

## INDIVIDUAL AND SEX SPECIFICITY IN THE ELECTRIC ORGAN DISCHARGES OF BREEDING MORMYRID FISH (*POLLIMYRUS ISIDORI*)

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### Summary

I monitored the electric organ discharges (EODs) of 14 *Pollimyrus isidori*, (Cuvier and Valenciennes) during an artificially induced breeding season, to examine sex and individual differences in reproductive fish. EODs were repeatedly recorded over an 11-day period to ascertain the stability of each individual's EOD and to make a quantitative assessment of sex differences. Within days, I found the individual's EOD to be constant from one EOD to the next. Over the 11-day sampling period, individuals were also quite stable and exhibited only slight changes in EOD duration and relative amplitude of the phases of the waveform. I found that the differences between individuals of the same sex were highly significant in measures of EOD duration and in measures of the relative amplitude of the phases. Differences between the sexes were also highly significant in relative amplitude but were not significant in duration. In a multivariate discriminant function analysis, I have found that individual fish can be correctly classified on the basis of temporal, relative amplitude and spectral cues in the EOD, despite slight changes in these parameters with time. The EOD exhibits characteristics of a good *signature* in the context of an information system.

### Introduction

The mormyrid fishes of Africa produce weak electric discharges (EODs) with an electric organ in the tail. EODs are detected with a distributed array of electroreceptors on the body surface, and EODs function in communication and orientation. Among mormyrid species there is great diversity in the waveform of the EOD; however, EODs are species-specific (e.g. Crawford and Hopkins, 1989), and in sexually reproductive adults there are differences between the EODs of males and females of some species (reviewed in Hopkins, 1986). These findings have led to the important hypothesis that the EOD functions in two critical forms

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of social behavior: species recognition and sex recognition (Hopkins, 1980, 1986). These hypotheses have been somewhat controversial because earlier investigators focused on the *rate* or the *temporal pattern* of discharge as the primary dimension used in social signalling (see review by Scheich and Bullock, 1974). This focus was partly due to the observation that the individual EODs were so brief that it seemed unlikely that they could carry useful information in their waveforms (discussed by Hopkins, 1980, 1986). Nevertheless, EOD-based sex recognition in mormyrids has now received experimental support in one species. Field studies of *Brienomyrus brachyistius* (Hopkins and Bass, 1981; Hopkins, 1983) showed that males could discriminate between male and female EODs on the basis of time-domain differences in the EOD. However, with over 200 species of described mormyrids, species that differ widely in EOD characteristics, morphology and ecology, it remains to be seen whether these findings for *B. brachyistius* are representative of the group. Is EOD-based sex recognition a general phenomenon in this group, and is it possible that EODs contain additional cues that may not have been considered in the earlier studies? In this paper I present EOD data from a longitudinal study of a different species of mormyrid, *Pollimyrus isidori* (Cuvier and Valenciennes, 1846), in an effort to address questions about the potential role of the EOD in both individual and sex recognition.

In the analysis of the significance of EODs in communication, it is useful to consider several distinct classes of questions. One concerns the ability of the animal to discriminate the features of the signal that are suspected to be significant. A second focuses on whether the putative signals actually carry the hypothesized information (e.g. sex or individual identity) when one takes into account the variability within and between individuals, over biologically meaningful time frames and group sizes. A third class of questions follows from the first two and deals with the extent to which the animals actually use the available, and discriminable, cues under natural circumstances. Answers to all three types of questions are important for evaluating the functional role of the EOD in natural communication. While some of these issues have been elegantly explored for EOD-based sex recognition in *B. brachyistius* (Hopkins and Bass, 1981), there has been essentially no effort to deal with the second class of questions mentioned above: in no species of mormyrid has the EOD been rigorously evaluated in terms of its potential information value during reproductive behavior. The longitudinal study of *P. isidori* described here was designed to contribute new data in this area: the features of the EODs of breeding fish were explored in detail to evaluate the potential role of the EOD in sex and individual recognition.

*P. isidori* has already featured in several important laboratory studies of mormyrid ethology (e.g. Kramer, 1978; Lucker and Kramer, 1981; Bratton and Kramer, 1989) and was one of the first species for which a quantitative argument was made for an EOD sex difference (Westby and Kirschbaum, 1982). Westby and Kirschbaum found that the relative sizes of the two positive phases (P1 and P2) in the triphasic EOD (see Fig. 1A) differed between females and males, and also that the frequency at the peak of the power spectrum was highest in females. However,

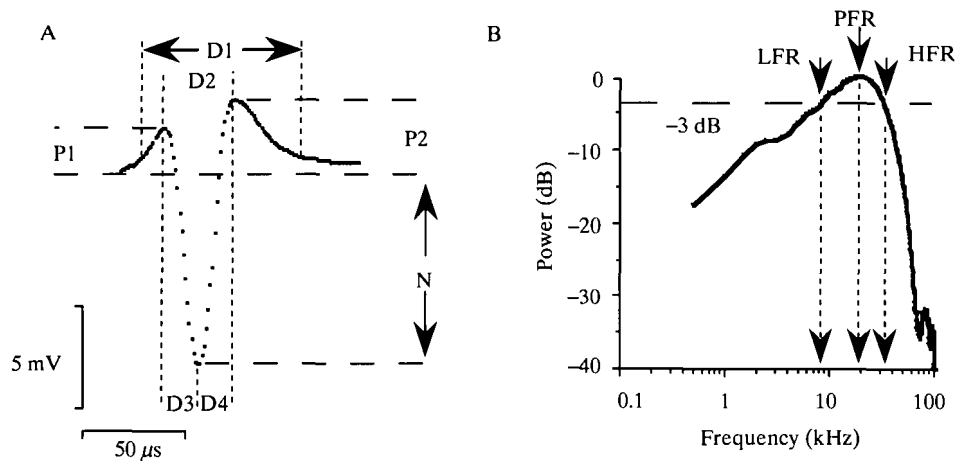


Fig. 1. EOD variables. Six measurements taken directly from the EOD waveform are illustrated on the left (A). D1–D4 are time intervals measured between different features of the signal. P1, P2 and N are the heights of the major positive- (P) and negative- (N) going phases of the triphasic EOD. A power spectrum, computed from a 2048-point fast Fourier transform, is shown on the right (B) with three frequency-domain variables indicated. LFR, low frequency; PFR, peak frequency; HFR, high frequency. The  $-3$  dB criterion is indicated by the horizontal dashed line 3 dB below the peak of the spectrum.

their conclusion that the EOD was an excellent candidate sex recognition cue has recently been challenged. Bratton and Kramer (1988) concluded that the *P. isidori* EOD could not be important in sex recognition because they found the EOD to be influenced by water conductivity (a natural ecological variable), and because there was overlap between the male and female distributions in the EOD characters they examined. Unfortunately, neither Bratton and Kramer nor Westby and Kirschbaum focused their research on fish that were breeding, or on fish living under relatively stable low-conductivity water conditions characteristic of natural breeding habitats ( $25\text{--}40\ \mu\text{S cm}^{-1}$ ; J. D. Crawford, personal observation). It is well known that breeding in mormyrids is seasonal and that reproductive hormones influence the electric organ and, consequently, the EOD (Bass and Hopkins, 1985). Because the appropriate data on breeding individuals have not previously been analyzed, it has been difficult to evaluate these competing ideas about the potential function of the EODs of *P. isidori*. The longitudinal study presented in this paper is based on adult breeding fish (Fig. 2), brought into reproductive condition through a simulation of a natural breeding environment. Consequently, the study provides new information that should help to resolve some of the controversy summarized above. To my knowledge, this is the first longitudinal study of the EODs of breeding mormyrids.

*P. isidori* is the only mormyrid that has been repeatedly bred in the laboratory, thus allowing a detailed study of its reproductive biology through visual, acoustic and electric eavesdropping (Crawford *et al.* 1986; Kirschbaum, 1987; Bratton and

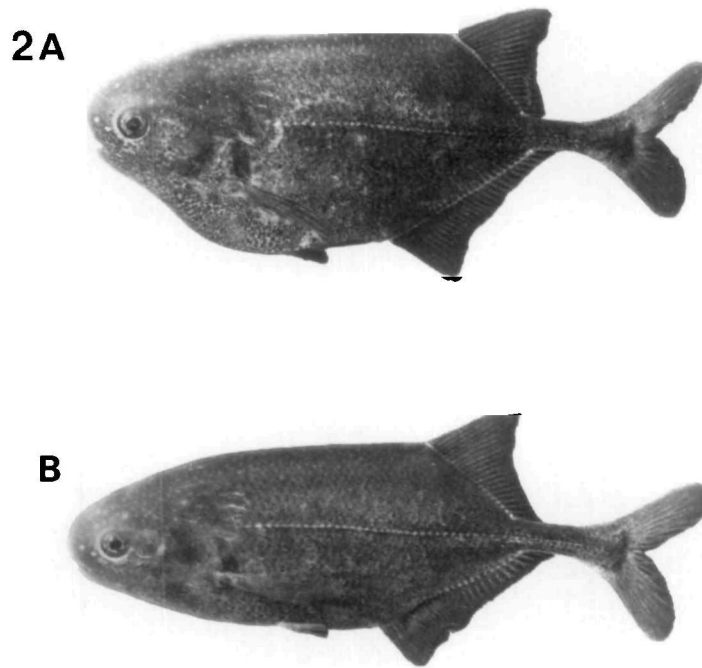


Fig. 2. Photograph of a breeding female (A) and breeding male (B). Note that the female was very near to spawning, and her body was noticeably distended by her swollen ovary. As in most breeding males, there was a clear indentation in the line formed where the anal fin meets the body. Female standard length=68 mm; male standard length=75 mm.

Kramer, 1989; Crawford, 1991). Males are territorial and build multiple nests from vegetation. They have a repertoire of five sounds, and three of these are used in courtship. Gravid females visit territorial males during the *sonic phase* of courtship. Males court females with an acoustic display of *grunts* and *moans* while females are on the territory, and a *growl* when they leave. During this phase of courtship, the male does not discharge his electric organ. However, the female discharges at a highly regular rate of about 20 EODs  $s^{-1}$ . In subsequent phases of courtship and spawning both sexes produce EODs but sounds are not produced. Spawning females deposit about 200 eggs on the male territory over a period of 6 h. The male places the eggs in his nests, evicts the female at the end of spawning, and guards the territory against all intruders until a gravid female arrives again. Females spawn serially through the rainy season at intervals of about 14 days, and sometimes mate with more than one male. The EODs analyzed in this report were recorded while the fish were engaged in these reproductive activities.

The EODs of reproductive *P. isidori* have been analysed below to address the following five questions. (1) Is there a true sex difference, as distinct from individual differences and differences that might arise spuriously from sampling a

signal that is variable over time (ANOVA)? (2) Are there significant differences between the EODs of different individuals, as distinct from sex differences (ANOVA)? (3) How reliably can individual fish be classified on the basis of their EOD characteristics (discriminant function analysis)? (4) Can a small number of mathematically uncorrelated new dimensions be derived, from the original EOD parameters, which account for the variance in the data (principal components analysis)? (5) In an information theoretical context, what is the information value of the signal (EOD) and how many individuals might be uniquely defined on the basis of the EOD (information theory)?

### Materials and methods

#### *Fish and fish care*

*Pollimyrus isidori* were imported to the United States from Nigeria, West Africa. Fourteen adults (50–85 cm standard length) were used in this study, including one laboratory-raised female (F501: offspring of two wild parents that were not used). The fish were maintained in laboratory aquaria (200 – 500 l), and fed a diet of aquatic worms (Tubificidae) and *Chironomus* larvae. Water temperature was  $28^{\circ}\pm 1^{\circ}\text{C}$ , pH 6.5–7.5, and conductivity  $35\pm 6\ \mu\text{S cm}^{-1}$  (s.e.).

Breeding was induced by creating a rainy season with the concomitant gradual decrease in water conductivity to a low plateau of  $35\ \mu\text{S cm}^{-1}$ . These methods are detailed in several earlier papers (Crawford *et al.* 1986; Kirschbaum, 1987). Briefly, several adult fish were placed in each large aquarium (usually one female and one male in a 200 l aquarium and two males and one or two females in a 500 l aquarium) with a starting conductivity of about  $200\ \mu\text{S cm}^{-1}$ . A rainy season was then simulated by periodic sprinkling of water into the aquarium (automatic recirculation of aquarium water), and by daily additions of deionized water. The daily water additions brought the conductivity down to  $35\ \mu\text{S cm}^{-1}$  over a period of roughly 6 weeks.

#### *Electric organ discharge recording*

EODs were recorded with carbon rod electrodes (length 6.0 cm; diameter 1.0 cm, electrode pair separated by 25 cm). For all recordings, the electrode pair was aligned with a fish, while it rested in a hiding place, such that the fish faced the positive pole and had its tail towards the negative pole. EODs were amplified with a differential amplifier (PAR 115, bandwidth 30 Hz–300 kHz) and digitized at a  $1.0\ \mu\text{s}$  sampling interval with 8-bit amplitude resolution. These conditions placed a  $1\ \mu\text{s}$  limit on temporal resolution (approximately 1 % of total EOD duration) and a limit of 1 in 256 for voltage (approximately 0.4 % for the vertical dimension). Recordings were made during a trough in the 24 h activity cycle (afternoon) so that multiple EODs could be recorded with constant electrode–fish geometry and minimal disturbance to the fish in its home tank. Previous studies have shown the mormyrid EOD waveform to be invariant with electrode orientation relative to the fish (Bass and Hopkins, 1985). EODs were also examined at other times of day

and night and in many different behavioral contexts, but no temporal or contextual variation in the EOD was noted.

In each recording session, six EODs were recorded from each fish, over a period of about 5 min. The 'armed' digital recorder (pulse logger, Helpware) was triggered by an EOD, the digitized EOD was then stored, and the digital recorder was 're-armed'. The next EOD to trigger the recorder was stored and so on until six digitized EODs were obtained. Six recording sessions were made, for each animal, at 48 h intervals. The resulting 502 EODs (14 fish  $\times$  6 EODs per fish per session  $\times$  6 sessions = 504 EODs, less two since a sixth was accidentally missed in two sessions) constituted the longitudinal data set presented in this paper. Data were collected over a 23-day period in October, 1988.

#### *Electric organ discharge characteristics*

Previous studies of sex differences in mormyrid EODs have focused on characteristics of the EOD reflected in the relative sizes of the various peaks in the waveform (e.g. Westby and Kirschbaum, 1982), the duration of the EOD (e.g. Hopkins, 1980; Crawford and Hopkins, 1989), and amplitude and phase spectra from fast Fourier transforms (FFTs) of the EOD (e.g. Hopkins, 1980; Hopkins and Bass, 1981). In this study, I used DEC computers and FORTRAN programs to make measurements similar to those used in these earlier reports; these measurements are illustrated in Fig. 1A,B. The measurements of peak sizes (P1, P2 and N) were normalized to the peak-to-peak amplitude of the signal, and expressed as a percentage. For all statistical analyses, the underlying ratios (e.g.  $P1 \div 100 = r_1$ ) were transformed by the arcsine transform [i.e.  $\sin^{-1}(\sqrt{r_1})$ ; see Zar, 1984]. Four measurements of duration were made (D1–D4), and these reflect the duration of the whole signal (D1) or the time between major features of the EOD. The total duration of the EOD (D1) was measured from the time when the EOD first departed from the baseline (i.e. 0 V) by 5% of the peak-to-peak voltage to the time when it last returned to the 5% criterion. The three other duration measures are simply based on the timing of the three peaks within the EOD. In the frequency domain, I measured the peak frequency of the power spectrum (PFR) and the two frequencies corresponding to 3 dB below the peak of the spectrum (LFR and HFR, Fig. 1B). Power spectra were constructed from 2048-point FFTs computed on a DTK computer with a digital signal processor (Microstar Labs 2400/5). These frequency measurements were limited by the inherent 488 Hz resolution of the FFT.

#### *Statistical and exploratory analyses*

I analyzed the data in several ways to identify individual and sex differences in the EODs of breeding fish and to explore the possible role of the EOD as a communication signal in *P. isidori*. Version 5.16 of the statistical analysis system (SAS) was used on an IBM 4341 computer to perform analysis of variance (ANOVA; PROCGLM in SAS). Correlation matrices, principal component analysis (PCA) and discriminant function analysis (DFA) were all performed with

Table 1. Correlation matrix for the 10 EOD variables illustrated in Fig. 1

	P1	P2	N	D1	D2	D3	D4	PFR	LFR	HFR
P1	+1.0									
P2	-0.80	+1.0								
N	+0.84	-0.98	+1.0							
D1	+0.29	-0.70	+0.63	+1.0						
D2	-0.14	-0.32	+0.29	+0.87	+1.0					
D3	-0.23	-0.18	+0.16	+0.78	+0.99	+1.0				
D4	-0.09	-0.40	+0.35	+0.90	+0.99	+0.92	+1.0			
PFR	+0.41	+0.12	-0.06	-0.69	-0.87	-0.86	-0.85	+1.0		
LFR	+0.60	-0.09	+0.15	-0.53	-0.78	-0.79	-0.75	+0.93	+1.0	
HFR	+0.30	+0.22	-0.16	-0.80	-0.96	-0.94	-0.95	+0.96	+0.86	+1.0

Day 3, one EOD per fish.

SYSTAT version 5.1 (Wilkinson, 1989), on a Macintosh SE/30 computer with a Motorola (68882) floating point coprocessor.

The first analysis performed was an ANOVA designed to evaluate sex differences and individual difference in the EODs collected during the entire study period. For this primary statistical analysis of the EOD, I selected a single measure of 'amplitude' (P1) and a single measure of EOD duration (D1) as dependent variables (see Fig. 1A). These measures were chosen because they describe clearly different dimensions of the signal. Some of the measurements shown in Fig. 1 were highly correlated with each other (see Table 1), particularly those with the same units; consequently, it would not have been particularly informative to include all of these as dependent variables in the ANOVA (see Neter *et al.* 1985). The objective of this ANOVA was simply to determine if there were significant sex and individual differences along different dimensions of the EOD. However, the ANOVA was followed by additional exploratory analyses which examined the other parameters measured, and descriptive statistics for all variables are provided in Table 2. These analyses provided insights to the possible involvement of the various EOD parameters in communication and provided some basis for the design of further experiments on communication in *P. isidori*.

The ANOVA was a three-factor, mixed-effects (i.e. different types of *independent variables*), hierarchical ANOVA with replication. Sex was treated as a fixed categorical variable, and ID (individual identity) was treated as a random categorical variable nested within Sex [ID nested within Sex: ID(Sex)]. Interactions between Day and ID and between Day and Sex were also included in the ANOVA model (Sex  $\times$  Day in Table 3). Hypothesis tests were based on type III sums of squares (SS), so that each independent variable would be evaluated after the variance due to the other independent variables had been removed (the analysis was not dependent on model order or balance in the data set). The significance of the main effects (i.e. Sex and ID) was ascertained by examining the variance associated with each effect (i.e. 'treatment or groups mean square', e.g.

Table 2. Summary statistics for female and male EOD variables

	P1*	P2*	N*	QWK	D1 ( $\mu$ s)	D2 ( $\mu$ s)	D3 ( $\mu$ s)	D4 ( $\mu$ s)	LFR (Hz)	PFR (Hz)	HFR (Hz)
Females ( $N=6$ )											
Mean	0.380	0.523	0.1047	-15.32	89.33	40.47	19.00	20.33	7116.67	15968.67	27788.83
s.d.	0.056	0.053	0.0050	11.21	24.24	06.55	02.37	04.41	2286.31	04502.70	04865.99
s.e.	0.023	0.022	0.0021	04.578	09.90	02.68	00.97	01.80	0933.38	01838.22	01986.53
Males ( $N=8$ )											
Mean	0.238	0.579	0.992	-34.63	87.25	44.20	21.50	21.87	5124.00	13013.50	24735.50
s.d.	0.079	0.027	0.027	08.99	18.58	06.53	02.33	04.26	1673.44	04055.56	04693.46
s.e.	0.028	0.009	0.009	03.18	06.57	02.31	00.82	01.50	0591.65	01433.86	01659.39

\*P1, P2 and N are in radians due to transformation;  $y = \sin^{-1}(\sqrt{r})$  where  $r$  is ratio.  $(100) \times (\sin y)^2$  back-transforms to percentage.

The number ( $N$ ) of individuals is shown for each sex.

In these computations, for each EOD variable, the average of six measurements (for each individual fish) was used to calculate the mean and variance (sampling day 3).

See Fig. 1 for definitions of variables.  $QWK = 100 \times [(P1 - P2)/N]$ , from Westby and Kirschbaum (1982).

s.e., standard error of the mean; s.d., standard deviation.  $s.e. = s.d./\sqrt{N}$ .

Note that the percentages P1, P2 and N were converted to ratios and normalized with the arcsine transform for these calculations, and are given in radians here. Percentages are given by  $100 \times (\sin x')^2$  where  $x'$  is the tabulated value in radians.



Sex MS) in relation to the variance introduced by other sources (denominator MS; in this analysis the denominator includes the within-individual variance, i.e. error MS, as well as other sources of variance). This relationship was quantified by the *F*-statistic, computed as the ratio of the MS for the effect (e.g. Sex MS) to the appropriate denominator MS (Zar, 1984; Neter *et al.* 1985). The denominator MS, and degrees of freedom (d.f.), were computed as suggested by Zar (1984, Appendix A). For analyzing sex differences, the denominator MS included variance associated with individual differences (ID) and variation among the different sampling periods (Day) as well as variance within individuals. The denominator effects list for Sex was ID(Sex), Day  $\times$  ID(Sex), Sex  $\times$  Day and Error. The denominator MS for individual differences included variance within individuals on each day as well as variance across days. The denominator effects list for ID(Sex) was Day  $\times$  ID(Sex) and Error (see Table 3).

Discriminant function analysis (Manly, 1986; Wilkinson, 1989) was used to ascertain how well a hypothetical receiver might be able to classify individual fish on the basis of a subset of EOD parameters. Parameters were chosen which may be accessible to a real fish (i.e. receiver). DFA uses multivariate observations on individuals (or 'groups') to compute the mean position of each individual in 'variable space' (see Manly, 1986; Wilkinson, 1989). The distances of subsequent observations, from each of these individual positions, are then determined. Each observation is classified as belonging to the individual to which it is closest. In this study, the success of the discriminant functions was evaluated by determining the proportion of EOD observations that were classified correctly in a DFA based on multiple EOD characteristics.

Principal components analysis (see Manly, 1986; Wilkinson, 1989) was carried out to determine whether a few components, derived from all of the EOD measures, might explain most of the variance in the data. This procedure was also used to transform the data so that the potential information in the EOD signal could be more completely explored. This procedure employs a set of variables, some of which may be correlated, such as those shown in Fig. 1, and uses them to derive principal components. These components are essentially orthogonal axes in multidimensional space: the values, for example representing single EODs along different components, are not correlated but are based on linear combinations of the original measurements. Each individual can then be described by its scores along the different principal components (six in the present case).

Drawing on information theory (e.g. Shannon and Weaver, 1949) and well-known multivariate analytical methods, Beecher (1989) has presented a particularly appropriate use of PCA in the study of communication signals. The methods used here follow directly from Beecher (1989). An important feature of the analysis is that the individual parameters (i.e. variables illustrated in Fig. 1) are weighted according to the amount of variability exhibited by individual animals in that parameter. In an information system, parameters with high intrinsic variability have low information value. The weighting was achieved by dividing all the values for a given parameter by the standard deviation for that parameter.

Standard deviations were computed from the error mean squares resulting from simple ANOVAs of the form:  $ID = \text{parameter}_i + \text{error}$ . The s.d. is then given by  $\sqrt{MS_e}$ . Following this transformation, a six-component PCA was performed on the covariance matrix for all the parameters illustrated in Fig. 1. The information measure  $H_s$  was computed as described in Beecher (1989) and is discussed further below.

#### *EOD characteristics and water conditions*

EODs were recorded within a narrow range of conductivities ( $35 \pm 6 \mu\text{S cm}^{-1}$ ) and temperatures ( $28.0 \pm 1.0^\circ\text{C}$ , s.e.), after the low conductivity plateau had been reached and the animals had begun spawning. By matching conditions between aquaria, influences of water conditions on EOD characters were minimized. In addition, each aquarium held a male and a female (in two cases there were two males per aquarium), so that nearly equivalent amounts of male and female data were obtained from each aquarium.

Analysis of variance revealed no significant differences between aquaria in temperature; temperatures were measured to the nearest  $0.1^\circ\text{C}$  each time EODs were recorded. Even though the aquaria were treated identically, there were small but significant differences between aquaria in conductivity; measured to the nearest  $0.1 \mu\text{S cm}^{-1}$  with a Cole-Parmer no. 12880 meter, calibrated to a  $147 \mu\text{S cm}^{-1}$  standard. However, the largest mean difference was only  $14 \mu\text{S cm}^{-1}$ . In the range of conductivities used here ( $25\text{--}45 \mu\text{S cm}^{-1}$ ), the EOD is relatively conductivity-independent (Bell *et al.* 1976; Bratton and Kramer, 1988), and the small conductivity fluctuations observed were found to influence the waveform characters measured in this study very little. A worst-case estimate for conductivity influences on P1 (more sensitive than D1) revealed a maximum effect of 1 or 2%. These calculations are based on the mean slope, from regressions of P1 on conductivity, for individual fish. This type of difference is much smaller than the significant differences discussed below.

#### **Results**

The EODs of individual breeding fish were extremely stable in form. I noted little short-term variability or dependence of the EOD on context or time of day. Consequently, I could readily distinguish many individuals on the basis of their EODs. Moreover, the type of sex differences originally described for non-breeding fish by Westby and Kirschbaum (1982) were also consistently seen among breeding individuals in this study. Breeding males produce an EOD with a greatly reduced P1 phase compared to females. Example EODs are shown in Fig. 3. Fig. 4 shows all the individuals plotted, on the coordinates P1 and D1 (see Fig. 1A), for a randomly selected day. Note that, although some effort is required to see the differences between the sexes and individuals in the raw EODs (Fig. 3), these differences appear quite clearly in Fig. 4. In this figure, the points corresponding to each of six EODs are plotted for each individual, and the

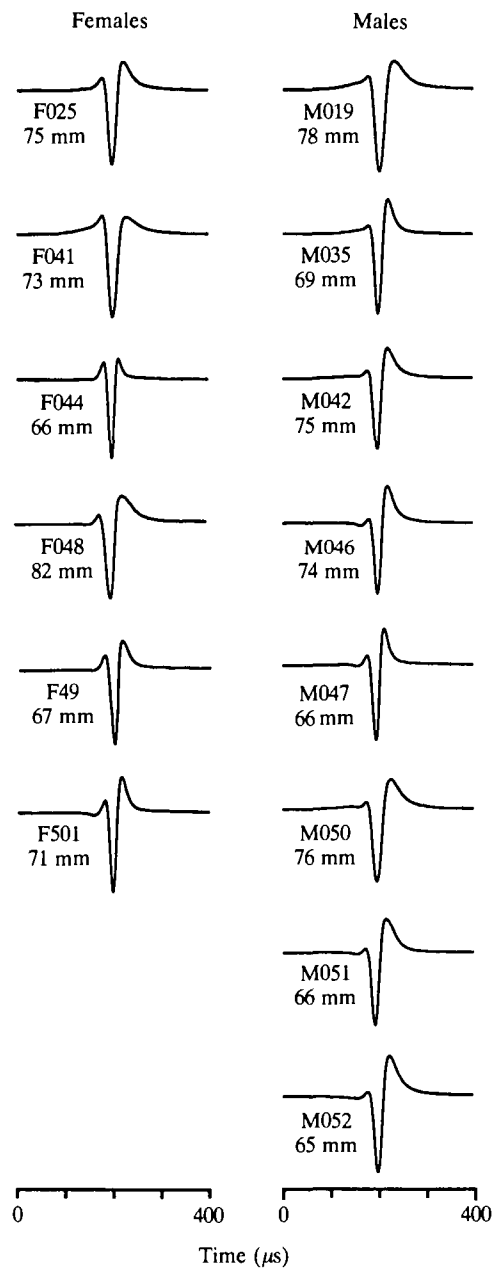


Fig. 3. Electric organ discharges of breeding *Pollimyrus isidori*. Each trace shows a single EOD as it would appear on an oscilloscope: time is shown in the horizontal direction, and voltage (not calibrated) in the vertical direction. Each EOD was scaled to the same peak-to-peak amplitude for plotting (the magnitude of the electric field is about  $5\text{--}10\text{ mV cm}^{-1}$  at a distance of  $10\text{ cm}$  in  $30\ \mu\text{S cm}^{-1}$  water). The identification and standard length of the fish that produced each EOD are given to the left of each trace.

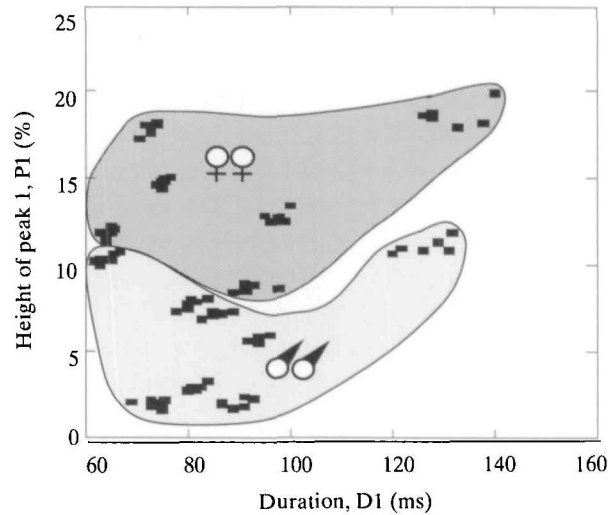


Fig. 4. Plot of all 14 individuals used in this study, as represented by their scores on two EOD variables: P1 and D1 (see Fig. 1). Each individual is represented by six points, corresponding to the six EODs recorded for each fish on this sampling day (sampling day 3). Note that all the individuals show up as distinct clusters of points, and the space enclosing the females (six individuals) does not overlap with the space enclosing the males (eight individuals).

individuals are clearly visible as distinct clusters of points. Individuals appear to separate along both dimensions, whereas the predominant sex difference is along the P1 dimension (vertical axis).

#### *Sex differences in EOD characters*

My analysis of the EODs of breeding fish revealed that there were clear and significant differences between the sexes, differences that the fish could use in sex recognition. In breeding *P. isidori* the relative size of P1 (Fig. 1A) was threefold larger in females compared to males (15 % of peak-to-peak amplitude compared to only 5 % in males; Figs 3 and 4). An ANOVA performed on the entire longitudinal data set confirmed that there was indeed a highly significant sex difference in P1, even after variance over time (Day) and individual variability (ID) had been taken into account ( $P < 0.0045$ , Table 3A).

This finding supports the earlier observation of Westby and Kirschbaum (1982) that there are sex differences in the waveform of the *P. isidori* EOD; application of the descriptive formula they used {i.e.  $[(P1-P2)/N] \times 100$ } to the EODs of breeding fish also revealed a marked sex difference (see QWK: Table 2). The results of this computation for the breeding fish were qualitatively similar to those of Westby and Kirschbaum, in that male scores were lower than females, but there were quantitative differences, which are probably attributable to large differences between the studies in water conductivity and differences in the physiological state

Table 3. Analysis of variance for EOD characters P1 and D1 (see Fig. 1)

Source of variation	SS	d.f.	MS	F ratio	F	P
<b>A. EOD waveform (P1)</b>						
Sex	1.81039	1	M1	M1/(M2+M5-M6)	0011.58	≤0.0045*
ID(Sex)	1.76073	12	M2	M2/M6	0084.43	≤0.0001*
Model	4.56047	83	M3	M3/M7	1085.55	≤0.0001
Day	0.08103	10	M4	M4/M5	0007.16	≤0.0024
Sex×Day	0.01132	10	M5	M5/M6	0000.65	≤0.4857
Day×ID(Sex)	0.08690	50	M6	M6/M7	0034.34	≤0.0001
Error	0.02116	418	M7			
<b>B. EOD duration (D1)</b>						
Sex	003320.68	1	M1	M1/(M2+M5-M6)	002.450	≤0.5000 NS
ID(Sex)	184742.00	12	M2	M2/M6	113.990	≤0.0001*
Model	224578.92	83	M3	M3/M7	450.630	≤0.0001
Day	002815.31	10	M4	M4/M5	001.450	≤0.2500
Sex×Day	001943.64	10	M5	M5/M6	001.439	≤0.2043
Day×ID(Sex)	006752.77	50	M6	M6/M7	022.490	≤0.0001
Error	002509.83	418	M7			

\* Highly significant main effect; NS, not significant.

Sex is the sex of fish for each observation ( $N=2$ ); ID(Sex) is the individual identity of fish for each observation ( $N=14$ , ID nested within sex); Model, independent variable=Sex+ID(Sex)+Day+Sex×Day+Day×ID(Sex); Day, day on which observation was made ( $N=11$  days); Sex×Day, interaction of sex with day; Day×ID(Sex), interaction of sex with ID; Error, variance in observations within each individual, within each day.

SS, sum of squares (type III); d.f., degrees of freedom; MS, mean square ( $MS=SS/d.f.$ ).

The effects lists for the denominator MSs were obtained from the type III expected mean square output generated by the 'Random' statement in the SAS procedure.

For Sex, this list included ID(Sex), Day×ID(Sex), Sex×Day and Error. For ID(Sex), the effects list included Day×ID(Sex) and Error. The arithmetic equivalence of these lists and the denominators indicated in this table are explained by Zar (1984, Appendix A).

of the fish employed. The absolute values for the breeding fish were offset by about  $-20$  relative to the Westby and Kirschbaum data (note that this is a measure without units, based on a ratio multiplied by a constant as shown above).

I found no significant difference between the sexes in EOD duration ( $P \leq 0.5$ , Table 3B). The male mean ( $85 \pm 6.7 \mu\text{s}$ ) and female mean ( $89 \pm 10.7 \mu\text{s}$ ) differed by only  $4 \mu\text{s}$  (computed from the entire data set, 36 observations per individual, 1 mean per fish). In contrast to Westby and Kramer, I found no differences between the amplitude spectra of male and female EODs, computed from fast Fourier transforms. The plotted spectra overlapped extensively, and peak power frequencies did not differ significantly ( $P=0.22$ ,  $t=1.288$ , d.f.=12; females= $16.0 \pm 1.8$  kHz, s.e.; males= $13.0 \pm 1.4$  kHz; analysis of EODs from sampling day 3, 1 mean per fish; Table 2).

*Individual differences in EOD characters*

The EODs produced by single individuals were highly stereotyped, and usually distinguished the individual from other animals with little ambiguity. Within a single day, most individuals were distinguished by one or more EOD characteristics, as illustrated in Fig. 4 for P1 and D1. This was also clear by inspection of longitudinal plots of EOD parameters over the duration of the study (Fig. 5): on any particular day, most individuals were clearly visible as distinct points, and did not overlap with other individuals. Note that on each sampling day, each individual was represented by a mean with standard error bars. The standard errors were small and in most cases are not visible in the plot. In EOD duration the standard errors, for the daily individual samples, were near the limit of the time resolution of the measurements ( $1 \mu\text{s}$ ). For P1 amplitude the standard errors were less than 0.5% (measured as a percentage of peak-to-peak amplitude).

Most individuals were also quite stable in their EOD characteristics from day to day. Over the 11-day observation period some individuals showed noticeable drift in EOD characters, but the magnitude of the drift was usually small compared to the differences between individuals (see Fig. 5). In both EOD characters P1 and D1, individual differences were highly significant, even when variability within and across days was taken into account ( $P < 0.0001$  for P1, Table 3A;  $P < 0.0001$  for D1, Table 3B). Thus, even over long periods (days), many individuals could potentially be identified on the basis of the EOD. The times at which different individuals spawned during this study are indicated by small arrows in Fig. 5A–D; no systematic shifts in the EOD at the time of spawning were apparent.

*Classification of individuals by EOD characters*

Individual fish can be recognized and classified accurately on the basis of their EODs. I used a discriminant function analysis to explore quantitatively the extent to which individual fish can be recognized on the basis of their EOD characteristics. A subset of six EOD characteristics was chosen for this analysis (see Fig. 1): P1, P2, D3, D4, LFR and HFR. These variables constitute three pairs of measures

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Fig. 5. Longitudinal plots of EOD waveform,  $[P1/(P-P)] \times 100$  (A and B), and EOD duration, D1 (C and D). Data from females are shown on the left (A and C) and for males on the right (B and D). Each individual is represented by six successive points connected by a line (see inset, lower right). Each point is the mean of six measurements ( $\pm 1.0$  s.e.; s.e. not visible on this scale in many cases) from a single sampling session (i.e. collected over a 5 min period). For the waveform measure (top), means were computed from arcsine-transformed ratios and back-transformed to percentage. The approximate time(s) when each individual spawned are indicated by individual identification numbers and accompanying arrows. Note that the relative amplitude of P1 was much smaller in male EODs (B) than in female EODs (A), and this was reflected in the measure of waveform used here (there was a highly significant sex difference in this quantity). There was no significant sex difference in EOD duration (C vs D). Note also that in both EOD measures most individuals can be distinguished both within and between days. Water conditions (temperature and conductivity) are shown for each animal in E.

corresponding to the following distinct categories: (1) relative size of peaks, (2) time intervals defined by major voltage transitions in the EOD, and (3) bandwidth in the frequency domain. Based on what is known about the physiology of these animals, it seems possible that the fish could have access to all of this information,

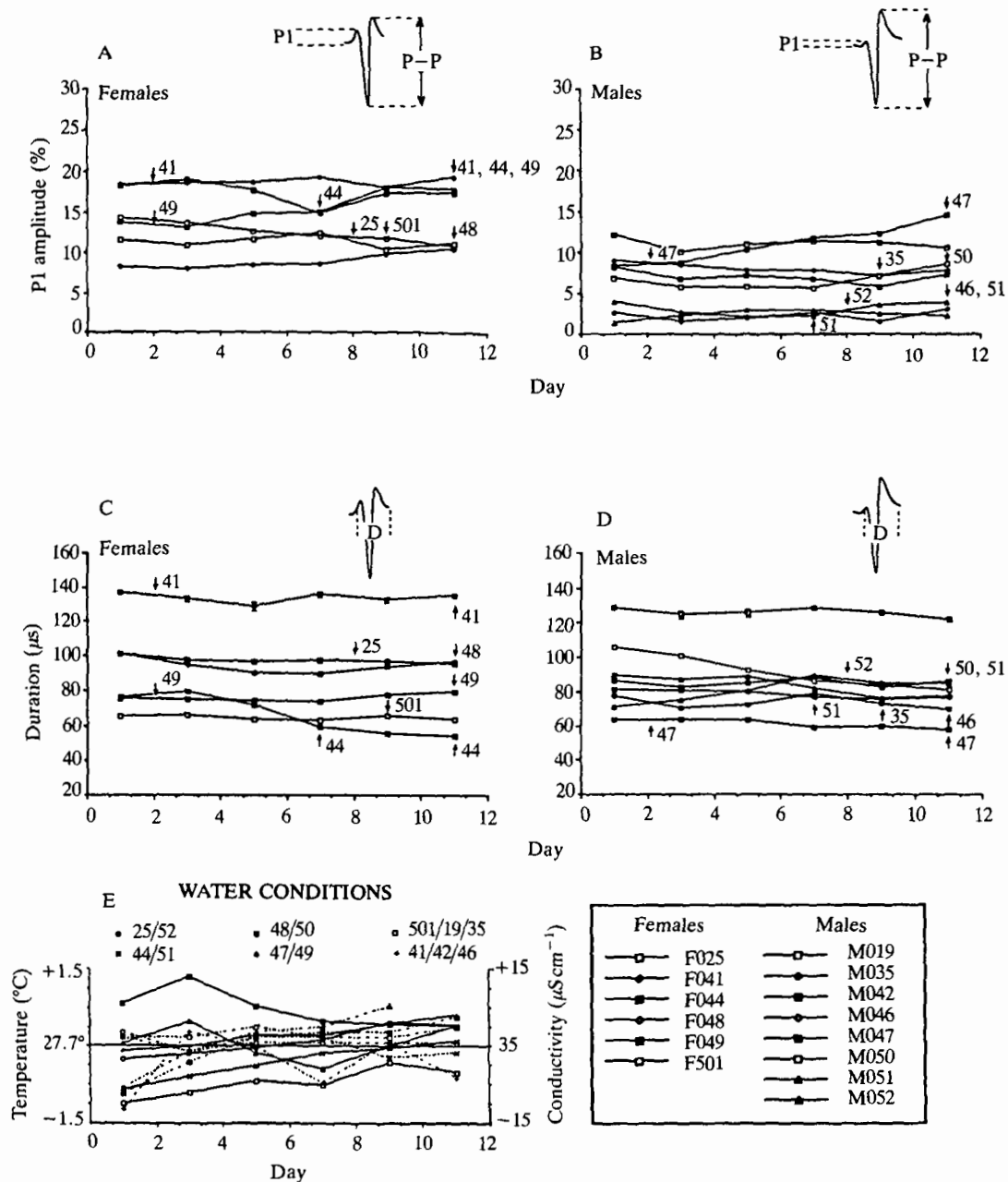


Fig. 5

but additional physiological and behavioral data are definitely needed to evaluate this (see Discussion).

Since individual animals are likely to be in frequent electric communication with their neighbors and potential mates, I postulated that information gathered during a particular set of interactions might be used to identify individuals during a future encounter. I evaluated this idea by computing discriminant functions for all individuals on the basis of their EODs recorded on a randomly selected day (sampling day 3 for the study). The question was then to determine how well the discriminant functions could classify individuals on the basis of EODs produced at a later time, approximately 48 h later (sampling day 3+1). This discriminant function analysis revealed that individuals could be classified extraordinarily well: 92.9 % of the classifications were correct (all individuals were classified correctly on the day used to compute the discriminant functions). The error rate (7.1 %) was due entirely to the incorrect classification of male 47 as female 501. This male had an EOD that was 'female-like' in the size of P1, and appeared virtually identical to the EOD of female 501 (see Fig. 3). Thus, a receiver capable of making, and remembering, these six measurements (or similar correlated measurements) could do very well at recognizing a small group of neighbors and potential mates. In the parlance of information theory, the EOD possesses *signature* information. This information might be used by the fish and could clearly be used by investigators wishing to track individuals.

#### *Principal components analysis*

Most of the variance in the EOD data can be explained by two dimensions derived from the original ten measurements made on each EOD. This was shown through a principal components analysis on all the EOD parameters shown in Fig. 1. Over 95 % of the variance in the original data set is explained by the first two principal components computed in this analysis (Table 4). Fig. 6 plots the individual fish (based on sampling day 3) as a function of their first two principal component scores. Note that as in Fig. 4, the individuals and the sexes are clearly distinct in the principal component plot. Even though many of the original EOD variables were highly correlated (Table 1), one can gain insight as to their contributions to these principal components that explain so much of the variance. This can be seen from the component loadings provided in Table 4. Principal component 1 explains more than half of the variance in the data, and the parameters with the four largest component loadings (i.e. absolute values) came from all three categories listed above in the discriminant function analysis: (1) relative size of peaks, (2) time intervals defined by major voltage transitions in the EOD, and (3) bandwidth in the frequency domain. All three categories seem to be 'important' insofar as they contribute to the information content of the EOD as a signal.

#### *Information value of the EOD*

The mathematical transformations implemented in the principal components



Table 4. Principal components analysis of EOD variables

COMP	EIGEN	%EXP	Component loading									
			HFR	LFR	P1	D2	PFR	D4	D1	D3	N	P2
1	562.3	58.2	-14.0	-10.8	-10.6	+6.7	-6.5	+4.3	+4.2	3.1	-1.6	1.5
2	364.8	37.8	-5.6	-0.3	12.4	3.6	-1.7	2.7	5.8	1.2	7.8	-7.8
3	22.2	2.3	-1.2	2.4	1.3	1.4	0.4	1.0	0.8	0.5	-2.2	2.2
4	7.5	0.8	-1.0	1.8	-1.1	0.1	0.0	-0.1	-0.8	0.2	0.9	-0.7
5	3.6	0.4	0.7	-0.0	-0.5	0.8	1.1	0.7	0.5	0.2	0.2	-0.3
6	2.3	0.2	0.1	-0.2	0.2	0.4	0.0	-0.6	-0.5	1.2	0.1	0.2

Each variable was normalized by its s.d., and the principal components analysis was then performed on the covariance matrix.

COMP, component number; EIGEN, eigenvalue; %EXP, variance explained by the principal component =  $[\text{EIGEN}_i / (\sum \text{EIGEN})] \times 100$ .

The component loadings are shown for each principal component and EOD variable.

The EOD variables are illustrated in Fig. 1.

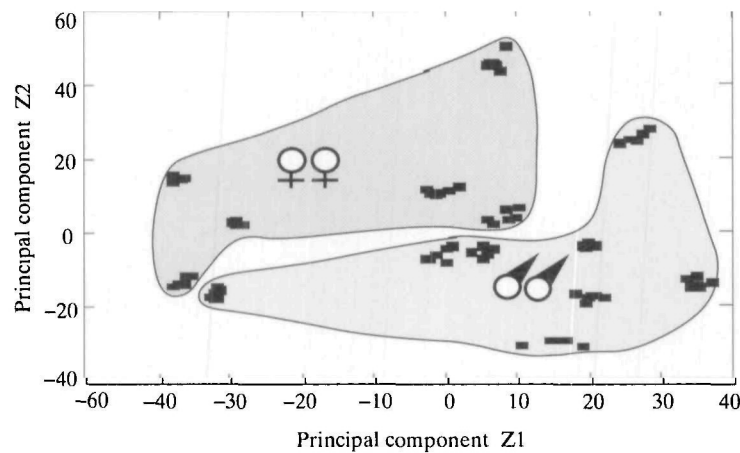


Fig. 6. Plot of all 14 individuals on a coordinate system defined by principal components 1 and 2. Note that these axes incorporate all ten EOD variables and that the two components used here explain more than 95% of the variance in the data. Analysis from sampling day 3, six EODs per fish.

analysis above make it possible to estimate the intrinsic information value of the EOD signal. This is because the PCA derived new dimensions that are completely uncorrelated, no new variance was introduced, and because the data were normalized so that original measurement units (e.g.  $\mu\text{s}$  vs  $\text{s}$ ) are no longer important. As a result, the component scores can be used to estimate the potential value of the signal from the variability in the transmitters' production of the signal and the differences between the signals produced by different transmitters. These

methods and ideas, derived from information theory, have been detailed by Beecher (1989). It has been suggested that the measure of *signature information* ( $H_s$ ) may prove particularly useful for comparing similar signals made by related species with interesting differences in behavior and ecology.

The information measure  $H_s$  was computed on the basis of one-way ANOVAs on the six principal components discussed above and presented in Table 4 (see Beecher, 1989; equations 13 and 14:  $H_s = \sum \log_2 \{ \sqrt{[(F-N-1) \div N]} \}$ , where  $N$  is observations per individual, or 6 in the present analysis, and  $F = MS_{ID} / MS_{error}$ ).  $H_s$  was 12 bits for the first five components, the last component (6) adding no additional information. Thus, in theory, the EOD has sufficient information to distinguish a very large number ( $2^{12} = 4096$ ) of individual fish (see Discussion, and Wilson, 1975; Beecher, 1989). However, additional theoretical considerations, as well as receiver characteristics and channel noise, would place the actual number of reliably identified individuals much lower than this. Nevertheless, an  $H_s$  of 12 bits for *P. isidori* compares favorably with the communication signals of several other vertebrate animals analyzed in the same fashion (Beecher *et al.* 1986); it should prove interesting to compare the EOD of *P. isidori* with the EODs of other mormyrid species using this sort of analysis. However, it should be emphasized that the exploitation of the information captured by  $H_s$  remains largely unexplored in *P. isidori*, or in any other mormyrid fish; the information is available in the EOD but we do not know how much of it is used by fish in their natural behavior.

### Discussion

In this study I have provided a detailed analysis of the *P. isidori* EOD as a potential signal in social communication. I chose to examine the EODs of breeding adults so that I could address important questions about sex recognition and other forms of communication that are likely to have a direct impact on the success of breeding adults. Two essential observations were made. First, there were indeed significant differences between the EODs of reproductive males and females, differences that could be used in sex recognition. Second, beyond the sex differences there were striking differences between the EODs of different individuals. These individual differences have not previously received much attention in studies of mormyrid fish, but may be important in the behavior and ecology of these animals; some of the characteristics that distinguish individuals, temporal characteristics, are already known to be salient for the electrosensory system in these fish. It is hoped that the data presented here will be useful in designing future behavioral and physiological experiments to yield new information about how these fish actually use the EOD in communication.

#### *The EOD as a sex recognition cue*

The data show that the electric organ discharge of *P. isidori* is sex-specific during breeding, under naturalistic conditions. These results support the suggestion of Westby and Kirschbaum (1982) that the EOD of *P. isidori* contains cues that could

be exploited for sex recognition. Although there is some minimal overlap in the male–female distributions of some of the waveform characters (e.g. P1), this should not be taken as strong evidence against the use of the EOD in sex recognition, as was advocated by Bratton and Kramer (1988). Overlap exists in many sexually dimorphic characters of vertebrate animals, even in characters as fundamental as the reproductive organs themselves (e.g. Catlin and Crawford, 1990). Under natural breeding conditions in which mate choices exist, it may be that the uncommon fish, which fall in overlap zones for EOD characters, are at some disadvantage. Moreover, if the receiving fish could extract multiple cues from the EODs of transmitting fish, then EOD-based sex recognition is likely to be more reliable. Bratton and Kramer also proposed that the effects of conductivity on EOD characters will prevent their use in sex recognition because conductivity is variable in the natural environment. However, in evaluating this idea, it is important to remember that communicating individuals will be under identical water conditions. While gradual changes in conductivity may produce small changes in EOD characteristics, the differences between the sexes will persist. Thus, despite the caveats of Bratton and Kramer, it is my conclusion that the EOD in this mormyrid meets several important criteria as a potential sex recognition cue.

#### *The EOD as an individual recognition cue*

Of the two EOD dimensions considered with ANOVA on the longitudinal data set, a true sex difference was found only in the relative amplitude of the peak P1 (i.e. vertical dimension), and not in the duration of the EOD (D1). However, the finding that there were also highly significant differences between individuals in both EOD duration and relative amplitude indicates that the EOD could play a role in sex recognition and mate choice in two distinct ways. First, if a fish were able to discriminate differences in P1 (or in correlated parameters), it could use this in true sex recognition through a direct analysis of a conspecific's EOD, as discussed above. Second, since both types of parameters can be reliable identifiers of individuals, a fish might use the EOD in individual recognition, and then only indirectly in sex recognition. In this scheme, the fish might use non-electric cues to learn the sex of another fish whose identity was known on the basis of its EOD features. Individual recognition is now well known in some vertebrate animals (e.g. Falls, 1982), and could play an important role in mate choice and neighbor recognition in mormyrids.

Although much work remains to be done on the possibility of EOD-based individual recognition, two additional lines of evidence suggest that this is a subject worthy of more attention. First, in a recent experimental analysis of courtship responses to playbacks of EODs, I have found that EOD characteristics apparently do not function in true *sex recognition*, even though electric signalling is clearly important in courtship (see Crawford, 1991). In this study, the hypothesis of true EOD-based sex recognition was tested. The data did not support this hypothesis and left open the possible importance of individual recognition. A

second line of evidence comes from additional behavioral studies, using a differential conditioning paradigm, which have provided preliminary support for EOD-based individual recognition in *P. isidori* (Graff and Kramer, 1989). Further behavioral research is required to evaluate these two potential roles for the EOD, but the available behavioral data point only to individual recognition in this species.

The possible utility of the EOD, as an individual signature, will depend on a variety of things, including the number of individuals that the receiver fish must recognize individually, the number of individuals that must simply be classified as 'non-targets' and the total number of EODs in the local population (see theoretical discussion in Beecher, 1989). Unfortunately, relatively little is currently known about the behavioral ecology of *P. isidori* from field studies. However, laboratory observations show that males are territorial. Based on my own observations, I would predict that under natural conditions the territory size might be about 1 m in diameter. Since electric communication is a relatively short-distance phenomenon (see Hopkins, 1988; Moller *et al.* 1989), inter-male communication may be dominated by interactions with a few individuals (4–6) holding adjacent territories. It is more difficult to predict male–female encounters because females probably interact with many males in the process of selecting mates, thus increasing the number of individuals detected by any given receiver fish. Nevertheless, the relevant number of individuals in a localized breeding area is probably reasonably small, especially in relation to the theoretical information content of the EOD, discussed above. Consequently, even if *P. isidori* were to use only a subset of the cues included in the analysis presented in this paper, the EOD could prove useful in individual recognition.

#### *Physiological basis of EOD analysis*

Little is known about how the EOD is actually represented in the central nervous system of *P. isidori*. However, behavioral and physiological studies on related mormyrids do provide some useful information. It is well known that the knollenorgan electroreceptive pathway is specialized for the reception of EODs of other individuals (as opposed to the animal's own EOD), and that the pathway encodes information about the times of major voltage transitions while being a relatively poor encoder of signal amplitude (reviewed in Hopkins, 1986; Amagai *et al.* 1988; Bell and Grant, 1989). Experiments with a mormyrid in a different genus, *Brienomyrus brachyistius* (Hopkins, 1981, 1983; Hopkins and Bass, 1981), support a model of EOD sex recognition based on a temporal code. In the following paragraphs, the *P. isidori* EOD is analysed in terms of what has been discovered in these previous studies.

The knollenorgans generate single phase-locked neural spikes in response to each of two major voltage transitions in the *B. brachyistius* EOD: since the knollenorgans are polarity-sensitive, the EOD is believed to be represented by the time difference between neural spikes arriving at the brain from one side of the electroreceptive body surface (response to first major V transition) and spikes

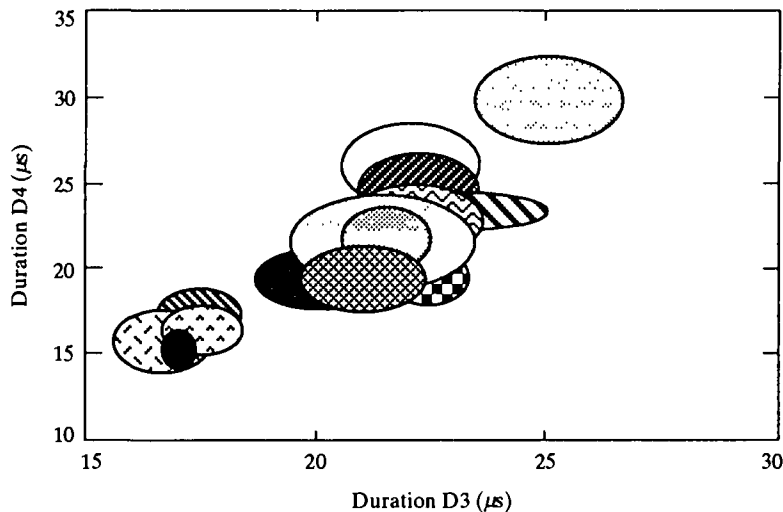


Fig. 7. Plot of 14 individuals in terms of durations D3 and D4. Each individual is represented by a single ellipsoid that encloses all six points for the individual. Data from sampling day 3 as in Figs 4 and 6.

arriving from the other side (second major V transition). Since the physiology of knollenorgans appears to be similar in *B. brachyistius* and *P. isidori* (see Hopkins, 1981; Raman, 1988), it is reasonable to consider the possibility that the EOD of *P. isidori* might be used in *individual recognition* through a similar mechanism (the data simply do not support a direct role for these temporal cues in sex recognition in this species). It should be noted that the application of this temporal code model (for *B. brachyistius*) to *P. isidori* is complicated by the fact that the *P. isidori* EOD is triphasic (but only biphasic in *B. brachyistius*), and by the fact that the EOD is an order of magnitude shorter in *P. isidori* (about  $100\ \mu\text{s}$  vs  $1\ \text{ms}$ ). Nevertheless, a population of knollenorgans might yield a CNS representation of the EOD consisting of spikes locked to P1, followed by N, and ending with P2. To illustrate temporal representation based on this model, the two time intervals corresponding to these three spike times (i.e. D3 and D4, Fig. 1A) are plotted for the *P. isidori* EODs in Fig. 7: ellipsoids were used to enclose all the values for each individual (6 measurements each, for sampling day 3). On the basis of these temporal cues, many individuals overlap, and individual recognition is thus constrained. A discriminant function analysis based on D3 and D4 (data from sampling days 3 and 4) showed that only half of the individuals were classified correctly on the basis of their EODs after 48 h (i.e. at least 4 of 6 EODs per individual were classified correctly; see Materials and methods and Results). The discriminant functions computed for sampling day 3 classified 73 % of the total 84 EODs correctly on day 3, but only 44 % correct on day 3+1. Based on these analyses, it is clear that *P. isidori* would not do particularly well by analyzing the EOD in the fashion suggested for sex recognition in *B. brachyistius*; considerable advantage would be

gained by extracting additional information from the EOD. Nevertheless, a few individuals might be recognized on the basis of these temporal characteristics.

With information about the frequency content of the signal and relative amplitudes of the peaks of the EOD, a receiving fish could potentially identify a reasonably large number of individuals. Studies of several mormyrids, including *P. isidori*, have shown that the knollenorgans are broadly tuned to different frequencies, and that they fall into two or more classes based on best excitatory frequency (Hopkins, 1981; Raman, 1988). These aspects of receptor physiology lend some support to the idea that *P. isidori* may have access to frequency cues such as those used in the discriminant function analysis and principal components analysis. Understanding the use of P1 and P2 in terms of present knowledge of the physiology is more problematic. Nevertheless, two possibilities are offered. First, since there is significant individual variation in peak size, it is conceivable that, on average, the EODs of some individuals are represented as two time intervals (P1 and P2 both relatively large), and those of others as a single interval (P1 quite small, as in many males). This could at least be the basis for a partial extraction of the information generally available in P1 and P2. A second possibility comes from the demonstration of Bell and Russell (1978) that the ampullary receptor system (low-frequency tuberous electroreceptors) may be stimulated by the d.c. component of the EOD, at least at relatively high EOD amplitudes. The *P. isidori* EOD is asymmetric about 0 V, and thus has a d.c. component. Since the d.c. component of the EOD will depend upon the sizes of the positive peaks (P1 and P2) and the negative peak (N), a receiving fish might gain some information by attending to this d.c. signal. However, use of the d.c. component as a cue is clearly complicated by the fact that its magnitude and sign will depend upon the distance and orientation of the receiver with respect to the sender (see additional discussion of the possible use of non-knollenorgan receptors for communication in Hopkins, 1986). Additional behavioral and physiological studies could advance this analysis greatly.

In conclusion, it appears that some of the controversy concerning the presence or absence of sex differences in the EODs of *P. isidori* has resulted from studies in which the fish were non-breeding, the water conditions were variable and/or unnatural, and in which the investigators expected perfect separation of the sexes on the basis of a single EOD character. The sex differences in the EOD of *P. isidori* are clear, and sex differences are probably a general characteristic of breeding adult mormyrids. A major challenge now is to evaluate carefully what information *P. isidori* actually extracts from the EOD. Perhaps the most striking finding presented in this paper is the great potential for individual recognition based on multiple features of the EOD. An experimental analysis of the exploitation of this signature information should be very interesting, and may provide new information about the electrosensory capabilities of these fish.

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