The Journal of Experimental Biology 210, 2244-2252
Published by The Company of Biologists 2007
doi:10.1242/jeb.003509

Electrical and behavioral courtship displays in the mormyrid fish *Brienomyrus brachyistius*

Ryan Y. Wong* and Carl D. Hopkins

Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853, USA

*Author for correspondence (e-mail: ryan.wong@mail.utexas.edu)

Accepted 19 April 2007

Summary

Mormyrid electric fish rely on the waveform of their electric organ discharges (EODs) for communicating species, sex, and social status, while they use the sequences of pulse intervals (SPIs) for communicating rapidly changing behavioral states and motivation. Little is known of electric signaling during courtship behavior because of two major difficulties: (1) the fish are not easily bred in captivity and (2) there is no reliable means of separating electric signals from several individuals in natural communication settings. Through simulating artificial rain conditions, we have successfully induced courtship and succeeded in breeding a mormyrid electric fish (*Brienomyrus brachyistius*) in the laboratory. We have also developed a system of video recording and editing combined with cross correlation analysis to precisely record and view behavior and separate EODs from two individuals in non-breeding and breeding contexts. Knowing the electrical and motor patterns during courtship allows for further exploration of topics such as mate choice and neural basis of pattern generation in these fish.

Here we describe nine common motor displays and 11 SPIs. Analysis of frequency of occurrences suggests that some SPI patterns are sex and season specific. We also observed electrical duetting called 'rasp matching' during courtship signaling among pairs; males and females exchange 'rasps' and 'bursts', respectively, in alternation. Our study employs new techniques to separate and document SPIs in the context of courtship. We show that some SPIs correlate with specific behavioral acts around the time of spawning.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/210/13/2244/DC1

Key words: Mormyridae, Brienomyrus, courtship, reproduction, electrocommunication, electric organ discharge.

Introduction

The weakly electric fish from Africa in the family Mormyridae, use their electric sense for communication and navigation (Hopkins, 1986b) (for reviews, see Kramer, 1990; Ladich et al., 2006; Moller, 1995; von der Emde, 1999). The waveform of the electric organ discharge (EOD) is highly stereotyped and contains information about species, sex and social status (reviewed by Carlson, 2002). The sequence of pulse intervals (SPIs) varies according to the behavioral state and motivation for aggression and courtship (reviewed by Carlson, 2002). This paper seeks to add to the limited knowledge of SPIs and behavioral motor acts involved in the context of courtship in mormyrids.

The behavioral work that has been done on mormyrid signaling has focused mostly on agonistic interactions because of their territorial behavior and the ease of observing their chasing and fighting. Some studies used mixed species interactions in which individuals could be distinguished by their divergent waveforms (Kramer, 1974; Kramer and Bauer, 1976). Other studies tethered an individual to a wire as a way of uniquely identifying its pulses in a mix (Bell et al., 1974; Graff, 1987; Hopkins and Westby, 1986). Still others controlled the location of an interacting pair (Moller et al., 1989; Terleph and Moller, 2003). As a result of these studies, we know about correlations between overt behaviors and associated stereotypic SPIs (reviewed by Hopkins, 1986a)

In spite of this work, little is known about SPIs during courtship behavior of mormyrid fish. Hopkins and Bass (Hopkins and Bass, 1981) first described an SPI during courtship, termed 'rasp', during their field study on *Brienomyrus* sp. from Gabon. However, because these experiments were conducted in the field where visibility was poor, the signals could not be correlated with behavior. Kirschbaum and Westby (Kirschbaum and Westby, 1975) reported the first successful breeding of mormyrids under laboratory conditions but did not describe any motor behavior. Crawford et al. (Crawford et al., 1986) documented courtship behavior of lab-bred *Pollimyrus isidori*, but concluded that courtship communication was largely acoustic-based and not through electric discharges as electrical signaling was suppressed during the male's acoustical courtship calls. The few SPIs that did occur during courtship in *P. isidori* were limited to changes from random discharges to a regularized discharge rate or cessations (Bratton and Kramer, 1989). Kirschbaum and Schugardt (Kirschbaum and Schugardt, 2002) reported success in breeding five additional different species of mormyrids, but did not report on courtship signaling. Although our laboratory in the past has successfully bred *B. brachyistius* in captivity, courtship behavior was not documented. Recently, Werneyer and Kramer (Werneyer and Kramer, 2005) described electric signaling and behavior during courtship in one pair of *Marcusenius macrolepidotus*. *M. macrolepidotus* showed no courtship behavior and produced only one putative courtship specific SPI (Werneyer and Kramer, 2005).

Although Kirschbaum and Schugardt (Kirschbaum and Schugardt, 2002) found that lowering the conductivity, rising water levels and the sprinkling of water over the surface induces reproductive behavior, successful spawning is difficult to achieve for some species. Each has different requirements for induction of spawning. Although we succeeded in breeding *Brienomyrus brachyistius*, an even greater challenge was to separate the EODs from more than one fish so that we could reconstruct each individual's patterns of discharge. Previous methods were too invasive to allow studies of courtship. However, with available technology, custom-written computer programs allowed for EOD discrimination by sex in freely behaving mormyrids. A similar method has recently been used (Werneyer and Kramer, 2005).

In this study, we reliably induced courtship while separating the EOD pulses in four pairs of *B. brachyistius*. We used two new methods to simultaneously record and view electrical and behavioral motor displays and automatically separated pulses based on sex-specific EOD waveforms. Video editing software allowed the merging of high quality EOD recordings with video recordings so that behavior and SPI patterns could simultaneously be viewed.

To separate a mixed signal, we used cross correlation analysis *via* a custom written program that was originally developed by Carlson (see Arnegard and Carlson, 2005). Briefly, a single EOD from each individual was digitized at 30 kHz sampling frequency and stored as a template for that individual. The combined signals from both fish were cross correlated, first with one and then the other individual's template. We reliably identified the two EOD types and assigned each to one individual or the other by comparing the two cross correlations. This method is accurate when there are clear differences in the duration of EOD waveforms. Because EODs in *B. brachyistius* are sexually dimorphic and the male's EOD is even more divergent from female's during breeding seasons, we were able to assign the sex to each EOD. Collectively, nine behavioral categories and 11 SPI patterns are described for *B. brachyistius*, some of which show sex and season specificity.

Materials and methods

Study subjects

Brienomyrus brachyistius (Gill 1862) is found naturally in western Africa with a patchy distribution ranging from Southern Senegal to coastal Gabon and Congo. Although no information is available about the breeding season for *B. brachyistius*, other mormyrids breed with the onset of the rainy seasons (Hopkins and Bass, 1981; Kirschbaum, 1995). Decreasing water conductivity, increasing water levels, and artificial rainfall on the surface all promote gonad growth in this and other species (Kirschbaum, 1979; Kirschbaum, 1987; Kirschbaum and Schugardt, 2002; Schugardt and Kirschbaum, 2004). The fish used in this study were wild-caught sexually mature males (N=4) and females (N=4) with a standard length greater than 110 mm. The fish originated from Nigeria, Africa *via* a commercial dealer. The two sexes were distinguished based on the presence of an anal fin notch on the male and absence of one on the female (Brown et al., 1996).

Stimulated breeding

In preparation for the study, each pair of fish was acclimated for 3 weeks in a 190 l aquarium kept at 25°C and on a 12 h:12 h light:dark cycle. The tank contained plastic tubes, driftwood, plants (*Vesicularia dubyana*) and cotton filter material. At the outset, water conductivity was maintained at high levels at 300–350 μS cm^{-1} to inhibit breeding. The conductivity was then gradually lowered over a 3-week period to 20–40 μS cm^{-1} by addition of deionized water. With the drop in conductivity, we observed the onset of courtship activity. The addition of water raised the water level in the tank approximately 2 cm per day until the tank was filled.

Behavioral and electrical recordings

We recorded electrical and behavioral activity during 30 min sessions on 10 different days equally divided into two phases: one at the end of the acclimation period (non-breeding) and one during the low conductivity periods after courtship commenced (breeding). We recorded *B. brachyistius* behavioral activity at night using infrared light illumination and a Sony Digital Video Camera Recorder (Model DCR-PC100). All recordings were done 2 h after the onset of darkness. Two chlorided silver wire dipole electrodes were placed on opposite sides of the tank to capture all electrical activity even as the fish swam around the tank. The signal was amplified 100× with a differential AC amplifier (A-M Systems, Inc., Everett, Washington, USA; model 1700) and band-pass-filtered from 0.1 Hz to 10 kHz. These signals were recorded on the two-channel audio track of the digital video recorder and simultaneously on a desktop computer using Cool Edit 2000 (Syntrillium 2000). The recording of the latter provided a clean low-noise recording. To minimize electrical noise during the recordings, all motors, pumps and unnecessary electronic equipment were shut off for the duration of the trial.

Each video recording was digitized using the video-editing software, Adobe Premier Pro (Adobe Systems 2003). We replaced the low quality audio track recording of the EODs with the high quality stereo recording from the computer after ensuring accurate alignment of the two signals. Realignment of video with digital audio tracks were accurate to 0.5 ms.

Signal separation

We used a custom software written in Matlab 6.1 (MathWorks 2000) by Carlson (see Arnegard and Carlson, 2005) to separate a mixed signal based on sex. The cross-correlation analysis is most effective when there is a distinct difference in EOD duration between male and female. The sexually dimorphic EOD waveforms of *B. brachyistius*, which become further divergent in the breeding season, allow us to use cross correlation to identify the sex of the fish that emitted a particular EOD. From a 30-s recording of a mixed signal recording, we selected one EOD from a male and one from a female as templates for cross correlation analysis (Fig. 1A). We then computed the cross correlation between the signals and each EOD template (30-s segments) (Fig. 1B,C). We calculated the square at each point to eliminate polarity of the original waveforms. We then compared the heights of the two resulting peaks and sex was assigned based on which one had a higher value (see Fig. 1B,C). As a final check, we inspected by eye to correct errors in mis-assigned EODs. We had incorrect assignments only when two EODs were similar in duration or when a pulse from each individual occurred almost at the same time. When correcting errors, we noted the patterns of amplitude changes in the original record, as well as the recent firing patterns. A plot of the SPI for each sex was made so that we could view any stereotyped temporal patterns (Fig. 1D,E).

Behavioral and sequence of pulse interval analysis

For each of the four pairs of fish, we observed and video-taped 30 min of behavioral and electrical activity on five different nights during both high and low conductivity conditions. From these 30-minute segments, we randomly selected three consecutive minutes of activity for detailed analysis, totaling 15 min per pair for non-breeding conductivities. We identified SPI displays and occurrences for stereotyped motor acts for each fish and tallied their frequencies (Fig. 2). During the stimulated breeding period, we selected to analyze records surrounding courtship events that included at least one ‘spawning’ motor act using the same methods described above (see Table 1 for motor act description). A courtship event started when the male and female were behaviorally interacting (excluding ‘hovering’) within approximately 7 cm of each other for at least 30 s and ended when that interaction stopped for at least 30 s. We included here analysis of 64.5 min of courtship activity (129×30-s segments) for all pairs. The results were then expressed as events per 15 min to be compared to rates of activities during the high conductivity period. We defined electrical and motor acts using subjective cues from the video and the patterns in the SPI. We used Carlson (Carlson, 2002) terminology for electrical displays. ‘Scallops’ were defined according to Serrier and Moller (Serrier and Moller, 1989). We distinguished ‘creaks’ from ‘scallops’ by the longer minimal interval lengths and the cessation periods preceding and following the ‘creak’. ‘Gradual increases’ were distinguished from ‘scallops’ by the long duration of returning to original interval lengths following the burst of pulses (2–6 s) and the greater number of EODs (>30).

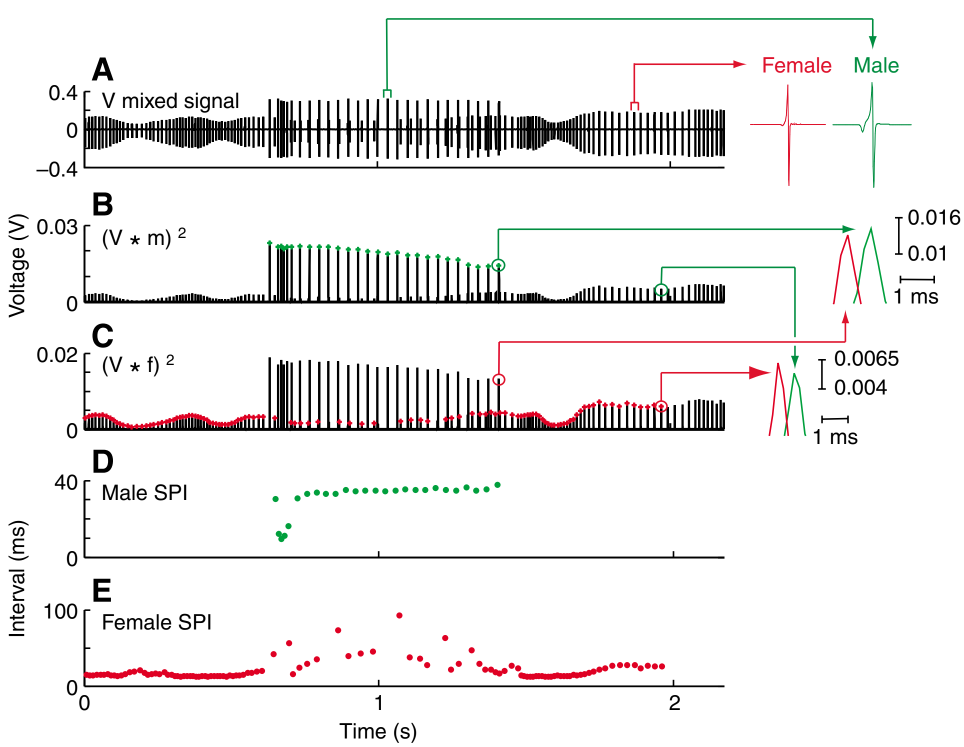

Fig. 1. Separation of a mixed electric organ discharge (EOD) recording into pulses from males and females using cross correlation analysis. (A) The original mixed recording voltage (*V*) *versus* time (s). Exemplar male and female EOD templates are expanded on the right. (B) $(V*m)^2$ as function of time. Where V is the original voltage trace, m is the male exemplar EOD and the operator * is the cross correlation. All squared coefficients are positive. (C) The same for the female template. To the right in A and B $(V*m)^2$ is compared with $(V*f)^2$ for a male EOD showing how the cross correlation is higher for the male (green) than female (red) model; C shows the same for a female EOD. EODs are subsequently identified for every pulse in the 2-s recording. (D,E) Inter-pulse intervals *versus* time for male and female, respectively.

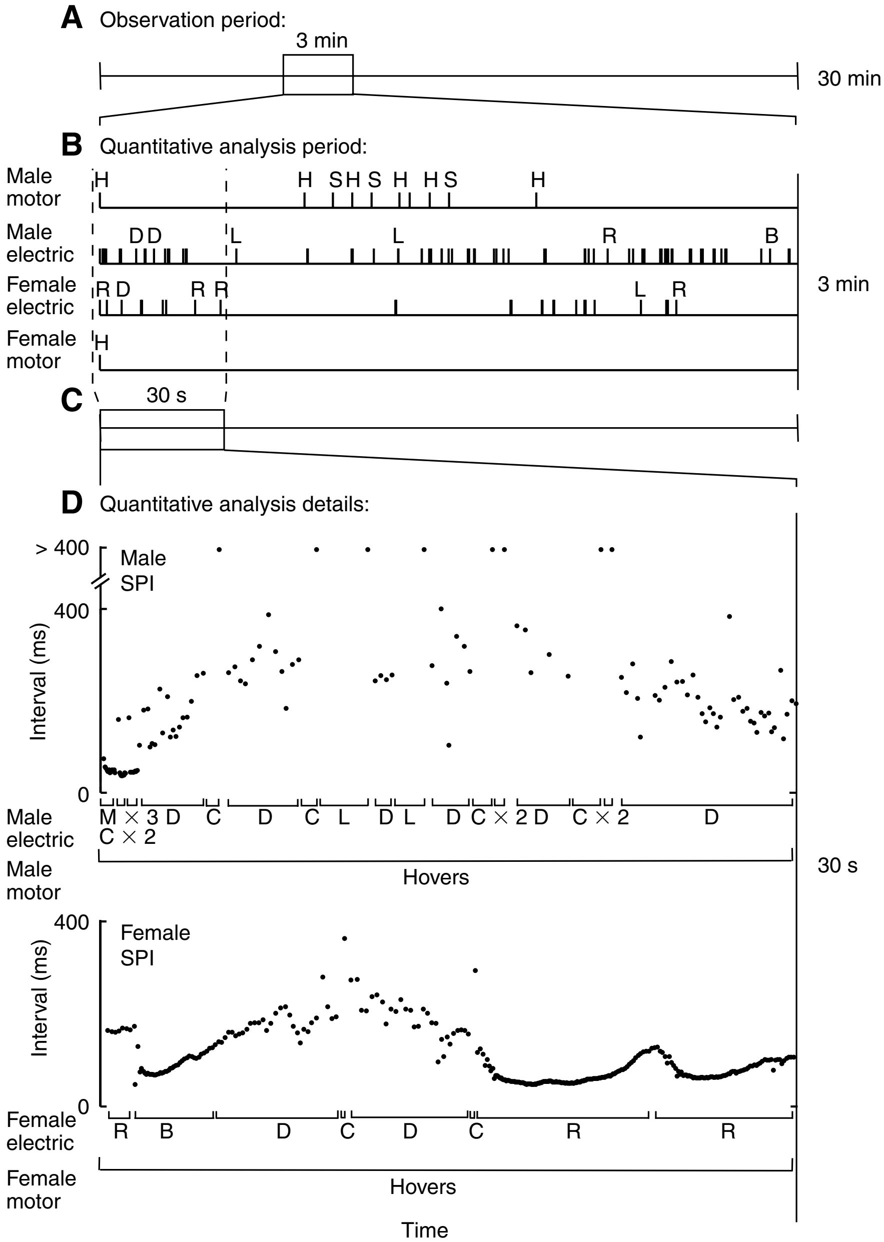

Fig. 2. Quantifying simultaneous stereotyped sequence of pulse intervals (SPIs) and motor patterns. (A) For each of the 30-min observation periods in the non-breeding conditions, three random consecutive minutes were analyzed. Note that depending on length of courtship bout(s), we analyzed varying lengths of time for each observation session. (B) In the 3-min segment, stereotyped motor and SPI patterns were identified and tallied for each sex. Each vertical bar indicates the starting time of the motor and SPI displays in each sex. Select displays are labeled: H, hovering; S, swimming; L, long cessations; R, regularization; B, slow burst. (C) A 30-s segment of a time (s) *versus* interval (ms) plot expanded in detail in (D) with SPI and motor patterns identified and bracketed for quantifying purposes. M, medium burst; D, random; C, short cessations.

Results

Stereotyped sequence of pulse intervals and behaviors

In total, we observed 11 stereotyped SPIs and nine motor acts during both breeding and non-breeding periods. The SPIs are described in Table 1 and illustrated in Figs 3–5. Behavioral descriptions of motor acts are given in Table 1. In a typical courtship display the male approached the female and 'lunged' at her. The female then 'swam' around the tank through obstacles with the male 'following' closely. Male and female may 'hover' close to one another. 'Head-to-tail circling' (HTC) occurred, followed by 'spawning'. Upon completion of 'spawning', the pair may start the sequence again with 'following' (see Fig. S1 in supplementary material). These acts are varied in sequence, duration and intensity. We did not find any eggs the morning after spawning. We saw all SPIs that we described (except 'scallops' and 'slow bursts') throughout the courtship displays.

Quantitative analysis of electrical and motor patterns

EOD activity varied greatly between the non-breeding and breeding periods. Using 2×2 repeated measures ANOVA with two levels of within factor (breeding season, nonbreeding season) and two levels of a second within factor (male, female), we found a significant increase in mean EOD firing rate for both sexes during the breeding season ($P<0.02$). There was no significant difference in the median EOD interval lengths or variances seen between the two seasons. We chose to omit male no. 3 (M3) and female (F)3 from the above analyses because F3 emitted few EODs during the nonbreeding season (see Fig. S2 in supplementary material).

There were both seasonal and sex differences in motor acts and SPIs. Tables S1 and S2 in the supplementary material show the frequency of behavioral motor acts and SPIs (events per 15 min), respectively, listed by sex and season. For seasonal behavioral differences, males 'HTC', 'spawned' and 'followed' more in the breeding season than the non-breeding season (Mann–Whitney *U*-test, $P<0.05$). Females showed increased behavioral activity for 'HTC', 'spawning' and 'probing' in the breeding period (Mann–Whitney *U*-test, $P<0.05$). 'Spawning' and 'HTC' were exclusively seen during courtship bouts. For SPIs, males and females both increased the number of 'fast bursts' (Mann–Whitney *U*-test, $P<0.05$) during courtship. Males increased the number of 'creaks', 'rasps', 'fast bursts' and 'short cessations' (Mann–Whitney *U*-test, $P<0.01$). Females had significantly more 'fast bursts' and 'gradual

Table 1. *Description of motor behaviors and sequence of pulse intervals*

	Description
Motor behavior	
Flee	An arching of the body and avoidance of a potential attack
Follow	The act of swimming closely behind a fish around and through objects in the tank
Head-to-tail circling	Male and female follow each other in tight circular motion with the head of each sex near the other's caudal fin
Hover	Stationary position with fins moving to stabilize position
Lunge	A fish swims rapidly, directly and deliberately at an opponent head first that causes the receiver to arch in an escape response
No activity	Laying on ventral side with no fin movement
Probing	Stationary or back-and-forth movement of body around an object leading with head or tail
Swim	Broad category where there is forward movement not directed at other fish
Spawning	Rapid undulation of body in vent-to-vent coupling position
SPIs	
Creak	Total duration of 2000–3000 ms and characterized by a cessation prior to slow discharges (100–300 ms) followed by a burst at about 50 ms and a subsequent cessation
Gradual increase	A fast burst followed by an increase of interval length to 60–100 ms over a period of 2000–6000 ms
Fast burst	Series of pulses with intervals of 10–30 ms lasting no longer than 1000 ms
Long cessation	No EODs for longer than 1000 ms
Medium burst	Series of pulses with intervals of 30–70 ms lasting no longer than 1000 ms
Random	No discernable SPI pattern
Rasp	Burst of 4–10 pulses with 10–20 ms intervals followed by longer intervals of 25–50 ms
Rasp matching	Although not an SPI by one individual, it is an electrical duet between the sexes during courtship. Male rasps or medium bursts would be followed by female fast bursts or *vice versa*. Varying duration of event and number of rasps, medium bursts and fast bursts produced
Regularization	Sustained EOD intervals (±10 ms) for longer than one second
Scallop	Sudden burst of 8–12 pulses of 10–20 ms from resting intervals (100–300 ms) and back
Short cessation	No EOD activity for 200–1000 ms. Shorter cessations included in this category when a clear stop and start of two SPI patterns is evident
Slow burst	Series of pulses with intervals of 70–100 ms lasting no longer than 1000 ms

SPI, sequence of pulse interval.
EOD, electric organ discharge.

increases' (Mann–Whitney *U*-test, $P<0.05$) in the breeding period while 'long cessations' were seen more during the non-breeding period (Mann–Whitney *U*-test, $P<0.05$). 'Creaks', 'rasps' and 'gradual increases' were only seen during courtship, whereas 'scallops' and 'slow bursts' were non-breeding season specific.

Within a season, there were sex-typical behaviors and SPIs. In the non-breeding season, males 'probed' more than females. A male was also seen to have 'no activity' during the breeding season while the females were always active. 'Lunges' were male specific and 'fleeing' was female specific in both seasons. The remaining behaviors were not preferentially displayed by either sex in the two seasons. For SPIs, aside from 'medium bursts', which were produced more by males, on average no other SPIs were emitted more by a sex during the non-breeding season. As for breeding season, 'rasps' and 'creaks' were male specific. Males also emitted more 'medium bursts' and 'short and long cessations' (Mann–Whitney *U*-test, $P<0.05$) in this season. The many cessations indicated the periods of time between other SPIs such as 'rasps' and 'medium bursts'. There were also many more 'fast bursts' by the female (Mann–Whitney *U*-test, $P<0.05$). The only female sex-specific SPI pattern was the 'gradual increase'.

Quantitative analysis of simultaneous electrical and motor patterns

With the ability to use video editing software in conjunction with SPI graphs based on sex, we could view the corresponding SPI patterns emitted during a particular behavior. Table S3 and Table S4 in the supplementary material show the number and types of SPIs emitted for a respective behavior, by sex for 60 and 64.5 min of analyzed non-breeding and breeding recordings, respectively. Although there were few behavioral patterns with a single associated SPI pattern (e.g. 'fast or medium bursts' with 'lunging' in males and 'fast bursting' with 'fleeing' in females), there were many behaviors associated with more than one SPI pattern. Similarly, there were few SPI patterns with a single associated behavior; females only 'regularized' and 'scalloped' when 'hovering' during non-breeding conditions and males only 'creaked' while 'spawning' during breeding conditions.

Discussion

Using new techniques to simultaneously view EODs and behavior as well as to separate EODs on the basis of sex from a mixed signal, we identified nine behaviors and 11 stereotyped

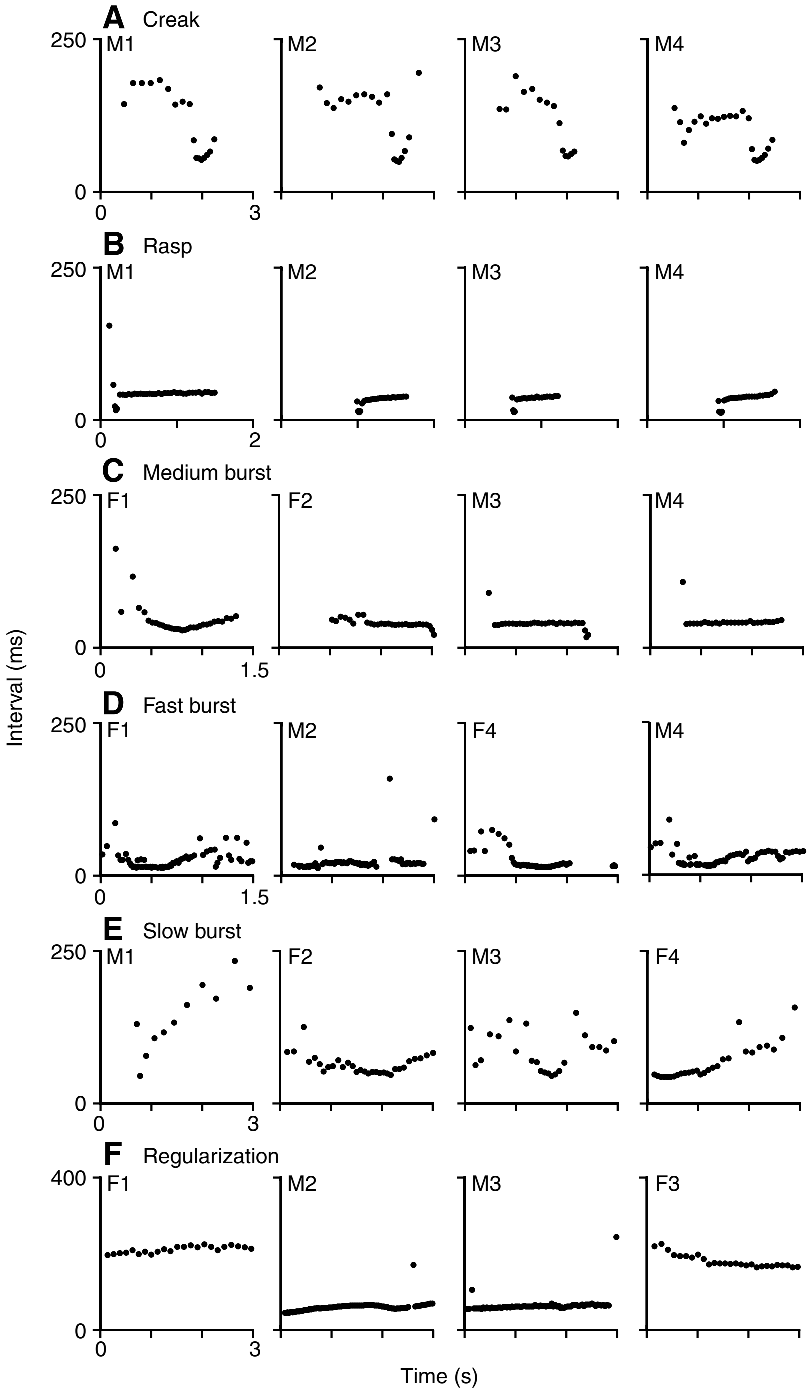

Fig. 3. Examples of (A) creaks, (B) rasps, (C) medium bursts, (D) fast bursts, (E) slow bursts and (F) regularizations from all pairs. The letter and number combination on the sequence of pulse intervals (SPI) plots indicate the sex (M or F) and pair that produced the SPI.

SPIs over non-breeding and breeding seasons. The non-breeding behavioral and electrical activity observed confirms previous findings in studies on agonistic interactions (Bell et al., 1974; Kramer, 1974; Moller et al., 1989). Males in this study were larger than females, were always dominant to the female and freely swam around the tank. While the male was patrolling, the submissive female tended to avoid the male by either hovering in a head-up posture or move toward the opposite side of the tank. While exhibiting either of these behaviors, some females were electrically silent for long periods indicating submissive behavior (Bell et al., 1974; Kramer, 1974; Moller et al., 1989). This strongly contrasted with the male as he frequently discharged and had higher numbers of ‘short cessations’. During the relatively infrequent encounters with the female, he would ‘lunge’ and chase the female while emitting a ‘fast or medium burst’. This behavior and electrical discharge frequency was also seen in *Gnathonemus petersii* in a dominance-subordinate relationship with another mormyrid (Bauer, 1972). There were also periods of times where both sexes ‘hovered’ near each other, emitted ‘scallops’ and ceased when there were behavioral interactions. ‘Scallop’ production during non-breeding conditions have previously been seen while fish were resting, and decreased in frequency during social interactions (Moller et al., 1989).

During courtship there was a marked increase in all motor and electrical activities. The male became tolerant of the female, which was indicated by prolonged and increased mutual behavioral interaction. The ritual included many permutations of initiating contact, ‘following’, ‘HTC’ and ‘spawning’. The lack of a rigid behavioral ritual suggests that motor patterns are not critical in eliciting receptivity of the female. This may also be the case in *M. macrolepidotus* as the female began spawning bouts almost immediately when given access to the male (Werneyer and Kramer, 2005). We did not observe any differences in behavior compared to the non-breeding conditions when the pairs were in breeding conductivities but not engaged in courtship.

Rather, SPIs more likely constitute honest signals. As seen in Table S3 and Table S4 in supplementary material, multiple SPIs were associated with a motor act during courtship, further suggesting that motor acts are not evaluated as critically as SPIs by each sex. ‘Rasps’ have been previously described during breeding seasons in the wild for *Brienomyrus* sp., and *B. brachyistius* and are believed to be a courtship signal (Hopkins and Bass, 1981; Carlson and Hopkins, 2004). This study lends support to that idea as ‘rasps’ were only produced by males during courtship activity. Notably, males produced many more ‘rasps’ and ‘medium bursts’ than any other SPI found during courtship and those SPIs were seen in all behaviors involving close proximity to the female. This suggests that these SPIs play a significant role

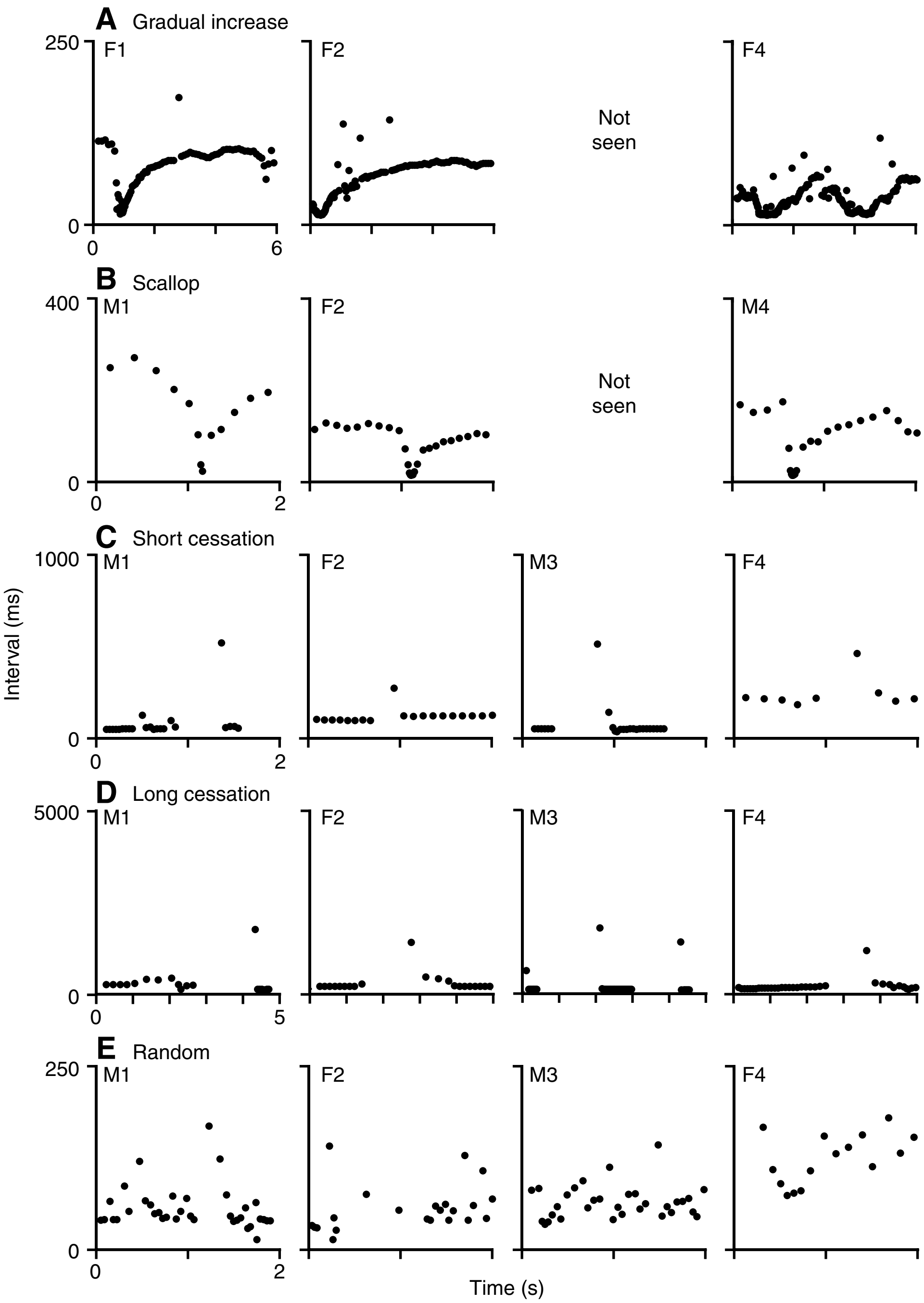

Fig. 4. Examples of (A) gradual increases, (B) scallops, (C) short cessations, (D) long cessations and (E) random from all pairs. The letter and number combination on the sequence of pulse intervals (SPI) plots indicate the sex (M or F) and pair that produced the SPI. 'Not seen' indicates SPI that was not emitted for that pair.

during courtship as the male's advertisement signal. It is possible that some aspect of the timing or characteristic of each SPI conveys fitness information. Furthermore, future studies should look at possible correlations between characteristics of the rasp and male quality (e.g. health, size, social status).

As soon as the male initiated courtship by 'lunging' or 'swimming' near the female, she continuously emitted EODs. Perhaps more interestingly, the female rarely had any 'cessations' and each SPI pattern merged continuously. The rate of 'fast bursts' increased over five times between seasons. Previously, bursts had mostly been described in all behavioral contexts besides courtship in mormyrids; they frequently served to indicate dominance and possibly to enhance electrolocation (Bell et al., 1974; Kramer, 1974; Kramer, 1976). While not dominant, the high frequency of discharging 'fast bursts' by females during courtship suggests they may also serve as a communication response in this species and *M. macrolepidotus* (Werneyer and Kramer, 2005). Hopkins and Bass (Hopkins and Bass, 1981) showed that while playing female EODs in the field to males during the breeding season, the males responded with 'rasps'.

There appeared to be a coordinated communication pattern between the male and female during courtship (Fig. 5). Although not as complex as the acoustical courtship in *P. isidori*, the observed *B. brachyistius* electrical courtship was more complex than that described in *M. macrolepidotus* (Bratton and Kramer, 1989; Werneyer and Kramer, 2005). Male 'rasps' or 'medium bursts' were followed by female 'fast bursts' or *vice versa*, which we termed 'rasp matching'. This duet was only seen during breeding conditions and when the sexes were interacting for long periods of time. Although not focused on in this study, 'rasp matching' contains some possible cues for future research to focus on, which include timing of each SPI relative to the opposite sex's SPI and the matching of frequencies. This electrical duetting between the sexes also can serve one of many possible functions such as a form of evaluation of the fitness of each sex, maintaining contact with the opposite sex, or indication of mutual interests as seen in avian acoustic duetting (Hall, 2004)

The data suggests that some SPI patterns were associated with a motor act during courtship. As mentioned previously, some SPIs occur in greater frequency with a particular behavior but 'creaks' were only produced by males during spawning.

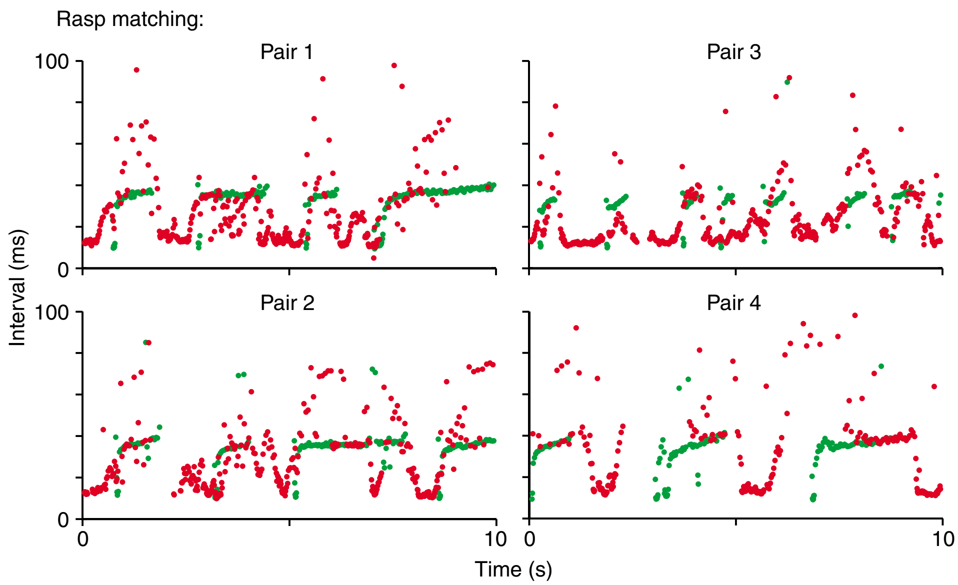

Fig. 5. Examples of rasp matching from all pairs. Those from males are in green and those from females are in red. Note the tendency for alternating pattern of rasps by the male and bursts by the female.

Although random SPIs were also seen during spawning, 'creaks' were not found with any other behavior. The general pattern of the 'creak' was seen in *P. isidori* and the one male *M. macrolepidotus* during their respective spawning bouts (Bratton and Kramer, 1989; Werneyer and Kramer, 2005). Similar to female *M. macrolepidotus*, *B. brachyistius* females predominantly emitted a 'medium burst' when spawning (Werneyer and Kramer, 2005). There were, however, occasional 'random' SPI by the female during this event. This could be a result of an unsuccessful courtship, either in terms of the male's failure to elicit receptivity from the female or poor positioning during 'spawning'. However, during successful 'spawning' events, in which both quiver their bodies and it lasts 2–3 s, the male and female emitted 'creaks' and 'medium bursts', respectively. The functions of these SPI patterns are currently unknown but they may facilitate or signal the release of gametes. Although no eggs were found the following day in this study, eggs were found in other species exhibiting similar SPIs to the 'creak' and 'medium bursts' in the vent-to-vent copulatory behavior (Bratton and Kramer, 1989; Werneyer and Kramer, 2005).

This study, and to our knowledge, one other study have successfully documented courtship in a mormyrid that only possesses the electric modality for communication (Werneyer and Kramer, 2005). Although this study reports courtship activity from four different pairs, we are confident that the overt behaviors and SPIs are characteristic of this species during courtship because of the consistencies of behavioral and electrical displays seen across the pairs between breeding and non-breeding conditions. Moreover, we observed a putative male advertisement signal ('rasp') in the laboratory, which has been seen in the wild during the breeding season, suggesting that we have induced similar breeding conditions as in the wild and likely similar communicative and behavioral responses (Hopkins and Bass, 1981). We acknowledge that our periods of data analyses were relatively shorter compared to the recorded periods. This may pose as a limitation to our understanding of the electrical and motor displays during breeding conditions while not engaged in courtship. However, we analyzed all the data during courtship and feel we have captured electrical and motor displays during those events. For periods outside of courtship, in each pair we observed similar behaviors seen in the non-breeding periods. With regards to non-breeding conditions, we observed similar behavioral displays throughout each of the observation periods for all pairs.

The importance of sensory modalities such as the auditory, olfactory, visual and mechanical sensory systems has been explored in many organisms in the context of courtship. In this study we have quantified and categorized overt behavioral and electric displays (SPI patterns) emitted during courtship in *B. brachyistius*. The variation differences of SPIs between sexes and individuals affect the actual broadcast, and by implication, the transmitted message. Furthermore, from this study and the few other reported studies of mormyrid courtship, we already see a broad range of complexity with respect to behavioral and electrical displays. The extent to which this range could be explained by ecological, evolutionary, physiological or phylogenetic constraints is unknown. Without knowledge of the overt behaviors and associated electrical activity during courtship, we cannot begin to explore issues such as mutual or reciprocal electrical signaling between sexes, sexual selection, honest electrical signals, and the neural mechanisms underlying SPI pattern generation and interpretation.

Funding was provided by Howard Hughes Medical Institute fellowship and CALS grant to R.Y.W. and NIH grant R01-C0062006 to C.D.H. We thank Bruce A. Carlson for use of the program allowing EOD separation by the cross correlation method, and Bruce R. Land for assistance with programming and data analysis. We are also grateful to Laurieanne Dent, Matthew E. Arnegard and B. Scott Jackson for helpful discussions.

References

Arnegard, M. E. and Carlson, B. A. (2005). Electric organ discharge patterns during group hunting by a mormyrid fish. *Proc. R. Soc. Lond. B Biol. Sci.* **272**, 1305-1314.

Bauer, R. (1972). High electrical discharge frequency during aggressive behavior in a mormyrid fish *Gnathonemus petersii*. *Experientia* **28**, 669-670

Bell, C. C., Myers, J. P. and Russell, C. J. (1974). Electric organ discharge patterns during dominance related behavioral displays in *Gnathonemus petersii* (Mormyridae). *J. Comp. Physiol. A* **92**, 201-228.

Bratton, B. O. and Kramer, B. (1989). Patterns of the electric organ discharge during courtship and spawning in the mormyrid fish, *Pollimyrus isidori*. *Behav. Ecol. Sociobiol.* **24**, 349-368.

Brown, B., Benveniste, L. M. and Moller, P. (1996). Basal expansion of anal-fin rays: a new osteological character in weakly discharging electric fish (Mormyridae). *J. Fish Biol.* **49**, 1216-1225.

Carlson, B. A. (2002). Electric signaling behavior and the mechanisms of electric organ discharge production in mormyrid fish. *J. Physiol. Paris* **96**, 405-419.

Carlson, B. A. and Hopkins, C. D. (2004). Stereotyped temporal patterns in electrical communication. *Anim. Behav.* **68**, 867-878.

Crawford, J. D., Hagedorn, M. and Hopkins, C. D. (1986). Acoustic communication in an electric fish, *Pollimyrus isidori* (Mormyridae). *J. Comp. Physiol. A* **159**, 297-310.

Gill, T. (1862). On the West African genus Hemichromis and description of new species in the museums of the Academy and Smithsonian Institutions. *Proc. Natl. Acad. Sci. USA* **14**, 134-139.

Graff, C. (1987). Recording electrophysiological signals from small moving animals: electrode fixation and low torque swivel for recording a weakly electric fish in a group. *J. Neurosci. Methods* **19**, 95-104.

Hall, M. L. (2004). A review of hypotheses for the functions of avian duetting. *Behav. Ecol. Sociobiol.* **55**, 415-430.

Hopkins, C. D. (1986a). Behavior of Mormyridae In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 527-576. New York: John Wiley & Sons.

Hopkins, C. D. (1986b). Temporal structure of non-propagated electric communication signals. *Brain Behav. Evol.* **28**, 43-59.

Hopkins, C. D. and Bass, A. H. (1981). Temporal coding of species recognition signals in an electric fish. *Science* **212**, 85-87.

Hopkins, C. D. and Westby, G. W. (1986). Time domain processing of electric organ discharge waveforms by pulse-type electric fish. *Brain Behav. Evol.* **29**, 77-104.

Kirschbaum, F. (1979). Reproduction of the weakly electric fish *Eigenmannia virescens* (Rhamphichtyidae, Teleostei) in captivity. *Behav. Ecol. Sociobiol.* **4**, 331-355.

Kirschbaum, F. (1987). Reproduction and development of the weakly electric fish, *Pollimyrus isidori* (Mormyridae, Teleostei) in captivity. *Environ. Biol. Fishes* **20**, 11-31.

Kirschbaum, F. (1995). Reproduction and development in mormyriform and gymnotiform fishes. In *Electric Fishes: History and Behavior* (ed. P. Moller), pp. 267-301. London: Chapman & Hall.

Kirschbaum, F. and Schugardt, C. (2002). Reproductive strategies and developmental aspects in mormyrid and gymnotiform fishes. *J. Physiol. Paris* **96**, 557-566.

Kirschbaum, F. and Westby, G. W. (1975). Development of the electric discharge in mormyrid and gymnotid fish (*Marcusenius* sp. and *Eigenmannia virescens*). *Experientia* **31**, 1290-1294.

Kramer, B. (1974). Electric organ discharge interaction during interspecific agonistic behaviour in freely swimming mormyrid fish. *J. Comp. Physiol. A* **93**, 203-235.

Kramer, B. (1976). Electric signaling during aggressive behavior in *Mormyrus rume* Mormyridae Teleostei. *Naturwissenschaften* **63**, 48-49.

Kramer, B. (1990). *Electrocommunication in Teleost Fishes: Behavior and Experiments*. New York: Springer.

Kramer, B. and Bauer, R. (1976). Agonistic behavior and electric signalling in a mormyrid fish, *Gnathonemus petersii. Behav. Ecol. Sociobiol.* **1**, 45-61.

Ladich, F., Collin, S. P., Moller, P. and Kapoor, B. G. (2006). *Communication in Fishes*. Enfield, NH: Science Publishers.

Moller, P. (1995). *Electric Fishes: History and Behavior*. London: Chapman & Hall.

Moller, P., Serrier, J. and Bowling, D. (1989). Electric organ discharge displays during social encounter in the weakly electric fish *Brienomyrus niger* (Mormyridae). *Ethology* **82**, 177-191.

Schugardt, C. and Kirschbaum, F. (2004). Control of gonadal maturation and regression by experimental variation of environmental factors in the mormyrid fish, *Mormyrus rume proboscirostris*. *Environ. Biol. Fishes* **70**, 227-233.

Serrier, J. and Moller, P. (1989). Patterns of electric organ discharge activity in the weakly electric fish *Brienomyrus niger* L. (Mormyridae). *Exp. Biol.* **48**, 235-244.

Terleph, T. A. and Moller, P. (2003). Effects of social interaction on the electric organ discharge in a mormyrid fish, *Gnathonemus petersii* (Mormyridae, Teleostei). *J. Exp. Biol.* **206**, 2355-2362.

von der Emde, G. (1999). Active electrolocation of objects in weakly electric fish. *J. Exp. Biol.* **202**, 1205-1215.

Werneyer, M. and Kramer, B. (2005). Electric signalling and reproductive behaviour in a mormyrid fish, the bulldog *Marcusenius macrolepidotus* (South African form). *J. Ethol.* **23**, 113-125.