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SPECIES SPECIFICITY OF ELECTRIC ORGAN DISCHARGES IN A SYMPATRIC GROUP OF GYMNOTOID FISH FROM MANAUS (AMAZONAS)

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SUMMARY We collected weakly electric gymnotoid fish in the vicinity of Manaus, Amazonas, in the Solimoes river (white water). We tried to find out whether Electric Organ Discharges (EODs) are species specific which is essential for their presumed role in recognition of conspecifics and reproductive isolation. We considered at least 43 valid sympatric species, some of them unnamed. All of these displayed stable EOD waveform patterns, most of them clearly distinct from the other species' EODs. Eleven species are of the pulse EOD type, 32 of the wave EOD type (one of the latter is intermediate). The EODs of pulse species were analysed (1) by EOD repetition rate at rest (variation from <1 Hz to 60 Hz), (2) by Fourier amplitude spectrum analysis of single EODs (Fig. 1; in these spectra, frequencies of peak amplitude ranged up to 2300 Hz). There was a significant, positive correlation between both parameters (Fig. 2). Identification of pairs of species with similar EODs by these parameters does not appear to be possible because of inter-individual EOD variations. In wave species there is conclusive evidence that EOD fundamental frequencies (= repetition rate of a complete EOD period) do not allow species identification: twenty-eight wave species displayed EOD fundamental frequencies from 300 to 1800 Hz (Fig. 3). This yields a hypothetical frequency band of 0.09 octave to signal species identity; the actual value of EOD frequency variations in Eigenmannia is much greater (1.2 octaves). Seven species of the family Apteronotidae displayed a new signal type: the main energy of the signal was contained in higher harmonics, and not at the fundamental frequencies (Figs. 6 and 7). For all wave species there was a significant, positive correlation between their dominant frequency (= the strongest signal component) and harmonic content of their EOD although individual species deviated considerably from what was predicted by the regression line (Fig. 8). Thus separation of species was greatly improved compared to the criterion of fundamental frequency (Fig. 3) but still appeared insufficient in a number of cases.
Therefore, in both wave and pulse species still other parameters must be involved in recognition of conspecifics.

INTRODUCTION

The weakly electric knife-fishes (Gymnotoidei) of South-America are nocturnal fish hiding during the day and dispersing widely during night (Lissmann, 1961; Lissmann and Schwassmann, 1965; Steinbach, 1970). As far as they are social, they reaggregate each morning at apparently the same spots although some fish made diurnal migrations of at least 100 m and from a depth of 10-20 m to the surface close to shore (Steinbach, 1970). Steinbach noted also that fishes of different species whose Electric Organ Discharge (EOD) characteristics (EOD frequency and waveform) appear to be identical can quite clearly recognize members of their own species.

Hopkins (1974) concluded that species-specific information is conveyed by the EOD of Eigenmannia (which is of the quasi-sinusoidal, continuous "wave" type (see, e.g., Fig. 3, left inset), as opposed to the "pulse" type where the EODs are separated by relatively much longer intervals (see, e.g., Fig. 1, upper left inset). Aggressive or courtship responses were elicited in Eigenmannia by playback of conspecific signals, or by sinusoidal electric stimulation in the frequency range of 200 to 700 Hz, only. This frequency range approximately corresponds to the normal range of EOD fundamental frequencies of Eigenmannia (240-600 Hz). Three other sympatric species of wave gymnotoids had either lower (50 to 150 Hz) or higher (750 to 1250 Hz) frequencies; responses of Eigenmannia to playback of their signals or to sine waves of their fundamental frequencies were either lacking or considerably less.

Gymnotoid weakly electric wave species are known to possess tuberous electroreceptors matched to the species' (or even to the individual's) EOD waveform: the electroreceptors are most sensitive to the species' (or to the individual's) fundamental EOD frequency (Bennett, reviewed in Bennett, 1971a, b; Scheich et al., 1973; Hopkins, 1976; recent review: Viancour, 1979a; Viancour, 1979b, c). Recent investigations demonstrated species-specific tuberous receptor tuning also in gymnotoid pulse species: the most sharply tuned tuberous electroreceptors were tuned approximately to stimulus frequencies corresponding to the peak power frequency of the Fourier spectrum of single EODs of the species (Bastian, 1976, 1977; Hopkins and Heiligenberg, 1978). However, in the last mentioned work two out of three coexisting Hypopomus species showed overlap of tuberous receptor characteristic frequencies although the peak power frequencies of the respective EODs were clearly separated by more than one octave. In pulse species, pulse repetition rate may also signal species identity (Black-Cleworth, 1970); this, however, presumably is a central and not a peripheral property (Scheich and Bullock, 1974).

The finding of a close match of tuberous electroreceptor tuning to the species' EOD fundamental (in wave fishes) or peak power frequencies (in pulse fishes) in most species investigated has suggested that gymnotoids in one geographic area may enjoy a relatively private communication channel for electrolocation and communication (other species' EODs are "filtered out" by primary afferents), and that the EOD may serve as a species identification signal (most recently expressed in Hopkins and Heiligenberg, 1978).

It is clear that the sensitivity of electroreceptors of a species for spectral cues and its EOD signal must undergo a coupled evolution. As far as the EOD is concerned, the above presumed functional roles rely on at least three crucial points: (1) the width of the occupied range of EOD frequencies in one geographical locality, (2) the number of species packed into that range, and (3) the individual- and species-specific variability of EOD spectral characteristics (or the range of EOD fundamental and peak power frequencies occupied by each species at a given temperature).

The most recent systematic review of the gymnotoids of Brazil by Fowler (1951) lists 36 species. At one specific locality in South America, considerably smaller number of species were found. Steinbach (1970) found 13 species downstream of the confluence of the Rio Branco and the Rio Negro, about 200 miles north of Manaus. From the same locality, Bullock (1969) reports two further species, one of which ("Sternarchogiton sp. (Adontosternarchus ?)") may be one of Steinbach's "Adontosternarchus sp. var.". Thirteen species of gymnotoids were found by Hopkins (1974) in the Rupununi district of Guyana (Amazon drainage), whereas Hopkins and Heiligenberg (1978) found 11 species in the coastal Guyanas in N.E. Surinam. The span of fundamental frequencies was 4.1 octaves in wave fishes (data from Steinbach, 1970) and 3.6 octaves in peak power frequencies of the EODs of pulse species (Heiligenberg and Bastian, cited in Hopkins and Heiligenberg, 1978), in fishes of the Amazon drainage system. Steinbach (1970) found ten wave species, so this amounts to a hypothetical EOD frequency span of 0.4 octave/species under the assumption of a regularly spaced distribution of the species' EOD frequency ranges over the entire frequency range. The frequency band occupied by Eigenmannia usually called virescens is much greater (approx. 1.2 octaves); this range overlaps (very probably extensively) with at least one further Eigenmannia species in Surinam (Hopkins and Heiligenberg, 1978). This jeopardizes the hypothesis that species identity can be signaled by the EOD fundamental frequency as a universal principle in gymnotoid wave fishes, except in those localities where no overlap in EOD fundamental frequency ranges with those of coexisting species occurs.

We observed a by far greater number of sympatric gymnotoid wave and pulse species than recognized hitherto in the vicinity of Manaus. We recorded the EODs of almost all gymnotoids found and tried to characterise species differences. Some of the fish are apparently unnamed. A complete list of species with descriptions of their external morphology and of their EOD waveforms is in preparation.

MATERIAL AND METHODS

We worked at the Instituto Nacional de Pesquisas da Amazonia (INPA) in Manaus, Amazonas, from February 28 to April 10 in 1978 (beginning of the rainy season). The fishermen of the ichthyological department of the INPA assisted in catching the fish from a small boat with large circular nets in a water depth of approx. 1-2 m. The fishermen were guided by our "fish detectors" (small electrode-amplifier-loudspeaker assemblies) with which we were able to detect the presence of electric fish from a distance of up to 3 m. These devices were also useful to crudely classify the signals as to EOD frequency and pulse repetition rate.

Most catches were done on the Solimoes (= Upper Amazon) during day near an island called Marchanteria (upstream of the confluence of the Rio Negro; white water). The electric fish had a strong tendency to stand in high grass (mainly Paspalum) so that closing the circular net at the bottom was impossible without first clearing the ground. Some very successful catches were done near the opening of a canal from the Solimoes to Lago Janauaca. Lago Janauaca proved very rich in gymnotoids but fishing during day was rather unsuccessful because the fish were hiding in a dense grass vegetation and escaped from the net. A few catches were performed during night close to Marchanteria with a large seine from a ship. We also observed gymnotoids with our fish detectors in streams and creeks in the vicinity of Manaus: Estrada Manaus-Itacoatiara, km 45; Reserva Ducke, Acara; Estrada Manaus-Boa Vista: Igarapé Lajes (km 135); Rio Preto da Eva, Rio Tarumazinho (km 25). Judging from the sounds produced by the fish detector these fish were low EOD rate Hypopomus sp., Sternopygus sp., Eigenmannia and high frequency Apteronotids, as well as high EOD rate pulsers (such as Gymnotus, Steatogenys or Rhamphichthys). These fish were not caught.

The fish caught in the Solimoes were brought to the laboratory in large, constantly oxygenated containers filled with Solimoes water. Some species proved extremely fragile and were wounded by the net; they usually died in a matter of a few hours. After coming home the fish were placed singly in a recording aquarium fitted with plants and a ceramic tube, one of which was accepted as a hiding place by most fish. When the fish was at rest the positive electrode was placed in front of its head, and the negative electrode (both carbon rods) behind its tail. The EODs were displayed on a battery-powered storage oscilloscope Tektronix 214. An also battery-powered Nagra IV-SJS tape recorder (20-35000 Hz at tape speed 38.1 cm/s) was used to record the EODs. In most cases, amplification by the input circuitry of the Nagra recorder was sufficient. In some cases a WPI-DAM6A preamplifier (battery-powered) was used (x 100, frequency range set at 1 Hz - 100 kHz). Temperature varied between 27 and 29°C, conductivity of the Solimoes-water was around 65-70 µS/cm.

Back in Germany, the tape-recorded EODs were digitized by a Nicolet 1074 instrument computer with Model SD-72/4A signal digitizer (9 bits) and Model SW-71A wide range sweep control at a sampling rate of 50kHz. Depending on the frequency content of the EODs, tape speed was reduced by 1/2 or 1/4 during playback. 2K of digital data were output on paper tape and transferred onto magnetic tape files in the computer center of the University of Konstanz. These data files were used as the input to a computer program written in Fortran IV which performed a Fourier analysis and calculated 1000-point amplitude spectra (for further details, see Kramer, 1979). These amplitude spectra were output by a digital plotter connected to the computer.

Identification of species proved extremely difficult or even impossible in some cases, especially in the genera Hypopomus, Rhamphichthys, Eigenmannia, and several apteronotid genera such as Adontosternarchus. In the beginning of our work the following literature was consulted: Eigenmann and Ward (1905), Ellis (1913), Fowler (1951), Boeseman (1952), Hoedeman (1962), and Mago-Leccia (1976, 1978). Soon it became clear that there is some confusion concerning gymnotoid taxonomy, partially caused by Ellis' (1913) paper who apparently had done some simplifications which were not justified (e.g., in the genera Eigenmannia and Sternarchorhynchus). In order to solve the taxonomic problems of our Manaus material we therefore had to look at the original descriptions (e.g., Valenciennes, 1847; Müller and Troschel, 1849; Steindachner, 1868, 1878; Peters, 1877; Eigenmann and Allen, 1942), and had to compare our material with the types found in different museums in North- and South-America, as well as in Europe.

Presented with basic problems such as these, and because of the great number of species encountered, we normally limited our investigation of electric signals to one individual per presumed species. We excluded wounded specimens and those having a regenerated tail. Animals whose EODs had been recorded were photographed alive and then fixed in formalin, identified by a tag.

RESULTS

The number of species we found was impressive. Our preliminary list of fishes we consider valid species includes at least 43 species.

1. Pulse Fishes

We found 11 species of pulse fishes whose names (mostly preliminary working names) are given in Fig. 2 (our "Rhamphichthys sp. 2" is not included in the figure). Hypopygus was obtained from a fish dealer and very probably is not from Manaus (presumably from Leticia/Columbia, upper part of the Solimoes); it is included for comparison, only. We do possess fixed specimens of Hypopygus from Manaus, however.

The first analysis we did was EOD repetition rate. One individual per

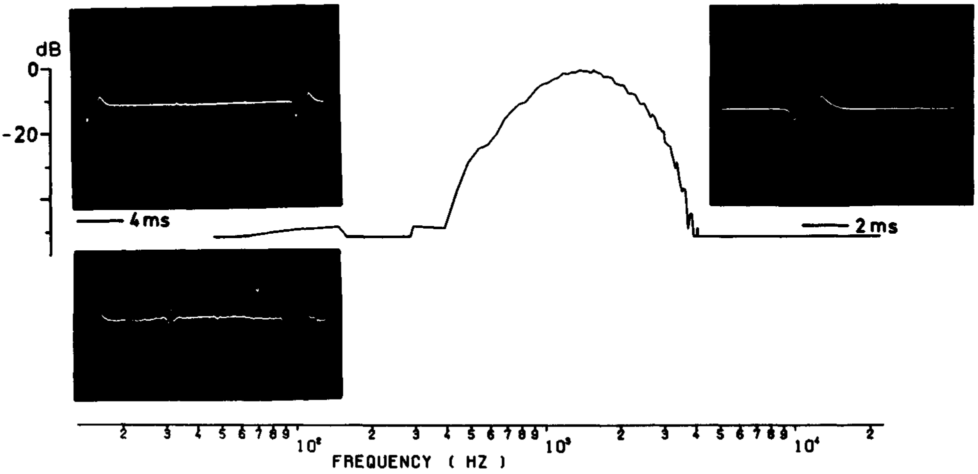

Figure 1: Amplitude spectrum of the pulse discharge of Rhamphichthys sp.3, Ordinate: intensity relative to the frequency of highest amplitude. Insets: show digitized waveforms of the discharges. Lower left: high magnification to show low amplitude potential in between discharges found in several pulse species. Time calibrations refer to insets.

Figure 2: Plot of EOD repetition rate vs. frequency of peak amplitude, as found by Fourier analysis of single EODs, in sympatric pulse fishes. The horizontal bars refer to the limits of the pulse rate distributions, the triangles indicate the modes of these distributions (measured as intervals with a high resolution digital computer). Two specimens of Hypopygus (boxes) are from a different location; pulse rate variation~~s~~ was as narrow as in Steatogenys. A least squares regression line shows the positive correlation between the modes of pulse rate distributions and frequencies of peak amplitude (the data for the allopatric Hypopygus are not included). Each point represents one individual.

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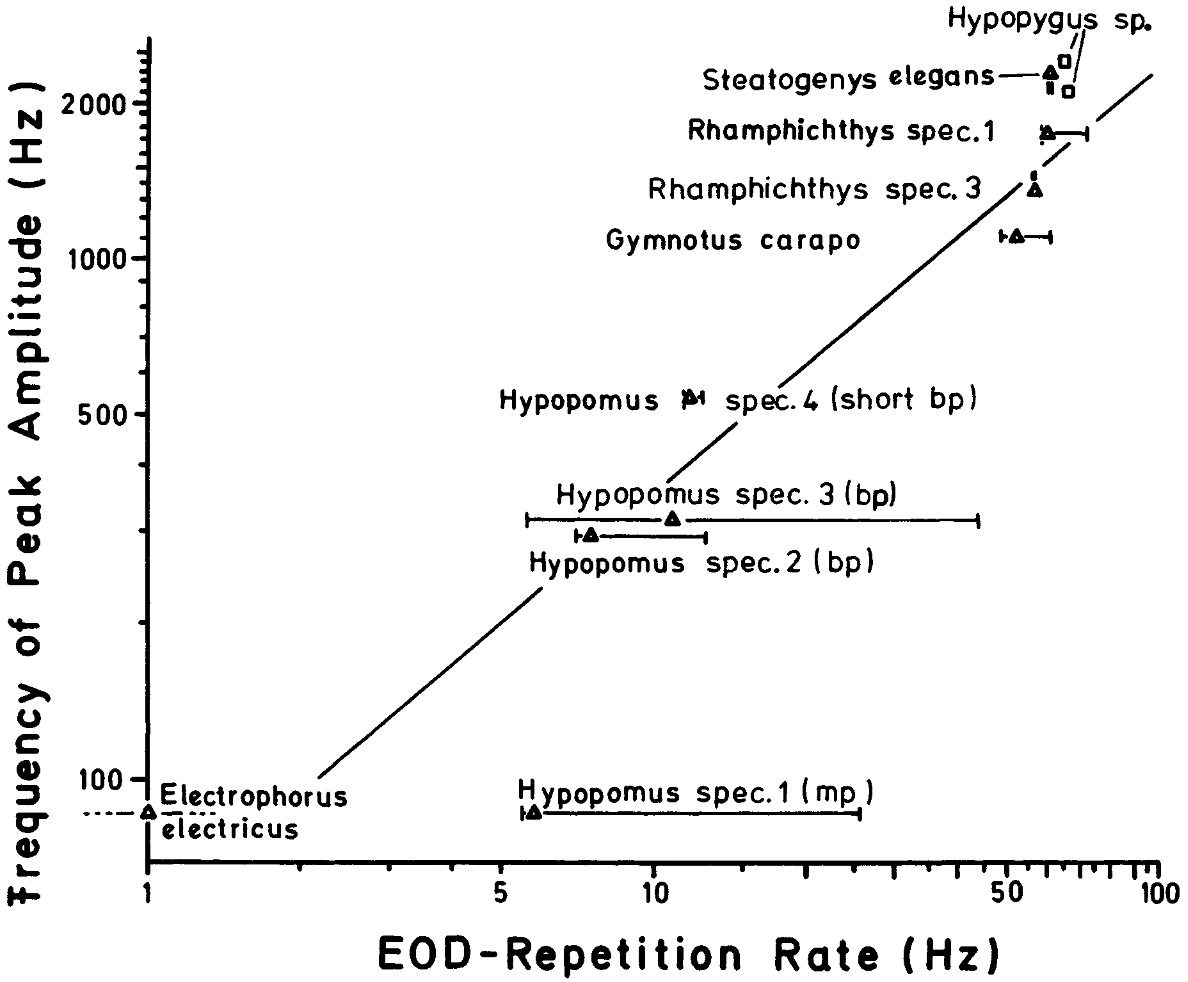

Hypopygus sp.
Steatogenys elegans
Rhamphichthys spec.1
Rhamphichthys spec. 3
Gymnotus carapo
Hypopomus spec. 4 (short bp)
Hypopomus spec. 3 (bp)
Hypopomus spec. 2 (bp)
Electrophorus electricus
Hypopomus spec. 1 (mp)
Frequency of Peak Amplitude (Hz)
2000
1000
500
100
EOD-Repetition Rate (Hz)
1
5
10
50
100

species was analysed for a few minutes during rest. EOD repetition rate varied from below 1 Hz (low voltage discharge of *Electrophorus electricus*) to approx. 65 Hz (*Hypopygus*) or 60 Hz (*Steatogenys elegans*). *Hypopygus*, *Steatogenys*, and *Rhamphichthys sp. 3* displayed an extremely low variation in successive EOD interval duration; the variation was considerably greater in *Rhamphichthys sp. 1* and in *Gymnotus carapo* although their EOD rates were also high. We distinguished four *Hypopomus* species with EOD repetition rates from approx. 6 to 12 Hz; two of them accelerated considerably from time to time. *Hypopomus sp. 4* (short-biphasic) was remarkable in that its EOD rate was fairly constant (more so than, e.g., that of *Gymnotus carapo*). From this analysis three groups of EOD repetition rates may be distinguished: (1) the electric eel *Electrophorus electricus*, (2) the *Hypopomus* group, (3) *Gymnotus*, *Rhamphichthys*, *Steatogenys*, *Hypopygus*. However, because of considerable overlap, no single species can be identified on the basis of its EOD repetition rate, save the electric eel at rest.

Steatogenys, *Hypopygus* and *Rhamphichthys sp. 3* did not show any EOD-rate changes related to motor behaviour.

Apart from differences in EOD rates, there were marked differences in EOD waveforms (the insets of Figs. 1 show the EOD of *Rhamphichthys sp. 3*). The Fourier amplitude spectra of single pulses revealed wide differences between most species. The monopolar, head-positive EODs of *Electrophorus* (weak discharge) and *Hypopomus sp. 1* were very similar and differed from all the other species' EODs. Their EODs displayed almost equal amplitude from D.C. to approx. 200 Hz. All the other species' EODs were essentially biphasic, most of them with additional pre- or afterpotentials of low amplitude (cf. Fig. 1). The amplitude spectra of all of these EODs had well pronounced peaks with the energy concentrated in a certain frequency band.

Fig. 2 is a double log plot of EOD repetition rate vs. frequency of peak amplitude of single EODs. There is a significant, positive correlation of 0.94 (Pearson's r; Spearman rank correlation coefficient $r_s = 0.99$; Kendall rank correlation coefficient tau = 0.97; all significant at $p < 0.01$). From the slope of the regression line of Fig. 2 it can be seen that the frequency of peak amplitude has a tendency to increase with EOD repetition rate as a power of approx. 0.8. As it is difficult to assign specific numbers especially to the ordinates of the entries for *Electrophorus* and *Hypopomus sp. 1*, these should probably better be left out from

from the analysis. Then the correlation coefficient r increases to 0.97 ($p < 0.01$). Also the slope of the regression line increases slightly to 0.85. This means that with a 100% increase in EOD repetition rate at rest, the frequency of peak amplitude increases only at a rate of 75-80%.

By also considering the parameter "frequency of peak amplitude", the entries for the various species in Fig. 2 are separated much better compared to an analysis of resting EOD rate alone.

2. Wave species.

Figure 3 shows the fundamental frequencies of most species found. Unfortunately, the EOD of *Eigenmannia sp. 2* was not recorded; the EOD of *Distocyclus goachira* was "off-scale" on this figure (extremely wide, head-positive 20 ms pulses, superimposed on a slightly negative baseline, repeated at a very regular rate of 15 Hz). Only the low frequency wave fishes *Sternopygus* (♀ ; cf. Hopkins, 1974b) and *Distocyclus conirostris* (Fig. 4) appear to be well separated from the rest of the group. The frequency band of 300-1800 Hz is occupied by 28 species, i.e., a theoretical 0.09 octave/species would remain for each species, if the individual species were spaced out over frequency at regular intervals.

In Fig. 3, fundamental frequency is defined as the repetition rate of a complete EOD cycle or period. This is the frequency of highest amplitude (referred to as "dominant frequency" in the following) of the Fourier spectrum in all those EODs marked by "$f_{dom} = 1/T$" (lower row of points). Harmonics show up in these spectra as odd and even integer multiples of the respective fundamental frequencies. EOD signals of this type are displayed by all the well-known species such as *Sternopygus*, *Eigenmannia*, and *Apteronotus albifrons*. Also *Distocyclus conirostris* (Fig. 4) and *Sternarchella schotti* (Fig. 5) display this type of EOD (although *Distocyclus conirostris* is remarkable because there is no energy at 2 x fundamental frequency). There exists another strategy, however: energy is "taken away" from the fundamental frequency and added to one or more of the higher harmonics. This is displayed by the EODs of *Sternarchorhynchus curvirostris* (Fig. 6) and *Sternarchella sp. 1* (Fig. 7) to a differing extent. *Sternarchella sp. 1* was the extreme in this respect; its EOD cycle repetition rate was 575 Hz (lowest in apteronotids), its dominant frequency was 5 times that fundamental frequency (2875 Hz). Its fundamental frequency component was very weak compared to its dominant frequency component (attenuation almost -40 dB). In the gymnotoid wave species

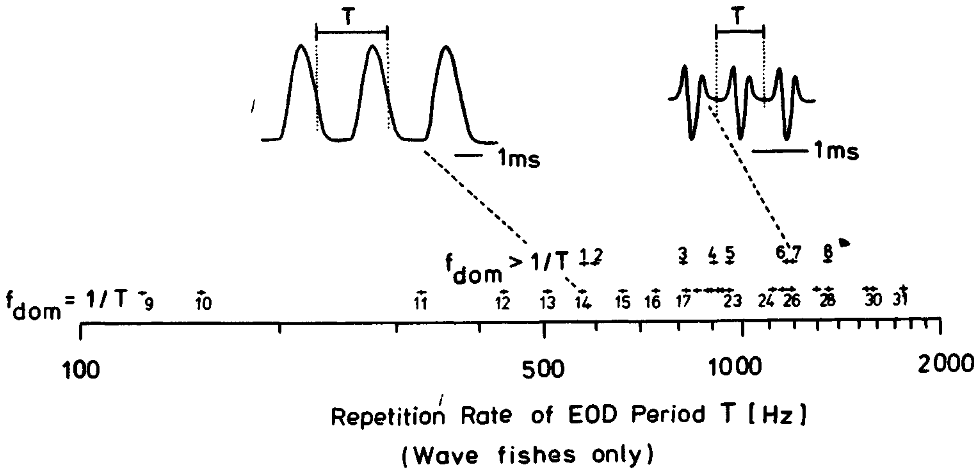
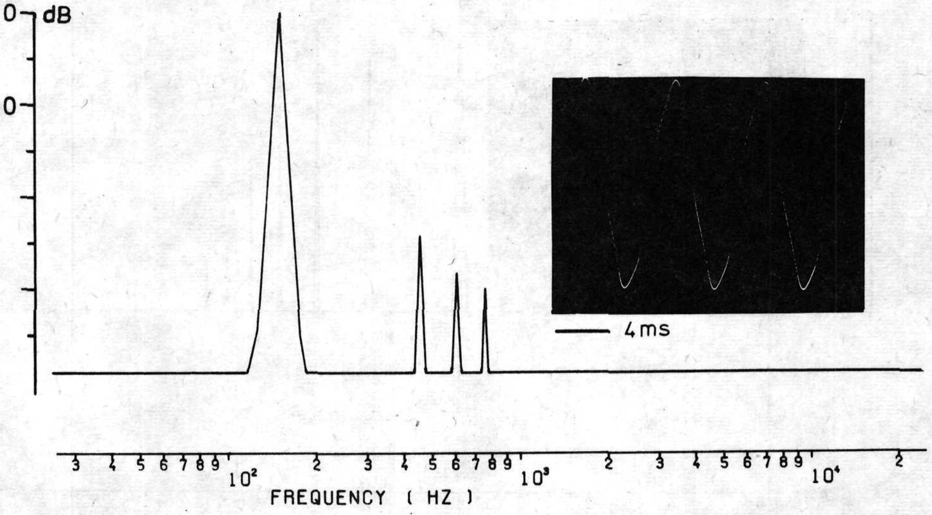


Figure 3: Distribution of EOD fundamental frequencies, measured as the repetition rate of an EOD period T in s (as indicated by the upper insets), of 30 sympatric wave species. Lower row of points: the fundamental frequency is also the dominant frequency ($f_{dom} = 1/T$); upper row of points: the dominant frequency is an integer multiple of the fundamental frequency ($f_{dom} > 1/T$). Left inset: EOD of Eigenmannia macrops = species no. 14 as an example of the wave type where $f_{dom} = 1/T$; right inset: EOD of Sternarchogiton nattereri = species no. 7 as an example of the wave type where $f_{dom} > 1/T$ 1: Sternarchella sp. 1; 2: Apteronotus sp. 2; 3: Sternarchorhynchus curvirostris; 4: Apteronotus sp. 1; 5: Adontosternarchus sp. 4; 6,8: two specimens of Porotergus gymnotus; 7: Sternarchogiton natteréri; 9: Sternopygus macrurus; 10: Distocyclus conirostris; 11: Eigenmannia virescens; 12: Eigenmannia lineatus; 13: Eigenmannia sp. 3; 14: Eigenmannia macrops; 15: Rhabdolichops troscheli; 16: Eigenmannia sp. 2; 17: Rhabdolichops axillaris; 18: Sternarchorhamphus macrostomus; 19: Adontosternarchus sp. 2; 20: Adontosternarchus sp. 5; 21: Apteronotus hasemani; 22: Adontosternarchus sp. 3; 23: Apteronotus albifrons; 24: Adontosternarchus sp. 1; 25: Oedemognathus exodon; 26: Apteronotus bonapartii; 27: Apteronotus anas; 28: Sternarchorhynchus oxyrhynchus; 29: Sternarchogiton sp. 1; 30: Sternarchorhynchus mormyrus; 31: Sternarchella schotti.



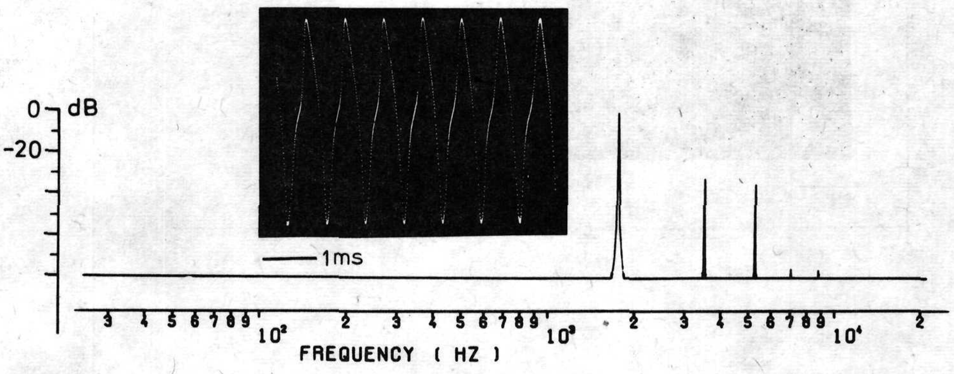
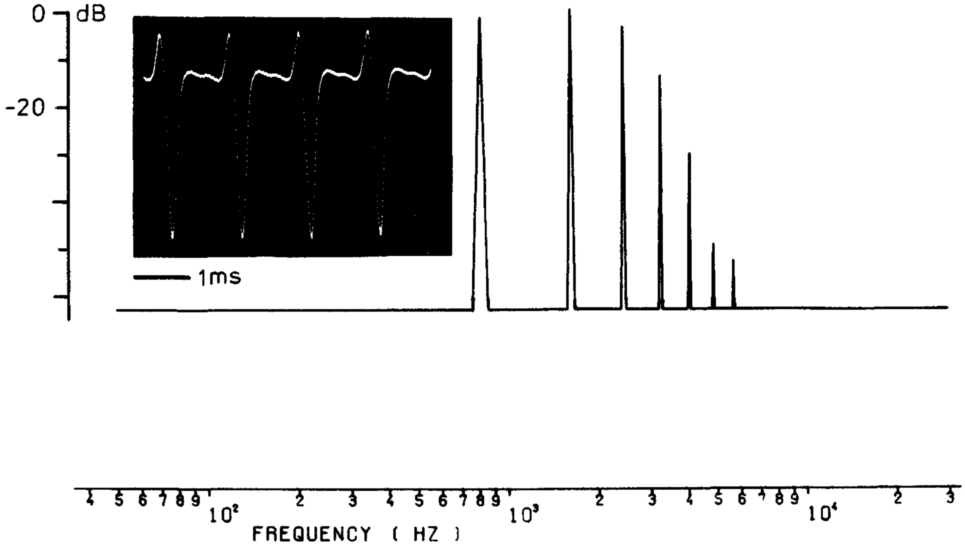


Figure 4: Amplitude spectrum of the wave EOD of Distocyclus conirostris. Ordinate: intensity relative to the frequency of highest amplitude. Inset: digitized waveform of EOD. Time calibration refers to inset.

Figure 5: As Figure 4, but for Sternarchella schotti.



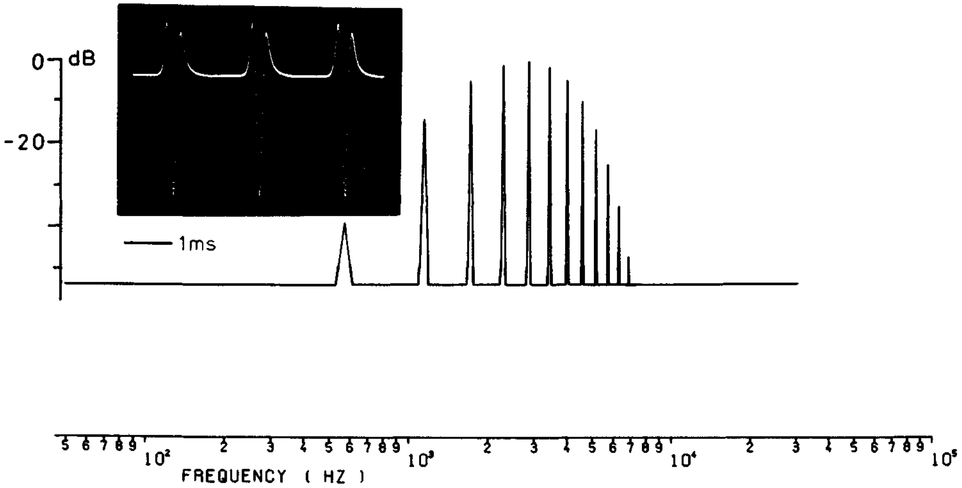


Figure 6: As Figure 4, but for Sternarchorhynchus curvirostris.

Figure 7: As Figure 4, but for Sternarchella sp. 1.

known up to now the fundamental frequency is the strongest signal component. Species such as *Sternarchorhynchus curvirostris* and *Sternarchella sp. 1*, besides showing that the fundamental frequency not necessarily is the strongest signal component, demonstrate that energy may be distributed over a frequency band of a considerable span. Unlike the EODs of pulse fishes with their broad amplitude spectra, in those wave fishes the energy is concentrated in discrete spectral lines of harmonic relationship. Three frequency lines representing a span of 1624 Hz (from 812 to 2436 Hz) are above the -10 dB attenuation level relative to the dominant frequency in *Sternarchorhynchus curvirostris*; the same figures in *Sternarchella sp. 1* are 6 harmonics over a frequency span of 2875 Hz (from 1725 to 4600 Hz).

Considering this signal structure it was felt that in order to characterise the EOD signal of these fishes the dominant frequency which is the main sine wave component of the signal, and not the fundamental frequency should be referred to. This was done in Fig. 8. Species are arranged on the abscissa according to their dominant frequencies irrespective of whether the dominant frequencies were the fundamentals or not. In order to further characterize an EOD signal as to whether it displays a strong or a weak harmonic structure (whether energy is concentrated at the dominant frequency as in *Distocyclus conirostris*, or distributed as in *Sternarchella sp. 1*) the amplitude of any dominant frequency was referred to as 100% and compared to the summed amplitudes of the other harmonics. This was called "harmonic content of EOD in % of dominant frequency" and essentially measures total harmonic distortion of the main sine wave, the dominant frequency. In a double log plot, Fig. 8. indicates the position of each species in this two-parameter space along with the species names. Also shown is the positive correlation between both parameters (Spearman's rank correlation coefficient $r_s = 0.74$; Kendall's rank correlation coefficient tau=0.58; both significant at $p < 0.001$).

The slope of the regression line (Fig. 8) indicates that the harmonic content of an EOD, as defined above, shows a tendency to increase with its dominant frequency as a power of almost 1.5. According to this, with a 100% increase of dominant frequency harmonic content would increase at a rate of approx. 180%.

By the graph presented in Fig. 8, a better separation of species compared to Fig. 3. is achieved. This would hold true even if all points

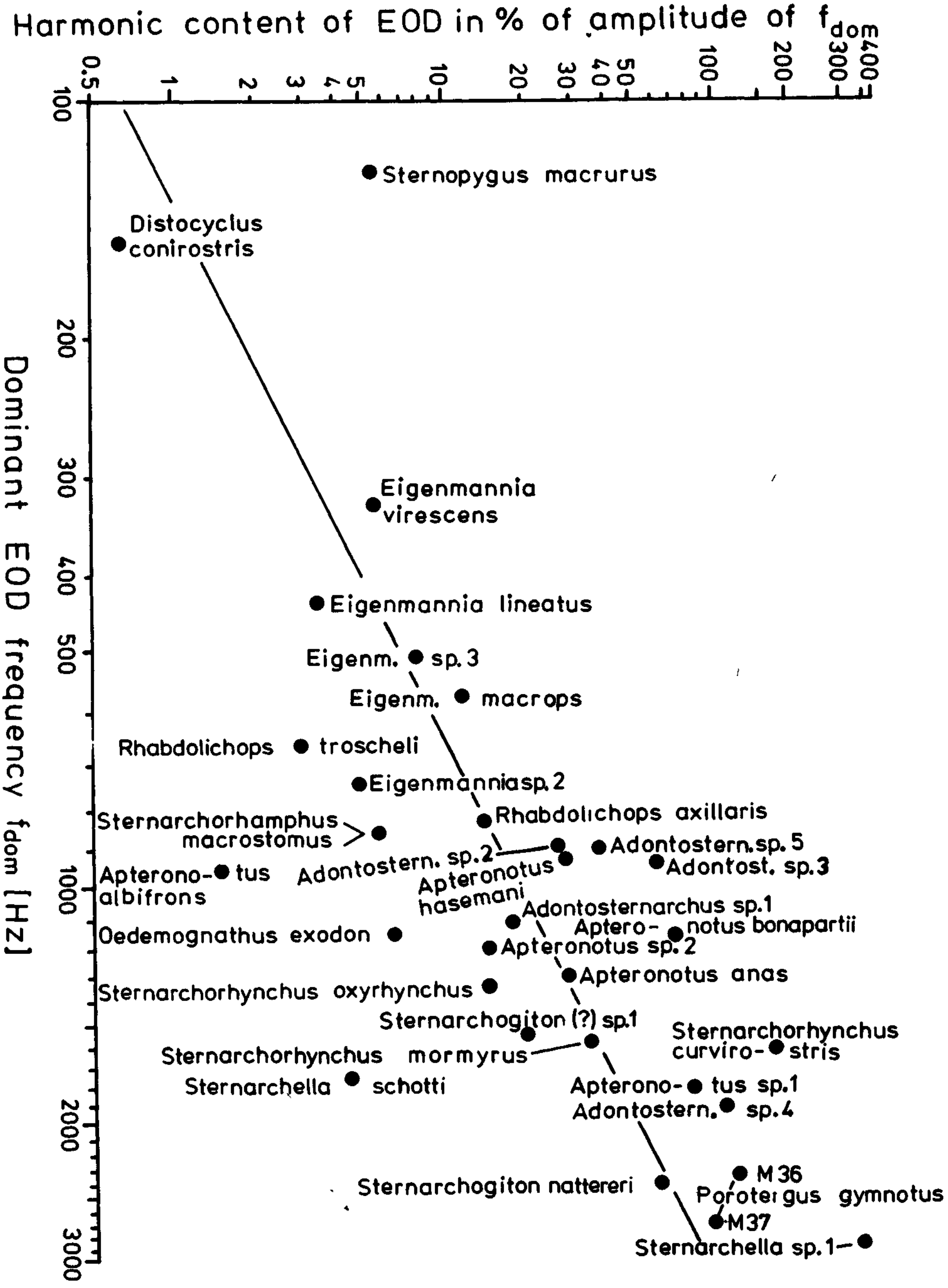


Figure 8: Double log plot of dominant EOD frequency vs. harmonic content of EOD for wave fishes. Each point represents one individual. A least squares regression line shows the positive correlation between dominant frequency of an EOD and its harmonic content.

would lie exactly on the regression line since the upper frequency limit of dominant frequencies is considerably higher than that of fundamental frequencies (2900 vs. 1800 Hz in our sample of species) so that the frequency span of 0.09 octave/species for fundamental frequencies mentioned above would increase to 0.11 octave/species for dominant frequencies. In addition, individual species may deviate considerably from the value predicted by the regression line. This is shown by a comparison of the EODs of Sternarchorhynchus curvirostris (Fig. 6) and of Sternarchella schotti (Fig. 5) which display similar dominant frequencies (1623 Hz and 1755 Hz, respectively) but vary enormously in harmonic content (172% and close to 4.5%, respectively; Fig. 8).

DISCUSSION

This study tries to investigate whether gymnotoid electric signals are species-specific. Our finding of new signal types and of many more species living together sympatrically than known before complicated what first appeared a simple problem. The reason for this undoubtedly is that we fished in the whitewater Solimoes, and not in the blackwater Rio Negro (on which Manaus is located). The lagoons and lakes formed in the varzea zone around the river bed (alluvial plain where the sediments brought along by the Solimoes from Andean headwaters settle after the annual flood) form one of the most productive biotopes in the Amazon system and support an immense number of species and individuals (Junk, 1973; Lowe-McConnell, 1975). There may - and probably do - exist still more gymnotoids than we were able to find. It is probably impossible to dress a "complete" list of species of our study area as conditions change constantly with rising and falling waters (13 m at the Central Amazon) which drastically alter the faunal composition (Lowe-McConnell, 1975). As this author points out further, at any one time many of the species at one site are likely to have recently arrived there, or to be moving away from habitats that have become unfavourable. Rare species, probably coming from populations established elsewhere, are likly to disappear soon unless joined by additional individuals. Roberts (1972) suggested that the brilliant colours of some species (e.g., the neon tetra) may help that individuals find one another and so to build up viable breeding populations. Do electric signals of gymnotoids serve a similar function (besides their well-known role in electrolocation; review in Heiligenberg, 1977)?

In almost each case where we had several specimens of a presumed species the EOD waveforms - either wave or pulse, or intermediate as in the case of Distocyclus goachira , mentioned above - were so similar to each other, and different from other species' EODs (with some possible exceptions; e.g., in the Eigenmannia group), that the strong feeling that EOD waveforms are Species specific just as any morphological feature~~s~~ is inescapable. The similarities in waveforms persisted also when there were marked differences in EOD fundamental frequencies between individuals of a presumed species (cf., e.g., Porotergus gymnotus, Fig. 3). In certain gymnotoid fish communities some species' EOD frequency ranges are reported to be unique so that their EODs may effectively function as a species identification or reproductive isolation mechanism, especially when the number of species is small, as in Hopkins' (1974a) study area in the Rupun~~i~~uni district of Guyana.

What is not at all clear is whether in localities where more species live together, displaying widely overlapping EOD fundamental frequency ranges (in wave species) or similar EOD peak power frequencies and EOD repetition rates (in pulse species), the electric signals of the various species may also serve a species identification function.Whether the hypothesis is correct or not also in these cases has to be investigated by appropriately designed behavioural experiments; mechanisms have to be elucidated by a comparison of signal with receptor properties.

In the absence of evidence of this kind concerning the gymnotoid fishes presented here some conclusions may be derived from properties of their EOD signals.

1. Pulse species.

In Fig. 2, the EODs of ten gymnotoid pulse species are arranged according to their EOD repetition rates and frequencies of peak amplitudes of their EODs. Most points lie rather close to a regression line showing that with increasing pulse repetition rates the frequencies of peak amplitude increase also (pulse durations decrease with shortening interpulse interval durations). This (incomplete: see below) sample of species represents a huge variation in both parameters which could very well convey species-specific information. However, Hypopomus sp. 2 and Hypopomus sp. 3 are separated so little in this graph that one may safely conclude that there is no difference. As their EOD waveforms were consistently different the possibility of species identification by EODs still exists in this pair of species.

In the rapidly firing group of fishes (> 50 pulses/s) considerable differences especially in the frequencies of peak amplitude of EODs were found (ranging from approx. 1200 Hz in Gymnotus to approx. 2300 Hz in Steatogenys); however, before any conclusions concerning identification of conspecifics by EODs may be drawn the intraspecific, inter-individual variation of EOD parameters has to be studied.

At least two additional species have still to be included in the upper right hand part of Fig. 2: our Rhamphichthys sp. 2 (EOD duration: 1.5 ms) and the local Hypopygus which died before recording. Given this situation, and the inter-individual variation in frequencies of peak amplitude of the EODs indicated by the two allopatric specimens of Hypopygus (Fig. 2), it appears at least doubtful whether recognition of conspecifics and reproductive isolation in pairs of species neighbouring in position in Fig. 2 would be possible by analysis of these two EOD parameters (pulse repetition rate, and frequencies of peak amplitude by matched, narrow-band tuberous electroreceptors). In the event that behavioural experiments should demonstrate that recognition of conspecifics out of heterospecifics with similar pulse EODs solely by resting EOD activity, unmodified by discharge rate changes, is a reality, either of two changes of the current hypothesis have to be accepted: (1) one or both parameters of Fig. 2 have to be replaced by other parameters, or (2) both parameters used in Fig. 2 play a role in recognition of conspecifics but one or more additional parameters have to be included in order to account for recognition of conspecifics from heterospecifics with similar EODs. In both instances the two parameters used in Fig. 2 would not allow unequivocal identification because of overlap between species.

Two parameters which may play a role in species recognition are (1) spectral bandwidth of EODs (e.g., the -10 dB bandwidths relative to the frequencies of peak amplitude, (2) temporal relationships between frequency components of EODs as expressed by their phase spectra. Heiligenberg and Altes (1978) demonstrated behaviourally that Hypopomus artedi discriminates stimulus pulses of identical Fourier frequency content but different spectral phase functions.

Spectral bandwidths may be analyzed by a set of electroreceptors tuned at different "best" frequencies (maximum of sensitivity). As the frequency spectra of pulses are broad distributions, differences in spectral bandwidths at some attenuation level relative to the frequencies of peak amplitude do not appear to be spectacular, however (e.g., Rhamphichthys sp. 3/ Gymnotus carapo: 1700/1400 Hz at - 10 dB; Hypopomus sp. 3/ Hypopomus sp. 2: 600/450 Hz at -10 dB). The available data do not justify the assumption that differences in spectral bandwidths are an important parameter in discrimination between similar EODs. So perhaps differences in spectral phase functions underlying what appears to be species-specific EOD waveforms in EODs with similar spectral frequency content are important in species recognition. This has yet to be explored.

2. Wave species.

There were two big surprises in our wave fish data. The first was that there were so many more sympatric species in our study area compared to the Rio Negro (Bullock, 1969; Steinbach, 1970) and different parts of Guyana (Hopkins, 1974a; Hopkins and Heiligenberg, 1978). The second was what appeared to be a new strategy in the evolution of EOD waveforms was discovered where energy is taken away from the fundamental frequency and distributed on higher harmonics (Figs. 6 and 7). These discoveries have some implications for the hypothesis of the function of EODs in species identification.

As put forward in the introduction the hypothesis of species-specific EOD fundamental frequencies was already weak using published information, only. For our wave fishes this hypothesis can safely be discarded (at least in a frequency range of 300-1800 Hz = 2.5 octaves including most wave species; Fig. 3). A frequency band of 0.09 octave/species is far too narrow to account for any intraspecific EOD frequency variations, even under the simplifying assumption of a regularly spaced distribution of species-specific frequency ranges. As said before, Eigenmannia commonly called virescens displays an EOD frequency range of 1.2 octaves. Species are spaced out much better in the two-parameter presentation of Fig. 8 where the harmonic content of the signals as a function of dominant frequency is considered. Species displaying the same dominant frequencies may differ drastically in harmonic content, and vice versa.

However, the separation of species does not appear good enough in a number of cases where the points lie closer than those of the two Porotergus gymnotus individuals (the ranges of intraspecific, inter-individual variations may be much greater than indicated by these two points, of course).

The two parameters used in Fig. 8.may play a role in crude classification of sensed signals. Finer discrimination between similar EODs, if existent, might be achieved similarly as argued above in the case of pulse fishes (1) by discrimination between different bandwidths relative to the dominant frequency at specified attenuation. Sampling over a continuous, broad frequency distribution as in pulse fishes, is replaced by stimulation of electroreceptors only at integer multiples of the fundamental frequency. (2) Spectral phase information which underlies the differences in waveforms of EODs with similar spectral frequency structure may be used.

Spectral bandwidth and harmonic content are, of course, coupled entities. In a first attempt to quantify the harmonic structure of signals their harmonic content was used since nothing is known about electroreceptor properties of but a few species (none of them displaying the new signal type where the dominant frequency is an integer multiple of the fundamental frequency, cf. Fig. 3). It would be particularly interesting to know more about the electroreceptors of this latter group of species. Are there also narrowly tuned electroreceptors the best frequencies of which closely match EOD fundamental frequency, as demonstrated in wave fishes displaying the "classic" signal type (Scheich et al., 1973; Hopkins, 1976), or are they rather tuned at EOD dominant frequency ? As further alternatives, are there different sets of electroreceptors, tuned at different harmonics of the EOD, or is there a specialized filter curve type characterized by a best frequency (corresponding to the dominant frequency of the EOD?) and increased sensitivity at higher and lower harmonics compared to frequencies between harmonics ? Hopkins (1976) found increased sensitivity of certain tuberous electroreceptors at 2 x best frequency in _Sternopygus macrurus_, _Eigenmannia virescens_ and _Apteronotus albifrons_; in _Sternopygus macrurus_ this was also found at 3 x best frequency (the best frequencies closely corresponded to EOD fundamental frequencies).

Species identification by means of resting EOD activity thus appears as a possibility which has yet to be proven - at least in pairs of species which produce more similar kinds of EOD waveforms (either pulse or wave). The possibility also exists that only a crude classification is achieved by EOD waveform properties and that finer discrimination is made by one of several categories of EOD maneuvers such as "SIDs" (sharp increases in frequency followed by decreases to the original level), "chirps" and "rises" etc. (Black-Cleworth, 1970; Westby, 1975a; Hopkins, 1974a and b), specific phase relationships between the EODs of partners (Westby, 1975b; Heiligenberg, 1974), the jamming avoidance response (recent review: Heiligenberg, 1977), and phase coupling (Gottschalk and Scheich, 1979). Of course, also the other sensory modalities may be - and probably are - involved in fine discrimination between species, such as mechanical and perhaps chemical stimuli (visual and acoustic stimuli do not seem to be important in this respect).

It is also necessary to characterize more precisely the preferred habitats of species during day and night and time of year (or water level). We did most of our fishing during day in a zone where species resting close to the river bank below the surface (such as Eigenmannia spp. and Hypopomus spp.) probably overlapped with species preferring somewhat deeper water with less dense vegetation (such as Rhabdolichops spp.; cf. Mago-Leccia and Zaret, 1978). If species displaying similar EODs were shown to live in different habitats species identification would be facilitated within one habitat. Accordingly, if preferred breeding habitats in the inundated varzea during the rainy season (Ellis, 1913; Hopkins, 1974b; Schwassmann, 1976; Kirschbaum, 1979) were shown to be different for species displaying similar EODs (and the number of species living together at that time smaller) such ecological differences might also enhance the degree of reproductive isolation.

Coexisting gymnotoid fishes have evolved such a multitude of apparently species-specific waveforms of electric signals that the hypothesis of their function in species identification and reproductive isolation remains an attractive one. Appropriate behavioural stimulation experiments, analysis of signal and receptor properties, and an investigation of habitat, temporal and geographic distribution patterns, and of character displacements should be carried out in order to prove or to disprove the idea.

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DISCUSSION

Q: In wave species there is a perfect correlation between harmonic content and dominant frequency of EODs. You are trying to arrange the different wave species along the regression line systematically in order to define species differences. The relevance of this for species recognition for reproduction depends on the frequency discrimination and capabilities of the individual fishes. What is known about these frequency discriminating capabilities and are you working on it ?

A: As far as I know not much is known about discrimination of electric signals varying in harmonic content by gymnotoid fish. We are working on this at Regensburg at the moment.

Q: Why do you think that these fish have to rely on their electric organ discharge for conspecific recognition and not on their various other senses they certainly possess ?

A: As these fish are nocturnal and hide during day vision probably is not involved in recognition of conspecifics. In addition, in a large part of the geographic distribution vision is heavily obscured by white or black water. Although gymnotoids are very sensitive to acoustical stimuli, they do not seem to produce sounds by themselves. Species recognition by mechanical or chemical stimuli is a possibility which has to be investigated.

Q: Does not the alternation in amplitude you showed in the EOD of one species depend on the fact that the fish had perhaps regenerated tails ?

A: When we had fish in bad condition (wounded or dying) amplitude variations of the EODs were also encountered. These were not regular, however. The amplitude variations in some apteronotid EODs I reported here were of perfect regularity: every other peak in EOD waveform was high or low. These fish neither died soon thereafter nor were they wounded. The variation in amplitude in Apteronotus is probably not due to a particular regeneration form; in Apteronotids only the caudal fin seems to regenerate and not the caudal peduncle, so one can question whether in these fish the electric organ regenerates at all.

Q: Are there many other species and individuals of fish in the same habitat as these electric fish ?

A: We do not know whether there were still more species of gymnotoids in our study area but we do believe there were. In addition, there were all the other diurnal and nocturnal families of fishes typical for the Amazon in huge numbers of species and individuals.

Q: Was only one pulse or wave used for Fourier analysis ?

A: In pulse species only one EOD was used for Fourier analysis. In wave species a number of waves was used depending on fundamental frequency of the EOD, tape-speed during play-back, analogue-to-digital conversion rate, and memory size.

Q: Even within one fish, pulse waveform shows fluctuation from discharge to discharge.

A: Fluctuation in pulse waveform from discharge to discharge in one individual was so slight that it was not visible with normal oscilloscope display techniques.

Q: What do you call fundamental frequency: the first harmonic or the repetition rate ?

A: Fundamental frequency here is the repetition rate of a complete EOD period (see Fig. 3).

Q: You have talked about the importance of phase information in two Hypopomus species. Your Sternopygus waveform was inverted so how sure are you about correct polarity ?

A: You are right concerning the Sternopygus waveform. The recording conditions of all records were noted and can be checked.

Q: There are very subtle differences in the amplitude spectra of some of these species. Do you have any information on individual differences ? It seems that some of the samples are so small (even only one specimen) that individual differences that we have observed in both pulse and wave gymnotids could completely outweigh these interspecific variations.

A: Intraspecific inter-individual variation of amplitude spectra is shown in Hopkins and Heiligenberg (1978). In Figs. 2 and 8, the two Hypopygus and Porotergus individuals give some impression of what intraspecific, inter-individual variation may be in our material. As I said, this shows that the separation of species according to the two parameters used is not sufficient for discrimination between species close to each other in Figs. 2 and 8.

Q: You spoke about significance in a Fourier transformed picture. Have you any statistical test that can be used on this type of calculation ?

A: I think your question aims at whether it is possible to test whether a specific frequency component shown in a Fourier amplitude spectrum (especially when weak) is a consistent component of the analysed signal, or noise. A statistical comparison of two distributions of such spectra, produced by the same recording and analysing equipment under identical conditions, one distribution with and the other one without the signal to be analysed should allow the test.

Q: In the pulse species you found consistent differences between the species based on your two criteria. Is it possible that this consistency is due to the limited time you had for the investigation and that with longer investigation you would have found more pulse species and thus overlap between them similar as in wave species?

A: I am not sure whether the differences between pulse species, shown in Fig. 2, are consistent for neighbouring pairs of fish (in one case I am sure that they are not). I agree that the situation would probably become even worse with continued search for more species. This again suggests that there are either additional or other parameters involved in species recognition, if existent, in the case of pairs of species displaying similar EOD waveforms.