individually-housed subjects, followed by blood collection.

Finally, on Day 15, 9 individually housed subjects were placed in the test tank for data collection. Blood was then collected from these subjects as well as from 1 additional unrecorded individually-housed subject. Blood was also collected from the subjects maintained for 15 days in the second group (n=5) aquarium.

Data Analyses. There were no apparent differences in EOD behavior before and after Day 5 subjects were moved from their individual aquaria. Also, there were no apparent differences in plasma hormone levels from Day 0 and Day 15 subjects moved to the test tank and those from which blood was collected immediately upon removal from the import bags or individual aquaria, respectively. Thus, the EOD data and/or plasma hormone levels from these subjects were included in the data analyses.

Mean phase 2 and 3 durations and PPSFs were computed for the subjects maintained in group and individual aquaria for each period of captivity. Mean plasma T and 11-keto T levels were also computed for group- and individually-maintained subjects for the captivity periods. Non-detectable hormone levels were given a value of 0.1 ng/ml, the smallest value detectable by the RIAs. One-way analyses of variance were performed to compare the effects of time in the laboratory on

each EOD measure. The Neuman-Keuls multiple comparison test was used to compare means following a significant ANOVA. The effects of captivity on plasma androgen levels were analyzed with the Kruskall-Wallis H test followed by the Mann-Whitney U test for multiple comparisons.

Results

Blood and Plasma Hormones.

The blood obtained from the Day 0 subjects was much thicker and darker red in color than blood obtained from the Day 5, 10, and 15 individually- and Day 5 and 15 group-housed subjects.

Figure 5 shows the effects of captivity on plasma T and 11-keto T levels in all groups. Captivity had a significant effect on T ($H = 35.51$, $p < 0.00001$). By Day 5 in the laboratory, plasma T levels of both individually- and group-housed subjects dramatically decreased from a Day 0 level of (means $\pm$ SEMs) 5.84 ± 0.59 ng/ml to 0.41 ± 0.05 ng/ml ($U = 165$, $p < 0.00001$) and 0.22 ± 0.05 ng/ml ($U = 105$, $p < 0.0002$), respectively. Plasma T remained at these new low levels through 15 days of laboratory captivity (Fig. 5a). On Day 5, plasma T levels for the group- housed subjects were significantly lower than for the subjects maintained in individual aquaria ($U = 65$, $p < 0.02$). However, by Day 15, T levels for these groups did not differ ($U = 21$, $p = 0.62$).

Similar captivity effects were found for plasma levels of 11-keto T ($H = 18.79$, $p < 0.002$) [Fig. 5b]. Plasma 11-keto T levels in individually- and group-maintained subjects dropped from a mean

(+ SEM) Day 0 level of 0.55 ± 0.08 ng/ml to Day 5 levels of 0.27 ± 0.06 ng/ml ($U = 142$, $p < 0.01$) and 0.14 ± 0.02 ng/ml ($U = 75$, $p < 0.001$), respectively. Plasma 11-keto T levels remained at these new low values through the remainder of the experiment (Fig. 5b). Similar to T on Day 5, 11-keto T levels for group-housed subjects were lower than those for fish maintained in individual aquaria on Day 15 in the laboratory (means $\pm$ SEMs = 0.14 ± 0.02 ng/ml and 0.30 ± 0.06 ng/ml, respectively, Fig. 5b), however, the difference in these distributions did not reach statistical significance ($U = 39$, $p < 0.19$).

A scatter plot of plasma 11-keto T and T levels for all subjects is shown in Figure 6. A significant Pearson correlation coefficient indicated a positive relationship between the two hormones [$r(N=58) = 0.76$, $p < 0.001$]. This relationship was probably underestimated because of the limitations in sensitivity of the RIAs in detecting very low hormone levels.

Day 37 plasma T levels in the 2 males and 2 females from Experiment 1 were also analyzed. Testosterone levels were higher in both females (1.66 and 1.44 ng/ml) than in the males (0.42 and 0.94 ng/ml). Both of these females exhibited longer phase 2 (0.29 and 0.26 ms) and phase 3 (0.15 and 0.15) durations, and lower PPSFs (2.8 and 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).

EOD Measures.

Figure 7a shows the effects of captivity on phase 2 of the EOD. Time in the laboratory did not significantly affect the duration of this phase [$F(3,39) = 1.21$, $p = 0.32$]. However, it is interesting to

note the slightly higher phase 2 durations for Days 5, 10, and 15 compared to those for Day 0 (Fig. 7a). This tendency was similar to that found in Experiment 1 for individually-housed subjects on Day 14 (Fig. 3b).

Mean phase 3 durations as a function of days in captivity are presented in Figure 7b. The duration of phase 3 gradually decreased over the 15 Days in the laboratory, and an analysis of variance revealed that this captivity effect was significant [$F(3,39) = 3.63$, $p < 0.025$]. The mean phase 3 durations on captivity Days 10 and 15 were significantly shorter than the Day 0 mean duration ($p < 0.05$ and $p < 0.01$, respectively).

Figure 7c shows the mean PPSFs for individually- and group-housed subjects for the captivity periods. The PPSF clearly increased as a function of the number of days in the laboratory. Analysis of variance indicated significant effects of captivity on PPSF [$F(3,39) = 2.88$, $p < 0.047$]. By Day 15 in captivity, the PPSF significantly increased from Day 0 ($p < 0.05$).

Discussion

The results of this experiment show that laboratory captivity dramatically alters the EOD behavior and plasma androgen levels in newly imported adult male G. petersii. Regardless of whether subjects were maintained individually or in groups, both plasma T and 11-keto T levels fell to near non-detectable levels by the end of 5 days in the laboratory, where they remained throughout the remaining 15 days of captivity. These changes in androgen were accompanied by changes in

the EOD. By the end of 5 days in the laboratory, phase 3 of the EOD had already begun to decrease in duration, and the PPSF to increase. These changes progressed over the 15 day study. These results can explain the loss of EOD sex differences in the laboratory. The EOD of male G. petersii is androgen-dependent (Landsman & Moller, 1988; Landsman, 1989b). Landsman (1989b) demonstrated that gonadectomized juveniles and adults implanted with androgens exhibited gradual increases in the durations of phases 2 and 3, and decreases in the PPSF over 30 days. The EOD changes in phase 3 and the PPSF, as a function of the amount of time subjects were maintained in the laboratory, were also gradual, only reaching statistical significance by day 10 for phase 3 and day 15 for PPSF. This suggests that captivity indirectly or directly acted on the gonads or hormone clearance rates causing a rapid and profound decrease in plasma androgen levels, which in turn resulted in the gradual changes in the EOD. In so far as the loss or reversal of the sex differences in phase and PPSF in Experiment 1 were due to changes in the males' EODs, it appears that rapid changes in endocrine state were responsible for the effects of captivity on EOD behavior. However captive females also exhibited significant changes in EOD phase durations probably due to increases in androgens.

Landsman (1989b) reported that the blood of G. petersii implanted with dihydrotestosterone or T was thick and viscous and very dark red in color compared to the thin, runny blood obtained from subjects implanted with estradiol. It is interesting that when endogenous androgens were high (Day 0 subjects), blood also was thick and dark red in color, while after the androgens decreased (by Day 5), blood

was very thin and light in color.

It is not surprising that phase 2 was not also quickly affected by the drop in plasma androgen levels as this phase appears to be much more responsive to low androgen levels (Landsman, 1989b).

General Discussion

Explanations for the effects of stress on reproductive processes have been based on rather severe laboratory experiments (Wingfield, 1988) using species already selected for their ability to breed in captivity or on the few species of feral animals which do in fact exhibit sexual behaviors while maintained in captivity (e.g., Moore & Deviche, 1988). Therefore, attempts to explain why wild-caught animals brought into captivity do not exhibit any sexual behavior are largely anecdotal.

The major finding presented in my experiments is that captivity in the laboratory inhibits gonadal hormones and causes the sex differences in a steroid-sensitive behavior to disappear or even to reverse. It is well known that noxious stimuli or adverse environmental conditions, including capture and captivity, affect both adrenal and gonadal hormones in many vertebrate species (e.g., in man, Dessypris, et al., 1976; rats, Rivier et al., 1986; birds, Wingfield, 1988; fish, Mazeaud & Mazeaud, 1981; Pickering et al., 1987; amphibians; Licht et al., 1983; Moore & Deviche, 1988). However, because most feral animals brought into the laboratory already do not exhibit sexual behaviors (Moore & Miller, 1984), it has been difficult to quantitatively examine the effects of captivity on reproductive behavior. The findings that I present are the first to systematically demonstrate the link between captivity-stress, hormones, and behavior in any species, and therefore directly address the question of how and why captivity effects hormone-sensitive sexual behavior. Even animals bred for laboratory use or "breeding fools" transferred from one

environment to another may, at least initially, exhibit effects similar to those which I describe.

My findings suggest that the concept of a laboratory habituation period prior to the onset of experimental or descriptive research on behavioral endocrinology as well as studies involving other systems associated with steroid-sensitive neurons must be re-evaluated. At least for electric fish, it appears that the longer subjects are maintained in the laboratory, the more they are altered both hormonally and behaviorally.

Further, these findings suggest that animals may exhibit changes in behaviors even when captured and studied in the field. Such inhibition of reproductive behavior, to the extent that these behaviors are hormonally-dependent, may be explainable by the same mechanisms which I have demonstrated for laboratory captivity.

Both males and females exhibited significant behavioral changes due to captivity. These changes were a result of dramatic decreases in both T and 11-keto T in males and possible increases in androgens in females. These findings do not agree with those found for the spotted seatrout (Safford & Thomas, 1987) in which 21 days of captivity resulted in endocrine changes only in females. Further, Bass (1986) reported that one male B. brachyistius reverted from male to female EOD waveform, while females showed no change in EOD following up to three months of captivity. It appears that there may be species-specific sex differences in response to captivity.

These captivity effects can explain major discrepancies between and within sex difference studies on other weakly electric fish species. For example, in one study, female Eigenmannia virescens were

reported to discharge at higher frequencies than mature males (Hopkins, 1974), while in another study, it was reported that males discharged at higher frequencies than females, but only during a pre-artificial rainy season (Westby & Kirschbaum, 1981). However, in the latter study, when the artificial rainy season was introduced, females upshifted their EOD frequencies and the sex difference was abolished. These authors claim that gonadal recrudescence was responsible for the lack of an EOD sex difference and concluded that this species probably does not use EOD frequency in communication of sexual identity. It is not clear how long the fish were maintained in the laboratory prior to the pre-rainy season data collection. Further, post-rainy season data were collected between 18 and 62 days following the pre-rainy season data collection. Because the EOD frequency in this species is steroid-sensitive (Leong, unpubl. data, in Meyer, Leong, & Keller, 1987) and these researchers did not include a control group which was not exposed to the rainy season condition, it is equally parsimonious to explain the EOD changes in terms of captivity effects. In another study, at the time of spawning, male E. virescens EODs were always lower in frequency than females' (Hagedorn & Heiligenberg, 1985). In the same study, first generation animals spawned more readily and regularly than their wild-caught parents, indicative of captivity-induced suppression of reproductive processes when feral animals are brought into the laboratory.

A sex difference in the initial negative wave of the EOD in Pollimyrus isidori (Westby & Kirschbaum, 1977) could not be replicated (Westby & Kirschbaum, 1982). The latter study, however, reported a "clear and unambiguous sexual dimorphism within the major phases of

the EOD itself" (p. 400), while Lückner & Kramer (1981) failed to find any EOD waveform sex difference in this species. Unfortunately, no studies have yet investigated the possibility of hormonal control of the EOD in this species.

Finally, no sex difference was found in the EOD waveform of Brienomyrus brachyistius (Bass & Hopkins, 1983, 1985; Bass, 1986) and the EOD in this species was not affected by methyltestosterone (Hopkins & Bass, 1983). However, a recent study of this species found a sex difference in the EOD of this species which was clearly under hormonal control (Landsman & Moller, unpubl. data).

The EOD of weakly discharging electric fish is extremely susceptible to environmental perturbations as indicated by the present studies as well as by studies involving severe manipulations such as extreme temperatures (Enger & Szabo, 1968) and water conductivities (Bratton & Kramer, 1986, 1988; Squire & Moller, 1982), saline injections (Hagedorn & Carr, 1985), extensive handling (Meyer, 1983), and confinement and restraint (Landsman et al., 1987). Thus, the electrocommunication system of weakly electric fish appears to be plastic and the EOD anything but a fingerprint (Moller, 1980a, b) for individual identity.

Figure 1. Mean weights ($\pm$ SEMs) for (a) group- and (b) individually-housed male and female subjects. Subjects maintained in groups (n = 7 per group) over the 37 day study were moved to their group aquaria from the initial group stock tank after 7 days in the laboratory. Subjects housed in individual aquaria for 23 days were moved from the group stock tank after 14 days in the laboratory.

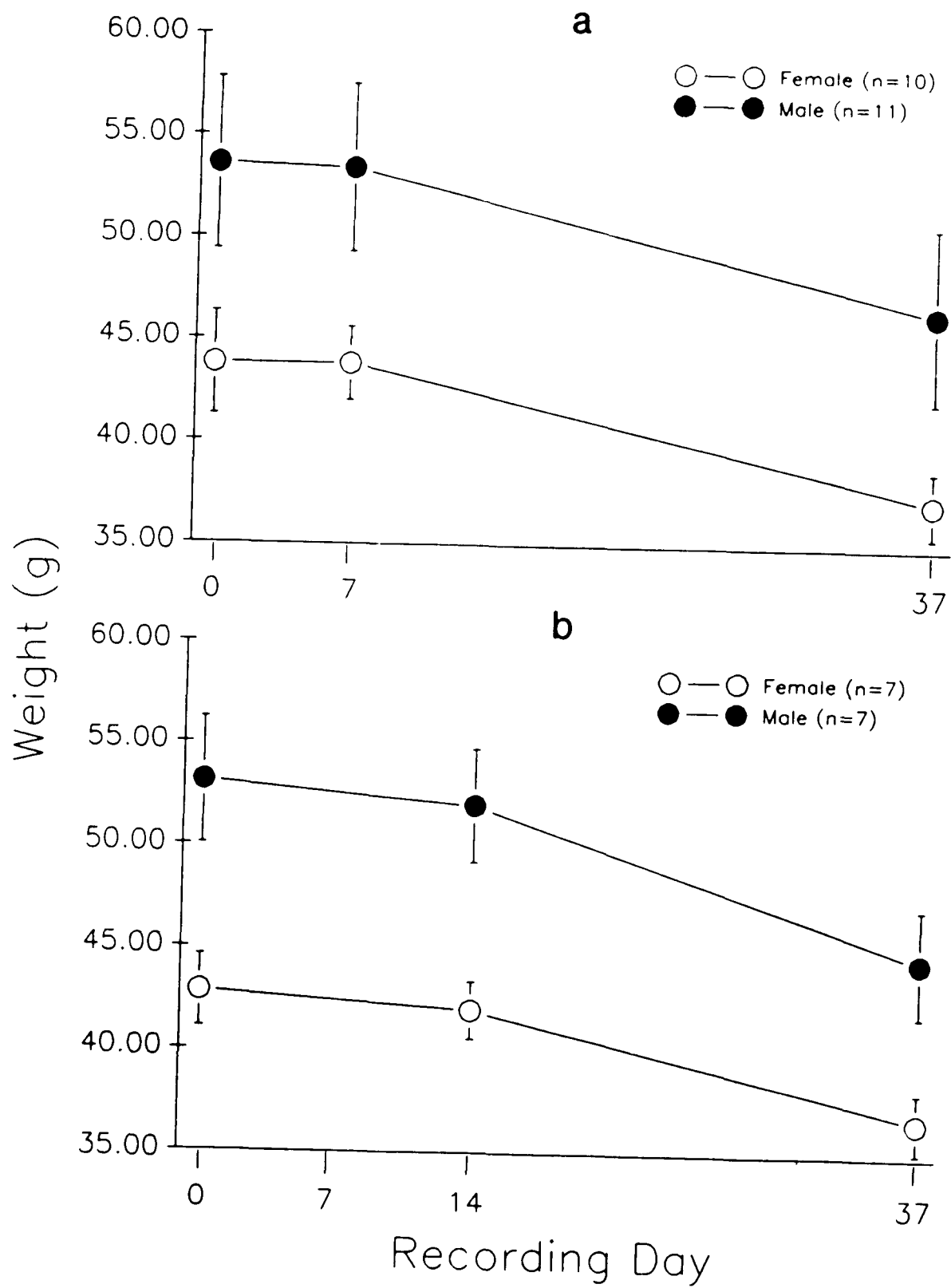


Figure 2. Mean PPSFs ($\pm$ SEMs) for males and females on Day 0 in the laboratory, and after 7 and 14 days of initial maintenance in a group stock tank, and finally after a) 30 days in group aquaria or b) after 8 and 23 days in individual aquaria, respectively.

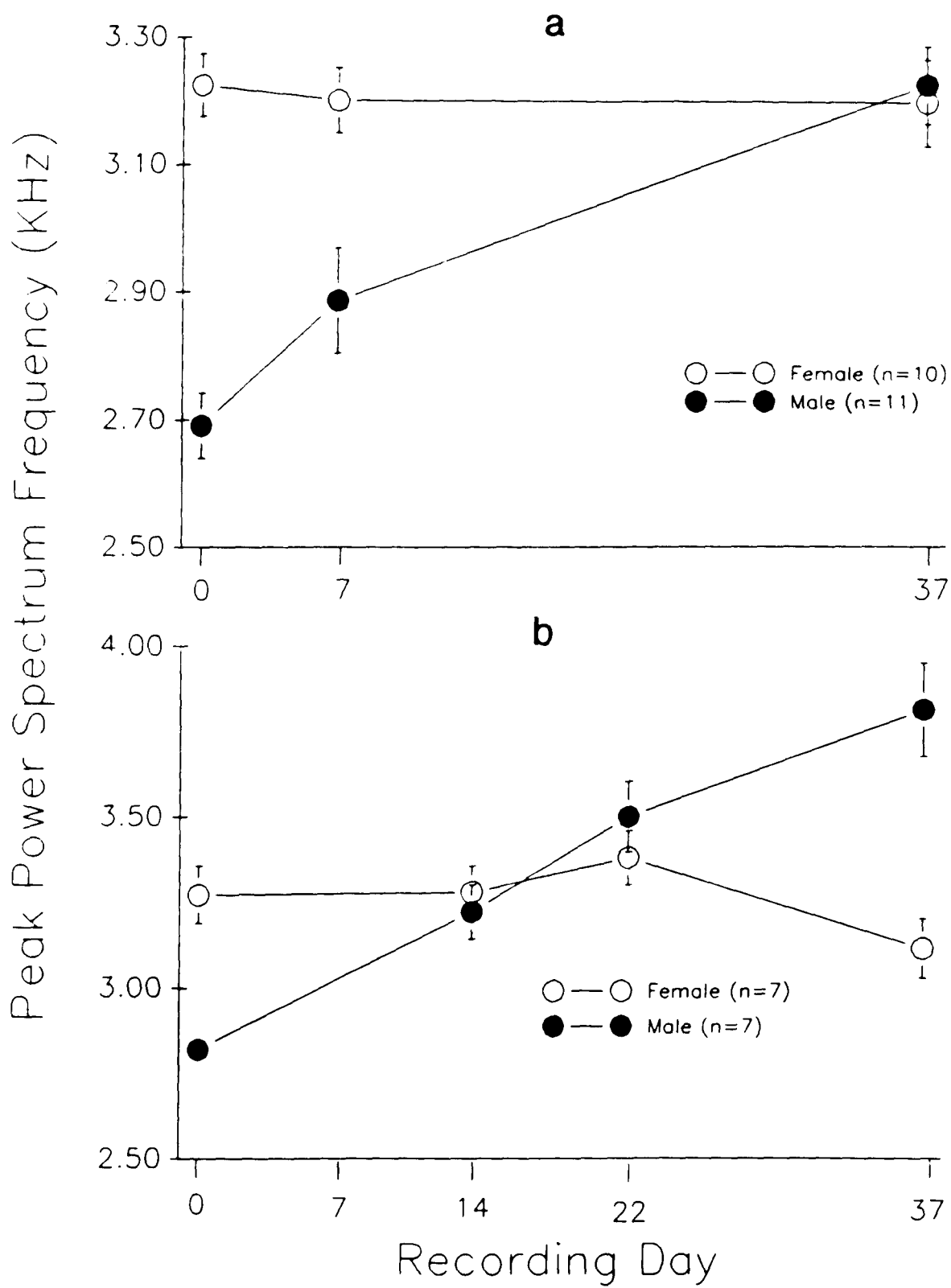


Figure 3. Mean phase 2 durations ($\pm$ SEMs) of the EOD for males and females on Day 0 in the laboratory, and after 7 and 14 days of initial maintenance in a group stock tank, and finally after a) 30 days in group aquaria or b) after 8 and 23 days in individual aquaria.

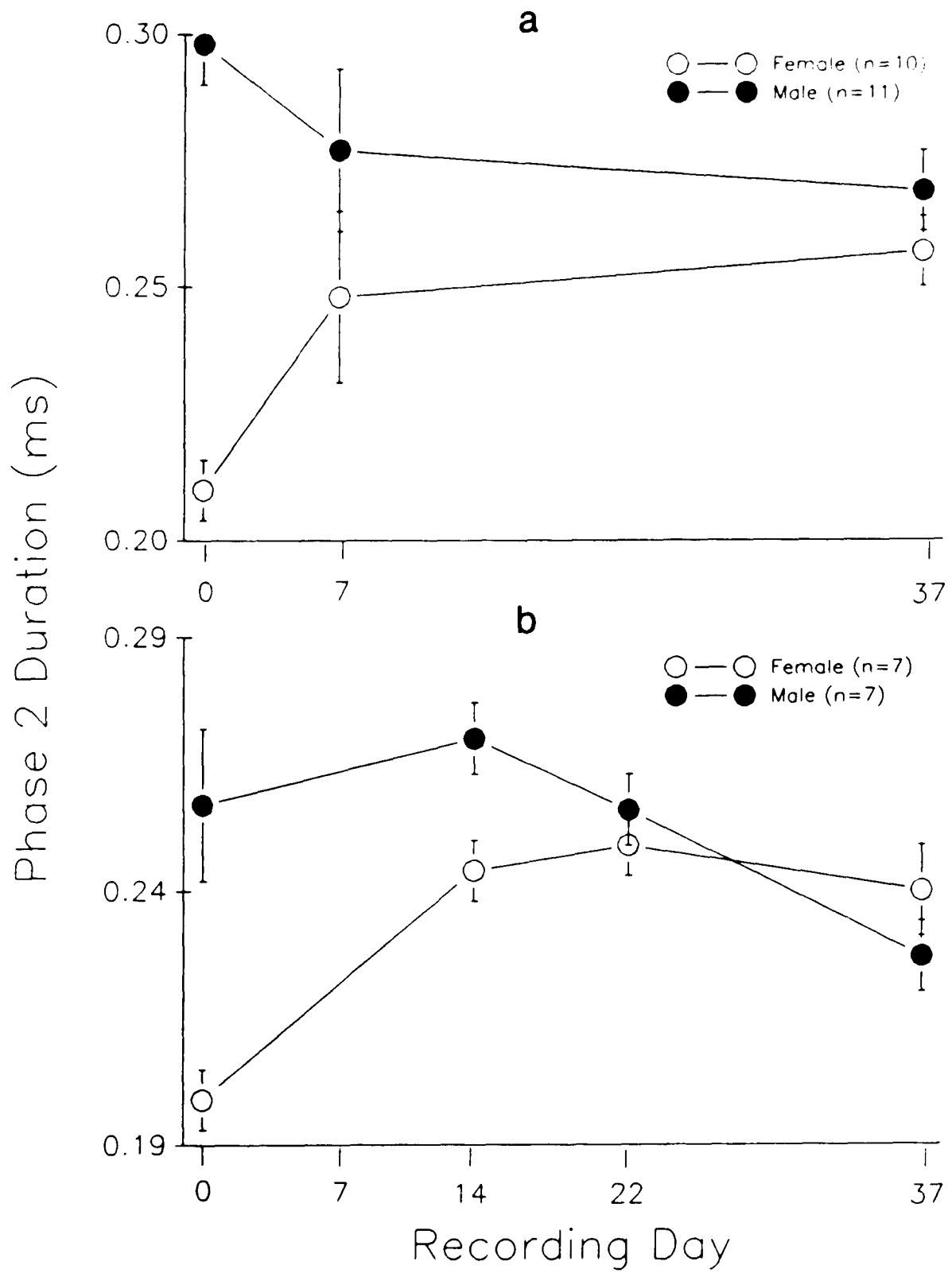


Figure 4. Mean phase 3 durations ($\pm$ SEMs) of the EOD for males and females on Day 0 in the laboratory, and after 7 and 14 days of initial maintenance in a group stock tank, and finally after a) 30 days in group aquaria or b) after 8 and 23 days in individual aquaria.

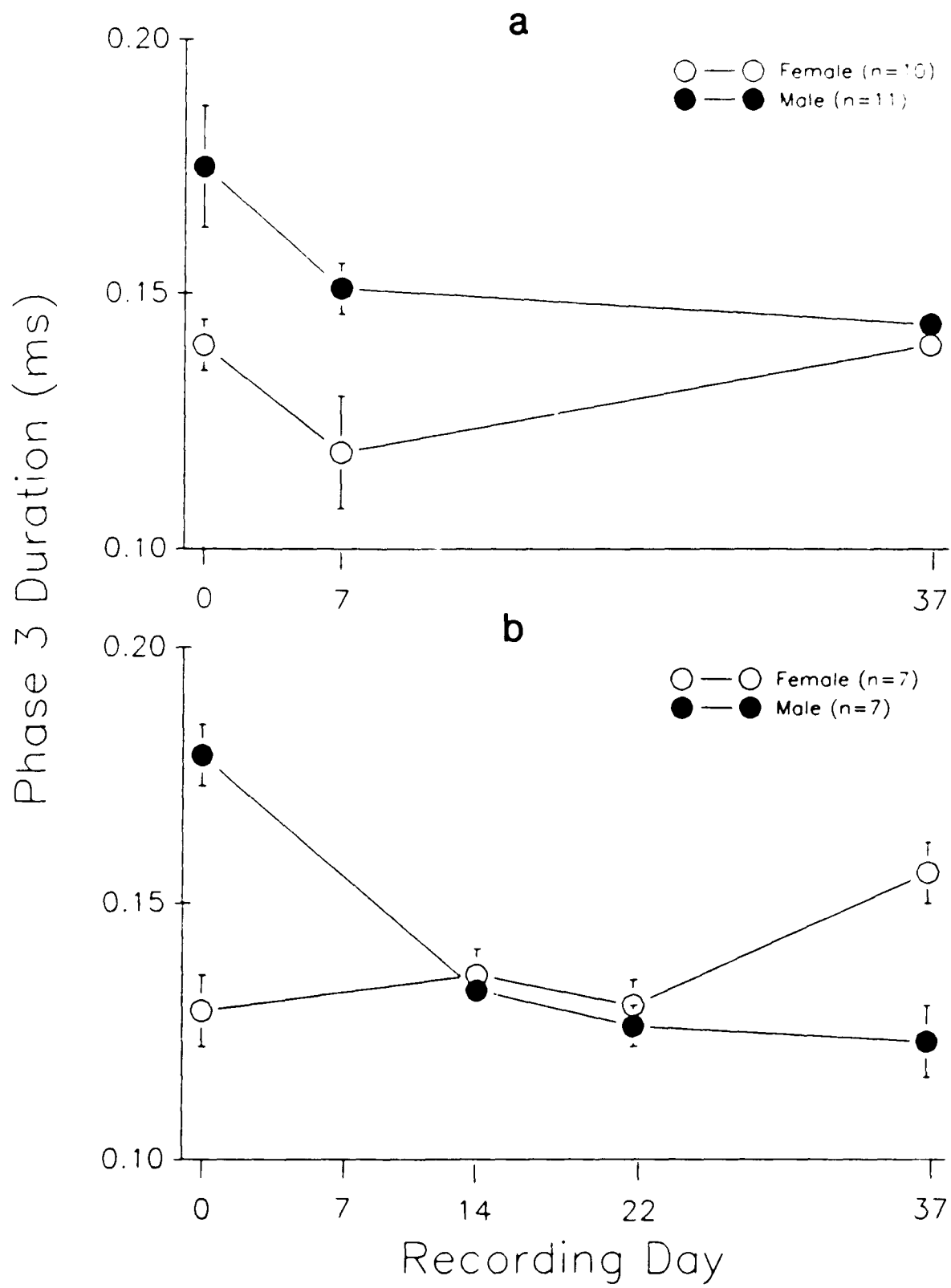


Figure 5. Mean ($\pm$ SEM) plasma levels of (a) T and (b) 11-keto T in adult male G. petersii on Day 0 and after 5, 10, and 15 days of captivity in individual aquaria, or after 5 and 15 days of captivity in group aquaria. Note dramatic decreases in both hormones after 5 days captivity regardless of whether fish were maintained individually or in groups. * $p < 0.001$ for T compared to Day 0 levels * $p < 0.005$ for 11-keto T compared to Day 0 levels

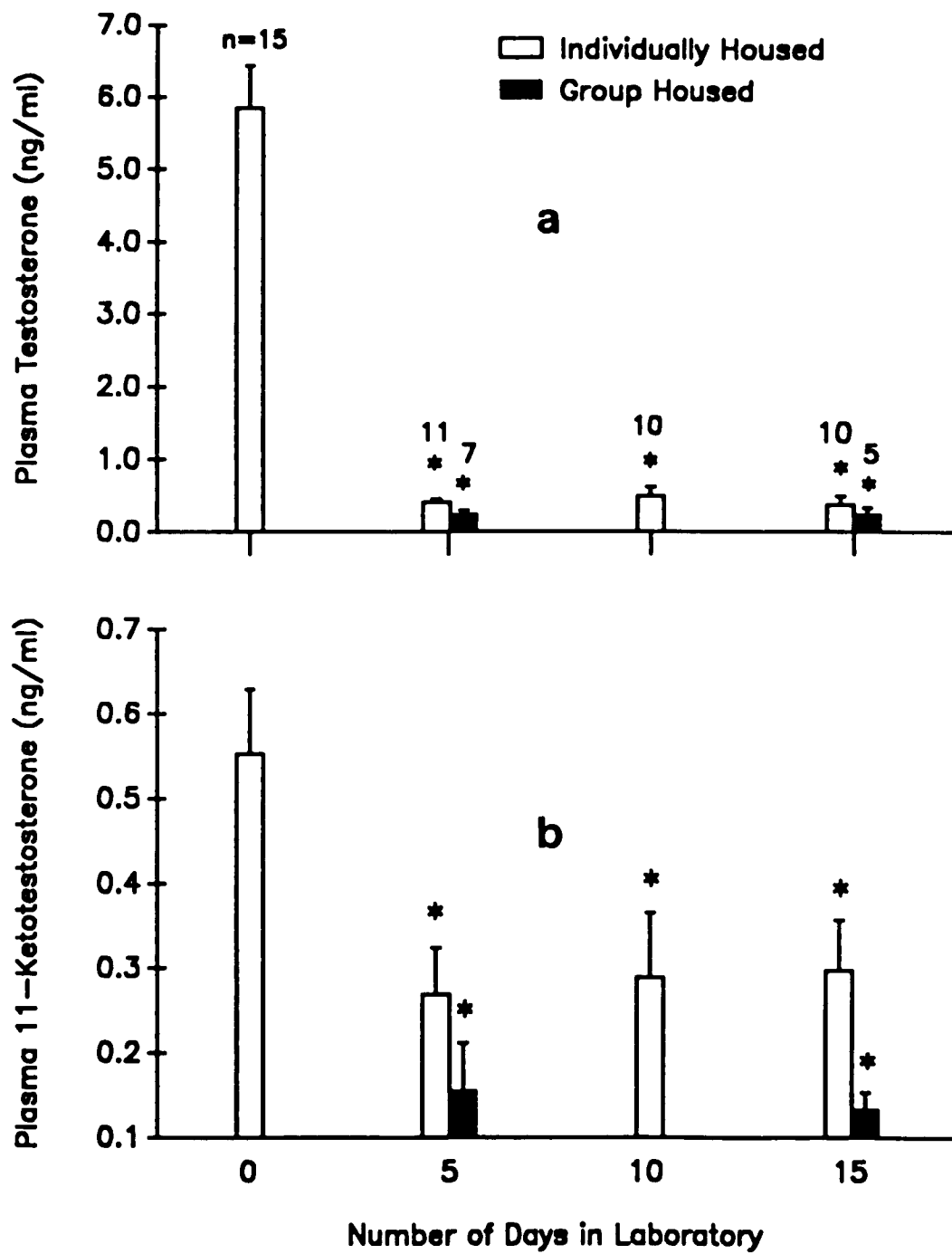


Figure 6. Figure 6 presents a scatterplot and regression line [$Y = 8.28 X + (-)0.58$] for plasma levels of T and 11-keto T for all subjects.

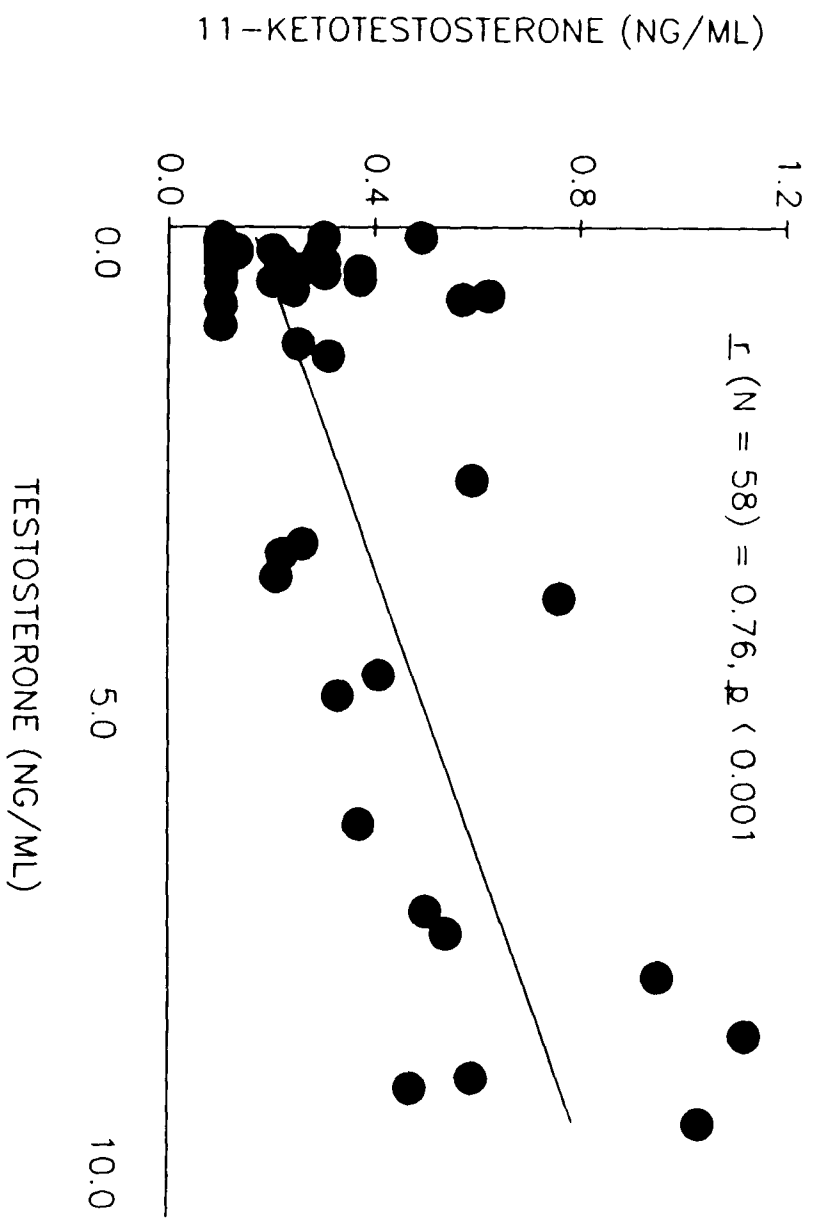
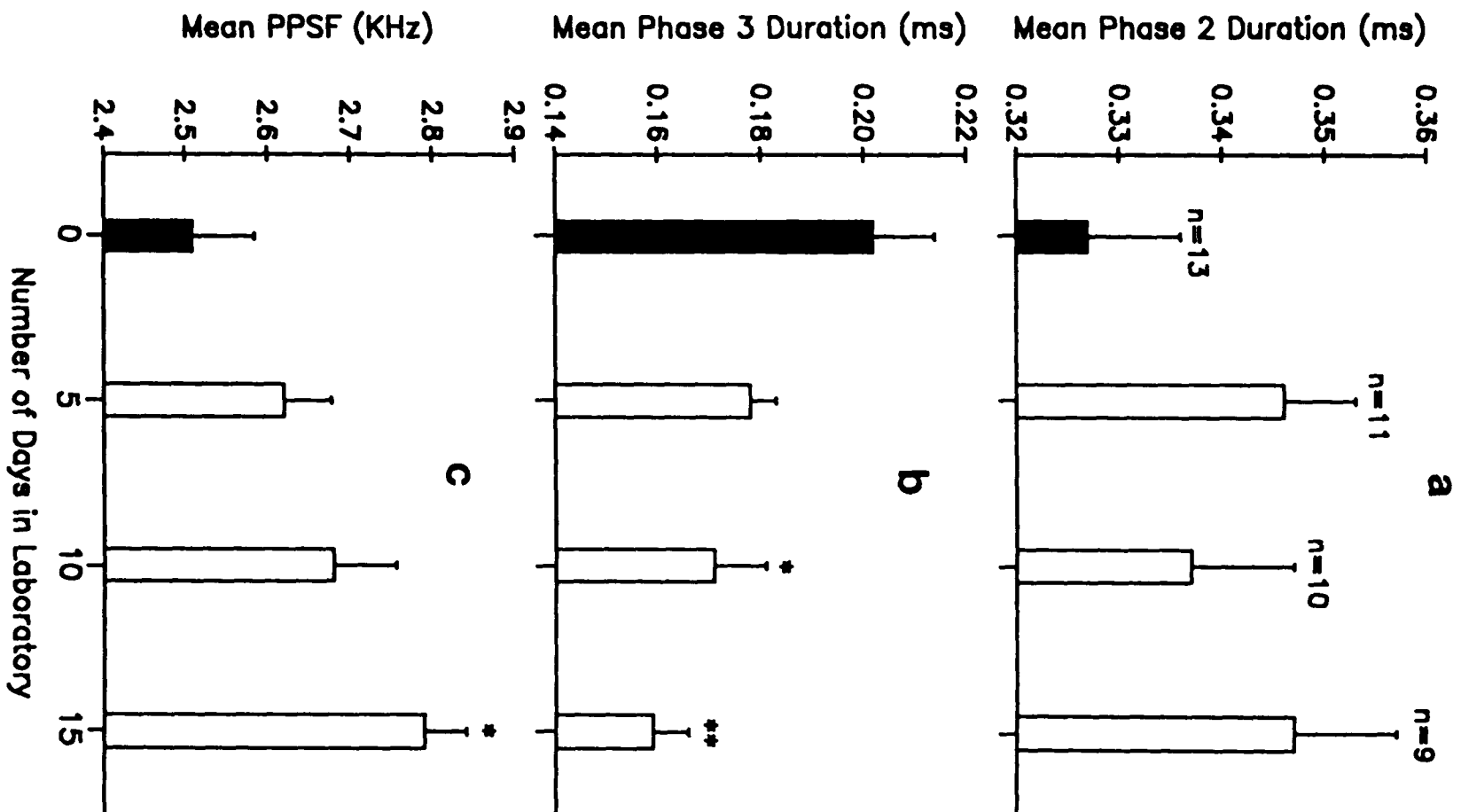


Figure 7. Mean ($\pm$ SEM) (a) phase 2 durations, (b) phase 3 durations, and (c) PPSFs for males on Day 0 and after housing in individual aquaria for 5, 10, or 15 days in captivity. * $p < 0.05$ ** $p < 0.01$



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PART VI: CAPTIVITY AFFECTS BEHAVIORAL PHYSIOLOGY:
PLASTICITY IN SIGNALLING SEXUAL IDENTITY

Abstract

Little is known about the effects of captivity on physiology and behavior in wild-caught terrestrial and avian species, and even less is known about these effects in fish. Studying the effects of captivity on reproduction is hampered because wild animals typically 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.

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 (1-3), the physiological causes underlying the behavioral differences found in field versus laboratory settings have only been surmised. The fact that wild animals typically do not show sexual behavior under captive conditions (1) 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 the signalling 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 (4, 5). 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 gymnotoid electric fish species (6, 7). 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 (8), indicating that these sexual characters are steroid sensitive.

In a previous laboratory study (5), 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 (6). The present studies were designed to investigate the endocrine basis for the EOD sex difference in G. petersii and to experimentally address captivity as a possible confound in laboratory research.

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. EODs of G. petersii have four phases (Fig 1a, left panel). Large sex

differences were obtained only in the durations of phases 2 and 3 (Fig. 2A), with males exhibiting longer phases than females [$F(1,57) = 12.24$, $p < 0.001$, phase 2 and $F(1,57) = 11.91$, $p < 0.001$, phase 3]. These sex differences in phase are reflected in the Fourier transformations (Fig. 1a, right panel) with males exhibiting significantly lower PPSFs than females [$F(1,57) = 16.86$, $p < 0.0001$] (Fig. 2A). These findings are opposite those reported in our earlier study (5).

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 (9). 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 phase 2 (both $p < 0.05$). 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 (Figs. 2A and 2B). Removal of the implants resulted in reversal of androgen-treated fish's EODs to pre-implant

phase durations and PPSFs. Finally, only T and DHT induced the adult male-like indentation in the dorsal margin of the anal fin (Fig. 1c).

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 same hormone effects were also obtained in gonadectomized adult males and females. This is the first report of a phase-specific, androgen-dependent sex difference in phase duration in EODs of weakly discharging electric fish.

Stress manipulations in vertebrates including fish result in alterations in plasma levels of adrenal and gonadal hormones (1, 2, 10, 11). 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 (5) were maintained in the laboratory in isosexual groups for approximately 3 weeks for adaptation purposes prior to data collection. This suggested that changes over time since the fish were caught, imported, and maintained under laboratory conditions could have altered the EOD sex differences. To investigate this possibility, 10 female and 11 male adult fish from the current sex difference study were randomly selected 7 days after the original recordings. EODs were collected and fish weighed, fin-clipped for later identification, and assigned to 50 gallon isosexual (n=7 for males and females) or heterosexual (n=4 males and 3 females) group tanks. Figure 3 shows the effects of this protocol on sex differences in EODs. At the start of this study, seven days after collection of the original sex difference data, the phase 2 sex difference was gone ($p = 0.07$), and within 30 days (Day

37) the phase 3 sex difference was lost. The mean male PPSF changed significantly, from (mean + SEM) 2.88 ± 0.08 KHz to 3.22 ± 0.06 KHz [t (df=10) = -3.70, $p < 0.004$], causing the sex difference to disappear. Clearly, captivity has dramatic effects on the phase-specific EOD sex differences in G. petersii, eliminating both the phase 2 and phase 3 sex differences as well as the PPSF sex difference.

Figure 4A shows plasma T and 11-keto T levels in newly imported adult male G. petersii at the beginning of the local rainy breeding season, and after 5, 10 and 15 days in the laboratory. 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. The effects were the same whether fish were maintained in group or individual aquaria. 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 [$F(3,39) = 3.63$, $p = 0.02$] over 15 days in the laboratory. The PPSF showed a corresponding increase [$F(3,39) = 2.88$, $p < 0.05$] 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 for the hormonally-dependent male EOD phases to decrease in duration (Fig. 3) and for the sex difference to disappear (13). However, females under laboratory conditions also appear to exhibit statistically non-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 (5).

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. These data imply that there are seasonal phase-specific duration sex differences in EODs of G. petersii.

These data can explain large discrepancies in the results of sex-difference studies in weakly discharging electric fish species. 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 (14) as well as for the non-reproducibility of sex differences in the laboratory (15), and identifies a possible confounding factor in all electro-communication studies performed under captive conditions in field and laboratory.

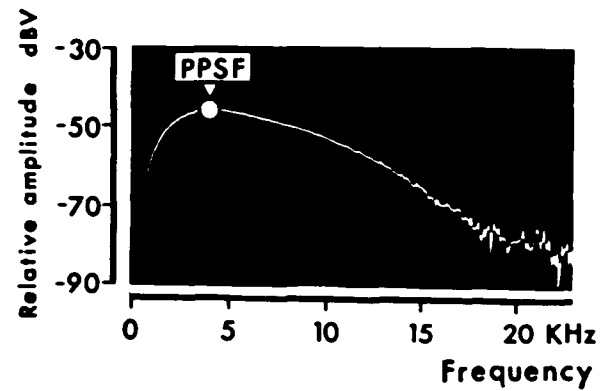
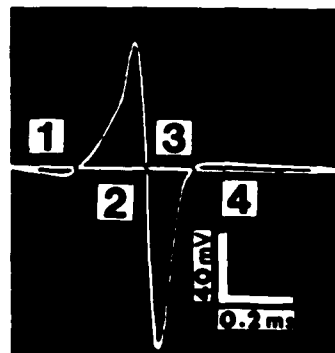
This is the first report to provide evidence that weakly discharging African mormyrid fish could use phase-duration cues of the EOD to communicate sexual identity (16). Phase-specific EOD plasticity suggests that individual EODs can communicate much about current motivational and endocrine status of the fish. Steroid hormones appear to exert their effects peripherally by altering

membrane properties in the electric organs of mormyrid and gymnotoid species (17). 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 system 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 (1). Even institutionalized humans exhibit behaviors that are similar in many respects to the aberrant behaviors exhibited in captive primates in zoos and laboratories (3), 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.

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. Arrows 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 (arrows) before (top) and 30 days following treatment with implanted T or DHT (bottom). Estradiol did not induce this indentation in juveniles or adult females.

a



b

Male

Female



c

TESTOSTERONE

DIHYDROTESTOSTERONE

PRE

PRE

POST

POST



Figure 2A. Phase-specific sex differences in EODs in G. petersii.

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'. ** $p < 0.001$ *** $p < 0.0001$

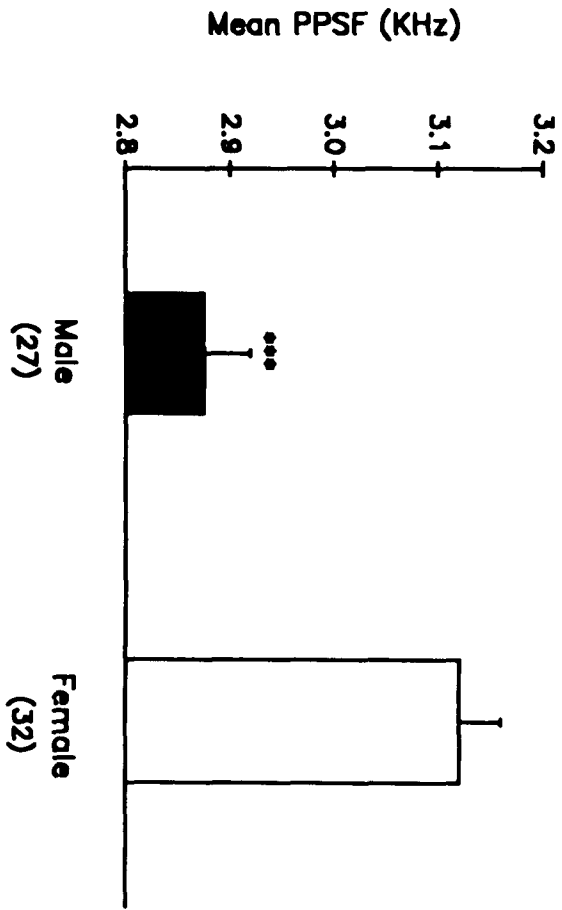
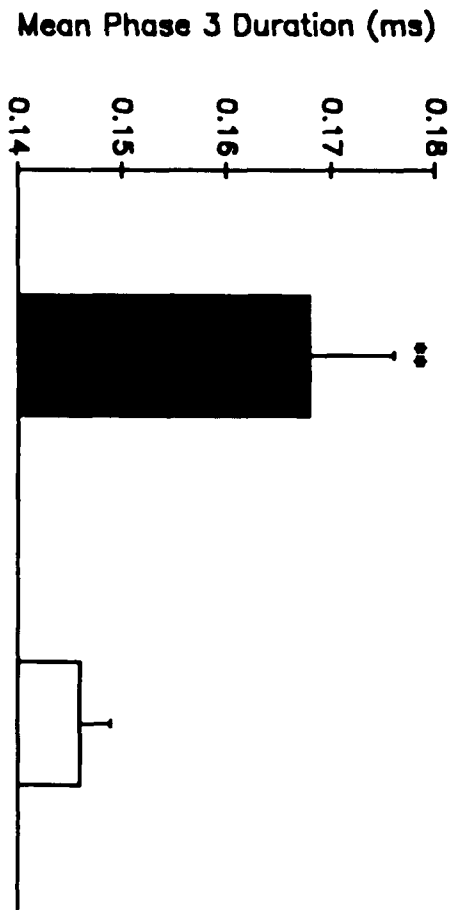
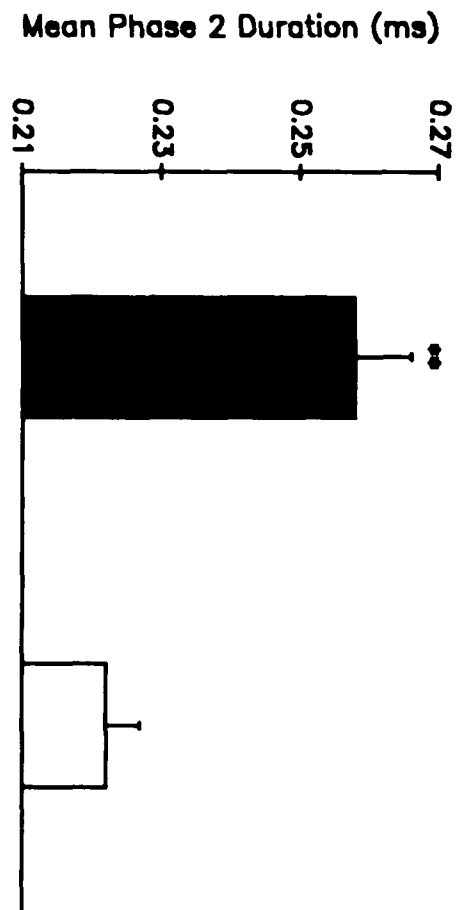
A

Figure 2B. 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 (9). (top) Phase 2. Only androgens increased the duration of this phase by the first 6 day post-implant block [F hormone $\times$ 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, $p < 0.00001$].

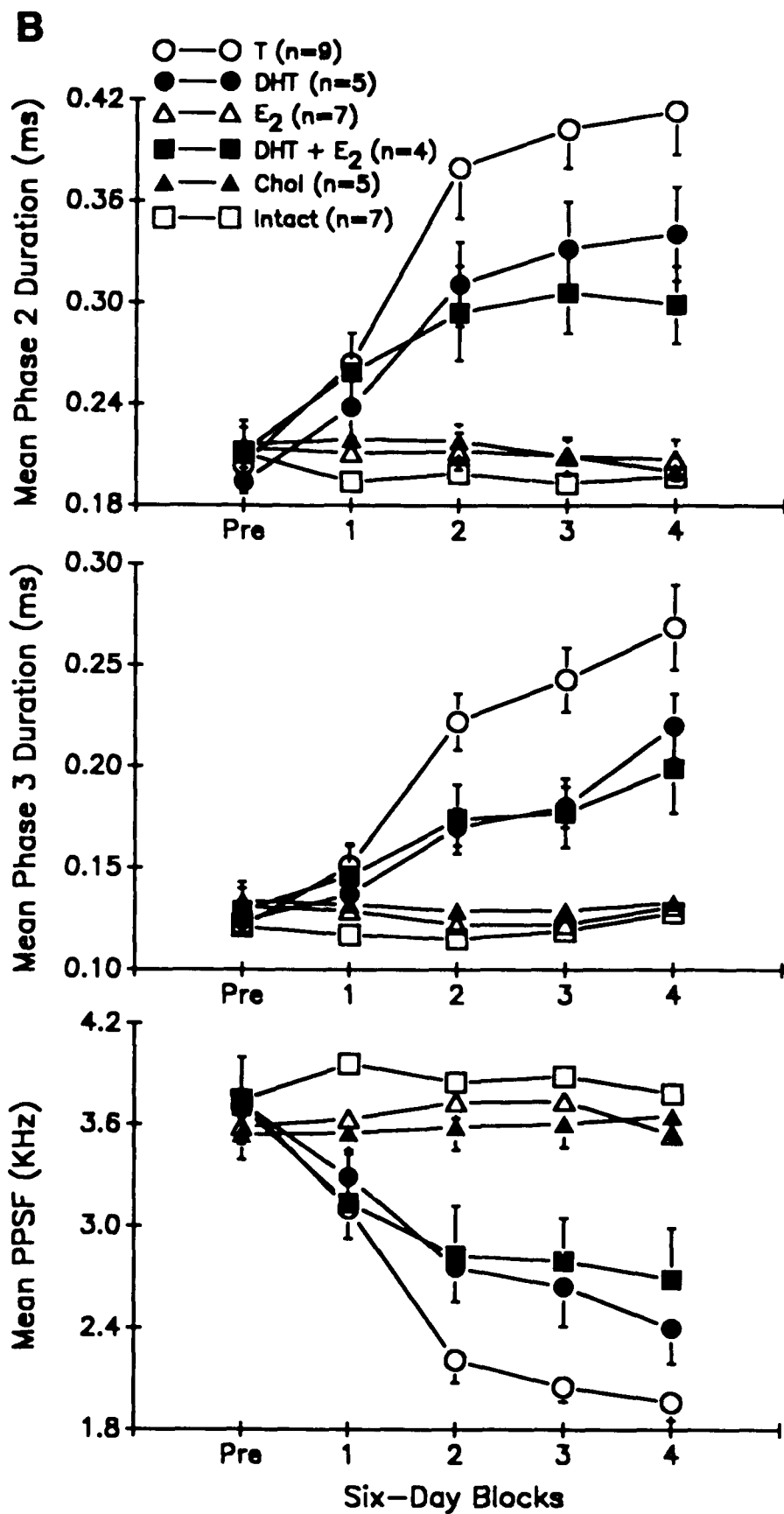


Figure 3. The effects of laboratory maintenance on sex differences in EODs in G. petersii. Fish were selected from a large group tank which housed only the fish used in the sex difference study and maintained in isosexual or heterosexual group tanks over a 30-day period, beginning 7 days after they were received in the laboratory. Mean male and female (A) phase 2 and (B) phase 3 durations, and (C) PPSFs. The lines connecting the Day 7 and Day 37 data points are included only to highlight EOD changes and may not accurately reflect the pattern of change.

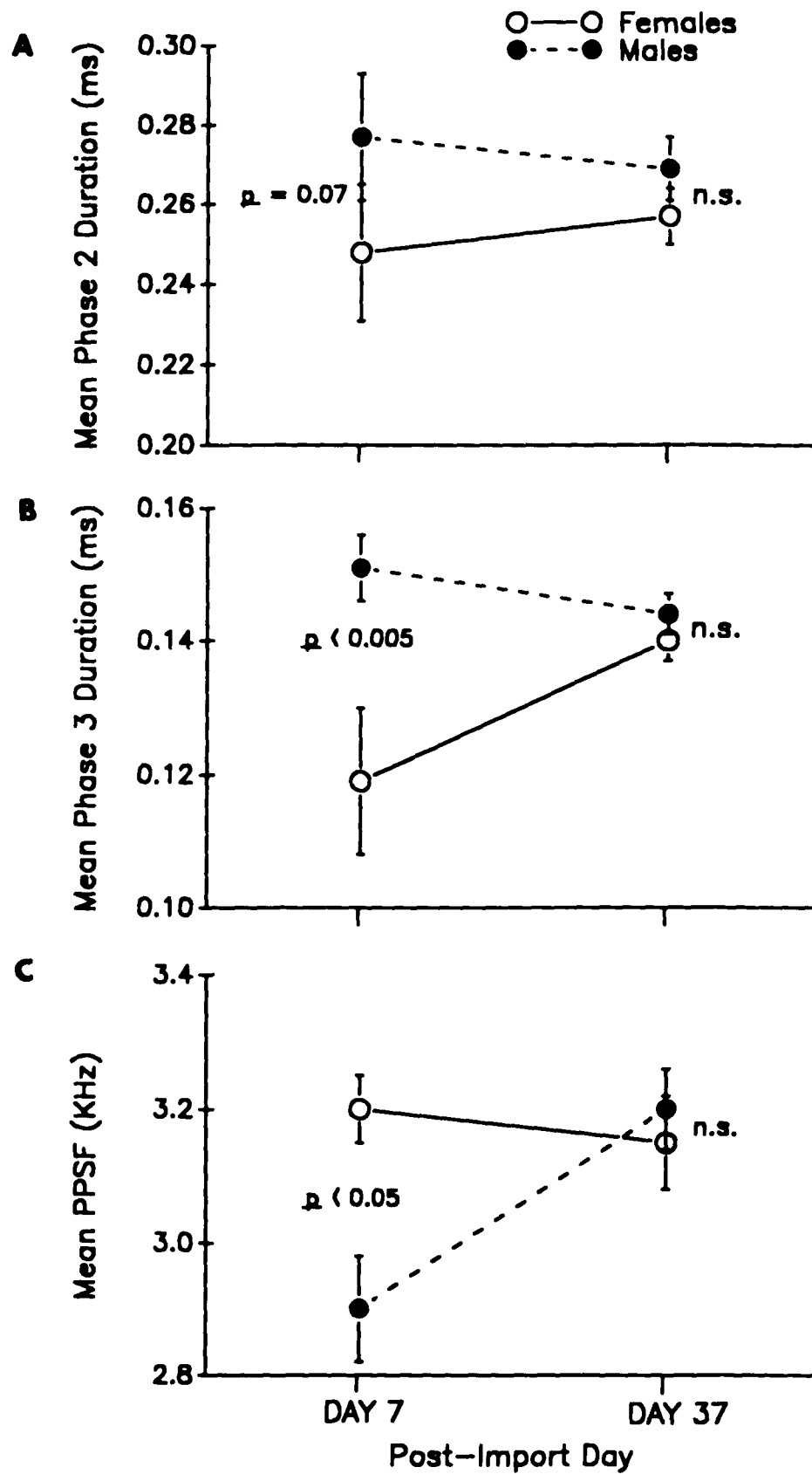


Figure 4A. Mean plasma T and 11-keto T levels in sexually mature adult male G. petersii obtained from Nigeria during the rainy breeding season (13). Five, 10, and 15 day plasma T (top) and 11-keto T (bottom) levels were lower than the 0-day levels ($*p < 0.001$ and $*p < 0.005$, respectively) regardless of whether maintained in groups with uncontrolled water conductivity or individually with conductivity maintained between 100 and 120 $\mu\text{S}/\text{cm}$. The effects of this decline in androgen on the EOD are shown in Fig. 4B.

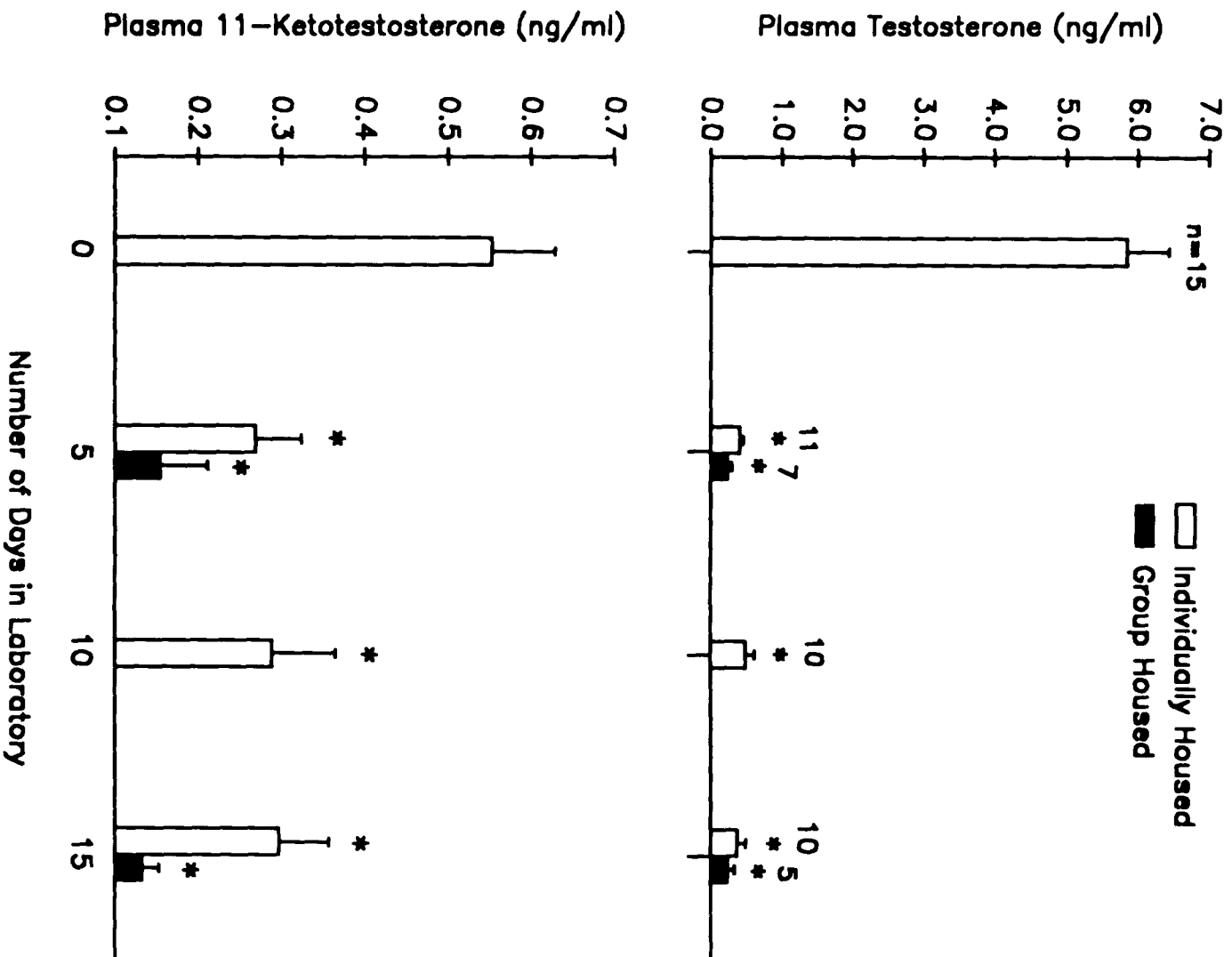
A

Figure 4B. EODs collected from the individually-housed fish. (top) Phase 2 was not significantly affected by the decline in androgen levels (shown in Fig. 4A) over the fifteen day study. This 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). Low levels of circulating T may be sufficient for maintaining the duration of phase 2. (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.

* $p < 0.05$ ** $p < 0.01$

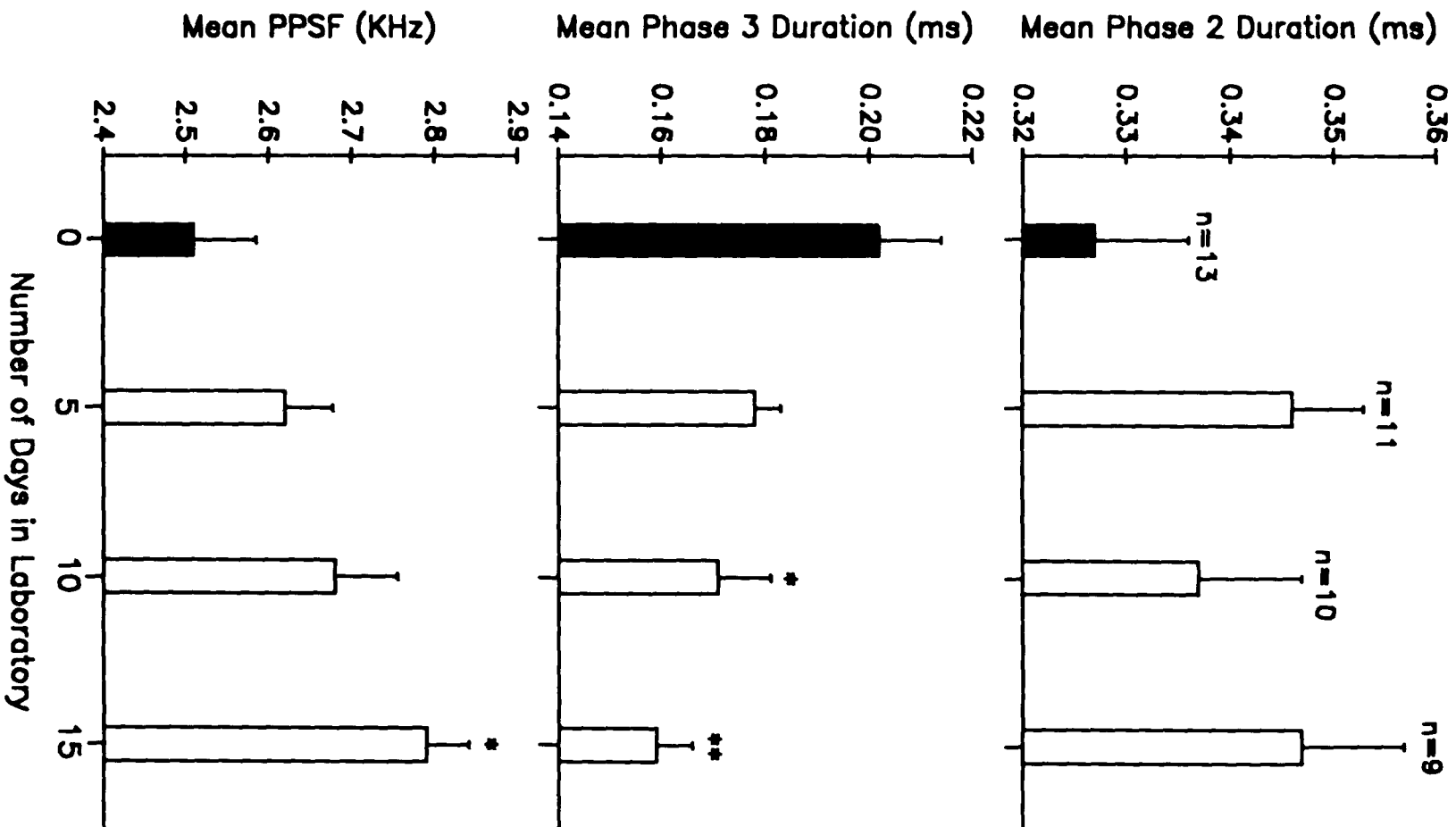
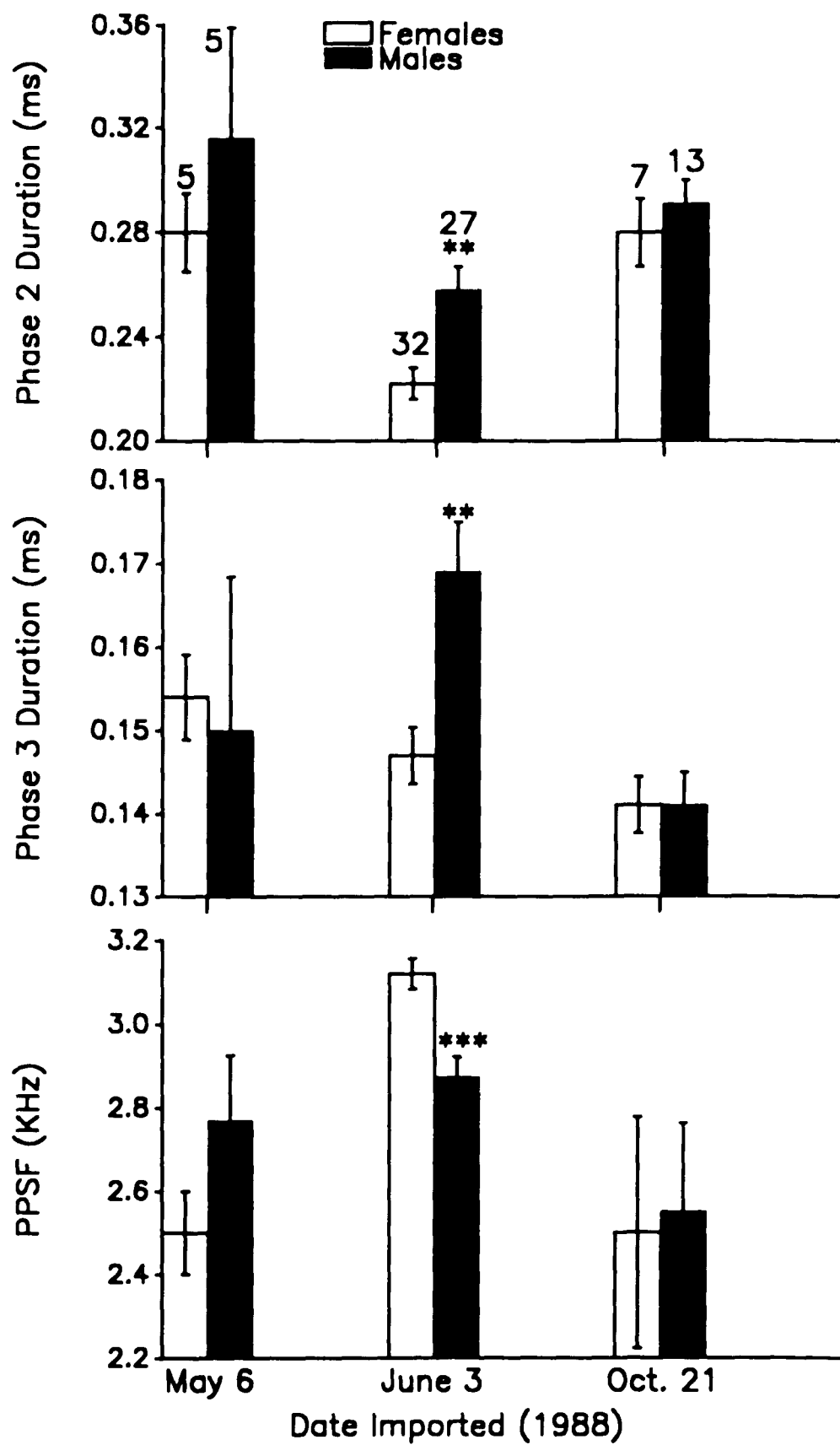
B

Figure 5. Seasonal sex differences in phase 2 (top), phase 3 (middle) and PPSF (bottom). In all cases, adult fish were brought directly to the laboratory and EOD data recorded on the day they were received from Nigeria by the importer. Numbers (top) indicate sample sizes.

****p** < 0.001 *****p** < 0.0001



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9. The care of animals was in accord with institutional guidelines. Following pre-implant data collection, fish were anesthetized (buffered tricaine methane sulfonate, 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 μm /lg b.wt. (0.4 μm /lg 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. Data were analyzed with two-way ANOVAs for mixed designs (hormone x 6-day blocks), followed by the Newman Keuls test for multiple comparisons. In other studies (data not shown), juveniles were implanted with lower hormone doses (1 μm /2.5g 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. 4), to 40.87 ng/ml, both well within the range reported for other fish species during reproductive season (R. Ozon in Steroids in Nonmammalian Vertebrates, D. R. Idler, Ed. (Academic Press, NY, 1972), pp. 328-389; A. Fostier, B. Jalabert, R. Billiard, B. Breton, Y. Zohar in Fish Physiology, W. S. Hoar, D. J. Randall, E. M. Donaldson, Eds. (Academic Press, NY, 1983), pp. 277-372. High-dose E₂ 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.

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12. EOD behavior was recorded first from all but two fish in the 'day 0' and one fish in 'day 15' groups. Fish were fed tubifex worms ad lib. 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. T and 11-keto T were analyzed in the same extracts. Radioimmunoassay (RIA) techniques (see H. Singh, et al., Gen. Comp. Endo. 72, 144, 1988) were validated in G. petersii using column chromatography, and 50- and 100- μ l aliquots or 10-, 25-, 50-, and 100- μ l aliquots from the reconstituted adult male captivity study and implanted fish samples, respectively, were measured in each RIA to test for parallelism. The T antiserum cross-reacted 11.0 % 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%.
13. Fish recorded on day of import are probably already hormonally-altered because of handling and transport stress (11).
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15. For example, Hopkins [C. D. Hopkins, Behavior 50, 270 (1974)] reported that mature female Eigenmannia virescens (gymnotoid) discharge at higher frequencies than mature males while Westby and Kirschbaum [G. W. M. Westby, F. Kirschbaum, in Adv. Physiol. Sci., vol. 31. Sensory Physiology of Aquatic Lower Vertebrates, T. Szabo and G. Czeh, Eds. (Pergamon Press, Budapest, 1981), pp. 179-194.] reported a sex difference in the opposite direction. A sex difference in the preceding negative wave of the EOD [G. W. M. Westby, F. Kirschbaum, J. Comp. Physiol. 122, 251 (1977)] in the mormyrid Pollimyrus isidori could not be replicated [G. W. M. Westby, F. Kirschbaum, J. Comp. Physiol. 145, 399 (1982)], and while Lucker and Kramer [H. Lucker, B. Kramer, Behav. Ecol. Sociobiol. 9, 103 (1981)] failed to find any EOD waveform sex difference in this species, Westby and Kirschbaum [J. Comp. Physiol. 145, 399 (1982)] reported a "clear and unambiguous sexual dimorphism within the major phases of the EOD itself" (p. 400).

16. We have just found in another mormyrid species, Brienomyrus brachyistius, that T and 11-keto T elongate only phases 2 and 3, and have no effect on the duration of phases 1 and 4. A hormone-dependent sex difference was also reported in EOD symmetry of the two phases of the diphasic EOD in a gymnotid (7).

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18. I thank D. Bowling, G. Greiss, T. B. Perera and C. Williams for technical assistance; P. Thomas, M. B. Hawkins, and S. Safford for radioimmunoassay training and assistance; C. F. Harding, P. Moller, R. G. Schaubhut and P. Zeigler for comments and suggestions on the manuscript. Supported by NIMH grant MH09664, NIH Training Grant MH15341, and two Grants-in-Aid of Research from Sigma XI.

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