Exp Biol (1988) 47: 227–238

**Experimental Biology**

# 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

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**Summary.** The electric organ discharge (EOD) of the mormyrid *Pollimyrus isidori* is a short pulse with three phases: (1) weak head positive (P1); (2) strong head negative (N); (3) weak head positive (P2).

1. At a stable water conductivity (100 μS/cm), which is near the upper end of the natural range in tropical Africa, there was a statistically significant difference between the sexes only in one of five EOD parameters, the P-ratio. The P1-amplitude was lower than the P2-amplitude (i.e. P1/P2 < 1) in males ($N = 10$), while, on average, the opposite (P1/P2 > 1) was true for females ($N = 14$). Because of wide overlapping we do not consider this sex difference to be a sexual dimorphism. The difference between males and females could be due to well-known biophysical and physiological reasons (discussed later) and need not be the result of intraspecific selection (such as female choice).

2. Water conductivity seriously affected the EOD waveform. The P-ratio decreased in 2/3 of our fish (16 out of 24), as conductivity increased from 5 to 200 μS/cm, causing 6 out of 14 females to change from a P-ratio of > 1 to a P-ratio of < 1, becoming more "male-like". P1 amplitude increased with decreasing conductivity in the EODs of 5 out of 10 males to a more "female-like" shape (P-ratio > 1). The P-ratio changed only slightly when above a conductivity of 200 μS/cm. The N-wave duration increased with decreasing conductivity, while the peak amplitude frequency of an EOD amplitude spectrum decreased.

3. Long-term stability was found to be poor in the EOD of 1 female (better in 2 other fish), which changed from a "female-like" waveform (P-ratio > 1) to a "male-like" waveform (P-ratio < 1 over the whole conductivity range) without apparent reason within 120 days.

4. The EOD waveform of *Petrocephalus bovei* did not show a sex difference. Decreasing conductivity affected the EOD of *P. bovei* in a similar way to most *P. isidori*: the P1-wave increased and the P2-wave decreased, while the N-wave broadened strongly.

5. The occurrence of multiple discharges per primary neural command signal at very low conductivities, indicates that *P. isidori* is adapted to conductivities above 17 μS/cm, and *P. bovei* to those above 5 μS/cm.

6. Because of both the lack of a distinct sexual dimorphism, and the dependence of EOD waveform on water conductivity, the EODs of both species do not appear to be the unequivocal signatures needed for sex recognition, or for the distinction from other species (if the EODs are of similar spectral amplitudes). Alternative mechanisms are known that would be better for these purposes.

**Key words:** Electric organ discharge – Pulse waveform – Intraspecific variability – Water conductivity – Communication signal

## Introduction

The weakly electric freshwater fish of the African family Mormyridae have been studied with regard to the waveforms of their electric organ dis-

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charges (EODs). Intraspecific, sexually dimorphic differences in waveform, and spectral peak frequency, in *Pollimyrus isidori* have been suggested, that might serve in identification during courtship (Westby and Kirschbaum 1982).

Sexually dimorphic EODs are also attributed to a few other mormyrids (see reviews by Bass 1986; Hopkins 1986), but these fish do not seem to be identified systematically: these are (1) "*Brienomyrus brachyistius* (long biphasic)"; (2) "*B. brachyistius* (triphasic)"; and (3) *Stomatorhinus corneti* (Hopkins 1981), alias *S. walkeri* (Moller 1980), and (4) "*Hippopotamyrus batesii* (triphasic)". *Gnathonemus petersii*, a species in which an intraspecific variability analysis was carried out, lacks a sex difference in EOD waveform (Kramer and Westby 1985).

Mormyrid EOD waveforms are believed to be reliable species characters (or, in the few suggested cases, sex characters), although there is considerable inter-individual, long-term, and temperature-correlated variability (Lücker and Kramer 1981; Westby and Kirschbaum 1982; Kramer and Westby 1985), which has barely been investigated.

The electric current produced by the electric organ of a mormyrid is subject to an external resistive load (Harder et al. 1964; Bell et al. 1976), that varies widely in natural African habitats due to fluctuating water conductivity (Gosse 1963; Bénech and Quensière 1983). The resistive load also affects the range of communication (Squire and Moller 1982).

The present study investigates the inter-individual variability of EODs in *P. isidori* and *Petrocephalus bovei* in a wide range of water conductivities, using the methods of Kramer and Westby (1985). The range of conductivities tested includes those found in the natural habitats. The aim of these investigations is to determine the reliability of the two species' waveforms, as a sex or species recognition cue. A short account of the results was given in Bratton and Kramer (1986).

## Materials and methods

### Fish used

The two mormyrids studied in this investigation are *Pollimyrus isidori isidori* (Valenciennes 1846) (10 males, 14 females, 6.6–8.8 cm total body length), and *Petrocephalus bovei* (Valenciennes 1846) (5 males, 5 females, 6.4–8.0 cm standard length). The maximum standard lengths are 9.0 cm in both species (Gosse 1984). The distribution of *P. bovei* is the Nile, Chad, Niger, Volta, Gambia and Senegal basins. The distribution of *P. isidori* is specified as the Nile, Gambia, Middle Niger, Volta and Chad basins, and coastal rivers of Ivory Coast (Gosse 1984).

The fish were imported from Ikea (Nigeria) by a tropical fish dealer. The *P. isidori* were housed in separate 45 l aquaria (water conductivity 100±10 μS/cm, temperature 27±2°C, pH close to 6.8), before being used in tests. Fish were taken from the imported stock, including three males and three females that were spawning during these experiments. The *P. bovei* were held in a community tank for 3–6 weeks (100±5 μS/cm, 27±2°C) before use. Aquaria were maintained on a 12 h/12 h light-dark cycle and the fish were fed daily on *Chironomus* larvae.

The sex of the *P. bovei* was determined by inspection of the anal fin bases, which is a reliable criterion in this species (and confirmed by gonad histology in 7 of the 10 fish; for histological techniques, see Lücker and Kramer 1981). This is an unreliable method in *P. isidori*, where only the largest and fully mature males showed the characteristic indentation of the base of the anal fin (Lücker and Kramer 1981; Kirschbaum 1987). The sex of the *P. isidori* was determined behaviorally, by ascertaining their EOD latency response type to free-running stimulus pulses of 8–10 Hz. A Preferred Latency Response (PLR) of around 12 ms is the male type response, while a preferred Latency Avoidance (PLA) is the female type response, and no response indicates juveniles (Kramer 1978; Lücker and Kramer 1981). In *P. bovei* only one latency type (PLA) is found in both sexes (Lücker 1982).

### *Variability of waveform at stable conductivity*

Fish were entered, 24 h before tests, into the experimental tank (74×39×41 cm high, filled with 100 l, at 27±1° C), and fitted with 2 carbon rod electrodes 47 cm apart (5 cm long, 6 cm from bottom; the "tank electrodes"), and a porous hilding tube (9 cm long, 4.5 cm inside diameter) centered between them. Water conductivity was 100±5 μS/cm, which is near the upper end of the natural range (see Discussion).

All EOD recordings were made with unrestrained, but stationary, fish resting in the porous tube. Four EODs per fish were recorded over a period of 1 h (1 every 15 min). EODs were differentially amplified (100×, 1 Hz–100 kHz bandpass), and digitized using a Vuko transient recorder (VK12-2 Scope memory), at a sampling rate of 2 MHz and with an 8 bit vertical resolution. Digitized waveform data were stored on disk, and plotted using a Hewlett packard HP85 or HP9825B computer linked to a plotter. Fast Fourier analysis (of 1024 points) was performed on centered EODs, digitized at 100 kHz sampling rate using an HP9825B computer and Vuko transient recorder.

Minor variations in the recorded waveform of individuals were noted with varying conditions which were therefore kept uniform. These details concern: electrodes used, distance between electrodes, and fish direction in the tube. A tape-recorder was not used in the present study, because, even with a high quality machine, the waveforms of mormyrid pulse-EODs are distorted due to the limitations of magnetic tapes (Kramer and Weymann 1987).

### *Conductivity effects on waveform*

A series of experiments were conducted on 24 *Pollimyrus isidori*, and 3 *Petrocephalus bovei* to ascertain the effects of water conductivity (external loading) on the waveform. A 45 l aquarium with carbon electrodes (40 cm apart) and a centered porous tube (15 cm long, 4.5 cm inside diameter) were used. To begin the experiment at the lowest conductivity possible, the tank was filled with de-ionized water at 26±1° C (pH varied from 8.2, at the lowest conductivity used, to 6.5; the mean pH was 7.2±SD 0.4). A fish was given 24 h to acclimatize to the water (1.0–1.8 μS/cm; readings taken before and after acclimatization) before the waveform was digitized and plotted. Four EOD waveforms were sampled for each stable conductivity during a period of 1 h, after which tap water (500 µS/cm, 27° C) was added to, or exchanged with, water in the tank. Fish were allowed at least 1 h to acclimatize to a new conductivity before the next measurement. Tests were also run from highest to lowest conductivity in 4 fish; a few measurement series were repeated after several days, with extended periods of acclimatization between each measurement (2–6 h). The results were unchanged from those previously obtained (as may be expected from Bell et al. 1976). Conductivity was measured with a WTW-LF-56 (Wissenschaftlich-Technische Werkstätten) conductivity meter and LTA 100/k electrode.

Further data on the neural command waves, post-synaptic potential waves preceding the EOD, and post-EOD occurring waves at low conductivity were recorded (using the carbon rod electrodes), either by direct photography from the oscilloscope, or digitized using a Nicolet 1074 digital analyzer (100 kHz sampling rate, 12 bit resolution) with models SD-71-B and SW-71-A plug-ins. Fish were given 24 h to acclimatize to the lowest conductivity before the first measurement was taken. When multiple discharges were observed, conductivity was slowly increased at a rate of approximately 5 µS/cm, until the last EOD of a multiple EOD series (if there were more than two) disappeared, or the amplitude of the secondary EOD decreased. To determine the threshold of the secondary EOD, tap water was added dropwise (at a very low rate) until the secondary EOD disappeared. For statistical tests we used Siegel (1956).

## Results

The EODs of *P. isidori* and *P. bovei* resemble an extracellularly recorded, nerve action-potential with three phases: (1) weak head positive (P1); (2) strong head negative (N); and (3) weak head positive (P2) (Fig. 1). These discharges are, however, much faster than nerve action-potentials: the duration of *P. isidori's* EOD is often less than 100 µs, and rarely exceeds 200 µs, while the duration of *P. bovei's* EOD is 200–250 µs (Fig. 7).

We used the amplitude ratio of the P-phases (that is, P1/P2) to characterize an individual's EOD waveform instead of the more complicated formula 100(P1-P2)/N (Westby and Kirschbaum 1982). Our simpler expression focuses on the suggested sex difference in P-phases (Westby and Kirschbaum 1982), and has the advantage of being independent of the N-phase amplitude which is influenced by water conductivity (see below). If our results were similar to those of Westby and Kirschbaum (1982) then the P-ratios of male *P. isidori* should be $<1$ and those of females $>1$.

Normalized plots of male and female digitized EOD waveforms (of *P. isidori*) were examined for differences in the amplitudes or durations of the individual phases of an EOD (Table 1). The average P-ratio of males was, indeed, $<1$, while it was $>1$ for females at 100 µS/cm. The difference was significant ($P<0.025$, Mann-Whitney *U*-test, two-tailed). However, the inter-individual variability was so great that the female range overlapped almost two-thirds of the male range. The lowest P-ratio in a female (0.37) was below the average male P-ratio (0.49). One of the females with a "male-like" P-ratio of 0.75 (and a peak amplitude frequency of 17.0 kHz, see below) has spawned repeatedly with two males.

P-ratio was not correlated with the size of males (7.0 to 8.5 cm, $N = 10$), with $r$ not significantly different from zero ($r = 0.029$, two-tailed). In females (7.0 to 8.5 cm, $N = 14$), this correlation was not significant either ($r = 0.46$).

The N-wave durations in fish of both sexes were not significantly different at 100 µS/cm (Table 1). Also, the means of the two other temporal parameters studied, the P1-P2 separation, and the P1-N separation (both in µs), did not differ significantly between the sexes ($P>0.10$).

The peak amplitude frequency of an EOD amplitude spectrum (see Fig. 5) tended to be higher in female EODs than in male EODs; the difference was, however, not significant ($P>0.10$, Table 1). The differences between individual fish exceeded a factor of 3 (8 versus 25 kHz) at 100 µS/cm. The range shown by females (10.5–25 kHz) overlapped almost totally with that

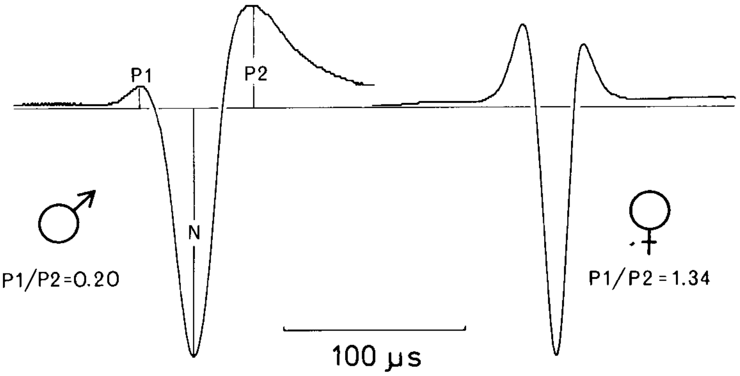

**Fig. 1.** *Pollimyrus isidori*. the EOD waveform of a male (*left*) and female (*right*) at a conductivity of 100 µS/cm (individuals selected for demonstrating a presumed sexual dimorphism, now known to represent only a statistical sex difference within an enormous intraspecific variability). *P1* first phase head positive, *N* head negative phase, *P2* second phase head positive. Note that the ratio of P-wave amplitudes is $<1$ in this male, and $>1$ in this female. *Ordinate* linear amplitude (baseline indicates 0 V). *Abscissa* time

**Table 1.** EOD waveform variability at 100 µS/cm in *Pollimyrus isidori* (as observed ranges and means ± SD)

| | Males ($N = 10$) | Females ($N = 14$) | Mann-Whitney $U$ |
|---|---|---|---|
| P1/P2 ratio | 0.04 – 0.94 | 0.37 – 3.33 | |
| Mean P1/P2 ratio* | 0.49 ± 0.26 | 1.17 ± 0.82 | 30.5 |
| 100(P1 – P2)/N | –49 – –2 | –23 – +13 | |
| Mean 100(P1 – P2)/N* | –19 ± 13 | –4 ± 13 | 31.0 |
| N duration (µs) | 23.1 – 37.8 | 17.3 – 31.6 | |
| Mean N duration** | 28.6 ± 5.3 | 25.8 ± 3.9 | 52.0 |
| P1 – N separation (µs) | 17.8 – 28.9 | 15.8 – 25.8 | |
| Mean P1 – N separation** | 23.0 ± 3.6 | 20.5 ± 2.7 | 45.0 |
| P1 – P2 separation (µs) | 33.8 – 60.9 | 28.9 – 52.4 | |
| Mean P1 – P2 separation** | 47.0 ± 9.5 | 41.8 ± 7.4 | 51.0 |
| Peak amplitude frequency (kHz) | 8.0 – 20.0 | 10.5 – 25.0 | |
| Mean peak amplitude frequency (kHz) | 13.4 ± 4.0 | 16.4 ± 4.4 | 42.5 |

* Difference significant at $P < 0.025$ (Mann-Whitney $U$-test, two tailed)

** Difference not significant ($P > 0.10$)

Definition of phases, see Fig. 1. N-wave duration measured as the time between zero-crossings. Peak amplitude frequencies determined from plots, see Fig. 5. For comparison the 100(P1 – P2)/N index (Westby and Kirschbaum 1982) is included

of the males (8–20 kHz). Three spawning males had EOD peak amplitude frequencies of 9, 15, and 20 kHz.

## *Effects of water conductivity on EOD waveform in P. isidori*

In order to determine the effect of water conductivity on the variability of the waveform of *P. isidori*, the fish were exposed to a series of different water conductivities (1.0–500 µS/cm). The P1/P2 amplitude ratio and the N-wave duration were studied while the test fish remained within the hiding tube of the experimental tank.

The P-ratio of two-thirds of our fish and the N-wave duration of all fish ($N = 24$), changed considerably over the water conductivity range tested. With decreasing conductivity below about 200 µS/cm, the P1-wave increased and the P2-wave decreased in most fish tested (16 out of 24), irrespective of sex (Fig. 2, see also Fig. 3a, b, d). In fish of either sex with a P-ratio below 1 at high conductivity ("male-like"), low conductivity caused the P-ratio to become > 1 ("female-like" in 14 out of 24 fish, 6 among these being females). For example, 1 female with a ratio > 1 at 1.2 µS/cm, had a ratio of 1 at 50 µS/cm, and a ratio < 1 at higher conductivities (0.75 at 500 µS/cm, Fig. 3b). The P-ratios of < 1 in 5 males and 3 females were almost independent of conductivity, although they showed considerable change in N-wave duration and P1-P2 separation, consistent with the other fish (Figs. 2, 3c).

The changes in N-wave duration were similar in all fish, showing a long N-wave at low conductivity (40.3 ± SD 8.9 µs, $N = 24$ at 5 µS/cm), and

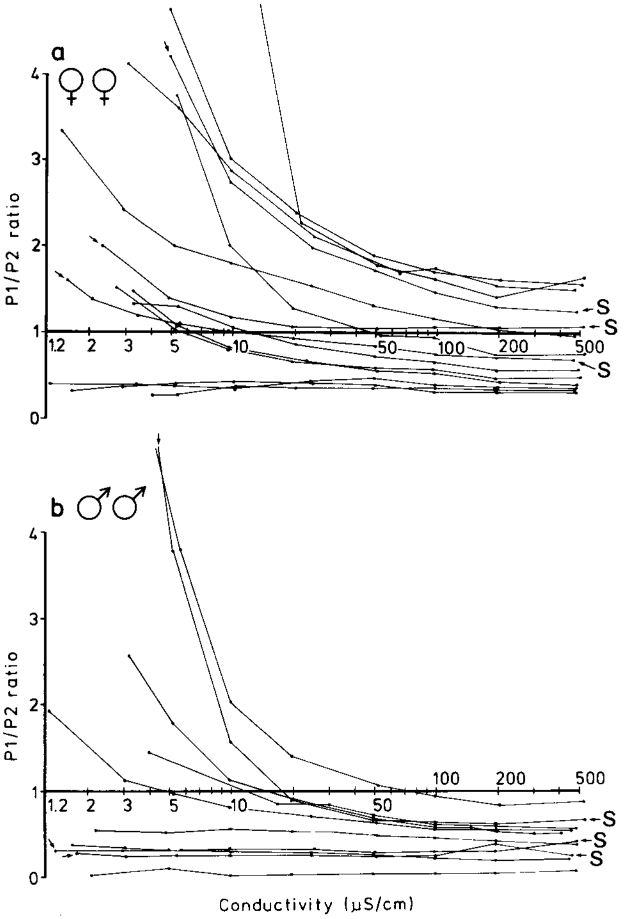

**Fig. 2a, b.** *P. isidori*. The P-ratio decreased in **a** 11 of 14 females and **b** 5 of 10 males as conductivity increased. The P-ratio remained stable above 200 µS/cm with most of the variation occurring below 100 µS/cm. *Abscissa* conductivity in logarithmic scale. *Ordinates* ratio of the two P-phase amplitudes (P1/P2) of an EOD. Three spawning males and three spawning females are indicated by *arrows*

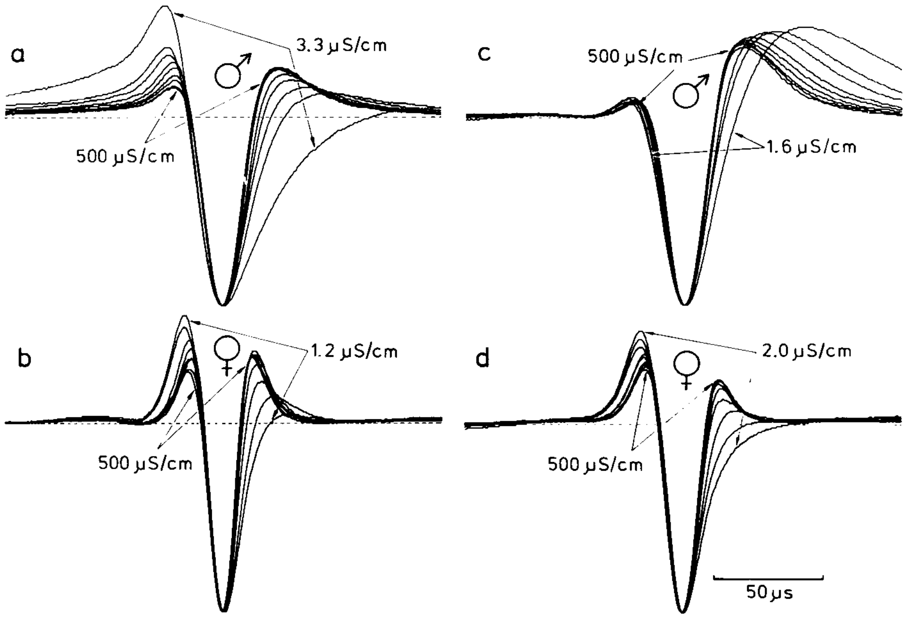

**Fig. 3a–d.** *P. isidori.* EODs of 4 fish recorded through a range of conductivities (≤2, 3, 5, 10, 20, 50, 100, 200, 500 μS/cm, extremes indicated by *arrows*), normalized and superimposed. **a** A male waveform that changed from a P-ratio > 1 to < 1 with increasing conductivity (observed in 5 out of 10 males). **b** A female waveform showed a similar change of P-ratio (observed in 9 out of 14 females). **c** The P1-wave of 5 out of 10 males was almost independent of conductivity, like that of 3 out of 14 females (not shown). **d** The waveform of some females (5 out of 14) did not show a P-ratio of < 1 even at the highest conductivities (500 μS/cm), unlike the males. Same time bar for all. *Axes* as in Fig. 1

a shorter N-wave at a higher conductivity (29 ± SD 6.2 μs, $N = 24$ at 500 μS/cm) (Figs. 3, 4). The peak amplitude frequency of an EOD amplitude spectrum shifted downward with decreasing conductivity; for example, from 12 kHz at 100 μS/cm to 8 kHz at 10 μS/cm, in 1 male (Fig. 5 b). P-ratio and N-wave duration remained relatively stable for most fish above 200 μS/cm, with most of the change occurring below 100 μS/cm.

The waveform of *P. isidori* at stable conductivities also showed a change with time, as observed in three individuals (Fig. 6). The P-ratio remained stable at a low value in male C, but decreased dramatically in female A (Fig. 6), within less than 120 days, from P-ratios of > 1 over the whole conductivity range, to P-ratios of < 1 (except the very lowest conductivity). In male B the P-ratio drifted upwards within 300 days.

### *EOD waveform and conductivity effects in* Petrocephalus bovei

Although belonging to a different genus, the mormyrid *P. bovei* looks so similar to *P. isidori* that the two species may be confused. *Petrocephalus isidori* (Sauvage 1880, cited from Daget et al. 1984) is synonymous with the modern name *Polli-*

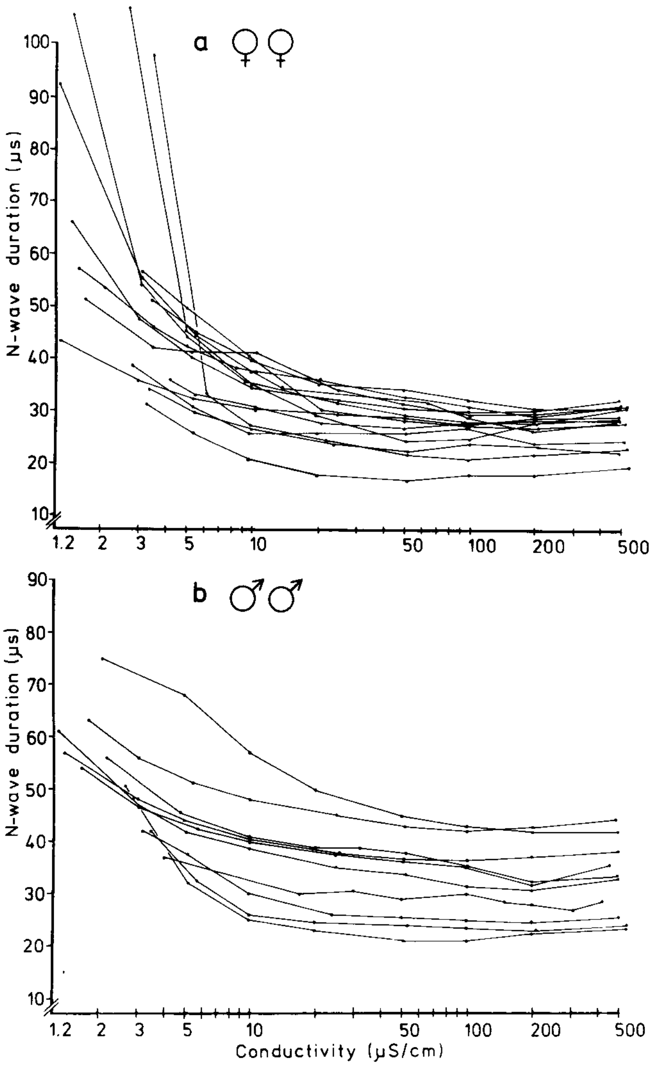

**Fig. 4a, b.** *P. isidori.* The N-wave duration increased at lowered conductivity in **a** females and **b** males. *Abscissa* conductivity in logarithmic scale. *Ordinates* duration of the N-wave of an EOD in μs

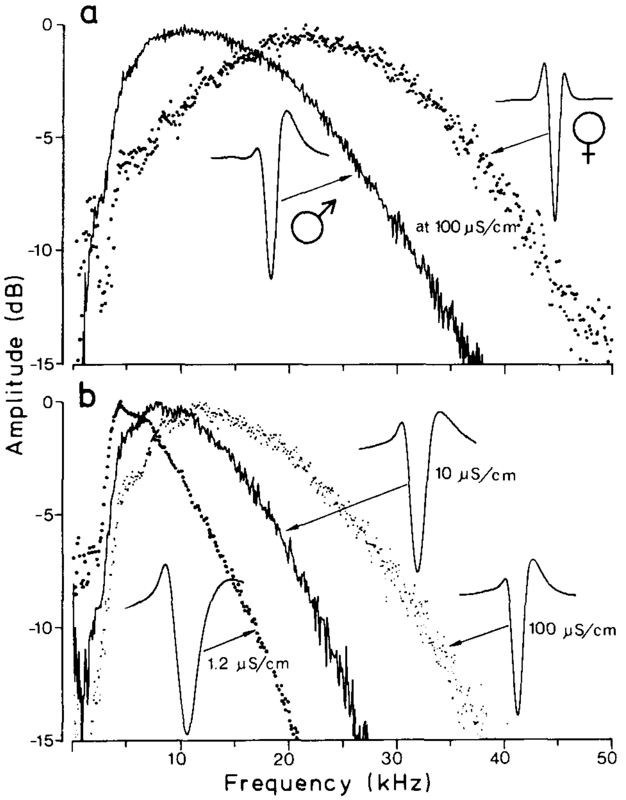

**Fig. 5a, b.** *P. isidori:* **a** Fourier amplitude spectra of a male (*line*) and female (*dotted*) EOD at 100 µS/cm. Examples selected as explained in Fig. 1 (same fish). **b** Three amplitude spectra of the EODs of the same male at different conductivities (ranging from 1.2–100 µS/cm). Note that the peak amplitude frequency decreases with decreasing conductivity. *Ordinates* amplitudes expressed as dB attenuation relative to the strongest spectral component of each EOD

*myrus isidori.* Their EOD waveforms are also of a related shape, although the duration of *P. bovei's* EOD (Fig. 7) is longer than that of *P. isidori* (Figs. 1, 3). We investigated *P. bovei* in order to find out: (a) whether there is an EOD sex difference; and (b) whether the EOD is subject to external load conditions (water conductivity) in a similar way as that of *P. isidori.*

The normalized waveforms of 5 males and 5 females showed no specific groupings by sex at a water conductivity of 100 µS/cm (Fig. 7a, b). Males overlapped females in all of the amplitude, temporal and spectral characteristics studied (partial overlap in P-ratio), and no evidence for two distinct male-female populations was found (Table 2).

In a similar way to *P. isidori,* decreasing conductivity caused the P1-wave to increase and the P2-wave to decrease, while the N-wave broadened (Fig. 7e). Unlike most *P. isidori,* the P1-wave amplitude was higher than the P2-wave, even at the highest conductivities, in all fish.

Stability of the waveform was also studied in individual fish over short and long term periods. From 9 recordings made within 90 min, no variation in the waveform was detectable in spite of the extremely fast (2 MHz) digitization (Fig. 7d). This same waveform stability was found in the other 9 fish. Over a 3 month period the waveform showed some variation in N-wave duration and P1 height (Fig. 7e).

### *Multiple EODs at low conductivity*

At conductivities below 17 µS/cm, most *P. isidori* ($N = 12$) had up to 3 additional EODs per primary discharge, separated by abnormally short intervals of 3.8 ms (Fig. 8a). The shortest EOD intervals, observed in isolated or socially interacting *P. isidori,* are 8–11 ms at higher conductivities (Kramer 1978; B. Bratton and B. Kramer, submitted). Each of these secondary EODs contained the P and N phases of the species. The neural command discharges (CMD 1–3), postsynaptic pulses

**Table 2.** EOD waveform variability at 100 µS/cm in *Petrocephalus bovei* (as observed ranges and means ± SD)

| | Males ($N = 5$) | Females ($N = 5$) | Mann-Whitney $U$ |
|---|---|---|---|
| P1/P2 ratio | 1.14 – 1.26 | 1.11 – 1.18 | |
| Mean P1/P2 ratio* | 1.20 ± 0.05 | 1.15 ± 0.03 | 5.0 |
| N duration (µs) | 32.0 – 46.0 | 34.0 – 42.0 | |
| Mean N duration* | 41.5 ± 5.9 | 37.4 ± 2.9 | 6.0 |
| P1 – N separation (µs) | 25.0 – 33.0 | 26.0 – 29.0 | |
| Mean P1 – N separation* | 29.3 ± 3.5 | 27.5 ± 1.4 | 8.5 |
| P1 – P2 separation (µs) | 48.5 – 77.0 | 56.0 – 62.5 | |
| Mean P1 - P2 separation* | 65.1 ± 11.9 | 57.7 ± 2.7 | 6.0 |
| Peak amplitude frequency (kHz) | 8.0 – 12.4 | 8.5 – 11.0 | |
| Mean amplitude peak frequency (kHz)* | 9.1 ± 1.8 | 9.7 ± 1.0 | 5.5 |

* Difference not significant ($P > 0.05$, Mann-Whitney $U$-test, two tailed)

Definition of phases, see Fig. 1. N-wave duration measured as the time between zero-crossings. Peak amplitude frequencies determined from plots, see Fig. 5.

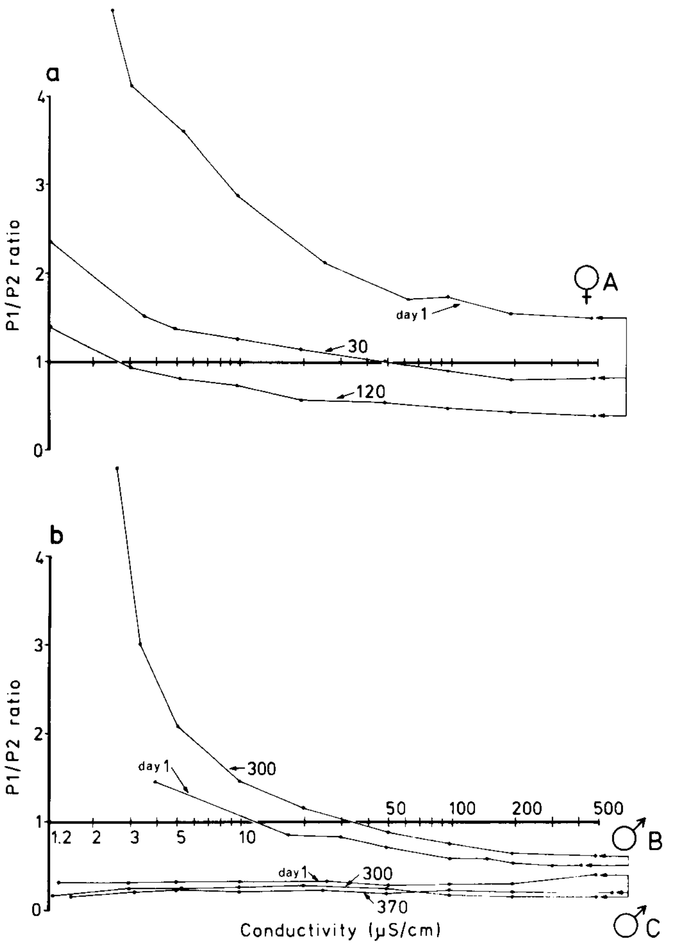

**Fig. 6a, b.** *P. isidori*. The waveform (shown as the P1/P2 ratio) of individual adult fish also changed with time in **a** female A, and **b** male B, while male C showed little change. The EOD of female A drifted to a seemingly "male waveform" over a period of 120 days. The EOD of male B drifted in the opposite direction over a period of 300 days. All 3 fish were housed in water of 100 μS/cm. *Axes* as in Fig. 2

(PSP1 and PSP2) and the stalk potential (ST, Fig. 8b), all preceding the EOD (Westby and Kirschbaum 1978), were also visible from recordings with the tank electrodes at conductivities between 1–17 μS/cm. No differences in the time between CMD-1 to CMD-3 or PSPs were found between male ($N = 6$) and female ($N = 6$) *P. isidori.*

At conductivities below 5 μS/cm, multiple discharges were also observed in *P. bovei* ($N = 3$). In *Brienomyrus niger* ($N = 2$), as many as 3 secondary EODs followed the primary EOD at conductivities below 2 μS/cm and they disappeared above 17 μS/cm.

## Discussion

### *Is the pulse EOD of P. isidori sexually dimorphic?*

Our results confirm the findings of Westby and Kirschbaum (1982), in that the EODs of male *P. isidori* exhibit a P1 phase which is weaker than the P2 phase (i.e. the amplitude ratio $P1/P2 < 1$), at a high water conductivity within the natural range (see below), while the EODs of females tend to show, on average, the opposite (i.e. $P1/P2 > 1$). However, in our fish we only found a *statistical difference* between the sexes, not a distinct *sexual dimorphism.* We feel the distinction between these two terms should be made in view of, for example, Wilson's definition (1975) of sexual dimorphism as "any *consistent* difference between males and females beyond the basic functional portions of the sex organs" (italics by present authors). Our 14 females overlapped 2/3 of the range of the 10 males. Certain females had P-ratios still smaller than the average P-ratio of males. In an additional sample of 4 females and 4 males, 2 fish of each sex had the "wrong" waveform type (Lücker 1982). Taken together, 11 fish out of 32 had the "wrong" waveform (at 500 μS/cm). There was no sex difference, hence no sexual dimorphism, in any of the other characters studied (N wave duration, P1-N and P1-P2 separation, and peak amplitude frequency, Table 1).

Unlike these results, the data of Westby and Kirschbaum (1982) showed almost no overlap be-

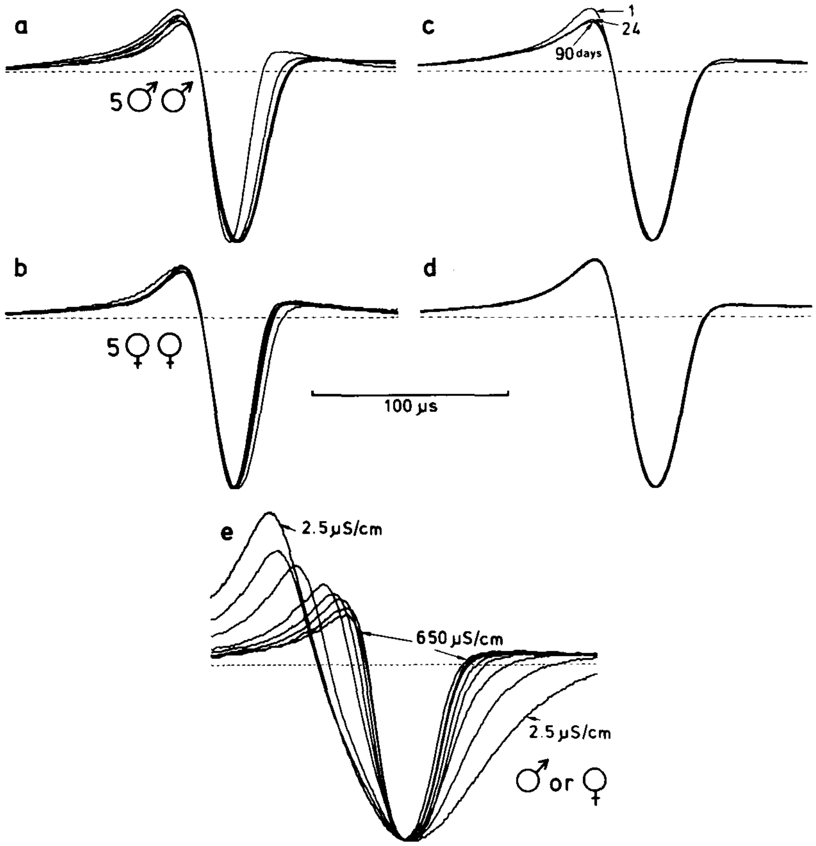

**Fig. 7a–e.** *Petrocephalus bovei:* **a–d** EOD waveforms at a conductivity of 100 µS/cm; *e* at a range of conductivities (2.5–650 µS/cm). **a** Five individual male waveforms normalized and superimposed. **b** Five superimposed female waveforms. **c** The variability in waveform of 1 fish over a period of 90 days (3 EODs at days 1, 24, 90, normalized and superimposed). **d** The stability of the waveform from 1 fish during 90 min (9 EODS 10 min apart, normalized and superimposed). *Axes* as in Fig. 1. **e** The waveform of a *P. bovei* changed in a similar way with conductivity as that of *P. isidori*, with a broadening of the N-wave but without the P2-wave amplitude increasing over the P1-wave

tween the sexes: out of 45 fish only 2 were (marginally) of the "wrong" waveform type as measured by the P-ratio (most fish at 800 µS/cm, 6 at 150 µS/cm, G. W. M. Westby, personal communication). Westby and Kirschbaum (1982) suggested a sexual dimorphism also in the spectral amplitude characteristics of EOD in *P. isidori*: the spectra of male EODs had lower peak amplitude frequencies than those of female EODs. Although the mean difference between the sexes was greater in our fish compared with that in Westby and Kirschbaum (1982), it was not significant because of an extensive overlap of the distributions (in spite of a greater sample size in the present study, Table 1). We conclude that there was no sexual dimorphism, and not even a difference between the sexes of a statistical nature, in the spectral amplitude data of our fish.

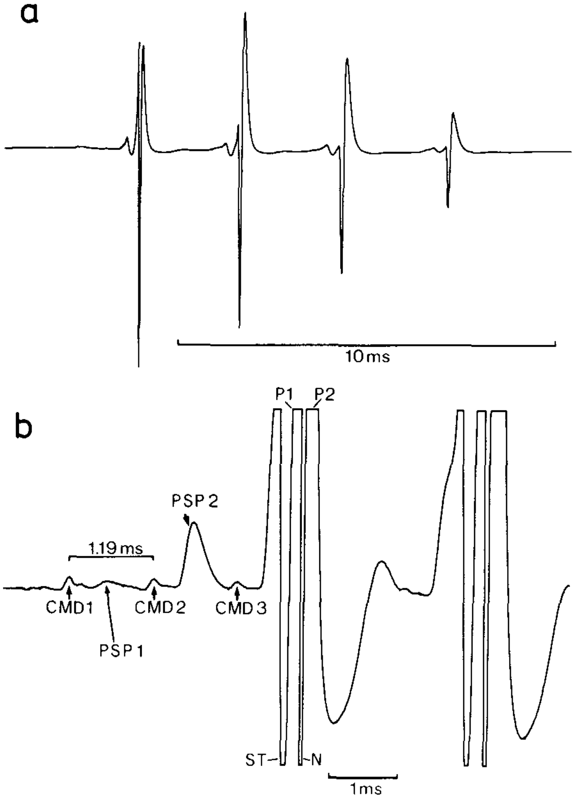

**Fig. 8a, b.** *P. isidori:* **a** Multiple EODs recorded (single sweep) at a conductivity of 1.3 µS/cm; **b** The neural command discharge waves (*CMD 1–3*) and postsynaptic pulses (*PSP 1–2*) at higher amplification, preceding the main EOD, recorded at 3.0 µS/cm with tank electrodes. The main waveform and stalk potential (*ST*) are shown clipped by digitizer saturation at high amplification (labelling conventions after Westby and Kirschbaum 1978)

We believe the reason why our results differ so much from those of Westby and Kirschbaum (1982) is primarily the different method of sexing: while we (and also Lücker 1982) used the behavioral criterion of EOD latency type, previously validated by gonad histology (PLR, PLA; Kramer 1978; Lücker and Kramer 1981), Westby and Kirschbaum (1982) used an 'anal fin test'. This is an observation of a reflex elicited by gentle mechanical stimulation of the base of the anal fin, believed to be present in all males but not in females (100% reliability). The reliability of their criterion was, however, verified only in those fish which successfully spawned and became parents; the proportion of fish maintained in community tanks where they could not breed is unspecified. It is this latter, probably greater, group in which the reliability of the anal fin test remains to be verified by an independent criterion. Another reason for differing results may be the 2 conductivities used by Westby and Kirschbaum (1982) (150 and 800 μS/cm).

The waveform of *P. bovei's* pulse EOD lacked a sex difference in all characters analyzed in the present report (as has also been shown to be lacking in *Gnathonemus petersii*, Kramer and Westby 1985). However, P. Moller (personal communication) found a statistical difference in peak power frequencies between the EODs of male and female *G. petersii*.

### *Dependence of the EOD on water conductivity*

The overlapping of P-ratios in *P. isidori* (both sexes) was augmented by changing water conductivities, which shift the waveforms of some fish extensively while changing others only marginally. At low conductivities certain male EOD waveform properties would resemble those of "females" (as proposed by Westby and Kirschbaum 1982), while at a higher conductivity certain female EODs would resemble those of "males". Also the frequency content, or spectral peak amplitudes of the EOD waveform, were shifted as water conductivity changed. This is probably due to the broadening of the N-wave with lowered conductivity in the first place. *P. bovei's* homomorphic EOD waveform was markedly affected by conductivity changes in a similar way to that of *P. isidori*.

The innervated posterior faces of the electrocytes of the electric organ, located in the caudal peduncle (anatomical data, Szabo 1961; Denizot et al. 1982), generate the first, or head-positive phase, of *P. isidori's* EOD. When a triplet of spikes (Westby and Kirschbaum 1978; CMD1-3 in Fig. 8b) arrive from the spinal electromotor neurons at the neuromuscular synapses on the stalks of the electrocytes, a depolarization then gives rise to a relatively long-lasting P-wave (head-positive phase, Bennett and Grundfest 1961), the P1 and P2 phases of the EOD. This current in turn causes the very brief and strong depolarization of the uninnervated anterior faces, hence a current in the opposite direction from the first phase (Bennett 1971; Bell et al. 1976). The waveform of the whole organ in this species is therefore the superposition of the first phase head-positive current, and the delayed second phase head-negative current (N phase, see Fig. 1). The waveform variation concerning the P-ratio found in *P. isidori*, was modelled in a computer simulation by varying the exact timing of the N-wave occurrence relative to the onset of the P-wave (Westby 1984). However, as seen here, a change of N-wave duration and waveform is also involved when conductivity changes (see Bell et al. 1976).

First phase current depends on the total internal and external resistivity. The internal resistance of the fish (tissues and skin) is controlled by species characteristics and hormonal influences (see Bass 1986), while the external resistance depends on the water conductivity and non-conducting boundaries in the electric near-field. The P-wave current has been shown to determine the latency of the N-wave, its amplitude and shape in *G. petersii* (Bell et al. 1976).

The P-ratio of some female and most male *P. isidori* did not change greatly with conductivity, presumably due to internal resistance being very high or low, and in which case the external load is not significant in changing the overall current load (see Bennett 1971; Bell et al. 1976).

Is the range of conductivities tested "physiological"? The occurrence of multiple EODs at abnormally short intervals (Fig. 8a) probably indicates that water conductivity was too low (i.e., an external resistance which was too high). Similar observations of multiple discharges have been made in other mormyrids, and were explained by an unphysiological self-stimulation of the electric organ in a medium of high resistivity (see discussion and literature in Bell et al. 1976). As the P-phase voltage is maximized by a high external resistivity, the current travels through a less resistive pathway (probably the spinal cord), thus re-exciting the electromotor neurons of the electric organ. We conclude that the electric organ of *P. isidori* is adapted to a water conductivity of greater than

17 μS/cm, while the electric organ of *P. bovei* still functions normally at conductivities of 5 μS/cm.

### *Physiological explanation of the EOD sex difference*

We propose that the statistical difference in P-ratio between male and female EODs of *P. isidori* could be due to well-known physiological reasons, and need not be the result of intraspecific selection such as female choice and male competition. We feel our explanation is the more parsimonious one.

Among the many effects of androgen hormones (e.g. testosterone), known since the last century, are the morphogenetic and the anabolic ones: the stimulation of growth and strength of skeletal muscles in vertebrates (see, for example, Eckert and Randall 1983; Blüm 1986). This has also been shown in the electric organs (which are derived from skeletal muscle) of androgen-treated female mormyrids (Bass 1986, p. 52), and in a gymnotiform pulse fish (Hagedorn and Carr 1985). Examples of a stronger EOD amplitude in males compared with females, are the gymnotiforms *Eigenmannia lineata* (Kramer 1985), and *Hypopomus occidentalis* (M. Hagedorn, in preparation, cited from Hagedorn and Carr 1985).

Under the influence of endogenous androgens, male *P. isidori* should develop a stronger electric organ, yielding, on average, a stronger P-wave current than electric organs of females. Therefore, the electrically evoked N-wave should be triggered at a shorter latency, relative to the onset of the P-wave, in males compared with females. The result would be a smaller P-ratio in males than in females (as modelled by Westby 1984).

The assumption of a tendency of electric organs in males to be stronger than in females, is supported by the observation of a considerably weaker and longer N-wave than normal at the lowest conductivities in several females, but not in males (Fig. 4). This indicates that, as the voltage of the P-wave was maximized by a very high resistivity of the medium, males – in contrast to some females – still generated a sufficient P-wave current to trigger a slightly longer, but normal N-wave. Bell et al. (1976) showed that such changes in second phase (N) waveform are due to variation in first phase current.

### *Ecological and behavioral considerations*

The following is a list of conductivity ranges from rivers in which mormyrids have been studied: 5.3–76 μS/cm in the rivers of the Congo Basin (Gosse 1963); 65–110 μS/cm in the Swashi River, Nigeria (Moller et al. 1979); and 17–100 μS/cm in the Ivindo River District, Gabon (Hopkins 1980). Because of the unphysiological self-stimulation of the electric organ at very low conductivities, any discussion of the effects of water conductivity on EOD waveform should focus on the conductivity range of 20–110 μS/cm in *P. isidori.* It is in this range that the EOD waveforms of 2/3 of our fish were considerably affected by conductivity, as measured by the P-ratio (in addition, there was an effect on the N-wave).

It has been suggested that temporal cues of sexually dimorphic EOD waveforms might provide a means of mate recognition (review: Hopkins 1986; Westby and Kirschbaum 1982; critically discussed in Kramer 1985, pp. 63–64). This is difficult to see in *P. isidori* since its EOD does not show a consistent difference between the sexes, and the EOD waveform of part of the population depends considerably on water conductivity.

Any discrimination of female and male EODs by tuned Knollenorgan-electroreceptors, using spectral cues, as suggested by Westby and Kirschbaum (1982), appears unlikely because of very broad receptor tuning (Hopkins 1983), and because of the absence of a sexual dimorphism in the spectral amplitudes of *P. isidori's* EOD. Also, spectral amplitudes are seen to be markedly affected by conductivity changes in our study.

As male and female ranges *overlap,* a simple shift, with conductivity, of a hypothetical innate releasing mechanism, could not compensate for conductivity-evoked EOD waveform changes. The fish might not be able to discriminate between the EOD waveform differences in these extremely fast, pseudo-stochastic click-like events (Kramer 1978), at least not within the range of the intraspecific variability (see below). If this were true, the process of evolution of a sexual dimorphism, by intraspecific selection, could not get started, or else the process is in its initial stage only. The perception of at least some of the fine detail of an EOD (beyond EOD frequency and intensity), was, however, demonstrated in the constant-frequency wave-EOD of the gymnotiform *Eigenmannia* (Kramer and Zupanc 1986), and a sexual dimorphism was actually observed in its EOD (Kramer 1985).

We consider it unlikely that the only statistical difference between male and female EODs of *P. isidori* is a useful cue for mate selection. The ambiguity of the EOD signal as to the sender's sex, is enhanced by seasonal and regional variations in the environment. Alternative cues are probably involved in mate selection in at least the three species investigated for the intraspecific variability of EOD waveform: *P. isidori, P. bovei* and *G. petersii.* These cues could be the sexually dimorphic EOD latency response (Kramer 1978; Lücker and Kramer 1981), or acoustic songs (Crawford et al. 1986, and our own observations, unpublished) in *P. isidori,* or inter-EOD time interval patterns (in all species), which, apart from motivational state (Kramer 1978, 1979), might also signal sex-specific information. We are currently studying these possibilities (Bratton and Kramer, submitted).

*Acknowledgements.* This work was supported by the Deutsche Forschungsgemeinschaft (SFB4/H1). We wish to thank Drs. D. Burkhardt, F. Kirschbaum (University of Cologne) and G. W. M. Westby (University of Sheffield) for critically discussing the manuscript, L. Cormick-Bratton for assistance with the manuscript and figures, Dr. U. Bonn for help with the histology, and B. Otto for assisting in the care of the fish.

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Received October 14, 1987/Accepted March 16, 1988

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## Note added in proof