

Effect of conductivity changes on the stability of electric signal waveforms in dwarf stonebashers (Mormyridae; *Pollimyrus castelnaui*, *P. marianne*)

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Abstract The dwarf stonebasher sibling species *Pollimyrus castelnaui* and *P. marianne* use differences in the electric organ discharges (EODs) for species recognition. As EOD waveforms are affected by water conductivity changes, the reliability of species recognition might be impeded due to natural variability in the environment. EODs of *P. castelnaui* ($N = 8$) and *P. marianne* ($N = 8$) under high (250 $\mu\text{S}/\text{cm}$) and low (25 $\mu\text{S}/\text{cm}$) conductivity were recorded and compared. Local peaks of the EODs of both species were significantly and predictably modified due to the conductivity change but species-specific differences were always recognizable. The duration of the EODs was not influenced by the conductivity change. Temperature alterations modified the duration in a linear relationship, allowing the determination of Q_{10} values (1.6 for *P. castelnaui*'s and 1.7 for *P. marianne*'s EODs). As the species-specific differences are not masked by conductivity effects, EOD discrimination seems to be a reliable species recognition mechanism under natural circumstances.

Keywords Electric organ discharge · Water conductivity · Q_{10} value · Species recognition · Weakly electric fish

Introduction

Biological communication signals are subjected to modifications by physical parameters of the medium (Endler 1992), a property especially obvious in the electric signals produced by weakly electric fish for electrolocation and communication (reviewed in Bullock et al. 2005; Kramer 1990; Moller 1995). The waveform and duration of the electric organ discharges (EODs) are influenced by water conductivity and temperature (Bell et al. 1976). Under constant conditions the waveforms of the African weakly electric fishes belonging to the family Mormyridae are highly stable but sudden conductivity changes elicit modifications of the amplitude and timing of the different peaks (Bratton and Kramer 1988; Kramer and Kuhn 1993). These changes of the EOD waveform affect the information content, as minute EOD waveform differences were documented to signal species identity and sex of the sender (Hopkins and Bass 1981; Arnegard et al. 2006). Reproductive behaviour in several species is triggered by a decrease in water conductivity (Kirschbaum 1975, 1995; Schugardt 1997) reinforcing the possible effect of conductivity induced EOD waveform modifications on species recognition.

The ability of the subtropical parapatric dwarf stonebasher sibling species *Pollimyrus castelnaui* (Boulenger 1911) and *P. marianne* Kramer et al. 2003 to distinguish between conspecific and heterospecific EOD waveforms was demonstrated in spontaneous choice, laboratory experiments (Markowski et al. 2008), supporting the suggested function of the EOD as a means of species recognition in this genus, not facilitated by the inter-discharge-intervals (Baier and Kramer 2007). The EOD waveforms of the two species differ in several parameters (Kramer et al. 2003; Markowski et al. 2008) with the main

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difference that *P. marianne*'s EODs possess three amplitude peaks while *P. castelnaui*'s EODs have five amplitude peaks (Fig. 1). As the habitat of *P. castelnaui* and *P. marianne* is subjected to changes in water conductivity caused by floods and rainfall (Kramer et al. 2003; Koekemoer 2003), also generating high conductivity gradients in small spaces (Wolski and Murray-Hudson 2006), the species-specific EOD waveform differences might be masked by environmentally caused effects. Here, the effects of temperature and conductivity changes, covering the whole natural range, on the EODs of dwarf stonebashers were studied, to evaluate the reliability of the EOD in the species recognition system.

Materials and methods

Tested *P. marianne* originated from the Upper Zambezi River (caught by F. H. van der Bank and B. Kramer in 1999 at Katima Mulilo, 17°32'31.9"S, 24°26'17.7"E, East Caprivi, Namibia) while the *P. castelnaui* were caught in the Okavango Delta (caught by F.H. van der Bank in 2002 at Makwena, 19°03'45.3"S, 22°23'24.5"E, Botswana). During the experiments fish were fed freshly-defrosted chironomid larvae. Light:Dark cycle was 12 h:12 h.

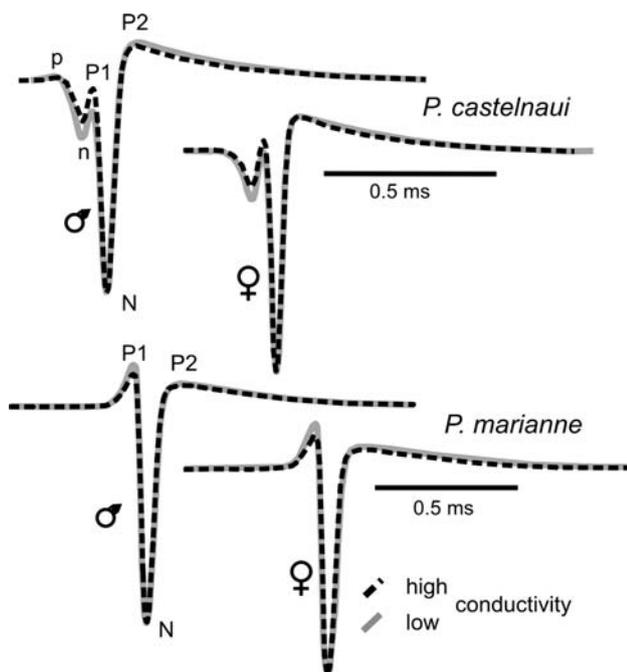


Fig. 1 Effect of decreased conductivity on the EOD waveforms of *P. castelnaui* and *P. marianne*. Shown are two EODs of a male and a female individual of both species, one recorded at a conductivity of 250 $\mu\text{S}/\text{cm}$ (grey line) and one at 25 $\mu\text{S}/\text{cm}$ (dashed black lines). The relative amplitudes of the peaks were changed but the waveforms are undoubtedly attributed to the correct species

Q_{10} values were determined for both species. Individually kept *P. castelnaui* ($N = 6$) and *P. marianne* ($N = 6$) were subjected to a gradual temperature increase (about 3°C in 24 h) from 19 to 29°C. The water was heated by an adjustable 150 W aquarium heater (Jaeger, Germany) installed in the tank. Conductivity was kept at $100 \pm 5 \mu\text{S}/\text{cm}$ during the gradual temperature increase. EOD waveforms were recorded with carbon rod electrodes connected to a custom-built amplifier (Elektronik Werkstatt Universität Regensburg) and stored on a digital oscilloscope with memory (Tektronix 420). EODs were recorded at 19, 21, 23, 25, 27, 29 $\pm 0.1^\circ\text{C}$. Eight single traces were recorded at each measurement. Analysis of the EODs was accomplished with custom-built routines in the software package Famos 4.0 (IMC). EODs were normalised on the negative main peak N (arbitrarily set to -1). The eight traces from one measurement were averaged. Local peaks were measured as proportion of the negative main peak (p, n: amplitudes of the weak positive and negative pre-potentials, only present in *P. castelnaui*'s EOD; P1, P2 amplitudes of first and second strong positive potentials, see Fig. 1). The duration of the EODs was measured at 1% of the negative peak amplitude N where the EOD rose from or fell to the baseline asymptotically. Least-squares-regression was employed to verify the linear relationship of the reduction in EOD duration with increasing water temperature in each tested individual. Q_{10} values were calculated by the formula:

$$\text{Log } Q_{10} = (\text{Log Dur}_{\text{High}} - \text{Log Dur}_{\text{Low}}) \times 10 / (T_{\text{High}} - T_{\text{Low}}),$$

Dur_{High} and Dur_{Low} give the EOD durations at the highest (T_{High}) and at the lowest (T_{Low}) temperature (Schmidt-Nielsen 1999; Dunlap et al. 2000). The duration values, used in the formula, were calculated by the linear regression coefficients for each individual.

Eight *P. marianne* and eight *P. castelnaui* were individually kept at a water conductivity of $250 \pm 5 \mu\text{S}/\text{cm}$, for 5 days at a temperature of $25 \pm 0.2^\circ\text{C}$. Water conductivity and temperature was measured with a WTW92 (Wissenschaftlich-Technische Werkstätten). Every day the EOD waveforms were recorded and analysed with the same methods as described above. After a 5-day period the conductivity in the tanks was lowered to $25 \pm 5 \mu\text{S}/\text{cm}$ and EODs were recorded 1 h after the conductivity change and on the following 5 days. As data on the conductivity changes in the natural habitat are scarce, only one sudden decrease in conductivity was employed to produce an effect, comparable to existing studies (Bratton and Kramer 1988; Kramer and Kuhn 1993). To compare the waveform parameters during high and low conductivity, means of the measurements from each individual during high and low conductivity treatments were calculated and compared by paired t tests (two-tailed, $\alpha = 0.01$) for the two species. To

check for possible adaptations of the EOD waveform to the conductivity over time, the first measurement after the conductivity change was compared to the last measurement ($\alpha = 0.05$).

Results

Temperature changes did not affect the relative amplitude of the peaks but did affect the duration of the EOD waveforms significantly (Table 1). The EOD duration of all tested fish showed a linear relationship with water temperature in the tested range (least-squares-regression; $r^2 > 0.9$ in all individuals, Fig. 2). The Q_{10} values calculated were 1.695 (mean) \pm 0.013 (SEM) for *P. marianne* and 1.642 ± 0.017 for *P. castelnaui*.

The relative amplitude peaks of both species' EODs showed significant modifications after the conductivity alteration but no significant change in the duration of the EOD waveforms was detected in either species (Table 2). In *P. marianne* the relative peak amplitudes *P1* and *P2* increased relatively to the *N* peak. Also in *P. castelnaui* *p*, *n* and *P2* increased in amplitude, while *P1* decreased in amplitude.

After the conductivity was changed the EODs were stable in all individuals and showed no adaptations during the course of the experiment between the first and last measurement at the lower conductivity (paired *t* tests; *P. castelnaui*: *p*, $p = 0.31$; *n*, $p = 0.34$; *P1*, $p = 0.69$, *P2* = 0.84 ; *P. marianne*: *P1*, $p = 0.52$; *P2*, $p = 0.61$). The species-characteristic features of the EOD waveforms were present under all conditions (Fig. 1).

Table 1 Influence of temperature on EOD waveform parameters

	Temperature					
	29°C		19°C		Paired <i>t</i> test	
	Mean	SEM	Mean	SEM	Mean difference	<i>p</i>
<i>P. castelnaui</i>						
<i>p</i>	0.0185	0.002	0.019	0.003	-0.0002	0.84
<i>n</i>	-0.24	0.052	-0.24	0.061	-0.003	0.82
<i>P1</i>	0.009	0.011	0.005	0.01	0.004	0.15
<i>P2</i>	0.172	0.063	0.173	0.047	-0.001	0.95
Dur	0.741	0.039	1.24	0.052	-0.496	>0.001
<i>P. marianne</i>						
<i>P1</i>	0.158	0.017	0.156	0.015	0.002	0.61
<i>P2</i>	0.106	0.017	0.105	0.007	0.001	0.75
Dur	0.961	0.048	1.632	0.075	-0.670	>0.001

N = 6 for *P. castelnaui* and *P. marianne*. Given are mean and standard errors (SEM) of the relative peak amplitudes (see Fig. 1) and the duration (Dur) of the EOD pulses in ms

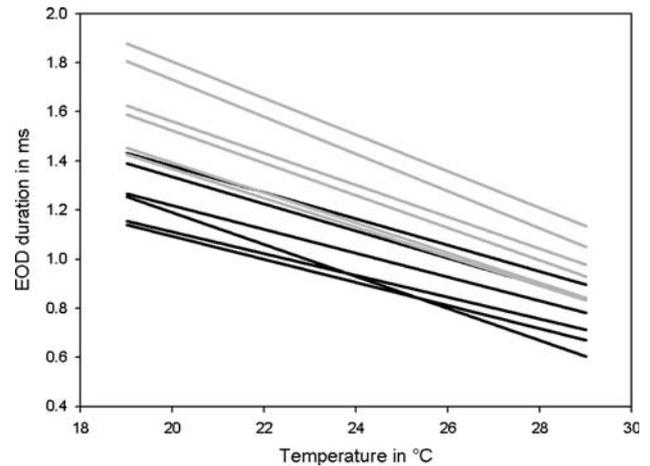


Fig. 2 Effect of increased temperature on EOD duration. Each line gives the least-squares regression for one individual. Black *P. castelnaui*, gray *P. marianne*

Table 2 Influence of conductivity on EOD waveform parameters

	High		Low		Paired <i>t</i> test	
	Mean	SEM	Mean	SEM	Mean difference	<i>p</i>
<i>P. castelnaui</i>						
<i>p</i>	0.013	0.002	0.018	0.002	-0.005	>0.001
<i>n</i>	-0.228	0.019	-0.299	0.023	0.071	>0.001
<i>P1</i>	-0.052	0.031	-0.171	0.039	0.119	0.002
<i>P2</i>	0.170	0.007	0.193	0.008	-0.023	0.010
Dur	0.893	0.041	0.849	0.041	0.044	0.325
<i>P. marianne</i>						
<i>P1</i>	0.129	0.015	0.174	0.017	-0.046	0.002
<i>P2</i>	0.128	0.015	0.173	0.018	-0.045	>0.001
Dur	1.241	0.029	1.247	0.100	-0.006	0.940

N = 8 for *P. castelnaui* and *P. marianne*. Abbreviations similar to Table 1

Discussion

Conductivity changes affected the EODs of the subtropical species *Pollimyrus adspersus* and *Petrocephalus boveii* in a similar way to that described for dwarf stonebassers, causing only moderate and predictable modifications (Bratton and Kramer 1988). Extremely low conductivities (>20 μ S/cm), beyond the naturally occurring range (Bossche and Bernacsek 1991), were needed to produce dramatic changes in the EOD waveforms of *P. adspersus* and *P. boveii* (Bratton and Kramer 1988). The EODs of the tropical species *Campylomormyrus rynchophorus* and *C. tamandua* were heavily distorted after a conductivity change and showed adaptation to the new environmental conditions after 30–48 h (Kramer and Kuhn 1993), while

the differences caused by the conductivity change were stable in the two species tested here. This difference in the susceptibility to water conductivity changes might be explained with the environmental variability the fishes have to face in their natural habitats. Tropical species live under more predictable and stable conditions than subtropical species (Bossche and Bernacsek 1991). Another difference between the *Campylomormyrus* species and the *Pollimyrus* and *Petrocephalus* species is that *Campylomormyrus*' EOD waveforms are principally biphasic, with an initial positive potential followed by a negative (*C. tamandua*'s EODs show only a very weak negative prepotential), while all other tested species have EODs with at least three phases, consisting of a positive potential superimposed by a very short negative potential (Hopkins 1999). Possibly tri- or polyphasic waveforms are less affected by alterations in water conductivity. In this context the effect of conductivity changes on the EOD waveform stability in principally biphasic mormyrid fishes from subtropical areas would be interesting.

As the EOD waveforms of *P. castelnaui* and *P. marianne* showed modifications due to conductivity and temperature changes, the possibility of reduced reliability in species recognition can not be excluded. Although it seems unlikely that the small and predictable differences would impair species recognition, as members of both species were able to detect conspecific waveforms in the spontaneous choice tests showing more variability (Markowski et al. 2008) than the differences caused by the conductivity change.

The relatively small differences between the EODs of male and female *P. castelnaui*, mainly caused by a stronger *n* peak in male fish (0.19 ± 0.01 in females and 0.21 ± 0.05 in males, Markowski et al. 2008), lie in the range of the modifications elicited by the conductivity change. This, however, does not mean that the sex difference is excluded as a reliable index, as all tested fish increased the amplitude of the *n* phase by a similar value (-0.07 ± 0.01) giving the same differences at different conductivities.

The Q_{10} calculated for the two species is comparable to the only reported value for a mormyrid fish (1.5 in *Gnathnemus petersii*, Kramer and Westby 1985). An effect of temperature changes on the species recognition system cannot be excluded but considering the high predictability of the changes and the only minimal changes in the structure of the amplitude peaks it might be considered unlikely. For a gymnotid fish (*Apteronotus leptorhynchus*) a more complex relationship of temperature and EOD characteristics was demonstrated, not only affecting the discharge frequency but also the amplitude. Moreover a significant difference between warming and cooling of the fish were documented on the Q_{10} (Dunlap et al. 2000). In

the present study only increasing temperatures were tested and the temperature change was applied more slowly (3°C per day against 0.25°C per minute) but comparability between *Apteronotus*, with its neuronally produced waveform type EOD and the pulse EODs of *Pollimyrus*, produced by modified muscle cells, seems limited. A broader base of comparison, with more species would be useful to elucidate the temperature dependence of the electric signals in different weakly electric fish taxa.

In conclusion, the EOD differences between the dwarf stonebasher sibling species seem stable enough to code for species identity under naturally occurring circumstances. Further studies, on the ecology and behaviour of these weakly electric fishes are needed, to evaluate the role of the complex electric communication system in speciation.

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