J Comp Physiol A (1991) 169:493–499

Journal of Comparative Physiology A Sensory, Neural, and Behavioral Physiology

Electric organ discharge frequency and plasma sex steroid levels during gonadal recrudescence in a natural population of the weakly electric fish *Sternopygus macrurus*

Harold H. Zakon[1+], Peter Thomas[2], and Hong-Young Yan[2*]

[1] Department of Zoology, University of Texas at Austin, Austin, TX 78712, USA
[2] Marine Science Institute, University of Texas, Port Aransas, TX 78373, USA

Accepted August 10, 1991

Summary. 1. *Sternopygus macrurus* were collected in Venezuela during the period of gonadal recrudescence in early or late dry season. Electric organ discharge (EOD) frequencies were recorded, blood samples were taken for analysis of steroid titers, and gonads were taken for determination of reproductive condition.
2. Mean EOD frequencies were significantly lower in males than in females in all samples. EOD frequency was inversely correlated with body length in males in late, but not early, dry season, and these parameters were never correlated in females.
3. Plasma levels of testosterone (T) and 11-ketotestosterone (11–KT), but not estradiol-17β (E_2), were inversely correlated with EOD frequency in males. No 11–KT was observed in plasma of females, and plasma levels of T and E_2 in females were comparable to those of males. Neither T nor E_2 were correlated with EOD frequency in females.
4. Testes collected in late dry season were more mature than those from early dry season; androgen levels and EOD frequency were correlated with testicular maturity. Ovaries collected in early dry season were immature, while those from late dry season were more mature. There was no relationship between EOD frequency and stage of ovarian development.
5. These results suggest that plasma androgens modulate EOD frequency in males during the reproductive season and that plasma E_2 has little relationship to EOD frequency in either sex.

Key words: Electric organ discharge – Electric fish – Androgens

[+] To whom offprint requests should be addressed

* Current address: Department of Zoology, University of Maryland, College Park, MD 20742, USA

Introduction

The weakly electric fish of South America (Gymnotiformes) and Africa (Mormyriformes) generate electric signals for electrolocation and communication with conspecifics. Some species of weakly electric fish produce electric organ discharges (EOD) that are a series of irregularly-occurring pulses; these are referred to as pulse fish. Other species, referred to as wave fish, produce a sinusoidal discharge. The EOD waveform is species-specific, and, moreover, sex differences in the EOD waveform of mature adults have been described in a number of species (Hopkins 1972, 1974; Bass and Hopkins 1983; Bass 1986; Hagedorn and Carr 1985; Hagedorn and Heiligenberg 1985; Landsman 1991; Landsman et al. 1990; Bratton and Kramer 1988). These sex differences in EOD waveform are behaviorally relevant in a number of species whose courtship behaviors have been studied (Hopkins 1974; Hopkins and Bass 1981; Bass and Hopkins 1983, 1985; Hagedorn and Heiligenberg 1985; Shumway and Zelick 1988).

In pulse fish, EOD pulse waveforms are similar in juveniles of both sexes and in adult females, but are typically longer in duration in mature males in breeding condition (Hopkins and Bass 1981; Hagedorn and Carr 1985; Shumway and Zelick 1988). In the wave fish *Sternopygus* and *Eigenmannia,* the EOD frequencies of mature males are lower than those of mature females (Hopkins 1972, 1974; Hagedorn and Heiligenberg 1985; Kramer 1985), whereas the opposite is true for the wave fish genus *Apteronotus* (Meyer et al. 1987).

In all cases studied to date, these sex differences appear to be under hormonal control. For example, gonadectomy increases the EOD frequency of males and lowers the EOD frequency of females in *Sternopygus* (Meyer 1983). Treatment of intact or gonadectomized fish of either sex with the adrogens testosterone (T), or

5 alpha-dihydrotestosterone (DHT) lowers EOD frequency, and EOD frequencies are raised with injections of estradiol-17β (E_2) (Meyer 1983; Mills and Zakon 1987). Additionally, injection of gonadally intact males, but not females, with human chorionic gonadotropin (hCG) causes increases in the levels of circulating androgens followed by a lowering of the EOD frequency, whereas this effect is not observed in gonadectomized fish (Zakon et al. 1990). Hormonal manipulations of other species of weakly electric fish also implicate gonadal steroids in the control of EOD characteristics (Bass and Hopkins 1983; Landsman 1991; Landsman et al. 1990; Meyer et al. 1987).

The above observations clearly implicate sex steroid hormones in modulating EOD frequency in *Sternopygus*. Yet, simultaneous measurements of EOD frequency and normal levels of these steroids have not been obtained from natural populations of *Sternopygus* or any other weakly electric fish. The purpose of this study, therefore, was to measure EOD frequency and the plasma levels of the two major teleostean androgens (testosterone and 11-ketotestosterone) and an estrogen (estradiol-17β) in individuals in a natural breeding population of *Sternopygus macrurus* collected during the period of gonadal recrudescence and to determine whether any relationship exists between the circulating levels of these hormones and the fish's EOD frequencies and gonadal condition.

Materials and methods

Individuals from a population of *Sternopygus macrurus* in the Llanos region of Venezuela were sampled in the early (January 3–14, 1986) or late (March 27–April 4, 1987, and April 19–23, 1989) dry season. In the Venezuelan Llanos the dry season begins in October or November and the rainy season begins in late April or early to mid-May (Ewell and Madriz 1968). These times were chosen based on Hopkins' (1974) observation that *Sternopygus* breeds in the late dry season in Guyana. Fish were collected from ponds or streams with a seine. Only fish greater than ~20 cm were used in this study.

Fish were taken from the net as rapidly as possible and placed in a large bucket of fresh pond or stream water where EOD frequency was recorded. A blood sample was taken, the fish's body length was measured (total length: from the tip of the snout to the tip of the tail), and its gonads were removed and fixed in alcohol-formalin-acetic acid (AFA) or 10% formalin.

EOD analysis. The EOD was amplified with a differential amplifier (Grass P–15), visualized on a battery-operated oscilloscope (Tektronix 214), and EOD frequency was measured with a Counter-Timer (Fluke 7260A). Temperature of the water in the bucket was measured with a digital thermometer and usually did not differ more than 1 °C from pond water temperature.

Since EOD frequency is temperature-dependent and water temperatures ranged from 26–31 °C, all values were converted to the EOD frequency expected at 25 °C using a temperature Q_{10} of 1.5 (Enger and Szabo 1968). We chose this temperature in order to compare our data with those of Hopkins (1974) who used this procedure. Nevertheless, all statistical tests were made using both original and transformed EOD frequency data, and the results were virtually identical.

Blood collection. Blood was removed from the vertebral sinus with a heparinized 25 gauge needle inserted through the tail within 15 min of capture (usually less than 10 min) to minimize stress-induced changes in circulating steroids (Safford and Thomas 1987). Blood was transferred to 1 ml propylene tubes containing a few grains of heparin and either centrifuged immediately (~800 × g) on a battery-operated D.C. centrifuge ('86, '87) or stored chilled in ice water for up to 6 h before centrifugation in a clinical centrifuge ('89). Plasma samples were transferred to 1 ml propylene tubes and stored on dry ice until they were brought back to the laboratory where they were stored at −15 °C until analysis.

To determine whether the blood collection procedure affected the steroid values obtained, blood was collected from 4 fish which had been maintained in the laboratory for over a year. Each sample was divided into two aliquots, one aliquot was centrifuged immediately and the other was kept in ice water for 6 h prior to centrifugation. Levels of T and E were found to be comparable to those measured in fish captured in the field and there were no significant differences in plasma steroid values obtained by these two collection procedures.

Steroid analysis. Steroid hormones were separated by column chromatography, and their titers were determined by radioimmunoassays validated for measurement in *Sternopygus* plasma as previously reported (Zakon et al. 1990). We determined that the E_2 antiserum (Radioassay Systems Laboratory) cross-reacted 22.3% with 16-ketoestradiol, 2.5% with estriol and 1.32% with estrone, and showed negligible cross-reactivity with androgens, progestins and corticosteroids. The assay could detect 2.5 pg E_2 per assay tube.

The T antiserum (Cambridge Medical Diagnostic) cross-reacted 28% with DHT, 17% with 11–KT and 1.5% with androstenedione. The assay could detect 1.25 pg T per assay tube.

The 11–KT antiserum (Helix Biotech Ltd.) cross-reacted 17% with DHT, 9% with T and 4% with 11β–OH-testosterone. The 11–KT assay could measure 5 pg steroid per assay tube.

Fifty to 100 µl of plasma were extracted with 2 ml hexane/ethyl acetate (70:30). The steroid extracts were dried under a stream of nitrogen and separated prior to radioimmunoassay by Sephadex LH20 chromatography (8.5 × 0.8 cm column, containing 1 g Sephadex LH20) using an elution solvent of iso-octane/toluene/methanol (62:20:15). The column was first flushed with 3 ml of solvent. A 2.75 ml fraction was then collected for the measurement of T. The next 0.5 ml of eluant, which contained small amounts of T and 11–KT, was discarded. A second fraction (2.25 ml) was then collected for measurement of 11–KT. Cross-contamination with the other androgens in both of the fractions was less than 10%. Each sample was assayed in duplicate for E, T and 11–KT by radioimmunoassay procedures described previously (Singh et al. 1988).

Histology. Fixed gonads were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The reproductive stage of the testes was classified according to Grier (1981). Testes primarily containing spermatogonia were considered in an early stage of development, those in which all stages of spermatogenesis were observed were considered to be in an intermediate stage of development, and those filled with mature sperm were considered to be in a late stage of development.

The stage of ovarian development was classified according to the criteria of Yamamoto and Yamazaki (1961). At all times during ovarian maturation, a range of oocyte developmental stages were present. The maturity of each ovary was classified according to the most advanced oocyte developmental stage observed. Oocytes in the late perinucleolar stage were small, had no yolk vesicles, and their nucleoli were situated in the periphery of the spherical nucleus. Oocytes in the primary yolk stage were characterized by yolk vesicles and globules appearing in the peripheral cytoplasm, a thickened follicular layer, and a polyhedral nucleus with randomly positioned nucleoli. The secondary yolk oocyte stage was characterized by an increase in the number of yolk globules in the inner cytoplasm resulting in an increase in size, and a rounded nucleus. The tertiary yolk stage was characterized by a further increase in oocyte size, by one or two well-formed rows of yolk vesicles in the oocyte periphery and by the clear presence of a micropyle at one pole of the oocyte.

Sectioning of ovaries containing mature oocytes beyond the tertiary yolk stage was unsuccessful, possibly because of their high yolk content and to inadequate fixation due to their large size. Oocytes from these fish were classified as beyond tertiary yolk stage.

Statistics. Data from males and females were compared by the Student's *t*-test with significance taken as $P<0.05$. Correlations were made between different variables using the Pearson correlation coefficient and these were judged as significant when $P<0.05$.

Results

Sampling biases

In all years more males were captured than females ('86: 66% males; '87: 65%; '89: 58%). The mean size of fish collected in the early dry season sample was smaller than the mean size of fish collected in the late dry season (Table 1). This may be related to our inability to sample the deeper parts of the ponds in the early dry season when the water level was higher. However, both sexes were of comparable size in each yearly sample (Table 1).

EOD frequencies

The EOD frequencies of males and females were significantly different in all 3 years (Table 1). Figure 1 illustrates the range of EOD frequencies of both sexes collected and bled in 1987. Note that while the EOD frequencies of males and females smaller than 40 cm show some overlap, the EOD frequencies of the largest individuals show none. These results were typical of both late dry season groups.

EOD frequency was significantly correlated with body length in males collected in the late dry season ('87: $r=-0.44$, $P<0.05$; '89: $r=-0.74$, $P=0.01$ sample sizes given in Table 1), but not in those collected in the early dry season ($r=-0.21$). No correlation was noted between length and EOD frequency in the females in any year.

Table 1. Sex, length, EOD frequency and number of fish in each year's sample (mean, s.d.). * indicates significant difference from corresponding male group ($P<0.05$)

	Early dry season	Late dry season	
	1986	1987	1989
Males			
Length (cm)	29.3 (4.0)	37.0 (8.0)	37.0 (12.7)
EOD freq. (Hz)	107.8 (7.1)	84.6 (23.2)	80.9 (22.3)
N=	14	21	15
Females			
Length (cm)	26.1 (5.3)	35.9 (7.37)	36.9 (8.5)
EOD freq. (Hz)	143.8 (42.6)*	129.3 (25.7)*	104.8 (24.9)*
N=	7	11	11

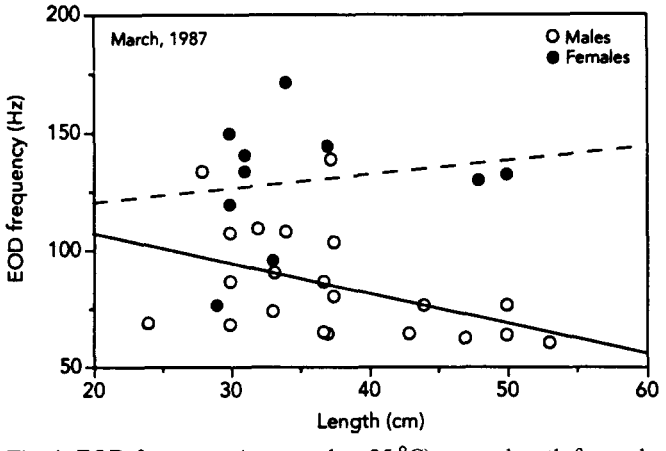

Fig. 1. EOD frequency (corrected to 25 °C) versus length for male and female *Sternopygus* captured in 1987 in the late dry season

Plasma sex steroid levels

Plasma T levels were not statistically different between the sexes in either late dry season group, but males had significantly more T than females in the early dry season group (Table 2). Both sexes had measurable E_2 in the plasma. Females showed statistically higher levels of E_2 than males only in the 1989 late dry season group, and no difference in the other years. As expected from a previous study (Zakon et al. 1990), female plasma had no 11-KT. Plasma levels of 11-KT in males were 58–80% higher than T levels.

T was significantly correlated with EOD frequency in males in both late dry seasons, and 11-KT was significantly correlated with EOD frequency in one late dry season (1987) group (Fig. 2). A correlation coefficient of the same magnitude was observed in the other late dry season group (1989). However, due to the smaller sample sizes in that year, it only approached statistical significance. There was no correlation between T or 11-KT and EOD frequency in the early dry season group. E_2 was not correlated with EOD frequency in males in any year. Neither T nor E_2 was correlated with EOD frequency in females in any of the groups.

Plasma levels of androgens T and 11-KT were highly correlated ('86: $r=0.78$; '87: $r=0.91$; '89: $r=0.86$) in males in all years, but no correlation existed between T or E_2 levels in any year. On the other hand, T and E_2 levels in plasma were significantly correlated in females in the 1987 late dry season group ($r=0.90$). T and E_2

Table 2. Plasma steroid levels in male and female *Sternopygus* collected during each reproductive season (mean, s.d.). * indicates significant difference from corresponding male group ($P<0.05$)

	1986	1987	1989
Males			
T	0.90 (0.32)	0.80 (0.99)	1.69 (1.47)
11-KT	1.45 (0.80)	1.48 (1.56)	2.61 (1.41)
E_2	0.19 (0.07)	0.15 (0.06)	0.39 (0.03)
N=	15	20	10
Females			
T	0.245 (0.13)*	1.42 (2.52)	0.91 (0.48)
E_2	0.164 (0.12)	0.28 (0.35)	0.95 (0.48)*
N=	7	11	10

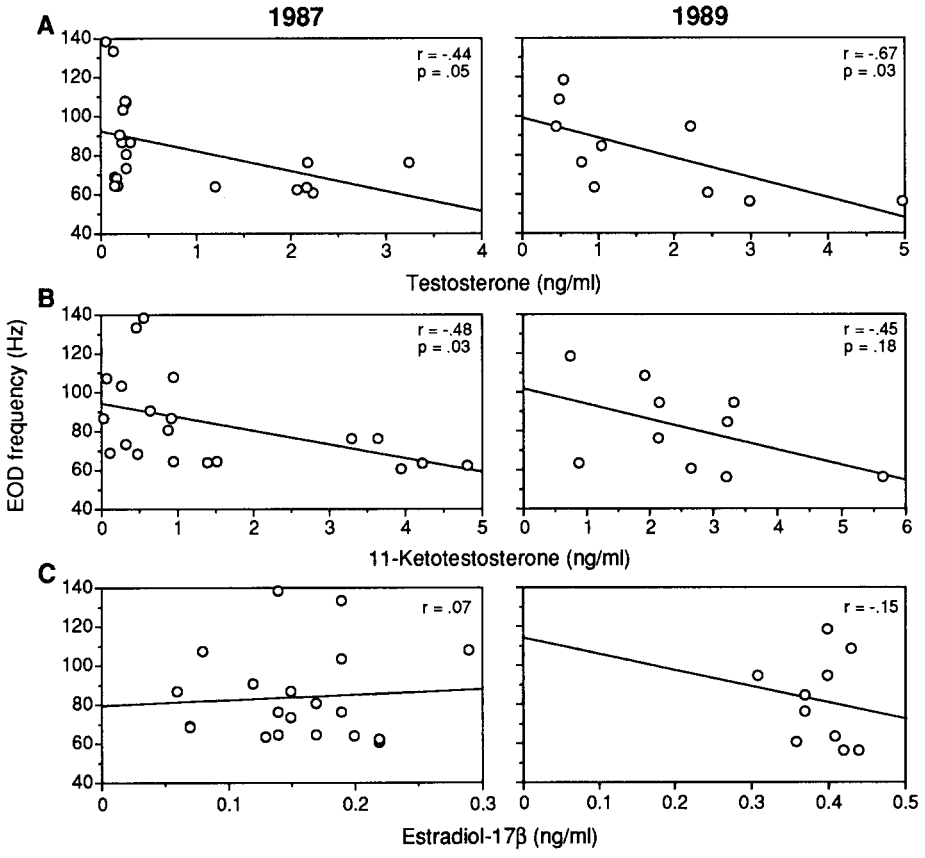

Fig. 2. EOD frequency versus plasma (**A**) testosterone, (**B**) 11-ketotestosterone and (**C**) estradiol-17β titers in male *Sternopygus* captured in the late dry season in 1987 (left column) or 1989 (right column)

were also correlated, but not significantly due to the smaller sample size, in the 1989 dry season female group ($r = 0.68$). These steroids were not correlated in the early dry season group.

Stage of gonadal development

Although no attempt was made to quantify the size of the gonads upon dissection in the field, it was obvious that the males from the early dry season had small testes, while those from the late dry season had larger testes.

Table 3. Number of *Sternopygus* collected each reproductive season at each stage of gonadal development. Testicular stages according to Grier (1981) and ovarian stages according to Yamamoto and Yamazaki (1961)

Sex	Stage	1986	1987	1989
Male	Early	3	5	0
	Intermediate	9	3	3
	Late	0	13	8
	Total $N=$	12	21	11
Female	Late perinucleolar	6	3	1
	Primary yolk	1	4	4
	Secondary yolk	0	0	4
	Tertiary yolk	0	4	0
	Beyond tertiary yolk	0	0	2
	Total $N=$	7	11	11

Histological sections revealed that the testes of males captured in the early dry season were in early or intermediate stages of development (Grier 1981). Testes from males captured in the 1989 late dry season were in intermediate or late stages of development, while those from males captured in the 1987 late dry season were in all 3 stages (Table 3).

The stage of testicular maturation was related to androgen levels insofar as mean levels of both androgens were almost twice as high in the 1989 late dry season group as in the early dry season group. The mean androgen levels of the 1987 late dry season group were similar to those of the early dry season group. However, the 1987 late dry season group showed the most variation in testicular stage, and androgen levels and levels of both androgens were significantly correlated with testicular stage within this group ($r = 0.51$, $P = 0.01$). There was no correlation between androgen level and testicular stage in the early dry season group and this correlation approached, but did not reach, significance in the 1989 late dry season group, again, probably due to the small sample size of this group.

EOD frequency was significantly inversely correlated with stage of testis development in the 1987 late dry season group, but not in the other groups. This is likely a result of the smaller variation in testis development in these latter groups compared with the greater variation in the 1987 late dry season group.

Ovarian condition varied between early and late dry season groups as well as within each group (Table 3). Not

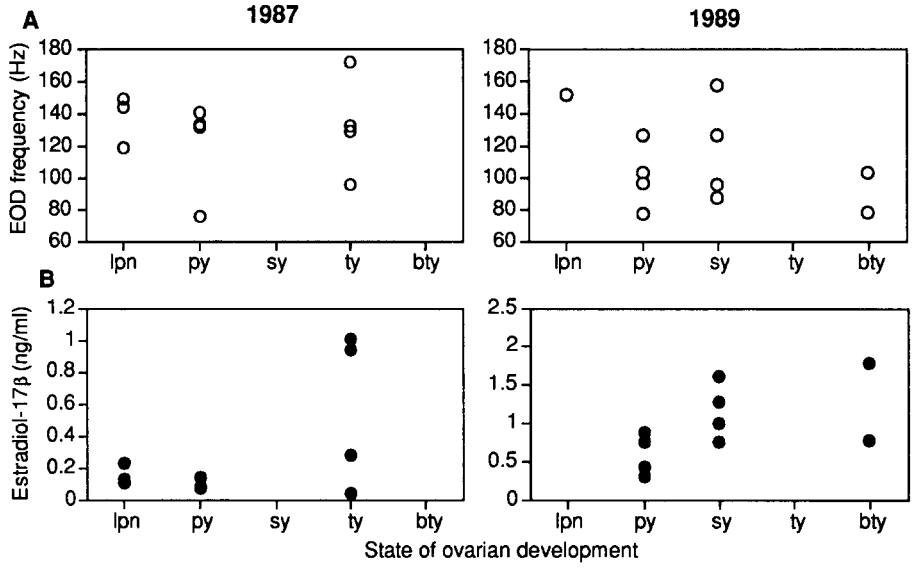

Fig. 3. EOD frequency (**A**) and plasma estrogen titer (**B**) as a function of state of ovarian maturation for female *Sternopygus* collected in 1987 (left column) or 1989 (right column). The categories along the abscissa denote progressively more advanced stages of oocyte maturation. *lpn* late perinucleolar stage, *py* primary yolk stage, *sy* secondary yolk stage, *ty* tertiary yolk stage, *bty* beyond tertiary yolk stage

surprisingly, ovaries from the early dry season were the least developed, while those from the late dry season were the most advanced. Scatterplots of EOD frequency versus ovarian stage show no obvious relationship between the two variables (Fig. 3A). On the other hand, plots of E_2 versus gonadal stage show that, in general, females with more mature gonads have higher levels of E_2 (Fig. 3B).

Discussion

Sex differences in EOD frequency

The results from this study corroborate other reports of a sex difference in the EOD frequency of *Sternopygus* recorded in the field (Hopkins 1972, 1974; Meyer 1983). Hopkins measured the EOD frequency of this species in Guyana, at the end of the dry season and at the beginning of the rainy season (April through June), and determined the reproductive status of the fish by visual inspection of the gonads in the field. He showed that the EOD frequencies of most nonreproductive and all reproductive (32–48 cm) females were in the range of 110–140 Hz, (2 out of 7 nonreproductive females were below 100 Hz), while all reproductively mature (26–60 cm) males had EOD frequencies between 50–90 Hz (all EOD frequencies adjusted to 25 °C with a temperature Q_{10} of 1.5). He reported only a single nonreproductive male with an EOD frequency close to 90 Hz. Sex differences were also observed in the EOD frequency of *Sternopygus dariensis*, a close relative of *Sternopygus macrurus* (Mago-Lecia 1978), collected during the late dry season in Panama (Meyer 1983).

We observed a negative correlation between EOD frequency and length in the males collected in the late dry season, as previously reported by Hopkins (1974). No correlation was observed between EOD frequency and length in males captured in the early dry season. However, since our sample did not include large individuals and it is those that have the lowest EOD frequencies, we cannot conclude that EOD frequency varies seasonally. Meyer (1983) has also reported a good correlation between length and EOD frequency in males ($r = -0.53$), and a weak one in females ($r = 0.26$), although the significance levels of these correlations were not tested. These observations show that the sex difference in EOD frequency is accentuated by the lowering of EOD frequency of large males, but with little or no change in female EOD frequency as a function of size or reproductive condition.

Relationship between plasma steroid levels and EOD frequency in males

The results of the present study show that there are significant correlations between plasma androgen titers, but not E_2 levels, and EOD frequency in a natural population of male *Sternopygus* collected during the period of gonadal recrudescence. We found that whereas males with low levels of T or 11-KT had a broad range of EOD frequencies, all males with high plasma levels of androgens had low EOD frequencies. Thus, while high levels of androgens appear to be a dominant factor in determining EOD frequency, other factors may influence EOD frequency in males with low androgen levels.

Previous laboratory studies have provided convincing evidence that androgens lower EOD frequency in *Sternopygus:* male EOD frequencies rise after gonadectomy; treatment of intact or gonadectomized fish of either sex with T, dihydrotestosterone (DHT) or 11-KT lower EOD frequency; injection of males with human chorionic gonadotropin increases plasma androgen titers, and this is followed by a decrease in EOD frequency (Meyer 1983; Mills and Zakon 1987; Zakon et al. 1990). The correlation between the plasma levels of androgens and EOD frequency reported in this study complements these laboratory studies and shows that naturally-occurring sex differences in EOD frequency in this species are likely to be mediated by endogenous androgens.

One possible interpretation of our results is that 11–KT, but not T, is the dominant steroid in determining EOD frequency in *Sternopygus*. T is present in the plasma of both sexes in comparable levels, but is only correlated with EOD frequency in males. Both T and 11–KT levels are highly correlated with EOD frequency in males. However, T and 11–KT levels are themselves highly correlated. Therefore, the high correlation between T and EOD frequency may be a result of the high correlation of T to levels of 11–KT rather than an indication of its potency to modulate EOD frequency. However, the specificity of the androgen receptor in *Sternopygus* is not known, and there is no evidence for marked differences in specificity of the receptor in teleosts for the various androgens present in teleost plasma (Callard and Callard 1987). In addition, T can be converted at the target tissue of teleosts to DHT (Pasmanik and Callard 1988; Callard et al. 1990). We have preliminary evidence that DHT is present in the plasma of mature male *Sternopygus* (Thomas and Zakon, unpubl.). Thus, while the correlation between EOD frequency and the two androgens measured in this study is good, the contributions of other steroids at the peripheral tissues cannot yet be ruled out.

Interestingly, plasma collected from males during the period of testicular recrudescence also contained small amounts of E_2. Low plasma concentration of E_2 have also been detected in males of several other teleost species (Fostier et al. 1983, 1987) but it is not known whether the hormone has a role in the control of the reproductive cycle in male fish.

Relationship between plasma steroid levels and EOD frequency in females

The relationship between EOD frequency and plasma sex steroid levels in females is less clear. In this study no correlation was found between T or E_2 and EOD frequency in females although E_2 titers were higher in fish whose ovaries were more mature (i.e. late perinucleolar stage vs. tertiary yolk stage). In many species of fish sex steroids gradually rise during ovarian recrudescence and peak prior to spawning (Campbell et al. 1976; Wingfield and Grimm 1977; Scott et al. 1980; MacKenzie et al. 1989; Thomas et al. 1987). In this study, females were sampled over a wide range of ovarian conditions from the late perinucleolar to the tertiary yolk stage and two fish were beyond the tertiary yolk stage. It is possible, then, that female *Sternopygus* show a relationship between EOD frequency and circulating sex steroid levels only at the latest stages of their reproductive cycle.

A second possible explanation is that another steroid hormone, in particular another estrogen, may play the dominant role in determining EOD frequency in females. In a previous study, Sephadex LH20 chromatography of female *Sternopygus* plasma revealed that a portion of the immunoreactivity did not always elute in the position of E_2, indicating the possible presence of other estrogens (Zakon et al. 1990). Therefore, in the present study we also performed a direct assay without column separation to determine total estrogens whenever sufficient plasma was available. Total estrogen levels in females, however, showed no relationship ('87) or, at best, a weak nonsignificant correlation ('89) with EOD frequency (Zakon, Thomas, Yan, unpubl.).

As noted above, there is overlap in the EOD frequencies of medium-sized males and females, but the largest most reproductively mature fish show no overlap in EOD frequency (Hopkins 1974; this study). Furthermore, EOD frequency is correlated with body length in males but not in females. It is conceivable, therefore, that the sex difference in EOD frequency in the most mature fish is solely caused by the lowering of male EOD frequencies while female EOD frequencies remain unchanged. This would be in keeping with observations made in both gymnotiform and mormyriform pulse fish in which juveniles and mature females produce the same EOD waveform while that of the males changes upon maturity (Bass and Hopkins 1985; Hagedorn and Carr 1985; Landsman 1991). Nevertheless, the EOD frequencies of female *Sternopygus* (Zakon, unpublished) and their close relative *Eigenmannia* (Hagedorn and Heiligenberg 1985), increase as these fish become gravid under simulated laboratory breeding conditions indicating that EOD frequency of females may shift upwards under certain conditions.

Although the results from this study suggest that there is no relationship between plasma estrogen and EOD frequency in females, another study has shown that EOD frequency increases after daily injections of E_2 in gonadectomized fish of both sexes (2.5–20 μg/g bw, resulting in unknown plasma levels) (Meyer 1983). This apparent paradox would be resolved if EOD frequency were dependent on local concentrations of E_2 produced by aromatase in the pacemaker nucleus. Since plasma levels of E_2 might not be indicative of the levels in the pacemaker nucleus, one might expect a poor correlation between EOD frequency and plasma E_2. On the other hand, an injection of E_2 that resulted in high plasma levels comparable to the local levels in the pacemaker might be sufficient to cause an increase in EOD frequency. In support of this idea, Pasmanik and Callard (1985, 1988) have shown that teleost brain possesses levels of aromatase 100- to 1000-fold higher than comparable regions in mammalian brain and that most of the estrogen in the brain is the result of local aromatization.

In conclusion, this study demonstrates a strong correlation between plasma androgen titers and EOD frequency in male *Sternopygus*, but indicates little or no relationship between plasma T or E_2 and EOD frequency in females. Further studies measuring the plasma steroid levels of hormones in females brought into breeding condition in the laboratory and examining the potential role of aromatization in the pacemaker nucleus must be conducted to determine whether the EOD frequency of *Sternopygus* is modulated by estrogens.

Acknowledgements. We would like to acknowledge the generosity of Drs. A.N. Popper and C. Hubbs for the loan of equipment; Beth Hawkins and Karen Dostal for performing the RIAs; Janet Young for making the figures; Dr. Eliot Brenowitz for reading an early version of this manuscript; Sr. Guillermo Feo for his invaluable help in the field; Sra. Edna Feo for her hospitality and motherly

care in Caracas; Dr. Donald Taphorn for his hospitality and the use of his facilities at UNELLEZ in Guanare, Venezuela and Mike Ferrari, John Patterson, Emily Guzman, and Drs. Alice Mills, Kip Keller and John Dye without whose help in the field this study would have been impossible. This study was funded by the following grants: NSF BNS–8606744, NIH R01 NS25513, NIH RCDA K04 NS01278 (HZ) and NIEHS E504214 (PT).

References

Bass AH, Hopkins CD (1983) Hormonal control of sexual differentiation: changes in electric organ discharge waveform. Science 220:971–974

Bass AH, Hopkins CD (1985) Hormonal control of sex differences in the electric organ discharge (EOD) of mormyrid fishes. J Comp Physiol A 156:587–604

Bratton BO, Kramer B (1988) Intraspecific variability of the pulse-type discharges of the African electric fishes, *Pollimyrus isidori* and *Petrocephalus bovei* (Mormyridae, Teleostei), and their dependence on water conductivity. J Exp Biol 47:227–238

Callard G, Callard I (1987) Sex steroid receptors and non-receptor binding proteins. In: Norris PO, Jones RE (eds) Reproductive endrocrinology of fish, amphibians and reptiles. Plenum Press, New York, p 355

Callard G, Schlinger B, Pasmanik M (1990) Nonmammalian vertebrate models in studies of brain-steroid interactions. J Exp Zool, Suppl 4:6–16

Campbell CM, Walsh JM, Idler DR (1976) Steroids in the plasma of the winter flounder (*Pseudopleuronectes americanus* Walbaum). A seasonal study and investigation of steroid involvement in oocyte maturation. Gen Comp Endocrinol 29:14–20

Enger PS, Szabo T (1968) Effect of temperature on discharge rates of the electric organ of some gymnotids. Comp Biochem Physiol 27:625–627

Ewell JJ, Madriz A (1968) Zonas de Vida de Venezuela. Ministerio de Agricultura y Cria. Caracas, Venezuela

Fostier A, Jalabert B, Billard R, Breton B, Zohar Y (1983) The gonadal steroids. In: Hoar WS, Randall DJ, Donaldson EM (eds) Fish physiology vol IXA. Academic Press, New York, pp 277–372

Fostier A, Le Gac F, Loir M (1987) Steroids in male reproduction. In: Idler DR, Crim LW, Walsh JM (eds) Proceedings of the third international symposium on reproductive physiology of fish. Memorial University Press, St. John's, pp 239–245

Grier HJ (1981) Cellular organization of the testis and spermatogenesis in fishes. Am Zool 21:345–357

Hagedorn M, Carr C (1985) Single electrocytes produce a sexually dimorphic signal in a South American electric fish *Hypopomus occidentalis* (Gymnotiformes, Hypopomidae). J Comp Physiol A 156:511–523

Hagedorn M, Heiligenberg W (1985) Court and spark: electric signals in the courtship and mating of gymnotid fish. Anim Behav 33:254–265

Hopkins CD (1972) Sex differences in signalling in an electric fish. Science 176:1035–1037

Hopkins CD (1974) Electric communication in the reproductive behavior of *Sternopygus macrurus*. Z Tierpsychol 35:518–535

Hopkins CD, Bass AH (1981) Temporal coding of species recognition signals in an electric fish. Science 212:85–87

Kramer B (1985) Jamming avoidance in the electric fish *Eigenmannia:* harmonic analysis of sexually dimorphic waves. J Exp Biol 119:41–69

Landsman RE (1991) Captivity affects behavioral physiology: plasticity in signaling sexual identity. Experientia 47:31–38

Landsman RE, Harding CF Moller P, Thomas P (1990) The effects of androgens and estrogen on the external morphology and electric organ discharge waveform of *Gnathonemus petersii* (Mormyridae, Teleostei). Horm Behav 24:532–553

MacKenzie DS, Thomas P, Farrar SM (1989) Seasonal changes in thyroid and reproductive steroid hormones in female channel catfish *(Ictalurus punctatus)* in pond culture. Aquaculture 78:63–80

Mago-Lecia F (1978) Los peces de la familia Sternopygidae de Venezuela. Acta Científica Venezolana 29:89

Meyer JH (1983) Steroid influences upon the discharge frequency of a weakly electric fish. J Comp Physiol 153:29–38

Meyer JH, Leong M, Keller CH (1987) Hormone-induced and ontogenetic changes in electric organ discharge and electroreceptor tuning in the weakly electric fish *Apteronotus*. J Comp Physiol A 160:385–394

Mills A, Zakon HH (1987) Coordination of EOD frequency and pulse duration in a weakly electric wave fish: the influence of androgens. J Comp Physiol A 161:417–430

Pasmanik M, Callard GV (1985) Aromatase and 5 alpha reductase in the teleost brain, spinal cord and pituitary gland. Gen Comp Endocrinol 60:244–251

Pasmanik M, Callard GV (1988) Changes in brain aromatase and 5 alpha reductase activities correlate significantly with seasonal reproductive cycles in goldfish *(Carassius auratus)*. Endocrinology 122:1349–1356

Safford SE, Thomas P (1987) Effects of capture and handling on the circulating levels of gonadal steroids and cortisol in the spotted seatrout, *Cynoscion nebulosus*. In: Idler DR, Crim LW, Walsh JM (eds) Proceedings of the third international symposium on reproductive physiology of fish. Memorial University Press, St. John's, p 312

Scott AP, Bye VJ, Baynes SM (1980) Seasonal variations in sex steroids of female rainbow trout (*Salmo gairdneri* Richardson). J Fish Biol 17:587–592

Shumway CA, Zelick RD (1988) Sex recognition and neuronal coding of electric organ discharge waveform in the pulse-type weakly electric fish, *Hypopomus occidentalis*. J Comp Physiol A 163:465–478

Singh H, Griffith RW, Takahashi A, Kawaichi H, Thomas P, Stegeman JJ (1988) Regulation of gonadal steroidogenesis in *Fundulus heteroclitus* by recombinant salmon growth hormone. Gen Comp Endocrinol 72:144–153

Thomas P, Brown NJ, Trent JM (1987) Plasma levels of gonadal steroids during the reproductive cycle of female spotted seatrout *Cynoscion nebulosus*. In: Idler DR, Crim LW, Walsh JM (eds) Proceedings of the third international symposium on reproductive physiology of fish, Memorial University Press, St. John's, p 219

Wingfield JD, Grimm AS (1977) Seasonal changes in plasma cortisol, testosterone and oestradiol-17β in the plaice (*Pleuronectes platessa* L.), Gen Comp Endocrinol 31:1–11

Yamamoto K, Yamazaki F (1961) Rhythm of development in the oocyte of the gold-fish, *Carassius auratus*. Bull Fac Fish Hokkaido Univ 12:93–110

Zakon HH, Yan H-Y, Thomas P (1990) Human chorionic gonadotropin-induced shifts in the electrosensory system of the weakly electric fish, *Sternopygus*. J Neurobiol 21:826–833