Chapter thirteen

Sources of plasticity in behavior and its physiology: sex, hormones, environment and the captivity model

Robert E. Landsman

13.1 COMMENTS ON BEHAVIORAL PLASTICITY

Behavioral plasticity (or variability) is the rule, not the exception, and in many instances, environmental perturbations are a major cause of variability observed in behavior. To the extent that changes or differences in environmental conditions persist, differences in response between and/or within members of a single species will persist in both field and laboratory. Consequently, the scientist who employs behavior as an end point in his/ her research must carefully assess alternative hypotheses to explain variability in results, and sometimes the elimination of outliers may be the elimination of the most valuable findings. Serendipity does not just occur, it is ferreted out by the reflective investigator.

The electric organ discharge (EOD) is highly variable, its waveform and frequency (or rate) being at the mercy of a number of parameters. Certain characteristics of the EOD are sex and hormone-dependent and are affected by maturational, developmental (Chapter 12) and a host of incidental, environmental factors (e.g. seasons, water quality, captivity). Variability in the EOD even results from distortion caused by objects close to the fish's body surface, providing the cues for active electrolocation (Chapters 5, 17).

The variability observed in the EOD emitted by both the African mormyrids and the South American gymnotiforms is an excellent indicator of fluctuations in these fish's aquatic habitat and should be considered as a prime example of plasticity and tire expression of individual variation in a behavior.

This chapter will focus on the topic of variability in the EOD of weakly electric fish resulting from factors such as sex, endocrine status, and environmental perturbations. I will present a rather critical view of the current state of this field by examining findings, dilemmas, contradictions, and possible resolutions presented by published data.

Throughout this chapter, I will use the following abbreviations denoting various hormones: T (testosterone), DHT (dihydrotestosterone), 11-KT (11-ketotestosterone), 17MT (17α-methyltestosterone, E_2 (estradiol), and the steroid hormone precursor CHOL (cholesterol). Measures of the individual EOD will be referred to as the duration and/or amplitude of the individual phases of the EOD (P1, P2, P3 and *P4),* and the peak power spectrum frequency (PPSF) of the fast Fourier transform associated with the EOD. As a rule, the shorter the individual EOD, the higher the PPSF; and conversely, the longer the EOD, the lower the PPSF. However, an increase or decrease in PPSF may result from changes in the durations of only specific phases of the EOD: statistically, the PPSF is also more related to the duration of some phases than to others (Landsman, 1993a,b). Measures of the rate or pattern of EODs will be referred to as SPIs (Chapter 8).

13.2 SEX-RELATED AND HORMONALLY INDUCED EOD PLASTICITY

Sex differences in EODs have been reported for both South American gymnotiform and African mormyrid species. Because the EOD is sensitive to gonadal hormones, sex-typical EODs can be reversed to resemble those of the opposite sex by gonadal manipulation and steroid hormone administration, and sex differences are highly correlated with season, gonadal maturity and reproductive state. Generally, the studies that suggest EOD sex differences were performed in the field, employed small samples, and are comprised largely of descriptive. non-statistical accounts of natural sex differences in EOD waveform, duration, arid/or SPIs for several gymnotiform and mormyrid species. These field-reported sex differences have rarely been reported in laboratory studies, and if so, only anecdotally or in one or two species which were bred in the laboratory. But both laboratory studies (reviews: Meyer, 1983; Bass and Hopkins, 1985; Meyer *et al.,* 1987; Mills and Zakon, 1987 Landsman *et al.,* 1990:) and field studies (Bass and Hopkins, 1983, 1985: Hagedorn and Carr, 1985) have employed hormone

manipulations to induce male- or female-like EODs. The majority of the field studies reporting sex differences show considerable variability and overlap between the sexes. The majority of studies involving hormone manipulation lack important control groups (i.e. CHOL.-treated fish to control for the effects of non-gonadal steroid hormones, fish administered blank implants to control for the effects of steroid hormones and CHOL implants and nonhandled fish to control for the effects of' all handling including surgical manipulations), and/or many include control groups of small sample size (e.g. n = 3) composed of both sexes and/or mixtures of juveniles and adults of both sexes. In many cases, studies used only methylated androgen, which does not naturally occur in fish, or DHT, which does not appear to be a major androgen in fish (although in the guppy, *Poecilia reticulata*, the ability of follicles to synthesize 5 α-DHT in vitro from precursors has been demonstrated by Venkatesh et al., 1991).

Endocrine studies on weakly electric fish commonly employ the methyl-lated androgen, 17-MT (Bass and Hopkins, 1983, 1984; Bass, 1986a; Landsman and Moller, 1988) which is more potent than T. Although MT 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 with the effects of T. Landsman and Moller (1988) implanted MT into juvenile and adult *Gnathonemus petersii* and found up to a fivefold increase in total EOD duration accompanied by large decreases in PPSF from 4100 to 400 Hz! In contrast, Landsman et al. (1990) employed T implants resulting in plasma levels of T in the range found in breeding males, and resulting in total EOD duration increases of up to 33%) with smaller decreases in PPSF to above I kHz (see also Fig. 13.11). Problems of hormone dose are compounded when the effects of MT are compared with the effects of non-methylated DHT or E_2 (e.g. Bass and Hopkins, 1983, 1984; Bass, 1986a). Thus, one must be careful in the interpretation of results obtained with MT unless substantiated with T or 11- KT, the two predominant androgens found in fish. DHT was found to be less potent than T in producing behavioral effects on the EOD, and did not have any effects on PI and P4 in juvenile *Gnathonemus petersii* (Landsman *et al.*, 1990). 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 (discussion: Harding, 1986). Further, many studies on electric fish employed different procedures for the administration of hormones. including injections, pellet implants and time-released silastic implants. Many of the above factors have added to the variability in findings on sex differences in and hormonal control of EOD behavior, and have made it particularly difficult to make generalizations regarding the sensitivity of the EOD to steroid hormones as well as to make comparisons of steroid effects across and within studies and species.

The remainder of section 13.2 will focus on those species in which there appears to be sufficient complementary data to warrant the claim for the existence of sex-related EOD differences and their sensitivity to steroid hormones. (When data based on small samples are cited, the number of subjects is provided.) However, except for one mormyrid species, *Gnathonemus petersii,* the differences between the sexes are overlapping and in many cases ambiguous: thus, the term 'sexual dimorphism' will not be used to describe these sex-related EOD characteristics.

Gymnotiformes

Some gymnotiforms exhibit a sex difference in their EOD waveform and/or frequency (Chapters 8, 12, 18). Mature female *Sternopygus macrurus* discharge at higher frequencies than mature males, while juveniles discharge at frequencies intermediate between the two (Hopkins, 1972, 1974a; Zakon *et al.,* 1991b; Mills *et al.,* 1992). When intact S. *macrurus* were implanted with silastic capsules containing DHT, the EOD rate decreased and EOD duration increased significantly compared with both pre-implant values and EODs of controls implanted with blanks (Mills and Zakon, 1987: *Mills et al.,* 1992). Two of the authors' controls, however, also appeared to show consistent changes in EOD frequency and duration over the experimental period, but in directions opposite to each other (shown in Mills and Zakon, 1987, figs. 8(b) and 10(b), respectively). Whether these data reflect effects of the implants themselves is difficult to interpret as this study did not include a non-implanted control group. Removal of the DHT implants from three subjects for *63* days resulted in EOD rates and durations comparable to pre-implant values, while three fish with sham removal of' DHT implants retained their lowest EOD rates and longest durations, suggesting that the steroid effects on the EOD are not permanent in this species.

A field study, in which EOD data were recorded and blood samples obtained from the same fish, indicated sex-specific relationships between EOD frequency and endogenous steroid levels in *S. macrurus* which suggested that androgens, but not E_2, modulate EOD frequency in this species (Zakon *et al.,* 1991b). Males exhibited lower EOD rates than females, and plasma levels of T and 11-KT, but not E_2, were inversely related to EOD rate in males, while plasma levels of T and E_2 in females were not related to FOD rate (Zakon *et al.,* 1991 b). Interestingly, the EOD sex difference in this species wits maintained across seasons over which T levels varied in both males and females, even though males with low levels of androgens had a wide range of EOD frequencies (Zakon *et al.,* 1991b). This suggests that (1) the male EOD may be influenced by factors other than androgen when T and 11-KT levels are low (Zakon *et al.,* 1991 b), and (2) the sex difference in

EOD characteristics in this species is maintained by unknown factors in addition to androgen.

Female *Sternopygus dariensis* also emit higher EOD rates than males (Meyer, 1983; Chapter 12). Meyer (1983) injected males and females with T, DHT, or E_2 in various doses ranging from 2.5 to 20.0 µg/g body weight. Following androgen injections, both sexes lowered their EOD rates. Fish with higher pre-treatment frequencies showed larger responses to the hormone treatment than fish discharging at lower frequencies. Unlike S. *macrurus,* fish treated with E2 showed frequency effects in the opposite direction to those *treated with* androgen. Further, castrated males showed an 11% increase and ovariectormized females an 18%) decrease in EOD rate. Hormone replacement reversed the effects of the surgery, while administering heterologous hormones to either sex increased the effects of gonadectomy. Thus, hormonal effects on the EOD rate of *S. dariensis* are not permanent, but rather are activational in nature. This means that the electric organ in this species may be bipotential in its ability to emit male- or female-like EODs, and it is likely that the presence or absence of gonadal hormones in adulthood determines the sexual characteristics of the EOD.

Eigenniannia virescens (E. lineata: Chapter 18) exhibits a sex difference in EOD rate in the same direction as *Sternopygus* (mature females possess a higher EOD rate than males) but with much overlap between the sexes (Hopkins, 1974b). Westby and Kirschbaum (1981), however, reported that sexually mature, but unripe male *Eigenmannia* produced a significantly higher FOD rate than females, but with considerable overlap, while immatures fell within a wide range of intermediate frequencies. As the fish became ripe, females shifted their frequency in the upward direction and in many cases surpassed the males' rates (Westby and Kirschbaum, 1981), suggesting hormonal involvement in the EOD rate of this species. These incongruent findings suggest at least three possible explanations: (1) some of Hopkins' (1974b) fish were gonadally ripe and producing hormones that influenced EOD rate, (2) the EOD rate is sensitive to seasonal changes in reproductive state (page 323), and/or (3) the EIOD rate is affected by captivity (page 336). Sex differences have also been reported in the waveform and harmonic content of *Eigenmannia* EOD activity, with males having a lower ratio of head-positive to head-negative EOD phase durations and a higher content of higher harmonics (Fig. 13.1; Westby and Kirschbaum, 1981; Kramer, 1985; Kramer and Otto, 1988). The sensitivity of the EOD to steroid hormones has not been adequately studied in this species to make any conclusions regarding the hormone dependence of the sex difference(s), although androgen injections purportedly decreased the EOD rate (unpubl. data, cited in Meyer *et al.,* 1987).

Compared with females, mature male *Brachyhypopomus occidentalis* (formerly *Hypopomus)* (a pulse-type gymnotiform) have broader tails

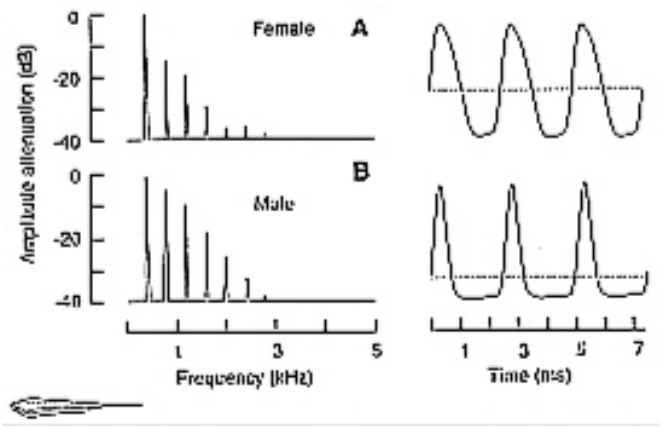


Fig. 13.1 Fourier amplitude spectra (left) and EODs (right) of female (A) and male (B) Eigenmannia lineata. Notice the lower ratio of positive to negative EOD phase durations (the identical EOD frequencies in both sexes seem to be coincidental), and the higher content of higher harmonics in the male's EOD compared with the female's signal. Zero potential level is indicated by dotted horizontal line. Modified after Kramer and Otto (1988).

containing electric organs with larger electrocytes that produce EODs with smaller PI/112 duration ratios and lower PPSFs (Hagedorn and Carr, 1985). If the size of the electrocytes accounts for both tail size and EOD sex differences, then it is not surprising that male PPSFs were significantly negatively related and female PPSFs positively related to tail width (Hagedorn and Carr, 1985) (see 'Notes on membrane effects', page 323). When females were injected with 5 pg/g of either DHT or E_2, DHT-treated fish developed larger, male-like electrocytes along with male-like EODs that were characterized by a significant increase in the duration of P2 and a 71% decrease in PPSF, while E_2-treated fish showed no change in electrocytes or EOD (Hagedorn and Carr, 1985).

Mature female Apteronotus (a gymnotiform with a neurogenic electric organ; Chapters 8, 18) spawned and raised in the laboratory exhibit lower EOD rates than males, i.e. a sex difference opposite in direction to that shown in other gymnotiform species, although considerable overlap between the sexes has been reported (Kirschbaum, 1983; Hagedorn and Heiligenberg, 1985; Meyer *et al.*, 1987). Silastic implants containing estrogen (E_2), but not those containing androgen (DHT), decreased the EOD rate as compared with blank implant controls (Meyer *et al.*, 1987). Since non-handled and CHOL-implanted controls were not included, and since all implants contained less than 0.5 mg of steroid and the actual dose

administered to each subject is unclear, a more detailed study is needed to make conclusive statements about the hormonal dependence of the EOD in this species. Interestingly, Meyer (1984) injected E_2, T, α- or β-DHT, or saline and reported *temporary* androgen-induced *in vivo* EOD frequency decreases, and *in vitro* decreases in pacemaker activity, and no E_2 effects. These findings appear to demonstrate that short-term EOD hormone sensitivity in this species is due to direct action of the hormones on the pacemaker. However, saline injection caused both significant short-term decreases and increases, and longer-term decreases in EOD frequency, although these decreases were significantly smaller than those caused by androgens. Because injections of saline also influenced frequency, an accurate interpretation of the steroid data would necessitate a non-handled control group, carried through the course of the study, and/or baseline data collected on the same subjects prior to beginning of the injection regime for statistical comparison. Also, because EOD data were only collected over the 7 day injection period, it is difficult to draw conclusions about long-term post-injection hormone effects.

Mormyridae

In their natural habitats, but also on a few occasions in laboratory-bred specimens, several mormyrid species appear to exhibit sex differences that are reflected in temporal (duration) and/or spectral features (PPSF) of the individual EOD, and sometimes expressed in the sequence of pulse intervals (SPIs) (Chapters 8 and 12; review: Zakon, 1993). EODs are steroid sensitive, and so these sex differences can be altered through administration of steroid hormones in all species investigated (reviews: Bass, 1986a; Landsman *et al.*, 1990; Landsman, 1993b: Zakon, 1993: Landsman and Moller, in prep). In mormyrids, EOD-related sex differences found in the field are elusive under laboratory conditions. Field studies have indicated that *Brienomyrus brachyistius* (long biphasic) (Bass and Hopkins, 1983), *Brienomyrus brachyistius* (triphasic) (Bass and Hopkins, 1983, 1985), and possibly *Stomatorhinus corneti* (Hopkins, 1980; Bass and Hopkins, 1985), *S. walkeri* (Moller, 1980: Fig. 8.9 (13)) and *Hippopotamyrus batesii* (triphasic) (one male and two females: Bass and Hopkins, 1985) may all exhibit sex differences in EOD waveform and/or duration. Males typically emit EODs that are two to three times longer (and thus exhibit lower PPSFs) than those of females (Moller, 1980; Hopkins, 1980, 1981a; Hopkins and Bass, 1981; Bass and Hopkins, 1983, 1985).

The species identification of *Brienomyrus is* not clear. Following the convention established in Chapter 8 (Fig. 8.11), fish studied on location or imported from Gabon will be identified as *B. brachyistius* 'biphasic', 'long biphasic', or 'triphasic' (Hopkins, 1980). Fish originating from collection

sites in Nigeria will be referred to as *Brienomyrus brachyistius* (Landsman and Moller, in prep). Other authors have referred to these or similar Nigerian imports as *Brienomyrus sp.* or *Brienomyrus sp.* 2 (Bass and Hopkins, 1984, 1985; Bass, 1986b; Bass and Volman, 1987). (For group comparisons of EODs across *Brienomyrus* groups and the effects of hormone treatment, Landsman and Moller, in prep.)

Although EOD sex differences have yet to be fully substantiated, the following species all have steroid-sensitive EODs: *Brienomyrus sp.* and *Brienomyrus sp.* 2 (Bass and Hopkins, 1984, 1985; Bass, 1986b; Bass and Volman, 1987), *Campylomormyrus tamandua* and *Hyperopisus bebe* (n = 1 fish of each species; Bass, 1986b), and *Stomatorhinus corneti* and *Hippopotamyrus batesii* (one fish treated with MT and one with T propionate, respectively) (Bass and Hopkins, 1985).

The following subsections contain a more detailed discussion regarding EOD-related sex differences and steroid effects in these species as well as in *Gnathonemus petersii* with an EOD-related sexual dimorphism demonstrated in the laboratory.

Brienomyrus brachyistius *(long biphasic) (Gabon)*

Male *B. brachyistius* (long biphasic) exhibit EODs of longer duration with lower PPSFs and different waveforms than females (Bass and Hopkins, 1983). 17-MT added to the water induced male-like EODs in intact (adult females, and in one juvenile male and female) or gonadectomized fish (one juvenile male and female, and one adult female) by increasing the EOD duration twofold with decreases in PPSFs (Fig. 13.2).

Androgen-induced effects on the EOD in this species appear to be temporary as the EODs of the intact androgen-treated fish reverted to the female type EOD over 24 days after treatment was terminated by placing the fish in fresh water (Bass and Hopkins, 1983). Intact (two juvenile males and one adult female) and one gonadectomized fish implanted with DHT pellets also exhibited male-like EODs, while E_2 had slight effects on the EOD of immature fish (males and one female) (Bass and Hopkins, 1983). Surprisingly, E_2 treatment also resulted in a downshift in PPSF and an increase in EOD duration; however, these changes were not as dramatic as those resulting from androgen treatment (Bass and Hopkins, 1983). Thus, it is possible that E_2 exerts only a partial masculinizing effect on the EOD because estrogen receptors might not yet be developed or functional in juvenile fish.

These findings implicated androgen as a mediator of maleness in the mormyrid EOD, and suggest that E_2 does not have a complete activational, masculinizing influence on the female EOD. Because DHT cannot be converted to E_2 by way of aromatase activity, and because E_2 has some masculinizing effects, the extent to which androgen is solely responsible for the

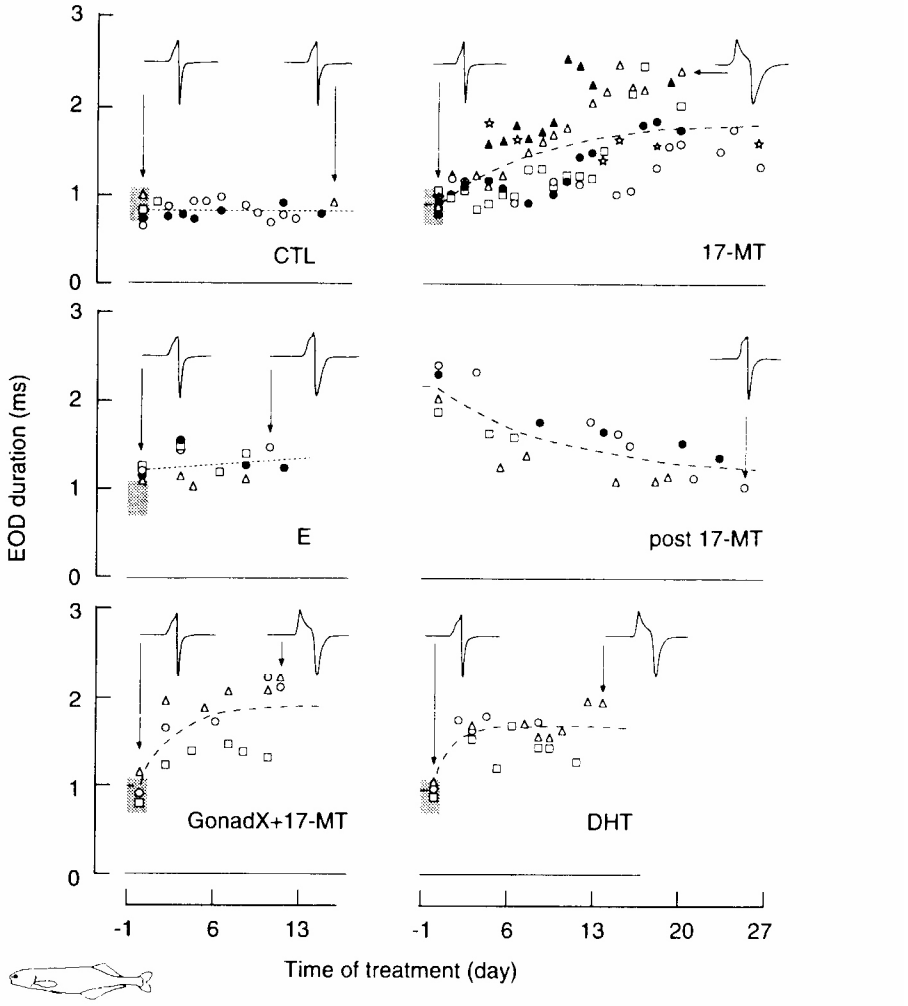


Fig. 13.2 Time course of changes in EOD duration during hormone treatment periods in Brienornyrus brachyistius (long biphasic); representative EODs are shown, each symbol is for one individual. The stippled area to the left of all but one plot is the range of EOD duration for immature and female fish for one standard deviation (0.161 ms) to either side of the population mean (0.908 ms, n = 25). Dashed lines represent a least-squares fit to the straight line (CTL, E) or an exponential curve (17MT, post 17-MT, GonadX + 17-MT, DHT). Changes in EOD duration were non-significant for the non-treated control (CTL) and estradiol (E) pellet-implanted subjects; but significant when powdered testosterone was added to intact (17-MT) or gonadectomized (GonadX + I 7-MT) fish, and after it was removed (post I 7-MT). Thin arrows point to individuals from which EODs were recorded. Modified after Bass and Hopkins (1983).

expression of maleness in the EOD of this species is not yet clear. The effect of CHOL on the EOD of *B. brachyistius* (long biphasic) has not been investigated (compare Bass and Hopkins, 1983, with Bass and Hopkins, 1985: p. 601).

Androgen-specific receptors have been found in the electric organs of adult mate *B. brachyistius* (long biphasic), and a possible sex difference in binding activity was suggested by preliminary data (Bass *et al.,* 1984). However, additional assays failed to confirm this result (Bass *et al.,* 1986b*).* Because a sex difference in androgen binding to receptors in the cytosol of efectrocytes could account for sex differences in the EOD waveform, more work in this area needs to be performed on other mormyrid species. Further, Bass *et al.* (1986b), using autoradiography, found ^{3}H-DHT binding cells in the brain adjacent to the relay cells of the medullary command nucleus. (These cells project to the spinal motor neurons that innervate the electrocytes of the electric organ; Chapter 16.)

Brienomyrus brachyistius *(triphasic) (Gabon)*

The E0D of *B. brachyistius* (triphasic) exhibits sex differences in waveform and duration (Hopkins and Bass, 1981*;* Bass and Hopkins, 1985). The sexually mature male EOD is usually double in duration with lower PPSFs and of distinctly different shape from that of females or juveniles (Fig. 13.3 (A)). However, maleness of the FOD varies with the size of the fish, and the EOD of large adult females overlaps with those of males (Bass and Hopkins, 1985).

Bass and Hopkins (1984) reported that both androgens, 17-MT and DHT, induced male-like EODs in females and juveniles, expressed in increased duration and decreased PPSFs (Fig. 13.3 (B)). Surprisingly, E_2 pellet implants or injections also increased EOD duration, lowered peak power, and induced the male-like waveform shape (Bass and Hopkins, 1985*). B. brachyistius* (triphasic) has not been subjected to treatment with CHOL or blank implants; it is thus difficult to assess the extent to which the EOD changes were due to surgery, implants, or general or specific hormone effects.

Interestingly, when five fish treated with 17-MT, dissolved in water, were placed in fresh water for *25* days, their hormone-induced male-like EODs did not completely revert to pre-treatment forms, suggesting that the effects of androgen on the EOD in this species may be relatively permanent (Bass and Hopkins, 1985). The authors claimed that this permanence is also supported by: (1) the EOD of mature males maintained in captivity for 3 months (n = 3) or 6 months (n = 1) did not revert to the female form (however, only the final day's EOD is presented; fig. 8 in Bass and Hopkins, 1985)*;* (2) one transitional male became more male-like, and one female with a male-like EOD (Fig. 13.3) became more female-like in captivity: and (3), when one mature male was castrated, its EOD remained unchanged (male-like).

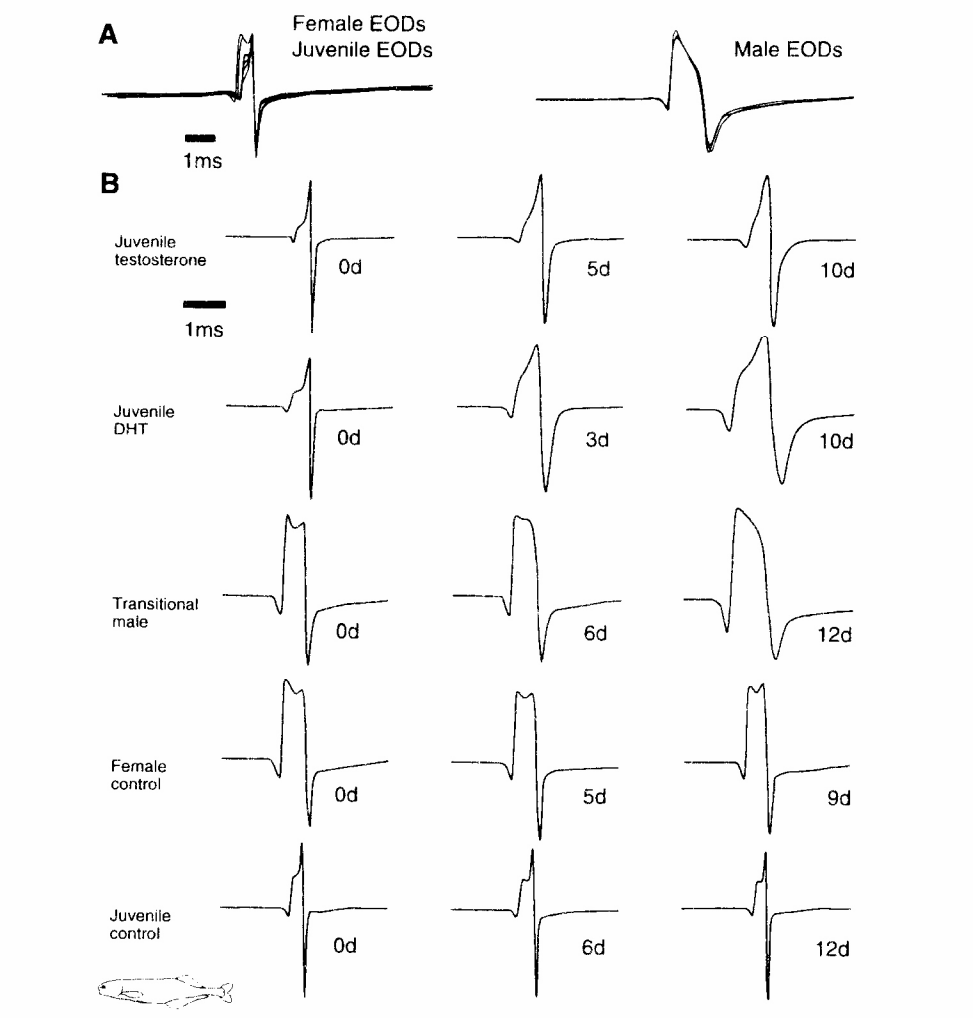


Fig. 13.3 (A) Oscilloscope tracings of EODs from *Brienomyrus brachyistius* (triphasic) recorded in the field. Notice the differences in shape and duration between female/ juvenile (n = 9) and male EODs (n = 3). (B) EODs of several individuals of *B. brachyistius* (triphasic): juveniles treated with 17α-methyltestostcrone (juvenile/testosterone) or 5α-dihydrotestosterone (juvenile/DHT) show a change in EOD duration over 10 days (10d). The EOD duration of a captive male with a 'transitional' waveform (transitional male) becomes more male-like in captivity (6d, 12d). In contrast to all of the above, the EOD waveform of captive females (female/control) or juveniles (juvenile/control) remains unchanged when kept in captivity for compareable times. Interestingly, the transitional male appears to be more female-like than the female control on 0d, while the EOD waveforms of neither of the androgen-treated juveniles assumes the adult male waveform (A) or the 12d transitional male waveforms. Note: while the EODs of captive males and females purportedly become more pronounced (Bass and Hopkins, 1984, 1985: Bass, 1986b), according to the authors, the female control shown did not change in captivity. Also note that DHT appears to have a more profound effect than 17-MT. Modified after Hopkins and Bass (1981) and Bass and Hopkins (1985).

Brienomyrus sp. (*syn*. Brienomyrus *sp. 2*) *(both imported from Nigeria*)

EODs from male and female *Brienomyrus* sp. maintained under laboratory conditions are almost identical (Bass and Hopkins, 1984). The fish's EOD has also been subjected to the influence of steroid hormones (Bass and Hopkins, 1984; Bass, 1986b). Bass and Hopkins (1984) reported that 17-MT, DHT, and CHOL, pellet implants lowered the PPSFs in gonadectomized and intact mates and females, with the effects of CHOL, being much smaller than those for both androgens. CHOL has also been shown to have effects on electrocyte characteristics in the male direction intermediate between controls and T-treated fish (Bass *et al.*, 1986b). Thus, a close inspection of methods and results sections and a comparison of Bass and Hopkins (1984) with Bass and Hopkins (1985) does not allow an unambiguous answer about the effects of CHOL, on the EOD in Brienomyrus sp. For example, in one study (Bass and Hopkins, 1984), CHOL-implanted gonadectomized females (n = 2) showed a 600 Hz drop in PPSF compared with a 500 Hz drop in gonadectomized controls; while in another study (Bass et *al.*, 1986a), CHOL-implanted intact females (n = 3) exhibited a final average PPSF at least 500 or 600 Hz lower than non-treated females and males, respectively. A well-designed study with the proper controls should be performed before the conclusion that "the effects of steroids on the EOD waveform are specific to gonadal steroids" (Bass and Hopkins, 1985, p. 601) can be made. Although Bass and Hopkins (1984) concluded that the EODs were also elongated by the steroid treatments, EODs are presented for only two androgen-treated fish and no quantitative data are presented.

Brienomyrus brachyistius *(biphasic)* (*Gabon*)

B. brachyistius (biphasic) purportedly has no EOD-related sex difference (Hopkins, 1980, 1981a). 17-MT added to the water of three juvenile males and three adult females did not affect the EOD (Bass and Hopkins, 1983). This is surprising since no other mormyrid species treated with androgens has failed to show some hormone-related effects on the EOD.

Brienomyrus brachyistius *(imported from Nigeria)*

Newly imported *B. brachyistius* exhibited a statistically significant sex difference in the P2/P3 duration ratio of their EOD, with males displaying lower ratios than females (Fig. 13.4 (A); Landsman and Moller, 1991; in prep.). Androgens and CHOL, but not estrogen, significantly influenced the durations of phases 2 and 3 of the EOD in laboratory-maintained fish not exhibiting the EOD-related sex difference, with androgen-treated fish exhibiting male-like EODs (Landsman and Moller, 1993, in prep.). Silastic

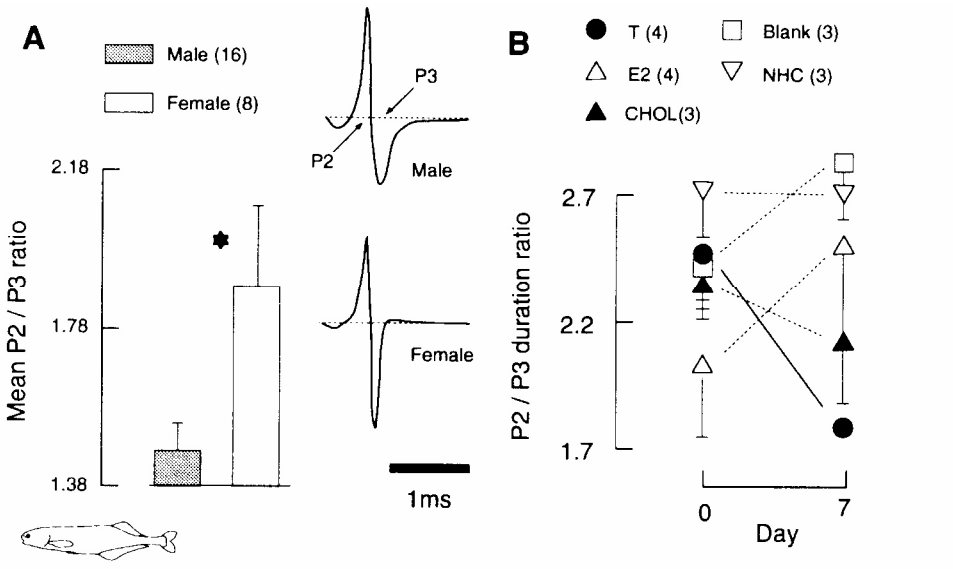


Fig. 13.4 (A) FODs of male and female *Brienomyrus brachyistius* (imported from Nigeria). Note the differences in EOD waveform and duration ratio of phase 2 (P2) to phase 3 (P3): mean + SEM P2/P3 duration ratio of male and female *B. brachyistius (** males had a smaller P2/P3 duration ratio than females: $t(22) = 2.32$, $P < 0.025$). (B) Effects of'silastic implants containing steroid hormones on the mean $\pm$ SEM phase 2/phase 3 duration ratios of the EOD in *B. brachyistius.* Three captive adult male and one female fish were gonadectomized and implanted with silastic capsules containing either testosterone (T), 11-ketotestosterone (11-KT, not shown in figure), 17-MT (not shown in figure), estradiol (E_2), cholesterol (CHOL) or no hormone (blank). NHC, non-handled controls. Sample sizes of surviving fish are indicated in parentheses. The data were analyzed using a two-way ANOVA (hormone x treatment day) followed by tests for simple main effects and Newman-Keuls tests. By 'Day 7' of treatment, only the naturally occurring androgens, T and 11-KT, caused a significant decrease in the mean duration ratio (hormone x treatment day interaction: $F(12,36) = 2.79$, $P < 0.01$; T: $F(2,36) = 5.03$, $P < 0.05$; 11-KT: $F(2,36) = 10.65$. $P < 0.01$). (Not shown: by day 13, the synthetic androgen, 17-MT, also exerted a significant effect on this ratio.) Modified after Landsman and Moller (1991 and unpublished).

implants containing T and 11-KT (11-KT: n = 1 male and 1 female; there was no difference in effects from T and 11-KT), 17-MT. and CHOL significantly increased the durations of both phases to different degrees (Landsman and Moller, unpublished). However, by day 7, only the two naturally occurring androgens, T and 11-KT, resulted in a significant decrease in the P2/P3 duration ratio compared with pre-implant ratios and with those of non-handled or blank-implanted controls. Neither E_2 nor CHOL exerted any significant effect on the sex-related duration ratio, while 17-MT caused a gradual decrease in this ratio by day 13 of treatment (Fig. 13.4 (B)).

Pollimyrus isidori

The triphasic EOD of *P. isidori* exhibits a sex difference in the *P1/P3* amplitude ratio (phases 1 and *3* are positive, phase 2 is negative). Males exhibit a smaller P1 and a larger P3 amplitude, and females a larger P1 and a smaller P3 amplitude (Fig. 13.5). This results in male ratios being lower than female ratios (Westby and Kirschbaum, 1982; Bratton and Kramer, 1988; Crawford, 1992). When artificially induced breeding seasons were introduced, females exhibited amplitude ratios that were threefold larger than males (Crawford, 1992). Westby and Kirschbaum (1982) and Crawford (1992) found almost no overlap between the sexes in this ratio, while others (Bratton and Kramer, 1988) found a largely overlapping, but statistically significant, sex difference.

While some reported consistent lower PPSFs for males than females (Westby and Kirschbaum, 1982), others found extensive overlap of PPSFs and no difference in EOD duration between the sexes (Bratton and Kramer, 1988; Crawford, 1992). Alteration of water conductivity conditions eliminated these sex differences (section 13.3), leading some authors to conclude that such differences did not function in species or sexual identification (Bratton and Kramer, 1988). To date, no published studies have investigated the potential of sensitivity to hormones in this species, so it is impossible to predict natural sex differences based on hormonal milieu.

Hippopotamyrus batesii

According to Bass and Hopkins (1985), the EOD waveform of one mature male *H. batesii* was twice as long as that for two mature females, when

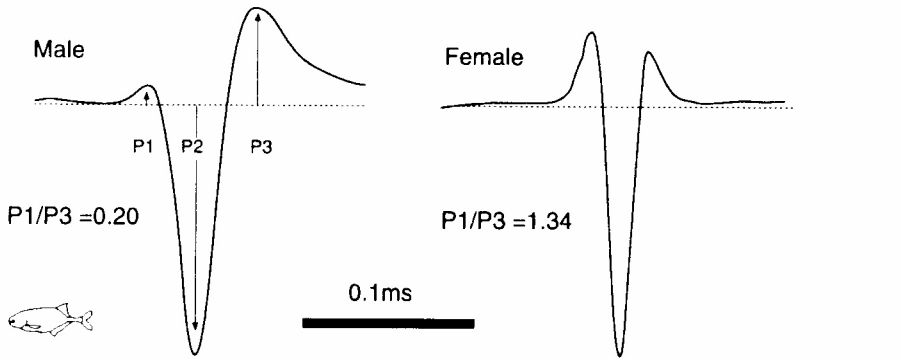


Fig. 13.5 EOD waveform of a male and female *Pollimyrus isidori* (conductivity: 100 µS/cm). Subjects were selected to demonstrate a presumed sex difference in EOD. P1, first head-positive phase; P2, head-negative phase; P3, second head-positive phase. Note that the ratio of P1 /P3 phase amplitudes is < 1.0 in this male, and > 1.0 in this female. Modified after Bratton and Kramer (1988).

data were recorded after an unspecified period of time following capture in Gabon and transport to France. Additionally, a review of the EODs of this species (sample size unspecified) from a previous field study (data collected by Hopkins in 1976; cit. in Bass and Hopkins, 1985) suggested a possible sex difference in EOD duration and PPSF (Bass and Hopkins, 1985). When immature *H. batesii* were implanted with pellets of *17-MT* (n = 1) and T propionate (n = 1), total EOD duration increased and PPSF decreased (Bass and Hopkins, 1985). No controls were included in this study.

Stomatorhinus corneti

Juvenile *S. corneti* undergo a transitional stage in the development of the adult EOD waveform (Bass and Hopkins, 1985). The adult male EOD appeared to be longer in duration than that of the female (Fig. 13.6). When juveniles and one female were treated with 17-MT, or T propionate (two juveniles, one female), their EODs showed dramatic downshifts in PPSF and increases in duration, both characteristic of male EODs (Bass and Hopkins, 1985). However, no cholesterol or blank implants were used, and only one untreated male served as a control.

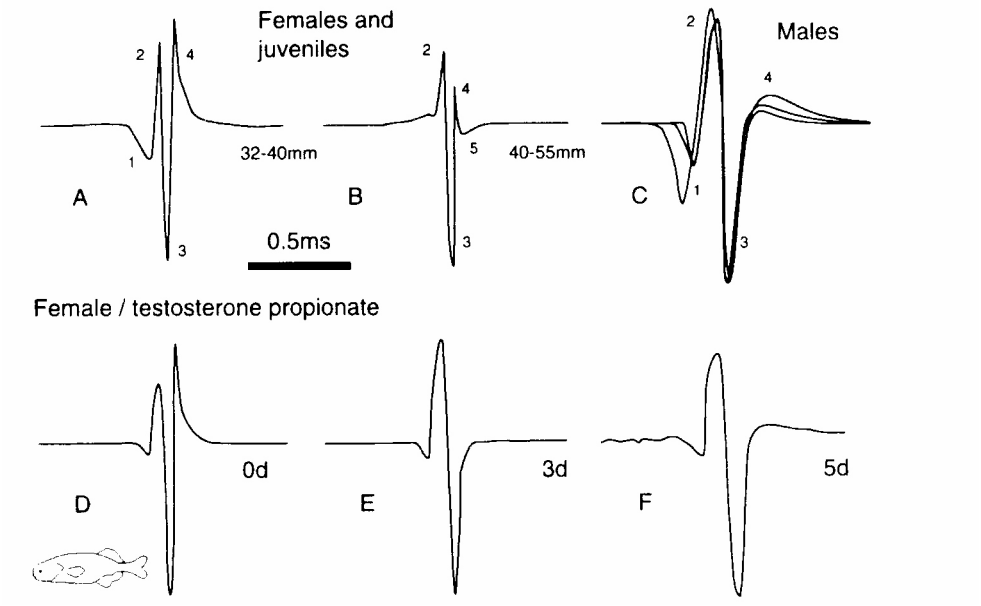


Fig. 13.6 EOD waveforms of *Stomatorhinus corneti*. Juveniles and females show two EOD forms depending on total body length (A, B, D). The EOD of mature males typically has a reduced second positive peak (point 4) and is longer in duration (C, three EODs superimposed). Testosterone propionate added to the water of a female induces a mature male-like EOD over a five day period (D-F). Modified after Bass and Hopkins (1985).

Gnathonemus petersii

Fish obtained during the Nigerian rainy breeding season and studied on the day they arrived exhibited EOD-related sexual dimorphisms: non-overlapping sex differences in the durations of phases 2 and 3 (the major positive and negative phases) and in the PPSF of the EOD (Landsman, 1993b). Males exhibited longer durations for both phases and lower PPSFs than females (Fig. 13.7; Landsman, 1993b). No sex differences were reflected in the duration of P1, P4, the total EOD or in the duration or amplitude ratios of P2 to P3.

The discovery of such a natural sex difference was surprising in light of two earlier incongruent reports by Kramer and Westby (1985), who did not find a waveform-related sex difference (section 13.3), and Landsman *et al.* (1987), who reported that males displayed higher PPSFs (and thus shorter EODs!) than females, provided the fish were unrestrained (section 13.3). These incongruent findings were resolved and will be discussed in section 13.4. The natural sex differences in the EOD in *G. petersii* are consistent with the results of exogenous hormone treatment in this species (Landsman and Moller, 1988; Landsman *et al.*, 1990).

Androgens affect the durations of P2 and P3 of the EOD in juvenile and adult *G. petersii*, and consequently affect the PPSFs (Fig. 13.8). Landsman and Moller (1988) and Landsman *et al.* (1990) demonstrated that both low and high doses of 17-MT, T and DHT (administered through silastic implants) significantly increase the durations of P2 and P3 in both gonadectomized juveniles (Figs 13.8, 13.9A) and gonadectomized adult males and females (Fig. 13.9B), while E_2 and CHOL have no effect on these phases (Figs 13.8, 13.9A, low- and high-dose). The androgens 17-MT and T decreased the PPSFs in both juveniles (Fig. 13.9A) and adults (Fig. 13.9B), while DHT had no effect on PPSF at low dose and was less potent than T at the high dose (Fig. 13.9A).

Surprisingly, E_2 caused a slight, but significant, increase in PPSFs in adults (Fig. 13.9B), but not in juveniles (Fig. 13.9A). This is interesting for a number of reasons, since it is the only reported E_2 effect on the EOD in mormyrid fish that worked in the direction opposite to that of androgens. Plasma E_2 levels in adults implanted with E_2 were about fourfold higher than in juveniles when both were administered the same dose of this hormone (Landsman *et al.*, 1990) (Fig. 13.10). This difference in plasma E_2 levels in *G. petersii* implanted with E_2 may have been a function of age-related differences in hormone metabolism rates (Harding, 1986). Thus, the effects on the adult EOD not present in juvenile EODs may have been a function of hormone levels and/or stage of receptor development.

A male-like EOD was induced in immature and adult *B. brachyistius* (triphasic) by treatment with E_2 (Bass and Hopkins, 1985) and, as

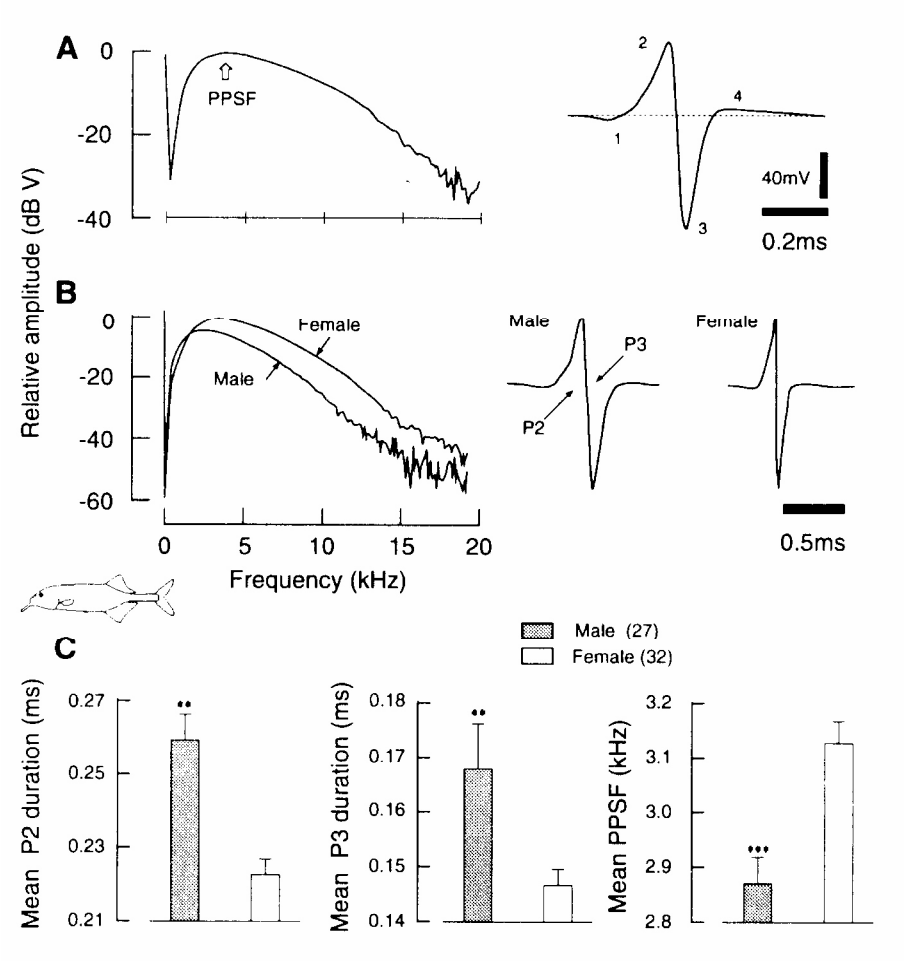


Fig. 13.7 (A) Representative Fourier transform and EOD waveform *of Gnathonemus petersii* illustrating peak power spectrum frequency (PPSF) and four EOD phases, respectively. (B) EOD-related sex difference in the EOD waveform (right) and associated Fourier spectrum (left) *of G. petersii*. Data were collected from 27 male and 32 female adult, gonadally ripe fish on the day they arrived from Nigeria (during the local rainy breeding season); EODs were recorded while fish were at rest in a porous ceramic shelter at the bottom of their individual aquaria. Males were identified by the distinct indentation at the dorsal margin of the anal fin. Male EODs are longer in duration, and the associated PPSFs are lower. (C) Quantitative data (means + SEMs). Males exhibit significantly longer mean P2 ($F(1,57) = 12.24$, $P < 0.001$) and P3 $F(1,57) = 11.91$, $P < 0.001$) durations than females. The mean male PPSF is significantly lower than the females' ($F(1,57) = 16.86$, $P < 0.0001$). **, $P < 0.001$; ***, $P < 0.0001$. Note that these findings are opposite to those reported by Landsman *et al.* (1987) and contrast with those of Kramer and Westby (1985) who reported no sex difference in this species, but are in accord with the effects of androgens on the EOD in this species (see Figs 13.8, 13.9, and text for details). Modified after Landsman (1991, 1993b) and Landsman *et al.* (1990).

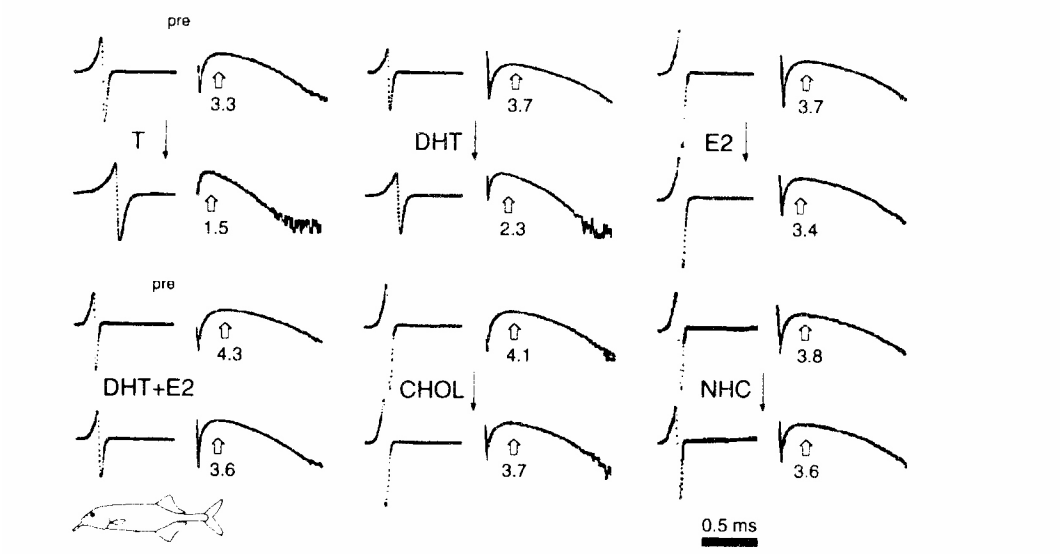


Fig. 13.8 Juvenile EOD waveforms and Fourier transforms before (pre) and 24 days following gonadectomy and implantation with high-dose implants (thin arrows). For explanation of EOD phases and hormone abbreviations, see Figs 13.7 and 13.9. Note the increase in duration of phases 2 and 3 and associated decrease in PPSFs (upward pointing arrows) only in fish treated with T, DHT, or DHT + E_2 (for methods and doses, Landsman *et al.*, 1990). Modified after Landsman *et al*. (1990).

discussed earlier, E_2 had a slight, but not statistically significant, effect on the EOD in the male direction in immature *B. brachyistius* (long biphasic) (Bass and Hopkins, 1983).

The effects of the androgens on P2 occurred much sooner than those on P3, indicating that the duration of P3 is less sensitive to androgen, at least in juvenile *G. peterii*. This can account for the finding that males in captivity showed decreases in endogenous androgen levels accompanied by decreases in the duration of P3, but not P2 (section 13.4). As T levels decrease, P3 is probably affected before P2, because the latter phase appears to be extremely responsive to even low doses of androgen.

The plasma T levels induced by the T implants were comparable to those found in males just imported during the rainy breeding season (Fig. 13.10; Landsman *et al.*, 1990). Males imported during the breeding season exhibited longer P2 and P3 durations and lower PPSFs than females imported at the same time. Newly imported males (caught during their breeding season) exhibit T levels around 6.0 ng/ml, while adult males held captive in the laboratory for 5 days exhibit near non-detectable levels (Fig. 13.16 (A); Landsman, 1993a,b) similar to those found in controls (Landsman *et al.*, 1990).

Although preliminary data suggested that the male-like characteristics

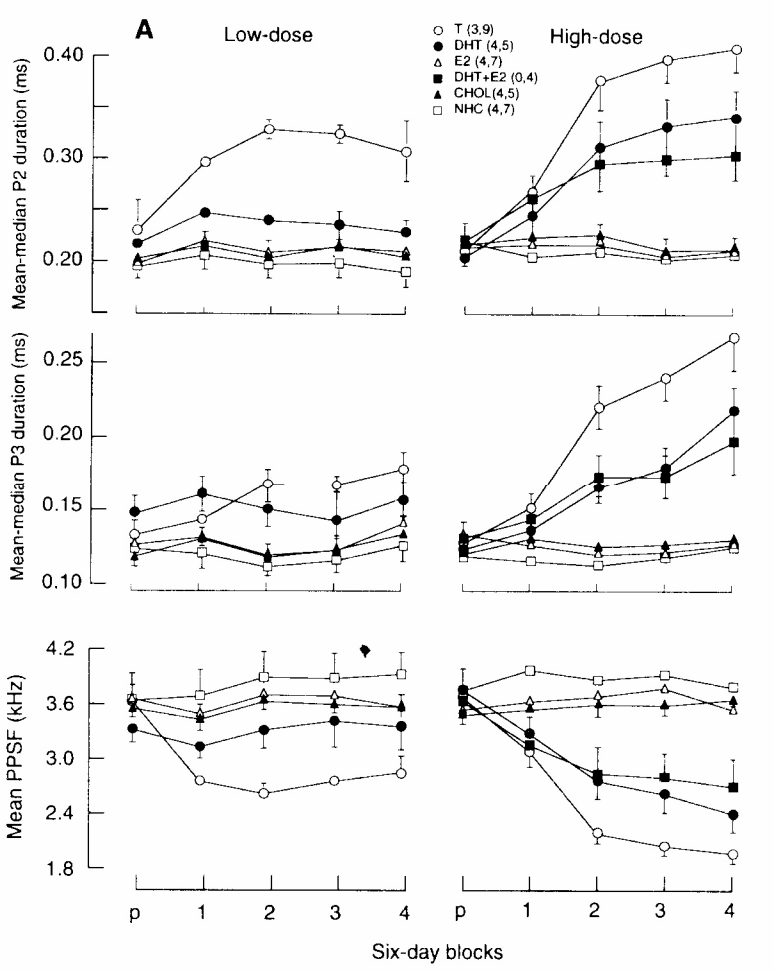
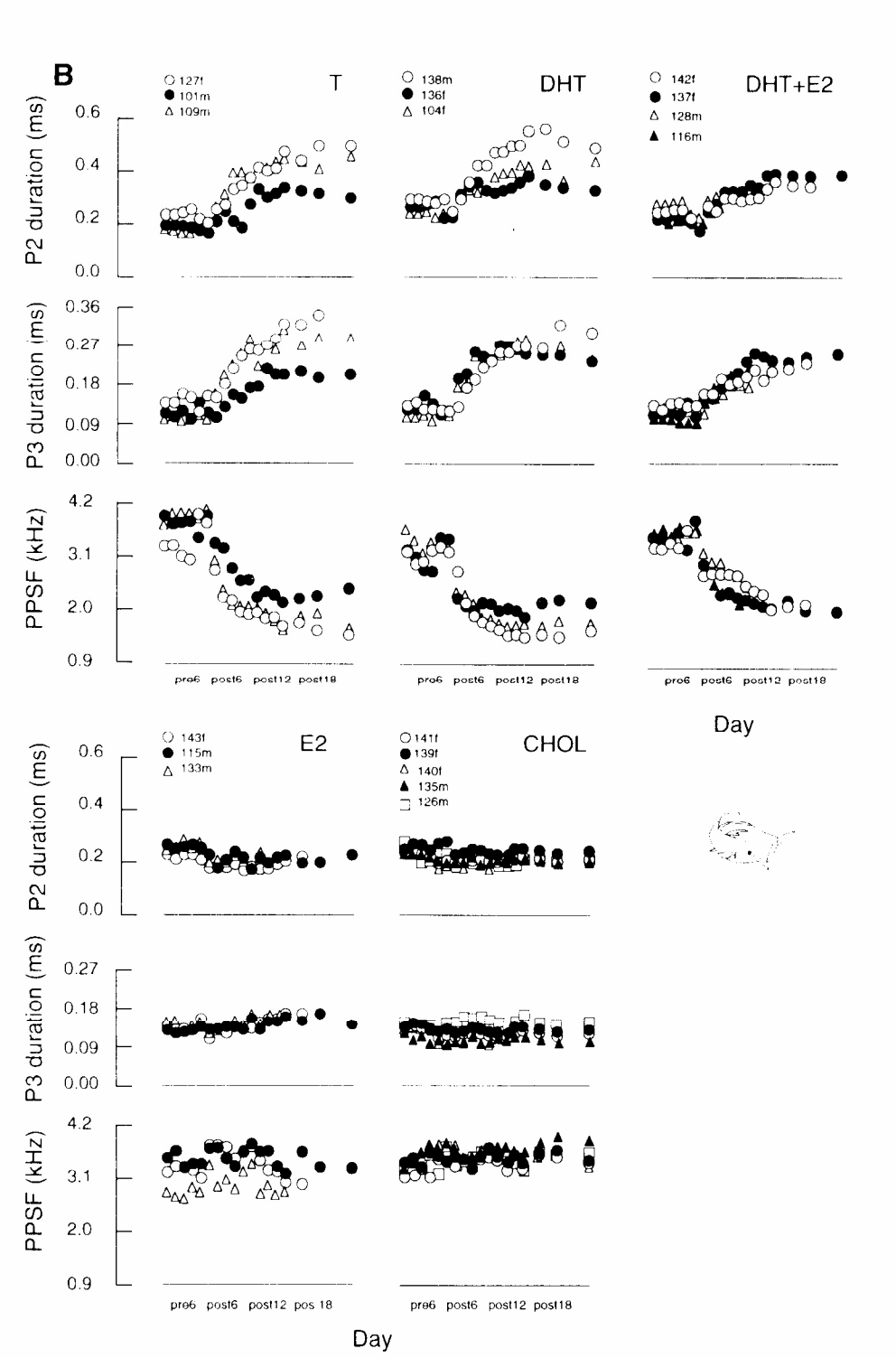


Fig. 13.9 (A) Effects of steroid hormone silastic implants on the durations of phases 2 (P2) and 3 (P3) and on the peak power spectrum frequency (PPSF) of the Fourier transform of the EOD in gonadectomized juvenile and adult *Gnathonemus petersii*. (A) juveniles administered low-dose (left) and high-dose implants (right). P2 durations, P3 durations and PPSFs: mean-median (± SEM) for a 6 day pre- (p) and post-implant blocks (1-4). (The mean of the medians was computed because the raw data were skewed.) Treatments: T, testosterone; DHT, dihydrotestosterone; E_2, estradiol; CHOL, cholesterol; NHC, non-handled controls; the two numbers following treatment condition (in parentheses) indicate sample size in low-dose and high-dose studies, respectively.

in the EOD in *G. petersii* induced by male hormones appear to be temporary (Fig. 13.11; Landsman *et al.*, 1990), further studies employing large sample sizes and proper controls will have to substantiate this possibility.


B
T
DHT
DHT+E2
E2
CHOL
P2 duration (ms)
P3 duration (ms)
PPSF (kHz)
pre6 post6 post12 post18
pre6 post6 post12 pos 18
Day

Notes on membrane effects

Inter- and intraspecific variability in EOD waveform and duration appear to be a function of variation in the electrical properties of the electrocyte membranes (Chapters 8, 16; Bennett, 1971a; Bass, 1986a,b; Mills and Zakon, 1987; Mills *et al.*, 1992; reviews: Zakon *et al.*, 1991b; Zakon, 1993). Mills and Zakon (1987) discussed the control and expression of EODs in gymnotiform and mormyrid fishes based on differences in membrane properties. The site of hormone action is on the medullary pacemaker neurons (PMN) and spiking membrane of electrocytes in wave-type fish, and on the electrocyte membrane (thickening) in pulse-type species. The locus of steroid action on the electrocyte is dependent upon the physiological properties of the electrocytes and thus varies across species. Steroid-sensitive sex differences in the morphology of electrocytes have been reported in a number of species and likely account for the steroid-sensitive sex differences found in the EOD (Bass and Hopkins, 1983; Hagedorn and Carr, 1985; Bass, 1986b; Bass *et al.*, 1986a: Mills and Zakon, 1987: review: Zakon, 1993). In such species, DHT masculinizes both the electrocytes and the EOD. Treatment with 17-MT and 11-KT increased the duration of all three phases of the EOD and caused an increase in the electrocyte width and anterior and posterior face surface areas in *Brienomyrus* sp. (Freedman *et al.*, 1989). Of all weakly discharging fish studied to date, *Sternopygus macrurus*, a wave-type species, is the only species in which electrocyte morphology appears not to be related to the waveform characteristics of the EOD; thus sex differences in electrocyte morphology cannot account for the sex differences in EOD waveform and frequency in this species (Mills *et al.*, 1992).

13.3 ENVIRONMENTALLY INDUCED PLASTICITY

Seasonal variables

Environmental variables exert a profound influence on the reproductive biology of weakly discharging electric fishes. Most influential are factors

---

Fig. 13.9 (B) Adult *G. petersii*. time course of hormone effects on P2 and P3 durations and on PPSFs for individual males and females (ID numbers and sex arc indicated) with high-dose implants across 6 pre- and 18 post-implant days. (Note: this study did not include low-dose treated adult subjects.) Only T increased the durations of either phase at low dose, while high doses of either of the androgens, T or DHT, increased the durations of both phases 2 and 3 in juveniles and adults. Only T decreased PPSFs at low dose in juveniles, while high doses of T and DHT decreased PPSFs in juveniles and adults. E_2 caused a slight, but statistically significant, increase in PPSFs in adults by post-implant day 6. Modified after Landsman *et al.* (1990).

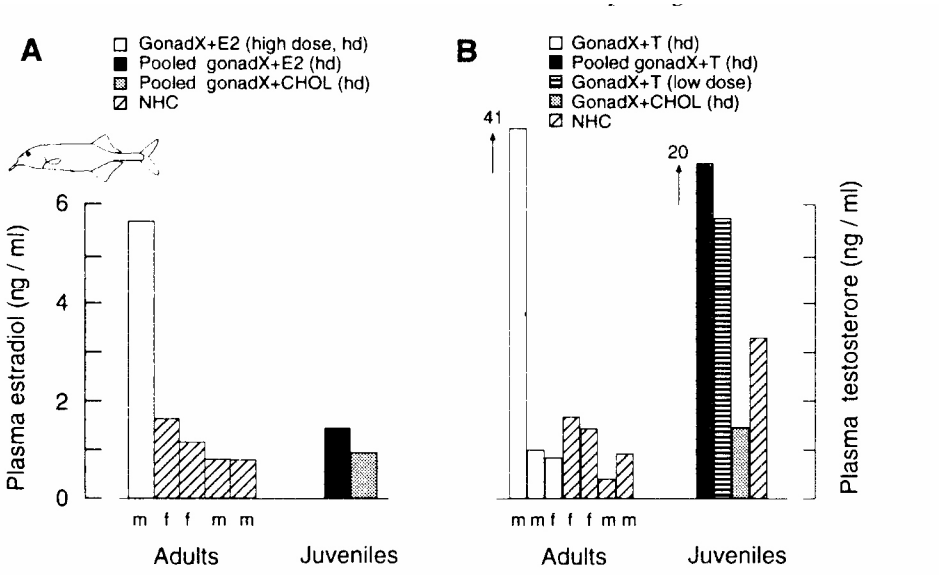


Fig. 13.10 Radioimmunoassay-determined blood plasma levels in adult and juvenile *Gnathonemus petersii* resulting from low-dose (1d) and high-dose (hd) hormone treatments (ordinate: same scale for both hormones). (A) Pooled and individual plasma estradiol (E_2) levels in gonadectomized (gonadX) E_2- and cholesterol (CHOL)-implanted fish and in non-implanted, non-handled controls (NHC). (B) Testosterone (T) levels in gonadX T- and CHOL-implanted subjects and in NHC. Blood was collected from all fish after 44 days of captivity in the laboratory, 24 days after gonadectomy and implant procedures were performed. (Plasma E_2 levels in pooled NHC juveniles were non-detectable and are not shown.) Pooled sample sizes: plasma E_2 levels, both E_2- and CHOL high-dose implants, n = 5 each; plasma T level, n = 9. Note that NHC subjects had T and E_2 levels comparable to those found in CHOL-implanted subjects. Also note that NHC adult males compared with the NHC adult females had lower plasma levels of T and E_2. Captivity in the laboratory probably altered hormone levels (section 13.4). Modified after Landsman (1990).

associated with the transition from dry to rainy season such as changes in conductivity, water level and rainfall (Chapter 12).

Gymnotiformes

Sternopygus dariensis collected during the late dry season in Panama were in reproductive condition and exhibited a sex difference with males discharging at lower frequencies than females (Meyer, 1983). However, although they were not in reproductive condition, fish collected during the rainy season exhibited an EOD-related sex difference. Male EOD rates were higher during the rainy season than during the late dry season; androgen-treated fish decreased their EOD rate, while estrogens increased it (Meyer, 1983), suggesting that the seasonal sex differences are a function of endogenous hormone levels.

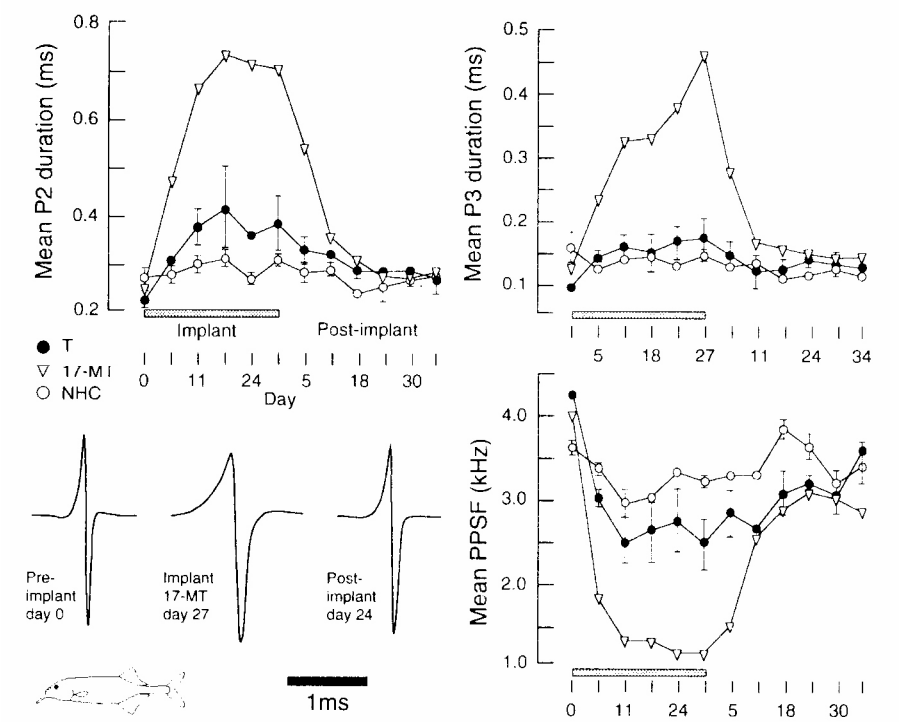


Fig. 13. 11 Androgen treatment increases the duration of phases 2 (P2) and 3 (P 3) and decreases the associated peak power spectrum frequency (PPSF) of the EOD in *Gnathonemus petersii*. Removal of the hormone-filled silastic implant results in EOD characteristics resembling those of pre-implant data (day 0). Numbers along abscissa indicate day of recording. Groups: testosterone (T, n = 2 fish), 17 α-methyltestosterone (17-MT, n = 1) and non-handled control (NHC, n = 2). After Voustianiouk and Perrotti (unpubl.). Note the exaggerated 17-MT effects compared with those of T.

S. macrurus breeds during the late dry season in Guyana (Hopkins, 1974a). Plasma androgens modulate the EOD frequency during the reproductive season (Zakon *et al.*, 1991b). In fish obtained from the Llanos region in Venezuela during one early and two late dry seasons, endogenous plasma T levels were significantly higher in males than in females only during the early dry season, while E_2 levels were significantly higher in females than males only in one late dry-season group. Plasma levels of T and 11-KT were highly related in males in all three groups, while 11-KT was absent in female plasma.

Zakon *et al.* (1991b) determined low to moderate significant inverse relationships between male plasma T level and EOD rate in both late dry-season groups (r^2 = 0.19 and 0.45, respectively), and between 11-K'I' plasma level and EOD rate in only one of the late dry-season groups (r^2 = 0.23). (r^2 values were calculated from data in Zakon *et al.*, 1991b.) Males with high T levels exhibited comparably low EOD rates. Such relationships

did not exist in early dry-season males, or between T level and rate in females, and E_2 and rate in males or females in all three groups. The average size of both males and females obtained during the early dry season was smaller than their same-sex counterparts obtained during the two late dry seasons; late dry season gonads were larger and more developed than those obtained from the early dry season. Androgen levels were directly, and EOD rates inversely, related to testicular maturity, while ovarian maturity was directly related to E_2 levels, but not EOD rate.

Mormyridae

EOD-related sexual dimorphisms in *G. petersii* were only observed in fish imported during their local breeding (rainy) season (Fig. 13.12). Male EODs have longer phases (P2 and P3), and a lower PPSF than those of females. Estradiol causes a slight, but statistically significant, increase in PPSF in adult *G. petersii* (Landsman *et al.*, 1990). In June, females exhibited their highest PPSFs, which may be indicative of higher estrogen levels during the breeding season (Fig. 13.12). There was no obvious difference in testis size between the seasons; ovaries in fish imported during the pre-breeding and

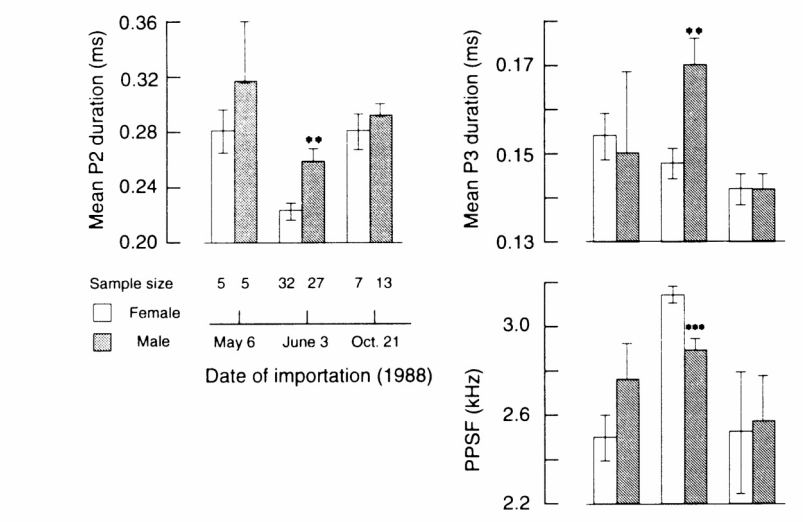


Fig. 13.12 Seasonal sex differences in mean (± SEM) phase 2 (P2) and phase 3 (P3) durations, and peak power spectrum frequency (PPSF) of the EOD in newly imported *Gnathonemus petersii*. Male EOD phases are longer and PPSFs are lower than females' during the rainy breeding season (June), but not during the pre- (May) or post-rainy (October) seasons. ** Denotes $P < 0.001$ and *** $P < 0.0001$ for June male vs. female values. Modified after Landsman (1991).

breeding seasons, however, were more developed than those examined in fish imported during the post-rainy season (Landsman, 1993b).

Similar to the gymnotiform *S. macrurus* (Zakon *et al.,* 1991b), sex- and seasonally dependent relationships are evident between various parameters of the EOD and between EOD and physical characteristics in the mormyrid, *G. petersii* (Landsman, 1993b). For example, subjects imported during the rainy breeding season, but not those imported during the pre- and post-rainy seasons, exhibited relationships between PPSF and the durations of P2 and P3. And in those subjects, PPSF was statistically more strongly related to P3 duration for females than for males.

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, 1974a; Westby and Kirschbaum, 1977, 1981, 1982; Lucker and Kramer, 1981; Kramer and Westby, 1985; Landsman *et al.,* 1987: Bratton and Kramer, 1988). When examining the mormyrid EOD for sexual differences under laboratory conditions, it is paramount to understand whether or not, under natural conditions, these differences are permanent or seasonal. Fish used in laboratory studies are usually obtained from local sources (importers, pet stores, or local aquaria), and in some instances collected and imported by the authors. Since, in most cases, no mention is made as to the season during which the animals were imported or the length of time they were maintained in the laboratory, it is not surprising that numerous reports are contradictory and incongruent with field data (reviews: Landsman, 1991, 1993a,b). When gonadal hormones are involved in the control of sexual behavior of seasonal breeders, ideally, the species' behavior and its physiological corollaries should be followed through an annual cycle.

Temporal and spectral characteristics of the EOD are steroid-sensitive and therefore indicative of the physiological state of the fish. Thus, the information conveyed during and outside the breeding season may be quite different given great differences in gonadal steroid levels. For example during the breeding season, when hormone levels are high, the duration of specific phases of the EOD is likely to carry information regarding sexual identity in *G. petersii*. When male and female EODs are identical during the non-breeding season, species identity, rather than sexual identity, may be their major function in social signaling.

Water conductivity

Water conductivity profoundly affects the fish's electrosensory motor system (Chapters 5, 8). This section will review the effects of water conductivity on the purported EOD-related sex differences.

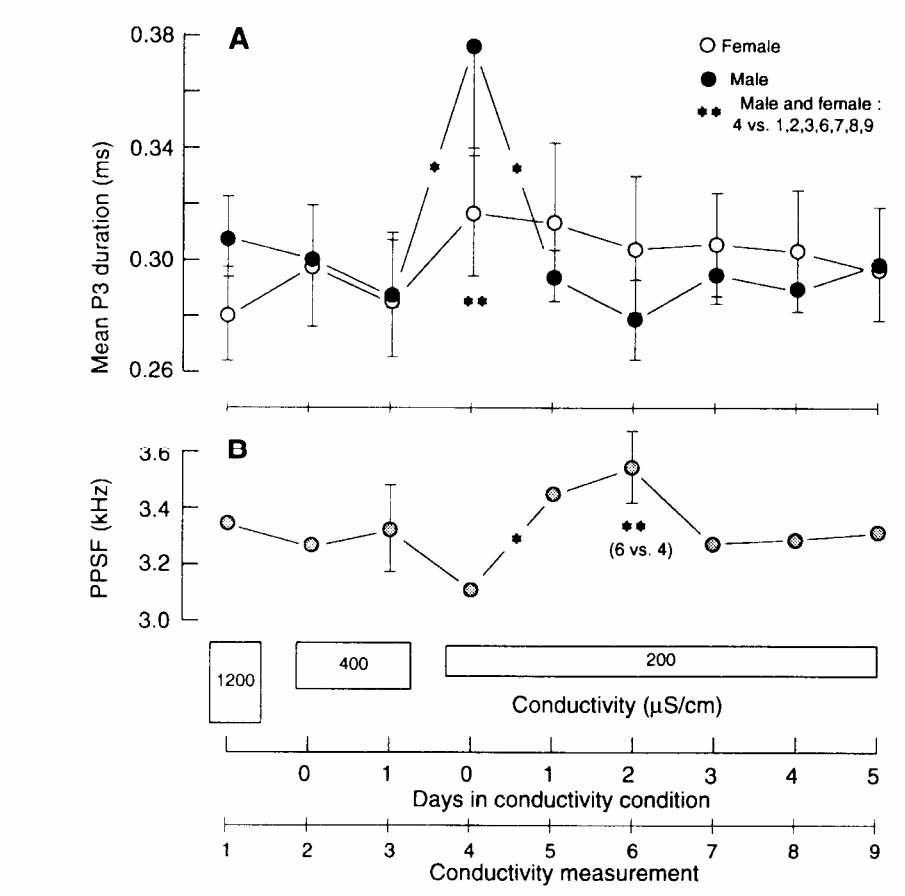


Fig. 13.13 Phase 3 (P3) durations (A) and peak power spectral frequencies of the Fourier transforms (PPSFs) of associated EODs (B) for male (n = 5) and female (n = 6) *Gnathonemus petersii across* conductivity measurements (consecutive numbers along x-axis: 1-9). (A) Mean (± SEM) P3 duration: days in conductivity condition are measured immediately prior to and 1 h following (0) a conductivity change, and then at the 24 h intervals (1-5) indicated (x-axis; days in conductivity condition). Water conductivity was lowered so as not to disturb the fish by simultaneously removing portions of the water and adding conditioned low-conductivity water to the individual aquaria by siphoning water through plastic tubes connected to the far ends of the aquaria. The conductivities under which data were recorded from all fish were (mean $\pm$ SD): 1200 $\pm$ 50, 400 $\pm$ 20 and 200 $\pm$ 10 µS/cm. A two-way ANOVA indicated a significant interaction effect between sex and conductivity ($F(8,72) = 2.26$, $P < 0.05$). Only phase 3 duration of male EODs, not that of females, were significantly affected immediately following the change from 400 to 200 µS/cm (conductivity measurement 4) ($F(8,72) = 4.91$, $P < 0.0001$). A significant conductivity condition effect was obtained for P3 duration for all subjects irrespective of sex ($F(8,72) = 3.90$, $P < 0.001$) with the mean duration for conductivity measurement 4 being longer than the mean durations for all other conductivity conditions except for measurement 5. No significant conductivity effects were obtained for phase 2 duration (data not shown).

Bratton and Kramer (1988) exposed male and female *P. isidori* to a wide range of conductivities (3-500 µS/cm) and reported that the overlapping, but statistically significant, sex difference in the EOD phase amplitude ratio (P1/P3: Fig. 13.5) was influenced, but not in all fish. When water conductivity was held stable for 24 h at about 100 µS/cm, the male ratio was smaller than that of the female (section 13.2). Below 100 µS/cm, male and female ratios became more female-like (> 1.0), and above 100 µS/cm, male and female ratios became more male-like (< 1.0). However, the male and female ratios in five males and three females that were less than 1.0 were not influenced by changes in conductivity. The results were the same whether conductivities were varied from low to high or from high to low. Anecdotal evidence from two males and one female showed long-term individual and intraspecific variability in P1 /P3 ratios. Bratton and Kramer (1988) noted that a decrease in conductivity caused a decrease in PPSF in one male, becoming more male-like according to the sex difference reported by Westby and Kirschbaum (1982).

Until the effects of exogenous hormone manipulations and/or endogenous levels of steroid hormones are known for *P. isidori*, it is difficult to determine the extent to which variability in the steroid-sensitive, sex- related characteristics of the EOD is due to direct conductivity-induced changes in electrocyte membrane properties alone or to an interaction between these effects and indirect changes in membrane properties induced by physiological factors such as endogenous hormone levels. Controls such as a non-manipulated group and a group subjected to water changes while conductivity was held constant would have controlled for the effects of conductivity and other factors on the EOD.

The EOD of *G. petersii* exhibits sex differences in the durations of P2 and P3, and in PPSF. Water conductivity affects EOD-related sex characteristics in this species: the duration of P3 (but not P2) and the PPSF (Landsman and Bowling, unpubl.). The fish were maintained in water of 1200 = µS/cm for 3 weeks, and subsequently tested in water of (mean $\pm$ SD): 1200 $\pm$ 50, 400 $\pm$ 20 and 200 $\pm$ 10 µS/cm. EODs were recorded immediately prior to and 1 h following a conductivity change, and again 24 h after the first

---

(B) Mean (± SEM) PPSFs for all subjects. A two-way ANOVA indicated a significant conductivity condition effect ($F(8,72) = 2.96$, $P < 0.01$). Mean PPSFs were significantly higher 1 day (conductivity measurement 5) and 2 days (measurement 6) following the change from 400 to 200 µS/cm compared with PPSFs immediately (1 h) following this change (measurement 4). No significant sex or interaction effects were obtained; statistically significant differences between means are indicated with * on line connecting the means, while ** below means indicate significant differences between means for days designated below stars (PPSF); ($\alpha = 0.05$). After Landsman and Bowling (unpubl.).

change, and every subsequent 24 h for 5 days following the second change.

Changes in conductivity interacted with sex, resulting in significant effects on the duration of P3 and the PPSF (Fig. 13.13). Males, but not females, showed a significant increase in the duration of P3 immediately following the change in conductivity from 400 to 200 µS/cm, while by 1 day post-200 µS/cm, the male P3 duration decreased significantly, back to its pre-200 µS/cm level (Fig. 13.13, top). The same change in conductivity caused an increase in PPSF for both sexes (Fig. 13.13, bottom).

Seasonal changes and short-term environmental perturbations can affect endogenous hormone levels in mormyrid fish which in turn can cause changes in their communication signals. Whether the demonstrated conductivity effects argue against the use of these signals in sexual recognition is currently being debated (see Bratton and Kramer, 1988, and Crawford, 1992 for two opposing views). Although the increase in P3 duration and decrease in PPSF in *G. petersii*, shown under lowered conductivity conditions, are consistent with the physics of biological membranes (Bennett, 1971b; Bell *et al.*, 1976), it does appear that conductivity effects exerted on the EOD are only in part due to direct physical action on the electrocyte membrane. The steroid-sensitive EOD phase 3 in *G. petersii* (one of the two phases which exhibit sex differences) is significantly influenced by conductivity. Because environmental perturbations affect endogenous hormone levels in this species (Landsman, 1991, 1993a), it is possible that variations in conductivity indirectly affect the EOD by inducing changes in the endogenous hormone milieu. This is supported by the fact that (1) P3 duration, but not P2 was affected by changes in conductivity, a finding that was not surprising because the duration of P3 compared with P2 in *G. petersii* is more sensitive to changes in androgen levels (Landsman *et al.*, 1990; Landsman, 1991); and (2) environmental perturbations that influence endogenous androgen levels affected the duration of P3, but not 2 (Landsman, 1991, 1993a). Also, the differential response of P3 in males and females (Fig. 13.13, top) could be explained by sex-dependent changes in endogenous hormone levels resulting from the conductivity manipulations. The large inter- and intra-individual conductivity-induced variability probably reflects what little is known about the interaction between physical and biological parameters in the determination of the EOD waveform.

Plasticity due to capture, handling and confinement

Successful reproduction requires an organism to synchronize its reproductive physiology and behavior with events in its environment (Moore and Marler, 1988). Consequently, interference with such events or with the

organism's adaptive abilities, possibly through stress caused by capture, handling and/or confinement, will inhibit reproduction.

Changes in environmental factors and laboratory manipulations are known to affect reproduction and alter the sex-related characteristics of the EODs in electric fish. This is probably why it is difficult to breed these fish in captivity. By manipulating environmental variables, however, several authors were able to induce a few species to breed in the laboratory (Chapter 12). Because sex differences in EODs are rarely seen in any wild-caught fish maintained under 'typical' laboratory conditions, it appears that the variables removed or introduced by bringing electric fish into captivity inhibit reproduction, at least in part, by changing their behavior. Consequently, while there are numerous field studies in which sex differences in the EODs of a number of species were observed, in only one species to my knowledge has a completely non-overlapping unambiguous EOD sex difference been reported in feral animals imported and brought into the laboratory (Landsman, 1991, 1993a).

In gymnotiforms, female *Brachyhypopomus occidentalis* (formerly *Hypopomus)* injected with saline as a control for hormone administration increased their PPSFs (Hagedorn and Carr, 1985), and *Sternopygus dariensis* of both sexes exhibited increases in EOD frequencies following handling compared with a non-handled control group (Meyer, 1983).

Landsman *et al.* (1987) reported that a sex difference in the waveform and possibly also duration of the EOD in the mormyrid, *G. petersii,* was eliminated when males and females were either confined or restrained (Fig. 13.14). When the same fish, however, were permitted to rest freely, males exhibited higher PPSFs than females (Fig. 13.14 (B)).

Restraints were used to reduce individual variation due to fish movement. In the 'confined' and 'restrained' conditions, both sexes emitted highly variable EODs which deviated from the normal PPSF range for either sex, suggesting that under aversive conditions, the EOD variations may reflect a change in message analogous to the alarm calls exhibited by other vertebrate species (Fig. 13.14 (C)). There was also a dramatic decrease in variability between individual PPSFs for both males and females in the 'free' condition compared with the relatively large variability between individuals in the 'restrained' condition (Fig. 13.14 (A)). 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.

These results suggest that EOD behavior is extremely malleable when fish are subjected to procedures that have been shown to be stressful, such as confinement and restraint. Stress in fish is typically assessed by measuring blood corticosteroid levels. Fish, similar to mammals, respond to exposure to stress with an increase in circulating corticosteroids and catecholamines

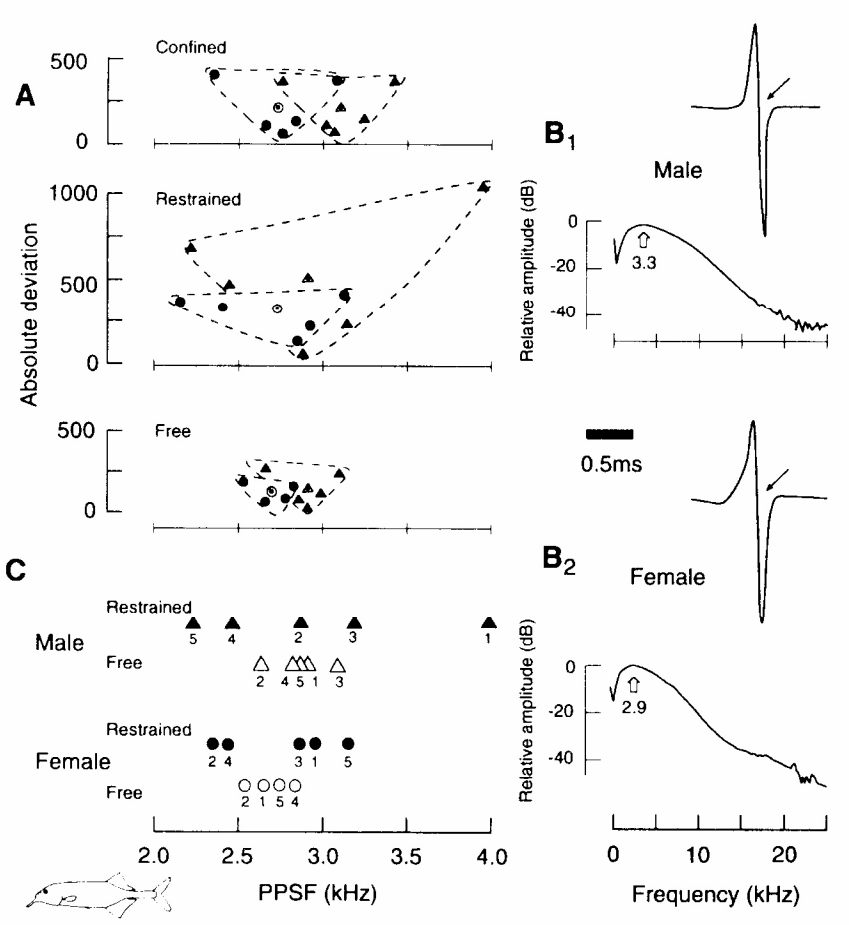


Fig. 13.14 Effects of confinement and restraint on the EOD in male and female *Gnathonemus petersii*. (A) EODs recorded from the same fish under 'confined', 'restrained' and 'free' conditions (temperature, 22.5°C; conductivity, 150 µS/cm). Absolute deviations (absolute difference between each subject's mean PPSF and the group mean for that sex) indicating the variability of individual average peak power spectral frequencies (PPSFs) (y-axis), are plotted as a function of the individual average PPSFs (x-axis). Each group mean (absolute deviation) is plotted as a function of its group mean PPSF. Black circles represent female individual means; open circles with point inside represent female group means; black triangles represent male individual means; open triangles with point inside represent male group means. The enclosure surrounding each sex group distribution illustrates the amount of variability of individual mean PPSFs. Each fish was confined in a suspended gauze envelope allowing some lateral mobility. After 15 min, for each fish, a mean PPSF was calculated from six separate readings. 'Confined' males tended to have higher PPSFs than females (means $\pm$ SE = 3102.4 $\pm$ 110.4 Hz and 2739.8 $\pm$ 117.8 Hz, respectively); however, this was only a trend, $t(8) = 2.25$, $P < 0.06$. The great variability between fish (illustrated by the vertical spread of the male and female distributions shown in (A), top) suggested the recording technique (which allowed the fish some degree of mobility) to be the source of the observed variability, obscuring any sex difference. Therefore, following a 5 day resting period, the fish were again individually placed into the test tank, this time either 'restrained' within the

as well as a change in gonadal hormone milieu (e.g. handling, confinement and restraint: Strange *et al.*, 1977; Mazeaud *et al.*, 1977; Mazeaud and Mazeaud, 1981; Pickering *et al.*, 1987; Safford and Thomas, 1987; Carragher and Pankhurst, 1991*;* pollutants**:** Ilan and Yaron, 1983: saline injection: Hegab and Hanke, 1986*).*

Gonadal steroid levels have effects on EOD rate and waveform duration and shape. Environmental influences on the EOD could be exerted through changes in plasma levels of gonadal and/or interrenal (stress) hormones. For example, cortisol could possibly affect the EOD either directly by influencing the electric organ electrocytes or indirectly by influencing gonadal steroid hormone levels. In freshwater tilapia (*Sarotherodon mossambicus*) and rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), increased cortisol has been shown to augment the entrance of sodium, calcium and

---

gauze envelope tied to a vertical support 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 discharge recordings were taken only when a fish remained in its shelter. Because 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 order so that each condition was separated by a 5 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 ((A), middle). Surprisingly, in the 'free' condition ((A), bottom), the male PPSF was located at a significantly higher frequency (mean $\pm$ SE = 2902.6 $\pm$ 73.1 Hz) than the female PPSF (mean $\pm$ SE = 2691.5 $\pm$ 60.6 Hz), $t(7) = 2.14$, $P < 0.05$, one-tailed; point biserial correlation coefficient: rpb = 0.63. Levene's test (Keppel, 1982), to assess differences in variability (absolute deviations), revealed a significant treatment effect, $F(1, 7) = 6.15$, $P < 0.05$, indicating greater variability between the individual means in the 'restrained' condition (mean absolute deviations were 492.8 for males and 324.5 for females) compared with the 'free' condition (male mean = 104.2, female mean = 93.5). Note the trend toward the expression of a sex difference and the intermediate variability of individual means in the 'confined' condition ((A), top) compared with the 'restrained' and 'free' conditions ((A), middle, bottom).

(B) EODs from representative *G. petersii* in the 'free' condition showing a male (B_1) and a female (B_2)) differing in PPSF (3.3 vs. 2.9 kHz) and EOD duration (shorter P3 duration in the male: arrows).

(C) Fate of individual mean PPSFs for male and female *Gnathonemus petersii* under 'free' and 'restrained' conditions. Under the 'free' condition, both sexes exhibited PPSFs between 2540 and 3100 Hz (males: 2660 to 3100 Hz; females: 2540 to 2820 Hz). Under 'restrained' conditions, 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). Under the 'free' condition, PPSFs are clustered; under 'restrained' conditions, PPSFs are dispersed to either end of the spectrum. Numerals identify individual fish. Modified after Landsman *et al*. (1987 and unpubl.).

chloride within muscle cells and to support retention of inter- and intracellular water (Assem and 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 and could possibly account for environmental effects on the EOD (see also Ferrari and Zakon, 1993).

Behavioral changes due to stress have been demonstrated in a number of other fish. Such 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 (*X. maculatus*), and then subjected the alpha member of each pair to either large dominating or smaller non-dominating fish. 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. The behavioral differences exhibited by subjects exposed to these aversive manipulations could be explained by changes in corticosteroid levels, as suggested by similar studies (Erickson, 1967; Noakes and Leatherland, 1977; Peters *et al.*, 1980).

When pumpkin seed sunfish (*Lepomis gibbosus*) were confined in small quarters, they established and defended territories (Erickson, 1967). The author found a negative relationship between aggressive behavior and interrenal volume, The highest-ranking swordtails (*X. helleri*) showed the least adrenocortical activity as measured by the nuclear diameter of adrenocortical cells (Scott and Currie, 1980). (However, Scott and Rennie, 1980, found that the nuclear diameter is only an approximate indicator of plasma cortisol level in *Coregonus lavaretus*.) Dominant *Oncorhynchus mykiss* (*Salmo gairdneri*) also exhibited lower interrenal activity than subordinates (Noakes and Leatherland, 1977). Most subordinate *Anguilla anguilla* exhibited enlarged interrenal cells and increased plasma cortisol concentrations, while dominant eels did not differ from controls on either measure (Peters *et al.*, 1980). These examples 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.

Manipulations such as the confinement procedures used on *G. petersii* may have constituted stressful events and resulted in a hormonal 'stress response' that, in turn, altered the EOD (Landsman *et al.*, 1987). It seems unlikely that the stress hormone, cortisol, was responsible for these effects,

because *G. petersii* implanted with open-ended silastic capsules containing this hormone did not exhibit changes in EOD characteristics (Landsman, unpubl.). (Cortisol levels have not been measured in weakly electric fish.)

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 DHT, E_2, and estrone 1-3 h later in the sea lamprey (*Petromyzon marinus*) and yellow eel (*Anguilla rostrata)* (Epple *et al.*, 1982). Progesterone (P) and T levels dropped within the first hour and then rose sharply, while androstenedione was not detectable. In pre-spawning 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 estrone did not change. The authors concluded that their results suggested that in vertebrates many steroid hormones are ‘stress hormones'. However, the data could also be interpreted as indicating that only agitation, the manipulation that increased cortisol, results in a stress response in these species. When spotted sea trout were maintained in captivity, after a 1 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; cortisol levels in females increased by day 1 post-capture, but returned to initial levels by day 21 (Safford and Thomas, 1987).

Chronic confinement for 1month as well as acute handling stress resulted in suppression of plasma levels of both T and 11-KT in sexually mature male brown trout (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 *et al.*, 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.

As sex differences in EOD behavior can be altered by sex-hormone manipulations, it is likely that the effects on the EOD-related sex characters caused by environmental variations and laboratory manipulations result from changes in endogenous gonadal hormone levels. Whether or not such hormone changes constitute a stress response is not clear.

13.4 CAPTIVITY-INDUCED PLASTICITY: THE MORMYRID CAPTIVITY MODEL

Captivity and reproduction

Environmental variation plays a role in electrocommunication. Here I will focus on the discrepancy between data on EOD-related sex-differences collected from fish in their natural habitats on location, and those collected from wild-caught specimens brought into the laboratory, as well as on between -laboratory discrepancies.

Our understanding of the mechanisms underlying the influence of captivity on reproduction has been limited because no adequate animal model has been identified in which to study the effects of captivity on both reproductive behavior and its underlying physiology. This is largely due to the fact that most feral animals brought into captivity fail to exhibit any sexual behavior (Moore and Miller, 1984). Manipulations that cause stress-responses and the effects of captivity disrupt reproduction across a wide variety of species. Laboratory manipulations (such as food deprivation, overcrowding, extensive handling, isolation, extreme temperatures, surgical procedures and injections) have demonstrated that severe stressors inhibit reproductive behaviors (e.g. Moore *et al,* 1982; Zoeller and Moore, 1982; Miller and Moore, 1983; Moore and Miller, 1983, 1984). To the extent that such reproductive behaviors are hormone dependent, it is generally assumed that the endogenous hormone changes resulting from such stress manipulations cause the behavioral change. However, these laboratory experiments employ severe manipulations and leave the animal no recourse other than a severe stress response (Wingfield, 1988). Feral animals captured and maintained in the laboratory also exhibit changes in reproductive physiology (e.g. in mammals, Rivier *et al.*, 1986; in birds, Wingfield, 1988; in amphibians, Moore and Deviche, 1988; in fish, Mazeaud and Mazeaud, 1981) and in reproductive behaviors (Elton, 1979; Erwin and Deni, 1979). However, little is known regarding the link between captivity, endogenous hormone milieu, and reproductive behavior in wild-caught animals (Moore and Miller, 1984).

It has always been assumed, but not demonstrated until recently (Landsman, 1991, 1993a), that some of the same hormonal changes that accompany laboratory stress-responses are responsible for the captivity effects on behavior and reproduction. Because various characteristics of the EOD behavior reflect physiological state in electric fish (e.g. endogenous hormone milieu), this behavior may be an excellent indicator for captivity effects. Electric fish may therefore serve as an excellent model in which to study the effects of captivity on reproductive behavioral physiology.

Captivity and mormyrid EODs

Anecdotal evidence based on only a few fish suggests that the EOD is altered over time in captivity. When two control female *S. corneti* were held captive in the field for 6 to 14 days, their average PPSFs decreased in the direction exhibited by males or androgen-treated females (Bass and Hopkins, 1985). But these changes in peak frequency were not statistically significant and were well within the range of normal female EODs (Bass and Hopkins, 1985). However, 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 PPSF. These authors suggested that the EODs of adult males are permanent, but have a modifiable appearance based on an individual's overall physiological state. Similarly, the EODs of captive male and female *B. brachyistius* (triphasic) became more pronounced, i.e. more male-like and more female-like, respectively, while those emitted by juveniles did not change (Bass and Hopkins, 1984, 1985; Bass, 1986b). In contrast, Bass (1986b) reported the opposite effect in one captive adult male *B. brachyistius* (long biphasic) that exhibited a reversal to the natural female waveform over time.

Incongruent findings regarding EOD-related sex differences have been reported across studies and appear to be a result of captivity effects. For example, in *G. petersii*, only freshly imported rainy-breeding-season fish exhibited sex differences in the direction predicted by results of studies employing exogenous hormone manipulations (Landsman and Moller, 1988; Landsman *et al.*, 1990). Fish imported and then maintained in the laboratory for periods of 3 to 4 weeks exhibited either a sex difference in the opposite direction to that predicted by hormone treatment effects (Landsman *et al.*, 1987) or no sex difference at all (Kramer and Westby, 1985).

Mormyrid captivity model

Landsman and Moller (in prep.) propose that a suitable model for the study of the effects of captivity on behavior and its physiological substrates would be a species that, both prior to and following its introduction into the laboratory, (1) exhibits an easily quantifiable overt behavior that clearly changes as a function of laboratory confinement, and (2) allows an equally easy access to and measurement of potential underlying physiological causes. Weakly electric fish seem to meet the requirements of such a model: they can be obtained from local fish importers; their EOD behavior is readily accessible with the use of recording electrodes; the EODs can then be quantified and analyzed with standard electrophysiological techniques (e.g. using oscilloscope displays, personal computers, spectrum analyzers).

These fish continually discharge under both field and laboratory conditions.

Mormyrids. in general, exhibit sex differences in their EODs that are observable in field but not laboratory studies. Even studies on the same or different species performed within and across laboratories have reported incongruent results regarding such EOD sex differences (review: Landsman, 1991). The EOD is extremely susceptible to environmental perturbations, as indicated by studies reviewed in the previous section. Research on two mormyrid species, *G. petersii* (Landsman, 1991, 1993a) and *B. brachyistius* (Landsman and Moller, 1993; unpublished) has experimentally demonstrated the profound effects that captivity exerts on the EOD, and can thus explain the variability and incongruences reported in previous studies focusing on EOD-related sex differences.

Within 2 h of arrival on the day that subjects were received from Nigeria during the local rainy breeding season, non-overlapping sex differences in the durations of phases 2 and 3 of the EOD, and in the PPSF were observed in *G. petersii* (Fig. 13.7; Landsman, 1993a,b). However, by 37 days of captivity in the laboratory, the sex differences were either abolished in fish maintained in group aquaria, or abolished (phase 2) and even reversed (phase 3 and PPSF) in fish maintained in individual aquaria (Fig. 13.15). Judging by the effects of this 37 day captivity period on the PPSF, the EOD phases for males were more influenced than those for females (Fig. 13.15B). This finding, together with the fact that the duration of the EOD in this species is extremely sensitive to androgens (Landsman *et al.*, 1990), led to the hypothesis that captivity altered endogenous levels of androgens, resulting in changes in EOD characteristics that are hormone sensitive (Landsman, 1991). This hypothesis was supported in a study which showed that regardless of whether males were maintained individually or in groups, both plasma levels of T and 11-KT (naturally occurring androgens in fish) rapidly and dramatically decreased to near non-detectable levels by day 15 in the laboratory (Fig. 13.16 (A); Landsman, 1991, 1993a). In the same subjects, the duration of P3 decreased, P2 did not change, and the PPSF increased over the 15 day captivity period (Fig. 13.16 (13)).

The finding that sex-related characteristics of the EOD change as a function of the amount of time in captivity can account for the incongruent results reported in previous work on EOD-related sex differences in *G. petersii*. In fish that had been maintained in the laboratory for approximately 4 weeks prior to data collection (Kramer, pers. communication, 1986), Kramer and Westby (1985) did not find any sex difference. In fish that were maintained in iso-sexual groups for approximately 3 weeks prior to data collection, Landsman *et al.* (1987) reported that male *G. petersii* exhibited higher PPSFs and shorter EODs than females, i.e. a sex difference opposite in direction to those found in field studies in related species

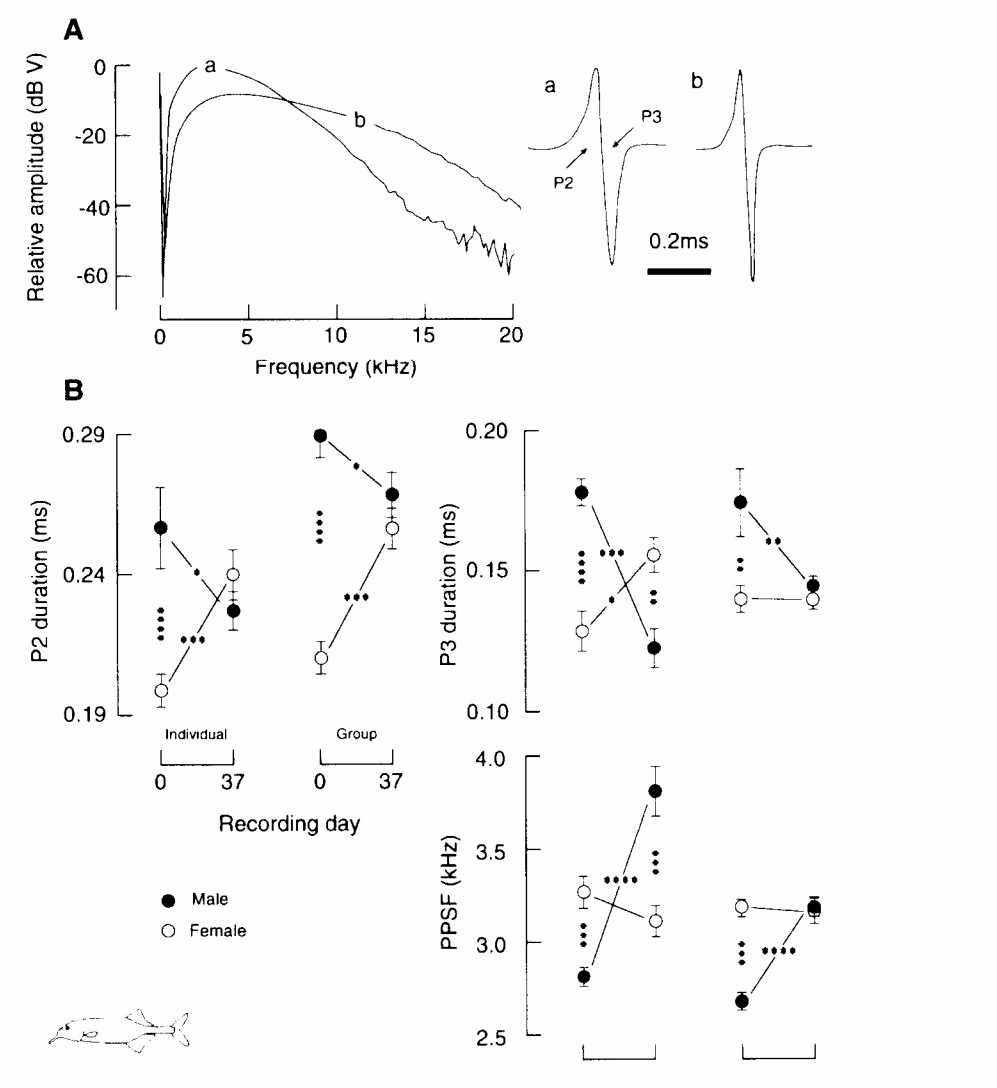


Fig. 13.15 Effects of captivity in the laboratory on sex differences in EOD phase durations and EOD-associated PPSFs in *Gnathonemus petersii*. (A) A Fourier transform and EOD recorded from a male on 'Day 0' (a) and from the same male after 37 days of laboratory captivity (b). Note decrease in EOD duration and shift in power spectrum following the captive period. Arrows and numbers refer to phases 2 and 3. (B) Quantitative data: means (± SEMs) of phases 2 (P2) and 3 (P3) durations, and PPSFs (males: n = 18; females: n = 17) for fish maintained in individual tanks (left panels; n = 7 males and 7 females), or group aquaria (right panels) with fish of the same sex (n = 7 males and 7 females) or mixed sexes (n = 4 males and 3 females). (Group data were pooled.) Asterisks (* $P < 0.05$; ** $P < 0.0$ 1; *** $P < 0.005$; **** $P < 0.0005$) indicate significant sex differences (vertical) and significant differences in means for each sex between days 0 and 37 (horizontal), respectively. All sex differences were either reversed or abolished by the end of the captivity period regardless of housing conditions. Phase 3 durations and PPSFs of males appear to be more affected by captivity than those of females (details of methods and statistical analyses: Landsman, 1991, 1 993a,b). Modified after Landsman (1991, 1993a).

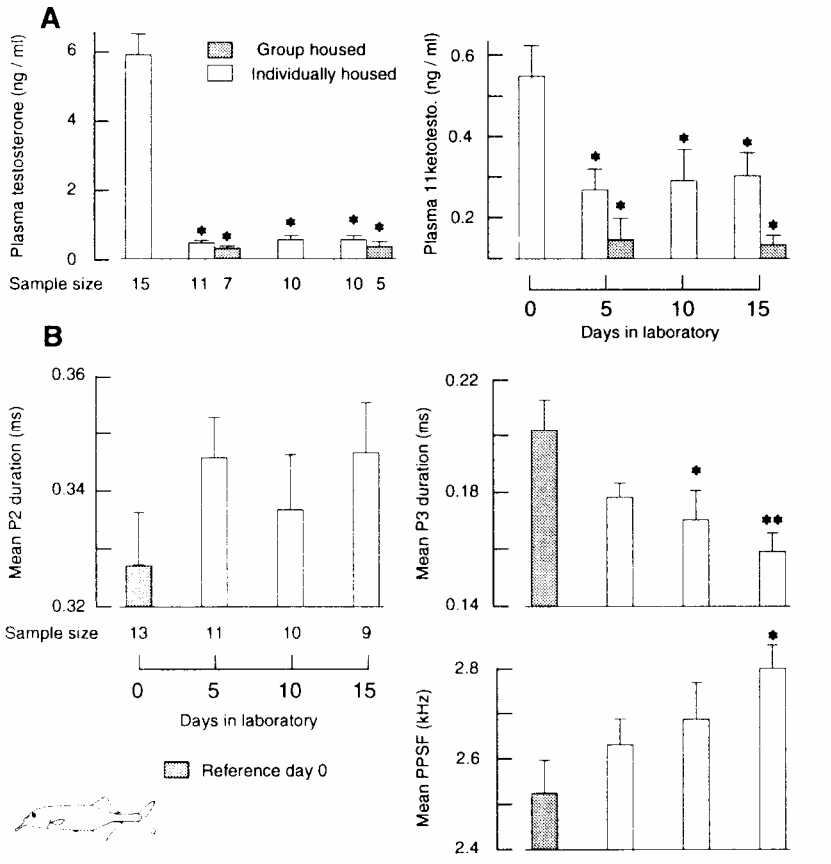


Fig. 13.16 Simultaneous effects of laboratory captivity on (A) plasma androgen levels, and (B) EODs in sexually mature male *Gnathonemus petersii* obtained from Nigeria during the rainy breeding season. (A) Mean (+ SEM) plasma levels of testosterone (T; left panel); and 11-ketotestosterone (right panel) on 'day 0' (day of import) 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 of captivity regardless of whether fish were maintained individually or in groups (*, $P < 0.001$ for T compared with 'day 0' levels; *, $P < 0.005$ for 11-ketotestosterone compared with 'day 0' levels). (B) Mean (+ SEM) P2 and P3 durations, and PPSFs for the same males on 'day 0' (day of import) and after housing in individual aquaria for 5, 10 or 15 days in captivity. No EOD data from two 'day 0' and one 'day 15' males were collected to control for handling effects on plasma hormone levels. Note decrease in phase 3 durations and increase in PPSFs with increasing time in captivity (*, $P < 0.05$ for phase 3 'day 10' value vs. 'day 0', and for PPSF 'day 15' value vs. 'day 0'; **, $P < 0.01$ for phase 3 'day 15' value vs. 'day 0' value.

(reviews: Bass, 1986a; Hopkins, 1986a; Kramer, 1990a), and opposite to those predicted by steroid hormone treatment (Landsman and Moller, 1988; Landsman *et al.*, 1990). Finally, a non-overlapping sex difference was found in *newly imported* fish, with males exhibiting lower PPSFs and

longer P2 and P3 durations than females (Landsman, 1993b), characteristics congruent with those found in hormone studies on this species and hormone and field studies on related species.

The effects of captivity on the EOD in *G. petersii* appear to be due to factors other than those considered to be 'stress-related'. The subjects maintained in individual aquaria showed more dramatic captivity-related EOD effects than those shown by subjects maintained in groups (Fig. 13.15 (B)). Subjects maintained in groups in this and in previous studies establish dominance hierarchies which involve much aggression (Bell *et al.,* 1974; Kramer, 1976a,b; Crockett, 1986). The subjects maintained in group aquaria displayed much biting and chasing over the entire experimental period, while individually housed subjects appeared calm and, when not scavenging for food, were at rest in their shelters (Landsman, 1993a). It would appear then that the subjects maintained in groups were more ‘stressed' than those maintained in the individual aquaria.

Further, as mentioned earlier, cortisol implants had no effects on the durations of individual EOD phases or the PPSF. In the same study, control fish implanted with cholesterol exhibited more variability in EOD characteristics than fish implanted with cortisol. This could, however, indicate a ceiling effect of cortisol on the EOD as it is possible that cortisol levels were already elevated in these subjects, and remained at high levels in the cortisol-implanted fish.

Testing the mormyrid captivity model

The EOD-related sex difference in *Brienomyrus brachyistius* (originating from Nigeria) is clearly under hormonal control (Fig. 13.4). Male *B. brachyistius* exhibit a lower P2/P3 duration ratio than females. Androgens decreased this ratio, while E_2 had no effect on this ratio. This steroid-dependent sex difference was present only in newly imported specimens, but was reversed, with females exhibiting lower ratios than males, following 20 days in captivity in the laboratory (Figs 13.17, 13.18).

The effects of captivity on the EOD in *G. petersii* and *B. brachyistius* can explain a number of inconsistencies regarding sex differences reported within and across studies involving electric fish. For example, in one study, female *E. virescens* were reported to discharge at higher frequencies than mature males (Hopkins, 1974b), while in another study, it was reported that males discharged at higher frequencies than females, but only during a pre-artificial rainy season (Westby and Kirschbaum, 1981). However, in the latter study, when the artificial rainy season was introduced, females increased their EOD frequencies and the sex difference was abolished. The authors claimed that gonadal recrudescence was responsible for the lack of an EOD sex difference and concluded that this species probably does not use

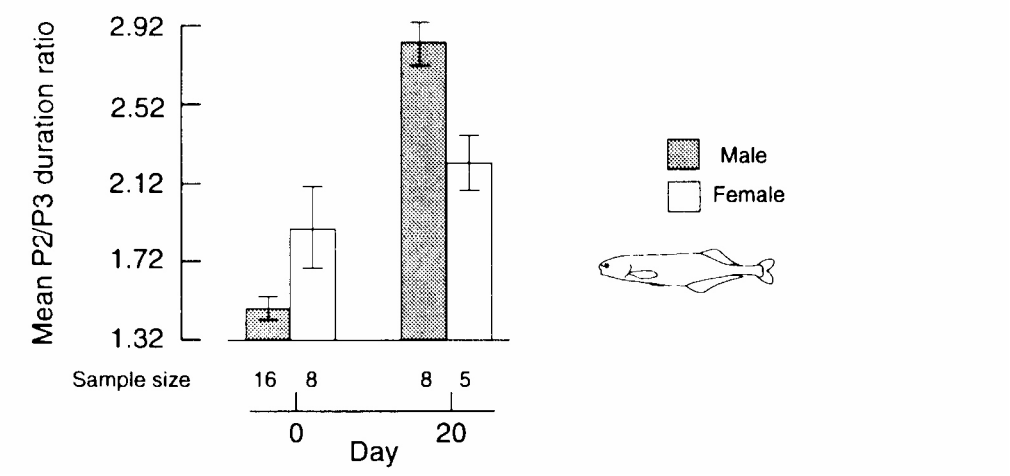


Fig. 13.17 Effects of captivity on the phase 2/phase 3 (P2/P3) duration ratio sex difference in the EOD of adult *Brienomyrus brachyistius*. On 'day 0' (day of import from Nigeria), males had a smaller mean (± SEM) P2/P3 duration ratio than females, $t(22) = 2.32$, $p < 0.025$. On 'day 20' (in the laboratory), males had a larger P2/P 3 duration ratio than females, $t(11) = 3.48$, $p < 0.005$. Modified after Landsman and Moller (unpublished).

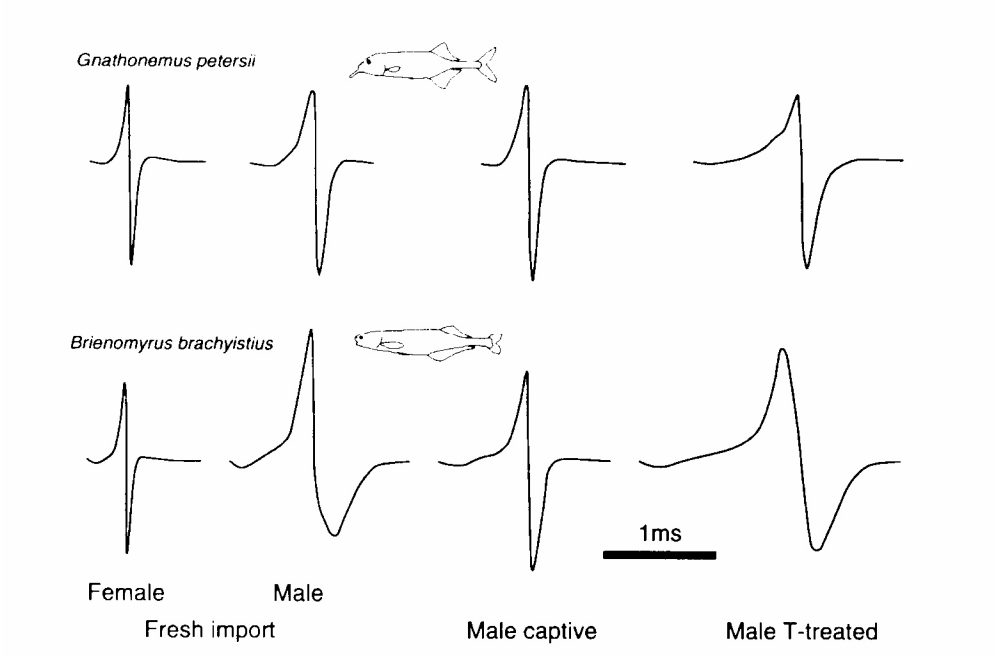


Fig. 13.18 Influences of captivity and testosterone (T) on the EOD waveform in male *Gnathonemus petersii* and *Brienomyrus brachyistius* (imported from Nigeria). For comparison, representative EODs from freshly imported specimens of both sexes and species are shown on the left. Note the shorter EOD phase durations of freshly imported females compared with those of freshly imported males of both species. The male's EOD resembles that of the female's after a period of captivity, but reverts to its original form (fresh import) following testosterone (T) treatment. Modified after Landsman and Moller (1993).

EOD rate 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 rate in this species is steroid sensitive (Leong, unpubl. data, in Meyer *et al.*, 1987) and a control group (not exposed to the rainy season condition) was not included, 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* EOD rates were always lower than female rates (Hagedorn and Heiligenberg, 1985). 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 negative phase (P2) of the EOD in *P. isidori* (Westby and Kirschbaum, 1977) could not be replicated (Westby and Kirschbaum, 1982). The latter study, however, reported a "clear and unambiguous sexual dimorphism within the major phases of the EOD itself" (p. 400), while Lucker and Kramer (1981) did not find any EOD-related sex difference in this species. No studies have yet investigated the possibility of hormonal control of the EOD in this species.

The EOD of weakly discharging electric fish proves to be an excellent behavior to study behavior-endocrine interactions and behavioral physiology. EODs provide a clear example of behavioral variability as a function of species, sex and of behavioral plasticity in response to changing environmental conditions. Many of the studies on these animals have been performed without adequate scientific designs or were confounded by captivity or other factors which resulted in disparate findings. But the plasticity of the EOD in response to a multitude of variables has led to the development of a viable model for the study of the effects of captivity on reproductive and behavioral physiology in vertebrates.

References

Arnold. S.J. (1977) The evolution of courtship behaviour in new world salamanders with some comments on old world salamandrids, in *The Reproductive* Biology of *Amphibians (eds* D.H. Taylor and S.I. Guttman), Plenum Press, New York, pp. 141-84.

Assem, H. and Hanke, W. (1981) Cortisol and osmotic adjustment of the euryhaline teleost, *Sarotherodon m*ossambicus. *Gen. Comp. Endocrinol.* **43,** 370-80.

Bass, A.H. (1986c) Species differences in electric organs of mormyrids: substrates for species-typical electric organ discharge waveforms. *J. Comp. Neurol.* **244,** 313-30.

Bass, A.H. and Hopkins, C.D. (1983) Hormonal control of sexual differentiation: changes in electric organ discharge waveform. *Science* **220**, 971-4.

Bass, A.H. and Hopkins, C.D. (1984) Shifts in frequency tuning of electroreceptors in androgen -treated mormyrid fish. *J. Comp. Physiol.* 15 5A, 713-24.

Bass, A.H. and Hopkins, C.D. (1985) Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes. *J. Comp. Physiol.* **156A,** 587604.

Bass, A.H. and Volman, S.F. (1987) From behavior to membranes: testosterone-induced changes in action potential duration in electric organs. *Proc. Natn. Acad. Sci. USA* **84**, 92958.

Bass, A.H., Segil, N. and Kelley, D.B. (1984) A steroid sensitive electromotor pathway in mormyrid fish: electric organ morphology, androgen receptor biochemistry and steroid autoradiography. *Soc. Neurosci. Abstr.* **10**, 927.

Bass, A.H., Denizot, J.-P. and Marchaterre, M.A. (1986a) Ultrastructural features and hormone-dependent sex differences of mormyrid electric organs. *J. comp. Neurol.* **254,** 511-28.

Bass, A.H., Segil, N. and Kelley, D.B. (1986b) Androgen binding in the brain and electric organ of a mormyrid fish. *J. Comp. Physiol.* **159A,** 535-44.

Bennett, M.V.L. (1971a) Electric organs, in *Fish Physiology, Vol. V* (eds W.S. Hoar and D.J. Randall), Academic Press, London, pp. 347-491.

Bennett, M.V.L. (1971b) Electroreception, in *Fish Physiology, Vol. V* (eds W.S. Hoar and D.J. Randall), Academic Press, London, pp. 493-574.

Bratton, B.C. and Kramer, B. (1988) Intraspccific variability of the pulse-type discharges of the African electric fishes, Pollimyrus isidori and Petrocephalus bovei (Mormyridae, Teleostei), and their dependence on water conductivity. *Exp.* ***Biol.*** **47**, 227-38.

Crawford, J.D. (1992) Individual and sex specificity in the electric organ discharges of breeding mormyrid fish (*Pollimyrus isidori*). 1. Exp. Biol. **164**, 79-102.

Elton. R.H. (1979) Baboon behaviour under crowded conditions, in *Captivity and Behaviour* (eds J. Erwin, T.L. Maple and G. Mitchell), Van Nostrand Reinhold, New York, pp. 123-38.

Erwin, J. and Deni, R. (1979) Strangers in a strange land: abnormal behaviours or abnormal environments? In *Captivity and Behaviors* (eds. J. Erwin, T.I. Maple and G. Mitchell). Van Nostrand Reinhold, New York, pp. 1-28.

Ferrari, M.B. and Zakon, H.H. (1993) Conductances contributing to the action potential of Sternopygus electrocytes. *J. Comp. Physiol.* **173A**, 28 1-92.

Freedman, E.G., Olyarchuk, J., Marchaterre, M.A. and Bass, A.11. *(1989) A* temporal analysis of testosterone-induced changes in electric organs and electric organ discharges of mormyrid fishes. *J. Ncurobiol.* **20**, 619-34.

Hagedorn, M. and Carr, C. (1985) Single electrocytcs produce a sexually dimorphic signal in South American electric fish, *Hypopomus occidentalis* (Gymnotiformes, Hypopomidae). *J. Comp. Physiol.* **156A,** 511-23.

Hagedorn, M. and Heiligenberg, W. (1985) Court and spark: electric signals in the courtship and mating of gymnotoid fish. *Anim. Behav.* **33**, 254-65.

Hannes, R.P. and Franck, 1). (1983) Diurnal variation of blood androgen and corticoid levels in male swordtails *Xiphophorus helleri. Zool. Jb. Physiol.* **87**, 337-41.

Harding, C.F. (1986) The role of androgen metabolism in the activation of male behavior, in *Reproduction: A Behavioral and Neuroendocrine Perspective. Ann. N. Y. Acad. Sci., Vol.* 474 (eds B.R. Komisaruk, H.F. Siegel., M.-F. Cheng and H.H. Feder), New York, pp. 371-8.

Hopkins, C.D. (1972) Sex differences in electric signaling in an electric fish. *Science* **176**, 1035-7.

Hopkins, C.D. (1974a) Electric communication in the reproductive behavior of *Sternopygus macrurus* (Gymnotoidei). Z. *Tierpsychol.* **35**, 518-35.

Hopkins, C.D. (1974b) Electric communication: functions in the social behavior of *Eigenmannia virescens. Behaviour* **50**, 270-305.

Hopkins, C.D. (1980) Evolution of electric communication channels of mormyrids. *Behav. Ecol. Sociobiol.* **7**, 1-13.

Hopkins, C.D. (1981a) On the diversity of electric signals in a community of mormyrid electric fish in West Africa. *Am. Zool.* **21**, 211-22.

Hopkins. C.D. and Bass, A.1 1. (19 8 1) Temporal coding of species recognition signals in an electric fish. *Science* **212**, 85-7.

Hopkins, C.D. (1986a) Behavior of' Mormyridae, in *Electroreception* (eds T.H. Bullock and W. Heiligenberg). Wiley, New York, pp. 527-76.

Ilan. Z. and Yaron. Z. (1983) Interference of o,p'DDD with interrenal function and cortisol metabolism in *Sarotherodon aureus* (Steindachner). *J. Fish Biol.* **22**, 657-69.

Keppel, G. (1982) *Design and Analysis. A Researcher's Handbook. 2*nd edn, Prentice Hall, Englewood-Cliffs, NJ. (see pp. 15 5-6).

Kirschbaum, F. (1983) Myogenic electric organ precedes the neurogcnic organ in Apteronotid fish. *Naturwissenschaften* **70**, 205-7.

Kramer, B. (1976a) The attack frequency of *Gnathonemus petersii* towards electrically silent (denervated) and intact conspecifics, and towards another mormyrid *(Brienomyrus niger). Bchav. Ecol. Sociobiol.* **1**, 425-46.

Kramer, B. (1976b) Electric signalling during aggressive behavior in *Mormyrus rume* (Mormyridae, Teleostci). *Naturwissenschaften* **63**, 48-9.

Kramer, B. and Otto, B. (1988) Female discharges are more electrifying: spontaneous preference in the electric fish, *Eigenmannia* (Gymnotiformes, Teleostei). *Behav. Ecol. Sociobiol.* **23**, 55--60.

Kramer, B. and Wcstby, G.W.M. (1985) No sex difference in the wavcform of the pulse type electric fish, *Gnathonemus petersii* (Mormyridae). *Experientia* **41**, 153031.

Landsman, R.E. (1990) *Seasonal, hormonally~controlled, phase-specific sex differences in electric organ discharges of the weakly electric fish, Gnathonemus petersii (Morrnyridae), and the effects of captivity on these discharges* (see also Psychol. Diss. Abstr., 1990, 1135 B), Phl) thesis, The City University of New York, New York, 272 pp.

Landsman, R.E. (1991) Captivity affects behavioral physiology: plasticity in signalling sexual identity. *Experientia* **47**, 11-8.

Landsman, R.E. (1993a) The effects of captivity on the electric organ discharge and plasma hormone levels in *Gnathonemus petersii* (Mormyriformes). *J. Comp. Physiol.* **172A**, 619--31.

Landsman. R.E,. (1993b) Sex differences in external morphology and electric organ discharges in imported *Gnathonemus petersii* (Mormyriformes). *Anim. Behav.* **46,** 417-29.

Landsman, R.E. and Moller, P (1988) Testosterone changes the electric organ discharge and external morphology of the mormyrid fish, *Gnathonemus petersii* (Mormvriformes). *Experientia* **44**, 900--3.

Landsman, R.E. and Moller, P. (1991) Laboratory captivity reverses sex differences in electric organ discharge in mormyrid fish, in *Proc. Fourth 1nt.* Symp. *Reprod. Physiol. Fish* 1991 (eds A.P. Scott, J.P. Sumpter, D.E. Kime and M.S. Rolfe), Norwich. UK, Fish Symp 91, p. 204.

Landsman. R.E. and Moller, P. (1993) Captivity and signal plasticity in mormyrid fish communication, in *Contributions of' Electrosensory Systerns to Neurobiology and Neurocthology. Proceedings of a Conference in Honor of the Scientific Career of Thomas Szabo* (eds C.C. Bell, C.D. Hopkins and K. Grant), *J. Comp. Physiol.* **173A,** 17 2 -3.

Landsman, R.E..]ou. S.H. and Moller, P. (1987) Stress obscures signalling of sexual identity in *Gnathonemus petersii* (Mormyriformes), in *Proc. Third Int. Symp. Reprod. P*hysiol. *Fish 1987* (eds D.R. Idler. L.W. Crim and J.M. Walsh), Memorial University of Newfoundland, St. Johns, p. 307.

Landsman, R.E., Harding. C,F.. Moller, P. and Thomas, P. (1990) The effects of androgens and estrogens on the external morphology and electric organ discharge waveform of *Gnathonemus petersii* (Mormyridae, Teleostei). *Horm. Behav.* **24**, 5 32-- 5 3.

Mazeaud, M.M. and Mazeaud, F. (1981) Adrenergic responses to stress in fish, in Stress and Fish (ed. A. D. Pickering), Academic Press, New York, pp. 49-75.

Mazeaud, M.M., Mazeaud, F. and Donaldson, E.M. (1977) Primary and secondary effects of stress in fish: some new data with a general review. *Trans. Am. Fish. Soc.* **3,** 201-12.

Meyer, J.H. (1983) Steroid influences upon the discharge frequencies of a weakly electric fish. *J. Comp. Physiol.* **153A**, 29-37.

Meyer, J.H. (1984) Steroid influences upon discharge frequencies of' intact and isolated pacemakers of weakly electric fish. *J. Comp. Physiol.* **154A**, 659- 68.

Meyer, J.H., Zakon, H.H. and Heiligenberg. W. (1984) Steroid influences upon the electrosensory system of weakly electric fish: direct effects upon discharge frequencies with indirect effects upon electroreceptor tuning. *J. Comp. Physiol.* **154A,** 625--31.

Meyer, J.H., Leong, M. and Keller, CIL (1987) Hormone-induced and ontogenetic changes in electric organ discharge and electroreceptor tuning in the weakly electric fish *Apteronotus. 1. Comp. Physiol.* **160A,** 383--94.

Miller, Lj and -Moore, F.L. (1983) Intracranial administration of corticolropin-like peptides increases incidence of amphibian reproductive behaviour. *Peptides* **4**, 729-311.

Mills, A. and Zakon, H.H. (1987) Coordination of' EOD frequencv arid pulse duration in weakly electric wave fish: the influence of androgens. *J. Comp. Physiol.* **161 A**, 41 '-30.

Mills, A., Zakon, H.H., Marchatcrre, M.A. and Bass, A.H. (1992) Electric organ morphology of *Sternopygus macrurus*, a wave-type weakly electric fish with a sexually dimorphic EOD. *J. Neurobiol.* **23**. 920-11

Moller, P. (1980) Electroperception. *Oceanus* **23**, 44-54.

Moore, F.L. and Deviche, P. (1988) Neuroendocrine processing of environmental information in amphibians, in Processing of Environmental Information in Vertebrates (ed. M.H. Statson), Springer, New York, pp. 20-45.

Moore, F.L. and Miller, L.J. (1983) Arginine induces sexual behaviour by acting on cells in the brain: corticosterone inhibits courtship behaviours of a male amphibian *Taricha granulosa*. *Peptides* **4**, 97-102.

Moore, F.L. and Miller, L.J. (1984) Stress-induced inhibition of sexual behavior: corticosterone inhibits courtship behaviours of a male amphibian *(Taricha grarmlosa). Horm. Behav.* **18**, 400-10.

Moore, F.L., Miller, L.J., Spielvogel, S.P., Rubisk, T. and Folkers, K. (1982) Luteinizing hormone-releasing hormone involvement in the reproductive behaviour of a male amphibian. *Neuroendocrinol.* **35**, 212-6.

Moore, M.C. and Marler, C.A. (1988) Hormones, behavior, and the environment: an evolutionary perspective, in *Processing of Environmental Information in Vertebrates* (ed. M.H. Stetson), Springer, New York, pp. 71-83.

Newton, B.I. (1982) Early stress effects on growth and adult behaviour in *Poecilia reticulata*. *Develop. Psychobiol.* **15,** 211-20.

Noakes, D.L.G. and Leatherland, I.F. (1977) Social dominance and interrenal cell activity in rainbow trout *Salmo gairdneri. Env.. Biol. Fishes* **2**, 131-6.

Peters, G., Delvanthal, H. and Klinger, M. (1980) Physiological and morphological effects of social stress in the cc], *Anguilla anguilla*. *Arch. Fischereiwiss.* **30**, 15 780.

Pickering, A.D., Possinger, T.G., Carragher, J. and Sumpter, J.P. (1987) The effects of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout *Salmo trutta*. *Gen. Comp. Endocrinol.* **68,** 249-50.

Rivier, C., Rivier, *1.* and Vale, *W. (1986)* Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. *Science* **231**, 607-9.

Safford, S.E. and Thomas, P. (1987) Effects of capture and handling on circulating levels of gonadal steroids and cortisol in the spotted trout, in *Reproductive P*hysiology *of Fish (eds* D.R. Edler and J.M. Walsh), University of Newfoundland, St. Johns, pp. 312.

Strange, R.I., Schreck, C.B. and Golden, J.T. (1977) Corticoid stress responses to handling and temperature in salmonida. *Trans. Arn. Fish. Soc.* **106**, 213-8.

Truscott, B., Walsh, J.M., Burton, M.P., Payne, I.T. and Idler, D.H. (1983) Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. *Comp. Biochem. Physiol.* **75**, 121 -30.

Westby, G.W.M. and Kirschbaum, F. (1977) Emergence and development of the electric organ discharge in the mormyrid fish, *Pollimyrus isidori*. 1. The larval discharge. *J. Comp. Physiol*. **122A,** 251-71.

Westby, G.W.M. and Kirschbaum, F. (1981) Sex differences in the electric organ discharge of *Eigenmannia virescens* and the effect of gonadal maturation, in *Advances in Physiological Sciences. Vol. 31: Sensory Physiology of Aquatic Lower Vertebrates* (eds T. Szabo and G. Cz6h), Pergamon Press, Akademiai Kiado. Budapest, pp. 179-94.

Westby, G.W.M. and Kirschbaum, F. (1982) Sex differences in the waveform of the pulse-type electric fish. *Pollimyrus isidori* (Mormyridac). *J. Comp. Physiol*. **145A,** 399-403.

Wingfield, J.C. (1988) Changes in reproductive function of free-living birds in direct response to environmental perturbations, in *Processing of E'nvironmental Inj6rination in Vertebrates* (ed. M.H. Stetson), Springer, New York, pp. 121-48.

Zakon, H.H. (1986) The electroreceptivc periphery, in *Electroreception* (eds T.H. Bullock and W. Heiligenberg), Wiley, New York, pp. 103-56.

Zakon, H.H. (1993) Weakly electric fish as model systems for studying long-term steroid action on neural circuits. *Brain Behav. Evol.* **42**, 242-51.

Zakon, H.H., Mills, A.C. and Ferrari, M.B. (1991a) Androgen-dependent modulation of the electroscnsory and electromotor systems of a weakly electric fish. *Sem. Neurosci.* **3**, 449-57.

Zakon, H.H., Thomas, P. and Yan, H.Y. (199 lb) Electric organ discharge frequency and plasma sex steroid levels during gonadal recrudescence in a natural population of *Sternopygus* macrurus. *J. Comp. Physiol*. **169A**, 49 3 -9.

Zoeller, R.T. and Moore, F.L. (1982) Duration of androgen treatment modifies behavioral response to arginine vasotocin in *Taricha granulosa*. *Horrn. Behav.* **16**, 23-30.