



Stereotyped temporal patterns in electrical communication

BRUCE A. CARLSON & CARL D. HOPKINS

Department of Neurobiology and Behavior, Cornell University

(Received 1 November 2002; initial acceptance 6 March 2003;
final acceptance 7 October 2003; published online 9 September 2004; MS. number: A9478R)

Mormyrid electric fish communicate using a fixed electric organ discharge (EOD) produced with a variable sequence of pulse intervals (SPI). Through a combination of spike train analysis techniques and multivariate statistics, we develop quantitative methods for analysing temporal patterns in the SPI in *Brienomyrus brachyistius*. Previous research has indicated the existence of distinct temporal patterns that play an important role in social behaviour, but these studies have relied upon subjective assessments of the SPI: no study has yet used quantitative methods for analysing temporal patterns, so it remains unclear whether there are categorically distinct displays or continuous variation. We demonstrate the existence of three modal classes of frequency modulations (bursts) that differ categorically in their temporal patterning and can therefore be appropriately termed displays. 'Scallops' are stereotyped, transient bursts to EOD intervals of 10–20 ms. 'Accelerations' are maintained decreases in EOD interval to values of 25–40 ms. Evidence indicates that 'rasps' combine a scallop followed by an acceleration. Analysis of signal characteristics and their production in relation to several variables, combined with previous research, suggests that accelerations function as aggressive signals, rasps as male courtship signals, and scallops as advertisement signals. This descriptive study is an important first step in the analysis of temporal patterns in the SPI, and provides a foundation for addressing general issues in animal communication. The relative simplicity of their electric communication displays suggest that mormyrids are an excellent model system for studying the evolution, function and mechanisms of stereotyped temporal pattern generation.

© 2004 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Temporal features encode a large portion of the information content in animal communication signals (Pollack 2001). The acoustic, vibratory and visual signals generated by many organisms are best described as time-varying patterns that fall into distinct modal classes that serve differing behavioural functions (Barlow 1977). Similarly, mormyrid fish communicate using a fixed electric organ discharge (EOD) that is produced with a variable sequence of pulse intervals (SPI; Hopkins 1986; Carlson 2002a). Mormyrid fish discharge their electric organs continuously for electrolocation of objects, even in isolation, which has made it challenging to quantitatively describe the variable patterns of discharges. Because of the difficulty of developing quantitative descriptors of the time-varying signals, the features that define variation in the SPI remain unclear. To date, no study has used quantitative methods

to catalogue the diversity of patterns within a single species to determine whether these patterns vary continuously or fall into distinct modal classes. In the current study, we develop methods for analysing the temporal patterning of EOD production, and use them to analyse the characteristics and diversity of frequency modulations (bursts) in a single species of mormyrid, *Brienomyrus brachyistius* (Gill 1862). While the current study is largely descriptive in nature, it is an important step in our goal of studying the neuronal control of the SPI.

Electric fish are well known for their ability to resolve temporal features of electric stimuli, with acuity in some species reaching microsecond precision (Kawasaki 1993). In mormyrids, this sensitivity is involved in sensory coding of precise timing in the EOD waveform (Xu-Friedman & Hopkins 1999), which shows species, sex and individual differences ranging from tens of microseconds to several milliseconds (Hopkins 1981; Friedman & Hopkins 1996; Carlson et al. 2000). The temporal features of the SPI, by contrast, vary on a timescale of tens of milliseconds to seconds (Carlson 2002a). Several studies have described a variety of phasic patterns in the SPI and linked them to social contexts such as aggression

Correspondence and present address: B. A. Carlson, Department of Biology, Gilmer Hall, P.O. Box 400328, University of Virginia, Charlottesville, VA 22904-4328, U.S.A. (email: bc6s@virginia.edu).
C. D. Hopkins is at the Department of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853-2702, U.S.A.

and courtship (for reviews see Hopkins 1986; Carlson 2002a). For instance, breeding males of an undescribed species of *Brienomyrus* (tentatively named 'VAD', see Sullivan et al. 2002) produce high-frequency courtship bursts termed 'rasps' (Hopkins & Bass 1981). During overt aggression, *Gnathonemus petersii* may produce 'pulse pairs' (Bauer 1972; Bell et al. 1974; Kramer 1974; Kramer & Bauer 1976), and several species produce maintained accelerations that have been given descriptive names such as 'burst', 'buzz', 'smooth acceleration', or 'sudden increase followed by a decrease in frequency (SID)' (Bell et al. 1974; Kramer 1974, 1976, 1979; Kramer & Bauer 1976; Hopkins 1986). 'Cessations' have been linked with both aggressive and submissive behaviour in several species (Moller 1970; Moller & Bauer 1973; Bell et al. 1974; Kramer & Bauer 1976; Kramer 1979; Moller et al. 1989). In *Pollimyrus isidori*, highly regular intervals are produced during courtship and spawning (Bratton & Kramer 1989).

These studies indicate that the SPI varies with social context, but descriptions of different patterns have been based on subjective assessments that are limited by the perceptual biases of human observers (Barlow 1977). The only rigorous quantification of a specific pattern that we are aware of is a study in *Brienomyrus niger* describing a transient burst termed a 'scallop' (Serrier & Moller 1989). Those authors demonstrated that scallops are stereotyped within individuals, and that different fish vary in the number of scallops they produce. This study was an important advance in quantifying the SPI as a signal, but the original categorization of the pattern was nevertheless determined subjectively, and therefore did not get around the problem of providing a specific definition for the behaviour that quantitatively distinguishes it from other modulations in the SPI. Without clear definitions of signal characteristics, it is impossible to address such basic issues as the diversity, evolution and function of specific patterns, as well as the mechanisms of their production and sensory encoding (see Carlson 2002a).

The goals of the current study were to: (1) develop a method for quantitative analysis of temporal patterning in the SPI; (2) catalogue the diversity of bursts in *B. brachyistius*; (3) determine whether these bursts vary continuously or can be categorized into specific displays; (4) define the attributes that characterize each category of burst; and (5) analyse burst production in relation to behavioural context. Because electrical signalling behaviour in mormyrids consists of spike-like all-or-none electrical potentials generated at varying intervals, it is analogous to spike trains of single unit activity in the nervous system. We therefore take an approach that has been used to discern temporal activity patterns in the spike trains of single neurons. By converting the SPI into a continuous estimate of spike rate termed the spike density function (SDF; Szücs 1998), we identify specific landmarks in the bursts. From these, we generate quantitative measures of timing, rate and changes in rate, and then analyse these variables using multivariate statistical techniques. Our results indicate that this is an effective approach to analysing and classifying temporal patterns of electrical behaviour in mormyrids, and it may prove useful in the study of other communication systems in which

temporal patterning is an important element of signalling behaviour.

METHODS

Study Subjects

Brienomyrus brachyistius has a widespread distribution in western Africa, from Senegal to Nigeria (Bigorne 1989). Although little is known about the ecology of this species or any other mormyrid, it is found in shallow creeks in densely forested areas and appears to breed during the rainy season, as do other species of mormyrid (Kirschbaum 1995; Carlson et al. 2000). We used mature male ($N = 16$) and female ($N = 16$) individuals from a laboratory stock composed of a mix of wild-caught and laboratory-bred fish. Individuals were sexed by their overall size, the presence of anal fin notches in males, and swollen abdomens in females (Kirschbaum 1995).

The fish were divided into three groups: (1) 'isolated', in which the fish were housed in isolation ($N = 6$ males, 6 females); (2) 'social', in which a single male and a single female were housed as a nonbreeding pair ($N = 5, 5$); and (3) 'breeding', in which a single male and a single female were housed as a breeding pair ($N = 5, 5$). Each fish (isolated) or pair (social and breeding) was placed in a 280-litre aquarium, with the temperature maintained at 25–27°C, on a 12:12 h light:dark cycle. The water conductivity of each tank was maintained at 150–200 $\mu\text{S}/\text{cm}$, except for tanks occupied by breeding pairs, in which conductivity was lowered to 10–15 $\mu\text{S}/\text{cm}$ by daily water changes using deionized water to induce breeding behaviour. To verify that individuals in the breeding group were in reproductive condition, while those in the social group were not, we scanned the tank bottoms every morning for the presence of fertilized eggs. All breeding pairs produced fertilized eggs, while none of the social pairs did. At the conclusion of each experiment, individuals were killed using anaesthesia (1000 mg/litre tricaine methanesulfonate; Sigma Chemical Co., St Louis, Missouri, U.S.A.) and their sex was verified by gross examination of the gonads. These procedures are in accordance with the guidelines established by the National Institutes of Health and were approved by the Cornell University Institutional Animal Care and Use Committee.

Signal Recording

In order to isolate the electrical output of single individuals, fish in the social and breeding pairs were separated for the duration of the recording by removable plastic barriers that divided the tank into separate compartments. However, fish in these groups were in complete social contact immediately before and after the recordings. Electrical recordings of individual fish were made using two pairs of chlorided silver wire electrodes, oriented perpendicular to each other, both amplified 10 000 times and band-pass-filtered from 10 Hz to 5 kHz with a differential AC amplifier (A-M Systems, Inc., Everett, Washington, U.S.A.; model 1700). The two-electrode

arrangement ensured that each EOD was detected regardless of the fish's horizontal angle. The two outputs were fed into separate Schmitt Triggers, which were set to produce pulses of 2 ms on each suprathreshold EOD. The outputs of the two Schmitt Triggers were sent to an event timer (Tucker-Davis Technologies, Gainesville, Florida, U.S.A.; model ET1), which recorded the time of occurrence of each EOD with a clock rate of 1 MHz. EOD times of occurrence were saved for later analysis using custom-made software on a PC.

For quantitative analysis of burst characteristics, we recorded 20 20-s (short) records from each fish over several days for a total of 640 records. For analysing burst production by context, we recorded 10- to 20-min (long) records from each fish once in the day (1000–1100 hours) and once at night (1900–2000 hours).

Burst Analysis

We analysed bursts from a random sampling of 40 short records using Matlab 6.1 (MathWorks 2000). We converted the SPI (Fig. 1a, b) to the spike density function (SDF) by converting EOD times of occurrence into a series of delta functions and convolving these with a Gaussian kernel function (Paulin 1992; Szücs 1998). This low-pass filters the data, resulting in a continuous function representing EOD rate in units of spikes per second (Fig. 1c, d).

The width of the Gaussian function, defined as four standard deviations, was set at 200 ms. We also used the first-order derivative of the SDF, the spike density derivative (SDD; Fig. 1e, f), to characterize changes in EOD rate (Szücs 1998). A primary advantage in using the SDF and SDD is that they provide a continuous measure of the temporal patterning of EOD activity that avoids problems associated with more traditional instantaneous or quantized measures of EOD rate (Paulin 1992; Szücs 1998).

We defined a burst as any portion of the SDF that exceeded the median SDF plus 1.96 standard deviations (Fig. 1c). For each burst, seven landmarks were determined from the SDF and SDD (Fig. 1d, f). We defined the peak (P) as the point where the SDF reached a maximum. The absolute minima immediately preceding and following P were located. The start (S) and end (E) were defined as a 5% peak-to-peak deviation from these points (Fig. 1d). We defined the peak rises as the positive peaks in the SDD that came immediately after S (R2) and immediately before P (R1), and the peak falls as the negative peaks in the SDD that came immediately after P (F1) and immediately before E (F2; Fig. 1f). In cases with an inflection in the rise or fall, R2 was distinct from R1, and F2 from F1, but in cases where the rise or fall was smooth, these landmarks were identical (Fig. 1f). Using these seven landmarks, we identified 20 variables that describe the temporal patterning in the SPI from each burst (Table 1).

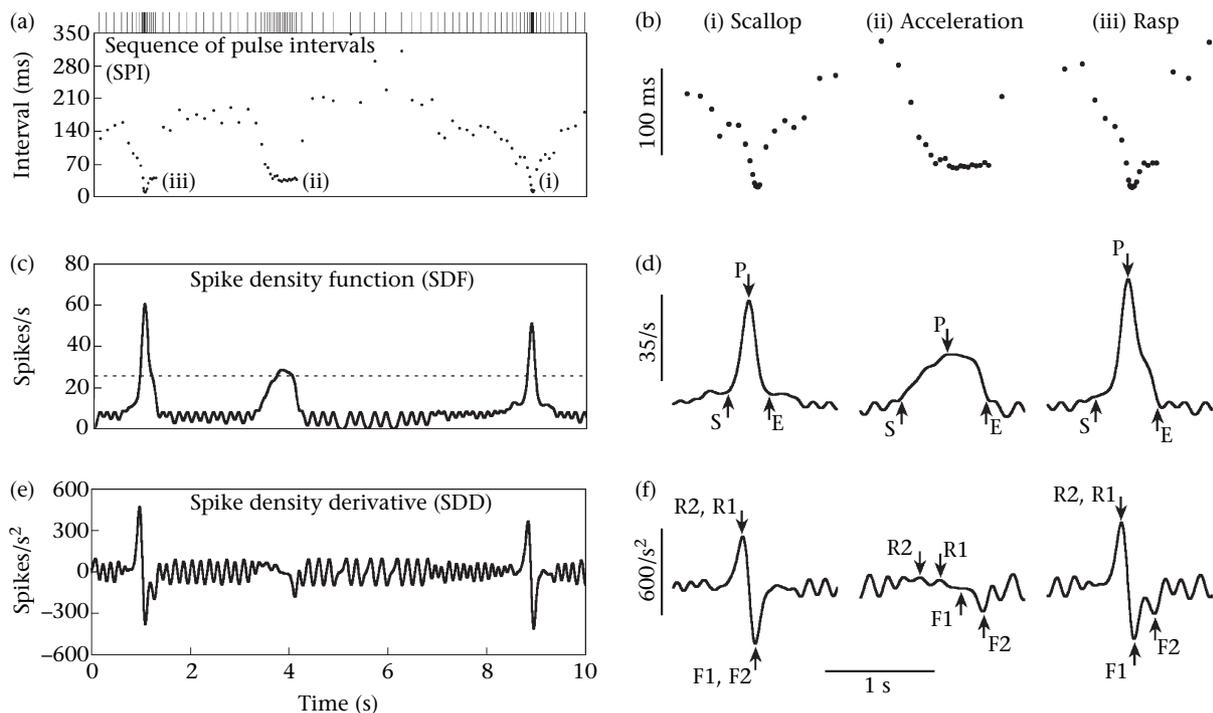


Figure 1. (a) Plot of the sequence of pulse intervals (SPI) from an isolated *Brienomyrus brachyistius*. The vertical lines above the plot represent delta functions indicating the time of occurrence of each electric organ discharge (EOD). Lower case Roman numerals indicate the occurrence of three different burst types, shown in (b). (c) Spike density function (SDF) formed by convolving the delta functions in (a) with a Gaussian curve (width = 200 ms). The dashed horizontal line marks the threshold for burst detection, defined as the median SDF plus 1.96 standard deviations. (d) SDF of the examples shown in (b). Arrows mark the start (S), end (E) and peak (P) landmarks. (e) Spike density derivative (SDD) resulting from the convolution in (c). (f) SDD of the examples shown in (b). Arrows mark the peak rise (R2, R1) and peak fall (F1, F2) landmarks.

Table 1. Twenty variables measured for each burst using the seven burst landmarks

Measure	Variable	Abbreviation
Spike density function (SDF)	SDF at start (S)	Sf
	SDF at peak (P)	Pf
	SDF at end (E)	Ef
	SDF at secondary rise (R2)	R2f
	SDF at primary rise (R1)	R1f
	SDF at primary fall (F1)	F1f
	SDF at secondary fall (F2)	F2f
Spike density derivative (SDD)	SDD at secondary rise (R2)	R2d
	SDD at primary rise (R1)	R1d
	SDD at primary fall (F1)	F1d
	SDD at secondary fall (F2)	F2d
Times of occurrence	Time at start (S) relative to peak (P)	St
	Time at end (E) relative to peak (P)	Et
	Time at secondary rise (R2) relative to peak (P)	R2t
	Time at primary rise (R1) relative to peak (P)	R1t
	Time at primary fall (F1) relative to peak (P)	F1t
	Time at secondary fall (F2) relative to peak (P)	F2t
	SDF areas	SDF area from secondary rise (R2) to primary rise (R1)
	SDF area from primary rise (R1) to primary fall (F1)	A2
	SDF area from primary fall (F1) to secondary fall (F2)	A3

Principal Components and Discriminant Function Analysis

All 20 variables from each burst were examined using principal components analysis (PCA), which does not make any a priori assumptions about group membership (Pimentel 1992). PCA uses linear combinations of all the variables to form a reduced number of orthogonal factors that maximally explain variation between cases. The meaning of each PCA factor can be interpreted by its eigenvalue (λ), which is related to the amount of variation explained by that factor, and by factor loadings, which represent the relative influence of a given variable on that factor. Clustering of points in PCA space indicate the existence of different categories. A primary advantage of using PCA is that it allows for the inclusion of a large number of variables that may or may not prove relevant, and then determines which of the variables contribute most to variation between the cases. Thus, PCA provides an objective measure of which variables are relevant for distinguishing between cases and categories.

After verifying the categorization of bursts using PCA, we used discriminant function analysis (DFA) to differentiate between burst categories (Pimentel 1992). DFA uses a priori group categorizations to create a reduced number of canonical roots from a linear combination of the variables such that within-group variance is minimized while maximizing variance between groups. DFA therefore provides a measure of how well separated the different

categories are in multivariate space, and like PCA, it provides information on the contribution of each variable to this differentiation. A primary advantage of DFA is that it produces a series of classification functions that can be used to evaluate novel cases and determine the relative probabilities of membership in the different categories. Each novel case is then assigned to the group with which it has the greatest probability of membership. To test the accuracy of these procedures, we randomly selected an additional 10 records from our library of 640 20-s records and categorized each burst using the classification functions. The PCA and DFA were done in Statistica 6.1 (StatSoft 2003).

We developed a series of Matlab programs to automatically analyse burst production by computing the SDF and SDD, detecting bursts using the threshold criteria, locating landmarks, and then evaluating the landmarks using the classification functions developed from the DFA. This allowed us to automatically detect and analyse bursts from the long records. These programs are available on request from the authors.

RESULTS

Subjective Description of Bursts

Electromotor output was typified by resting intervals of around 100–300 ms that were punctuated by the frequent production of bursts (Fig. 1a). We subjectively recognized three distinct burst types on the basis of their appearance in interval versus time plots and by their sounds when played through an audio monitor, and named these bursts after previous descriptions of similar bursts in other species (Fig. 1b). ‘Scallops’ (after Serrier & Moller 1989 for *B. niger*) are highly stereotyped, transient bursts that begin with a sudden drop in interval to values of 10–20 ms, followed by an immediate increase back to resting intervals. ‘Accelerations’ (similar to displays reviewed in Carlson 2002a for several species) were typified by less intense, sustained decreases in interval to values of around 25–40 ms. Unlike scallops, accelerations were quite variable in both their duration and in the minimum intervals reached during the burst. ‘Rasps’ (after Hopkins & Bass 1981 for *B. VAD*) were sudden, sharp decreases in interval to values of 10–20 ms followed by several intervals of around 25–40 ms before increasing back to resting intervals. Thus, rasps appeared to have two distinct phases, an initial scallop-like phase, followed by an acceleration-like phase.

Principal Components and Discriminant Function Analysis

Our random sampling of 40 20-s records contained a total of 269 bursts, of which we subjectively designated 103 accelerations, 79 rasps and 87 scallops. The first PCA analysis included all three types and resulted in six factors that explained 85.90% of the variance. The majority of the variance was explained by the first two factors ($\lambda_1 = 7.09$, 35.46% and $\lambda_2 = 3.44$, 17.19% of variance, respectively). Factor 1 was bimodally distributed, and completely

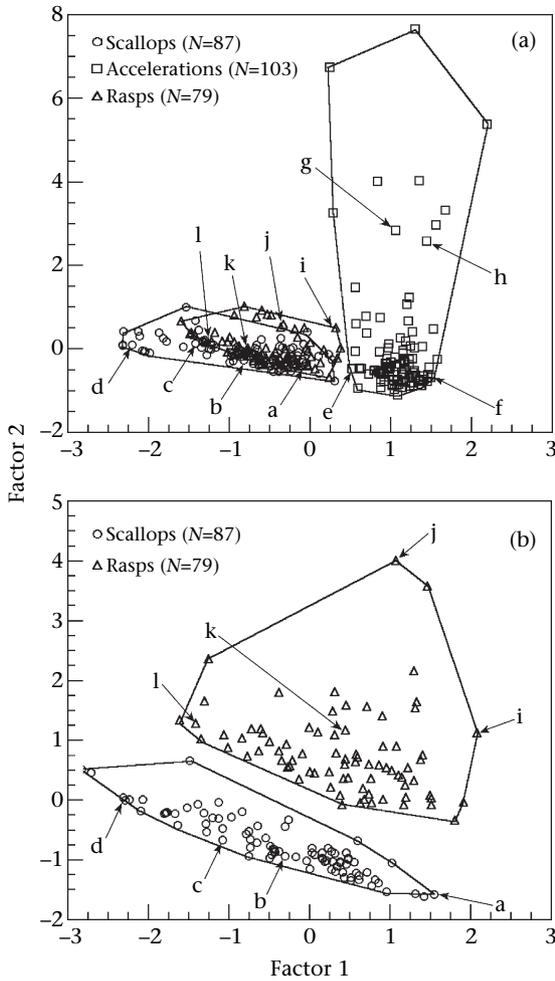


Figure 2. Principal components analysis (PCA) of EOD bursts. (a) Factors 1 and 2 from a PCA including all bursts. (b) Factors 1 and 2 from a PCA excluding accelerations. Lower case letters identify representative examples shown in Fig. 3.

separated accelerations from rasps and scallops (Fig. 2a). The variables that loaded most heavily (≥ 0.7) onto this axis included Pf, R1f, F1f, R2d, R1d, F1d and F2d (Table 2), confirming that scallops and rasps are distinguished from accelerations in reaching greater EOD rates with more rapid onsets and offsets. There was also wide variation within scallops and rasps across factor 1 (Fig. 2a). For both types of burst, variation within factor 1 was related to differences in the number of EODs near the peak of the burst, with more negative values corresponding to a greater number of EODs, and therefore larger SDF values (Fig. 3a–d, i–l). There was less variation within accelerations along factor 1 (Fig. 2a), although smaller values corresponded to shorter EOD intervals, and therefore larger SDF values, during the burst (Fig. 3e–h).

Factor 2 was unimodally distributed and did not discriminate between burst types, but rather primarily explained variation within accelerations (Fig. 2a). The variables that loaded strongly (≥ 0.7) onto factor 2 included St, R2t and A1 (Table 2). Thus, variation within accelerations along factor 2 was primarily related to differences in burst duration (Fig. 3e–h).

Table 2. Factor loadings for the 20 variables used in the PCA that included all bursts and the PCA that excluded accelerations (absolute values ≥ 0.7 are highlighted in bold)

Variable	PCA with all bursts		PCA excluding accelerations	
	Factor 1	Factor 2	Factor 1	Factor 2
Sf	0.4140	0.0716	-0.2511	-0.0270
Pf	-0.9473	0.2332	-0.8507	0.4134
Ef	0.2545	0.2160	-0.2704	-0.1010
R2f	-0.6750	-0.0511	-0.6189	0.1769
R1f	-0.8326	0.4209	-0.8158	0.2590
F1f	-0.8189	0.3051	-0.5427	0.7267
F2f	-0.5396	0.2070	-0.6402	-0.5512
R2d	-0.8667	-0.0561	-0.6288	0.2945
R1d	-0.9561	-0.0160	-0.7626	0.4366
F1d	0.9107	-0.0159	0.8357	0.1887
F2d	0.7508	0.0218	0.5334	0.5274
St	-0.2726	-0.8997	-0.1210	0.0612
Et	0.0863	0.6240	0.2956	0.7932
R2t	-0.3221	-0.8920	-0.2568	0.0164
R1t	-0.3085	0.1193	-0.0088	-0.6228
F1t	0.2876	-0.0464	-0.7107	-0.3306
F2t	0.1485	0.2718	0.3718	0.8464
A1	0.2655	0.9049	0.2104	-0.0314
A2	-0.5644	0.1815	-0.8439	0.3928
A3	0.1220	0.2754	0.3782	0.8441

See Table 1 for definition of variables.

Because scallops and rasps were not separated by the first two factors of the first PCA, we did a second PCA on all bursts except for those classified as accelerations. This resulted in five factors that explained 87.39% of the variance, with factors 1 and 2 explaining the majority ($\lambda_1 = 6.28$, 31.42% and $\lambda_2 = 4.42$, 22.09% of variance, respectively). Scallops and rasps were completely separated along the first two factors, with most of the separation occurring along factor 2 (Fig. 2b). The variables that loaded strongly onto factor 2 (≥ 0.7) included F1f, Et, F2t and A3 (Table 2), verifying that the burst offset is the primary difference between scallops and rasps. There was wide variation along factor 2 within rasps, with larger values corresponding to longer durations of the second, acceleration-like phase (Fig. 3i–l). Factor 1 provided only marginal separation between scallops and rasps, primarily explaining variation within burst types (Fig. 2b). Several variables loaded strongly onto factor 1, including Pf, R1f, R1d, F1d, F1t and A2 (Table 2), and similar to the first PCA, more negative values corresponded to a greater number of EODs near the peak of the burst (Fig. 3a–d, i–l).

Using DFA on the same 270 bursts produced two canonical roots that together explained 100% of the variance ($\lambda_1 = 10.74$, 64.16% of variance, $\chi^2_{40} = 1131.2$, $P < 0.01$; $\lambda_2 = 6.00$, 35.84% of variance, $\chi^2_{19} = 499.26$, $P < 0.01$). There was a high degree of discrimination between the three burst types (Fig. 4a; overall Wilk's $\lambda = 0.0122$; $F_{40,494} = 99.67$, $P < 0.0001$). Several variables contributed significantly to variation between burst types, including Pf, R1f, F1f, F2f, R1d, F2d, Et, F1t, F2t and A2 (partial $\lambda = 0.494$ – 0.972 ; $F_{2,247} = 3.572$ – 126.6 , $P < 0.05$). This confirms the PCA results, indicating that several features that describe the temporal patterning of the SPI distinguish the three burst types. Our random sampling of

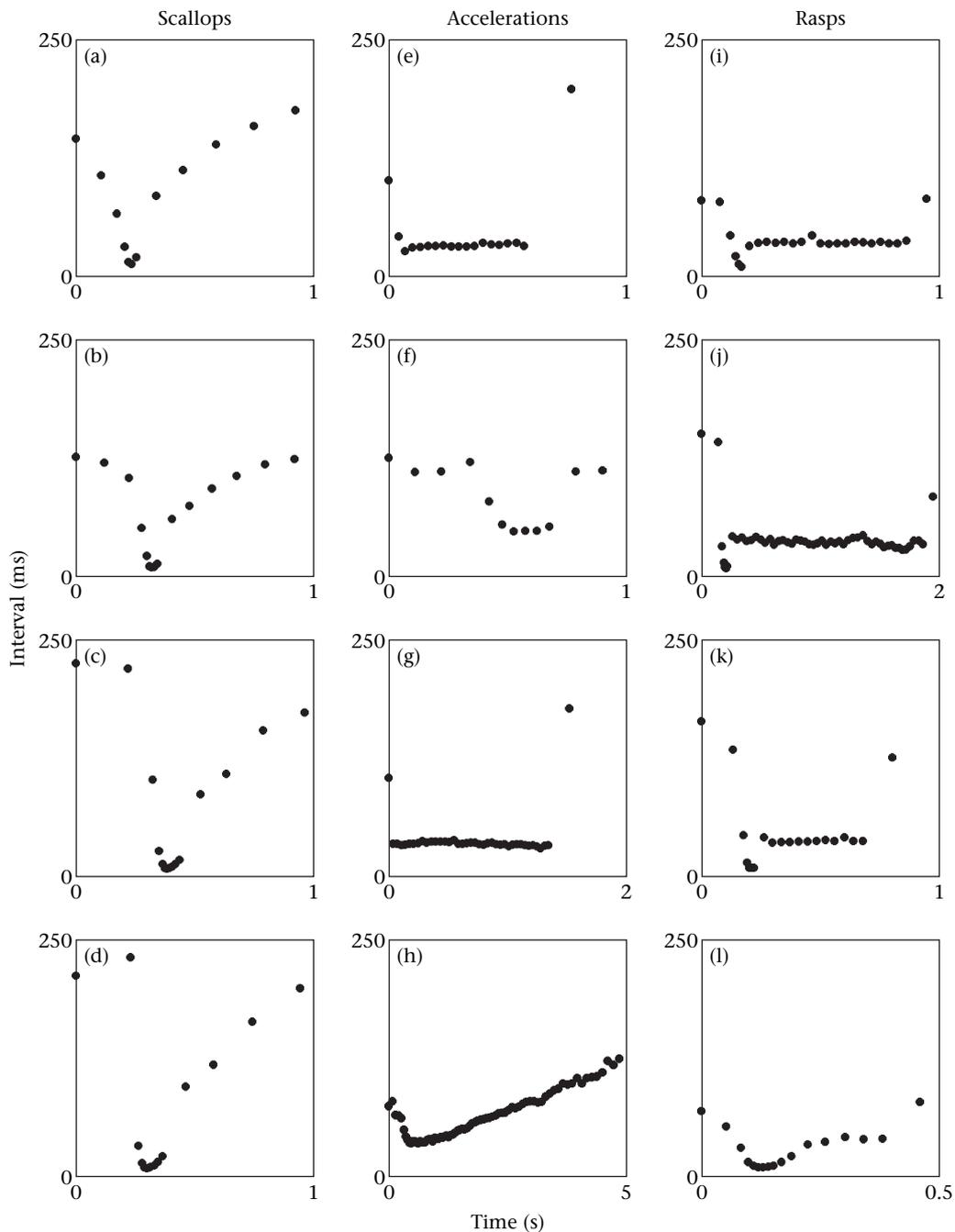


Figure 3. Four examples each of scallops (a–d), accelerations (e–h) and rasps (i–l). Within each class, the four examples are taken from four individuals. The letter corresponding to each example references its location within the PCA plots in Fig. 2.

10 additional records resulted in a total of 86 bursts, of which we subjectively designated 46 as accelerations, 18 as rasps and 22 as scallops. All 86 test cases were correctly assigned to burst type with near unity probability (Table 3).

Stereotyped Temporal Patterns within Bursts

The PCA and DFA results demonstrate that the bursts fall into three distinct categories based on differences in several variables that describe the temporal patterning of EOD output. To verify the importance of the sequence of

intervals in defining burst type, as opposed to the overall distribution of intervals, we randomly chose an additional 20 short records and randomly shuffled the order of intervals from each (Fig. 5). This created records with identical EOD interval distributions, but completely artificial SPIs, resulting in ‘random’ bursts lacking the temporal patterning of real bursts. Using the same threshold criteria on the resulting SDFs, this resulted in a total of 101 random bursts from the 20 records.

Under the original DFA model, the random bursts did not form three distinct clusters as did the real bursts, but rather formed a single cluster located intermediate to the

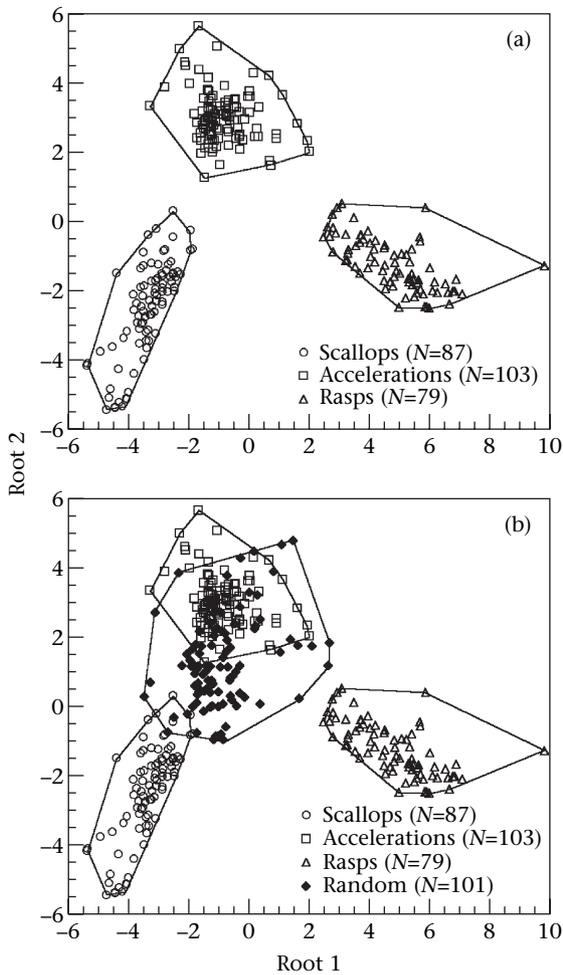


Figure 4. (a) Discriminant function analysis (DFA) of natural bursts. (b) DFA from (a) plus the values from ‘random’ bursts resulting from SPI randomization as shown in Fig. 5.

three natural clusters (Fig. 4b). There was minimal overlap between the random bursts and scallops or rasps, although there was a high degree of overlap with accelerations. Of the 101 random bursts, 85 were classified as accelerations, 14 as scallops and two as rasps (Table 3). For root 1, scallops and rasps were significantly different from the random bursts (Tukey HSD test: $P < 0.00001$), while accelerations were not (Tukey HSD test: NS). For root 2, all three bursts were significantly different from the random bursts (Tukey HSD test: $P < 0.00001$). These results show

that scallops and rasps are well defined by specific temporal patterns of EOD production, whereas accelerations are relatively indistinguishable from random increases in EOD rate.

Quantitative Description of Bursts

Using the DFA classification functions to discriminate bursts in the long records (64 20-min records), we detected a total of 3170 scallops, 3481 accelerations and 1204 rasps. Table 4 summarizes quantitative measures of several variables taken for each of the three burst types.

Our subjective observations of the three burst types suggested that rasp production might involve a combination of scallop and acceleration production (Fig. 6a). If this hypothesis is correct, the first phase of a given fish’s rasps should be similar to its scallops, whereas the second phase of its rasps should be similar to its accelerations. To test this hypothesis, we did two additional PCAs using all of the bursts detected in the long records, one that included landmarks from scallops and the first phase of rasps, and a second that included landmarks from accelerations and the second phase of rasps. For the first PCA, we used the following four landmarks from the raw data: (1) instantaneous frequency at the start of the burst; (2) instantaneous frequency at the peak of the burst; (3) time from start to peak; and (4) instantaneous frequency change from start to peak. This resulted in two factors that explained 77.93% of the variance ($\lambda_1 = 2.12, 53.15\%$, $\lambda_2 = 0.99, 24.79\%$). For both factors, there was a significant correlation between an individual’s mean rasp and scallop values (Fig. 6b, c). For the second PCA, we defined the start of the second phase of each rasp as the start of the burst and used the following six landmarks from the raw data: (1) instantaneous frequency at the start of the burst; (2) instantaneous frequency at the end of the burst; (3) time from start to end; (4) instantaneous frequency change from start to end; (5) mean instantaneous frequency; and (6) coefficient of variation in instantaneous frequency. This resulted in three factors that explained 81.88% of the variance ($\lambda_1 = 2.07, 34.45\%$, $\lambda_2 = 1.78, 29.66\%$ and $\lambda_3 = 1.07, 17.77\%$). For each factor, there was a significant correlation between an individual’s mean rasp and acceleration values (Fig. 6d–f).

To better quantify burst variation between individuals, we performed three additional DFAs, one for each burst type, using the same SDF landmarks as the original DFA.

Table 3. Probabilities of classifying test burst cases and random bursts to the three burst types using discriminant function analysis

Burst type	Number of test cases	Means (SD) of calculated probabilities of classification to the indicated burst		
		Scallop	Acceleration	Rasp
Scallop	22	0.992 (0.020)	0.008 (0.020)	0 (0.000)
Acceleration	46	0 (0.000)	1.000 (0.000)	0 (0.000)
Rasp	18	0 (0.000)	0.002 (0.007)	0.998 (0.007)
Random	101	0.143 (0.309)	0.8359 (0.323)	0.021 (0.122)

Values in bold represent correct classification rates.

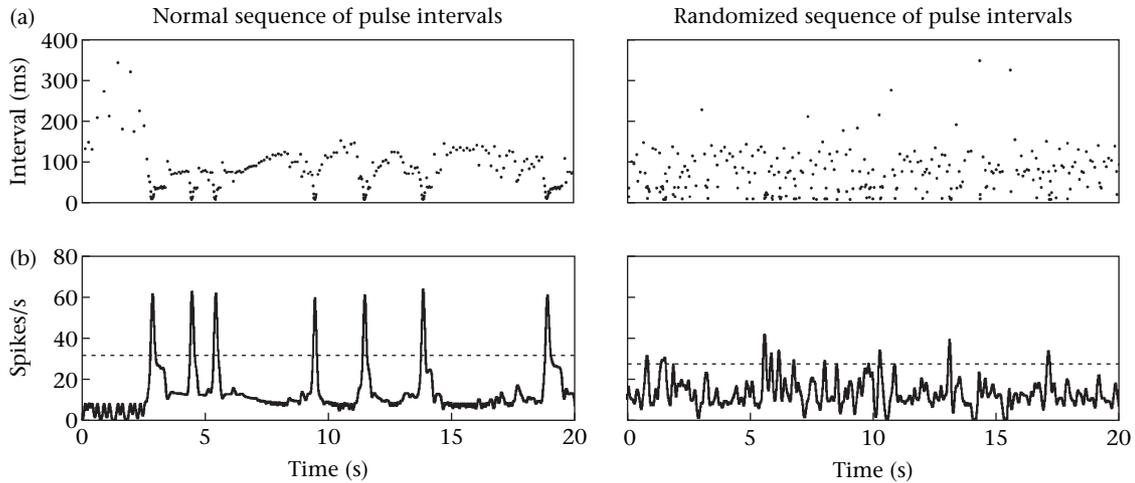


Figure 5. Randomization of the sequence of pulse intervals (SPI). (a) Plots of the SPI from a 20-s record, showing the original, normal sequence and the randomized sequence resulting from randomly shuffling the order of electric organ discharge (EOD) intervals. (b) Spike density function (SDF) plots from the SPI data shown in (a), with thresholds for burst detection shown as dashed horizontal lines.

For these analyses, individual identity was used as the independent variable, allowing us to assess the degree of individual specificity. With 32 individuals, assuming no individual specificity, there should be by chance, a mean probability of correct classification of 3.12%. For accelerations, scallops and rasps, overall probabilities of correct classification were 26.52%, 38.71% and 42.73%, respectively, verifying that all three burst types show individual specificity.

Burst Production by Context

Analysis of the long records revealed several trends in the rates of burst production (Fig. 7). Some individuals did

Table 4. Quantitative characteristics of bursts from raw data on the sequence of pulse intervals (SPI)

Burst type	Measure	Mean \pm SE
Scallops ($N=3170$)	Rise time	224.09 \pm 10.678 ms
	Fall time	245.50 \pm 6.1300 ms
	Peak instantaneous frequency	85.311 \pm 0.2565/s
Accelerations ($N=3481$)	Duration	1272.3 \pm 27.706 ms
	Mean instantaneous frequency	27.489 \pm 0.1346/s
	CV in instantaneous frequency	0.3620 \pm 0.0038
Rasps ($N=1204$)	Rise time	272.86 \pm 21.173 ms
	Duration of second phase	504.64 \pm 36.193 ms
	Peak instantaneous frequency	78.699 \pm 0.7315/s
	Mean instantaneous frequency of second phase	20.549 \pm 0.2376/s
	CV in instantaneous frequency of second phase	0.1095 \pm 0.0032

not produce any scallops, with numbers produced during each recording ranging widely from 0.000 to 16.90 per min. Overall, scallops were frequently produced by both sexes in all conditions, during the day and night. The only significant difference observed was an interaction effect between day/night and sex (ANOVA: $F_{1,26} = 8.924$, $P < 0.007$). Every fish generated accelerations, with the number produced per recording ranging from 0.200 to 13.29 per min. Acceleration production did not vary significantly from day to night or between the sexes, but there was a significant increase from the isolated to social to breeding groups (ANOVA: $F_{2,26} = 5.75$, $P < 0.009$). Similar to scallops, some individuals did not produce any rasps, with numbers produced during each recording ranging from 0.000 to 13.29 per min. Rasp production varied significantly across several factors. Males produced significantly more rasps than females (ANOVA: $F_{1,26} = 29.33$, $P < 0.0002$), and rasp production increased significantly from the isolated to social to breeding groups (ANOVA: $F_{2,26} = 6.31$, $P < 0.006$). In addition, there was a significant interaction effect between sex and day/night (ANOVA: $F_{1,26} = 4.415$, $P < 0.05$), as well as between sex and social housing condition (ANOVA: $F_{2,26} = 11.56$, $P < 0.0003$).

DISCUSSION

Previous studies have described various modulations in the SPI and linked them with specific behavioural contexts, such as courtship and mating, aggression and submission (for a review see Carlson 2002a). While these studies showed an association between social behaviour and the SPI, in the absence of a quantitative approach, it had remained unclear whether such modulations actually represented stereotyped displays, and no study had yet catalogued the diversity of patterns for a single species. The current study is the first to analyse variability in bursts and tackle the question of what actually constitutes a display in the SPI. We confirm the existence of at least

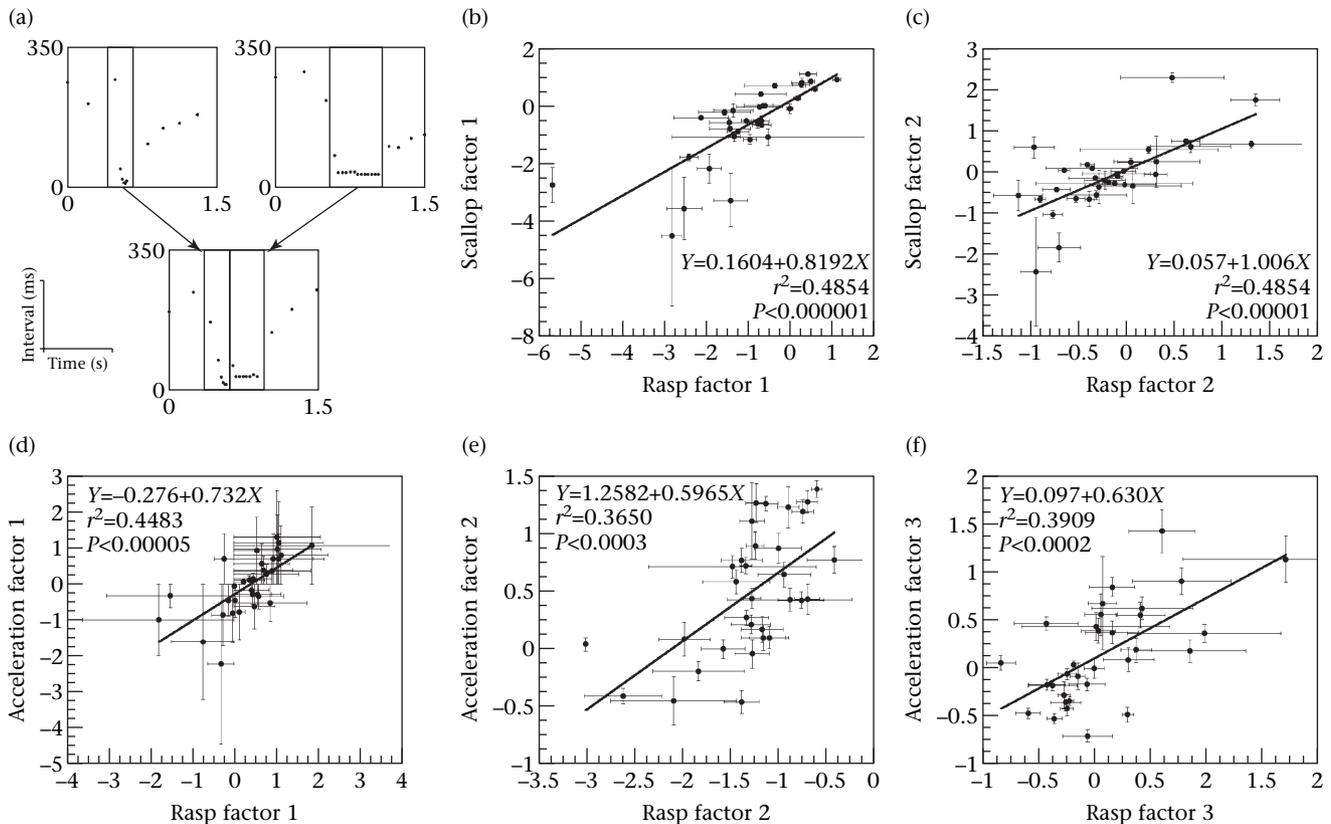


Figure 6. (a) Comparisons between a scallop, acceleration and rasp from a single individual. Subjectively, rasps appear similar to a combination of the other two displays, with an initial scallop-like portion followed by an acceleration-like portion. (b, c) Relationship between scallops and the first phase of rasps using the first two factors of a PCA analysis. Each point represents the mean value of a single individual, with error bars representing the standard errors. (d–f) Relationship between accelerations and the second phase of rasps using the first three factors of a PCA analysis. Each point represents the mean value of a single individual, with error bars representing the standard errors.

three modal classes of bursts in *B. brachyistius*, which we have termed scallops, accelerations and rasps. Although there was substantial variation within burst types, we also found quantitative, categorical differences between them, suggesting that they may appropriately be termed displays (Barlow 1977). These displays differ in several variables that describe the temporal patterning of EOD production, and we have verified that two of the bursts, scallops and rasps, are distinguished by unique, stereotyped temporal patterns. Thus, information contained within animal communication signals may be encoded into relatively simple temporal activity patterns, such as information about the external world is encoded by sensory systems as temporal patterns of action potentials (Lestienne 2001).

Mormyrids constitute an extremely diverse and speciose group (Taverne 1972). The methodology developed in this study can now be applied to catalogue the diversity of patterns across a whole range of species. Comparisons can be made across taxa, in terms of variation in display repertoires, and quantitative differences within displays, to ask important questions related to how these patterns have evolved in relation to ecological and phylogenetic factors. By objectively categorizing displays and quantifying variation within them, it will be possible to address their functional significance, in terms of the information they provide and how receivers use that information. For

instance, display rates and quantitative display variation can be studied in relation to overt behavioural interactions between fish, and linked to specific characteristics of the sender and receiver. Social conditions can be manipulated to observe the effects on signal production and signal characteristics. Playback experiments can address the behavioural responses of fish to different displays and variation within displays. From a mechanistic perspective, we can ask questions about how the production of different displays is controlled by the electromotor system (Carlson 2003; Carlson & Hopkins 2004), and how different displays may be differentiated by the electrosensory system (Xu-Friedman & Hopkins 1999). Variation in signalling behaviour across species can be linked with the evolution of these neural circuits.

Stereotyped Temporal Patterns in Animal Communication

The analogy between electrical signalling behaviour in mormyrids and single neuron spiking activity has proven quite effective in analysing the quantitative characteristics of electromotor patterning. This relates partly to the unique characteristics of the behaviour: an all-or-none electrical potential produced with variable patterning is

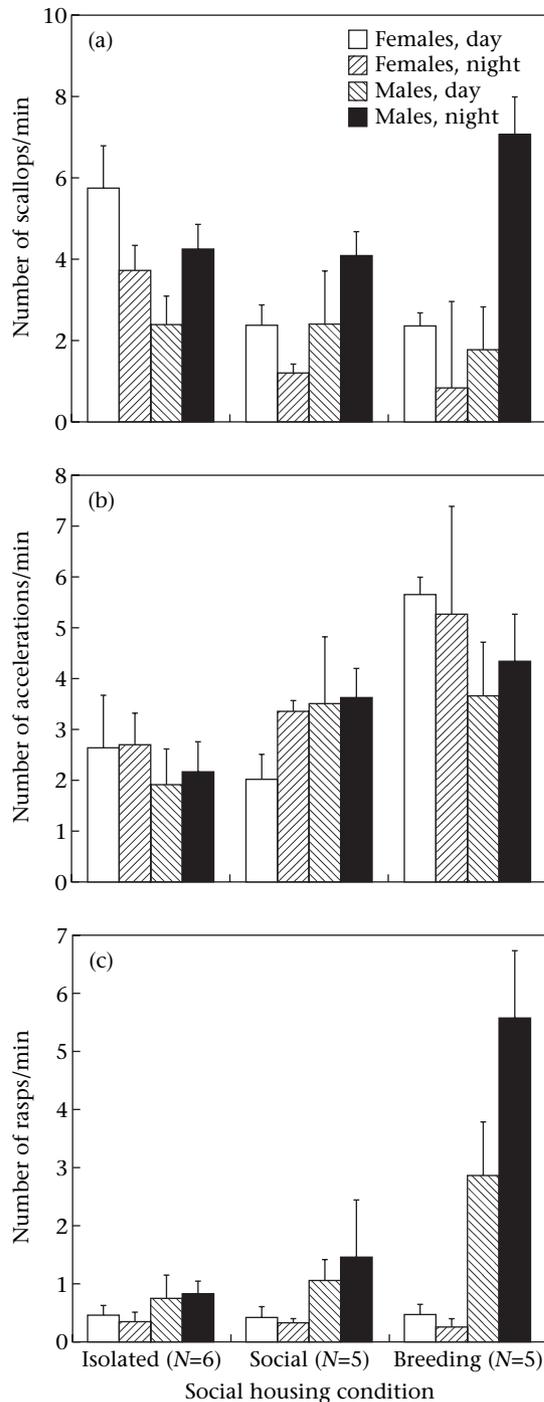


Figure 7. Mean \pm SE rates of burst production for scallops (a), accelerations (b) and rasps (c) in relation to sex, day/night and social housing condition. Sample sizes shown for each group represent the number of males and females in each group.

identical to the primary mechanism of information transfer in the nervous system. However, the signalling behaviour of many organisms in different modalities involves an all-or-none feature produced with a given temporal pattern. For instance, the calling songs of many crickets and frogs are composed of highly stereotyped sound pulses produced repeatedly (Blair 1958; Alexander 1962),

and many fish and birds produce sounds that consist of a single, repeated element (Evans & Evans 1999; Kihlslinger & Klimley 2002). Similarly, the luminescent signalling behaviour of many firefly species consists of a stereotyped pulse of light produced with a specific pattern (Lloyd 1977). The quantitative approaches developed in the current study lend themselves to the analysis of such systems. Even in more complex signalling systems with multiple elements, extensions of this technique could still be used. Bird song and bat communication calls, for example, are often characterized by a number of different syllables produced in various combinations (Kanwal et al. 1994; Brenowitz et al. 1997). Although there is no single repeated element, each syllable type tends to be highly stereotyped, and signalling behaviour could therefore be described as a series of co-occurring temporal patterns, one for each syllable.

The approach employed in the current study could therefore be used to analyse the temporal patterning of signalling behaviour in a wide variety of different systems. One advantage of such an approach is that it converts data on temporal information into a common currency. We can therefore analyse diversity in the temporal patterning of signals and look for functional convergence not only within and across taxa, but across modalities as well. In this regard, recent studies have provided quantitative methods by which the temporal patterning in different spike trains may be compared to obtain a scalar measure of dissimilarity (Victor & Purpura 1997; van Rossum 2001). Such methods could be employed in the study of signalling behaviour to provide robust measures of temporal patterning similarity.

Functional Significance of Stereotyped Temporal Patterns in Electric Fish

Although the current study did not directly address the functional significance of various displays, their quantitative features and production in relation to different contexts suggest specific hypotheses concerning their function. In addition, observational studies in other species that have looked at modulations in the SPI in relation to social interactions shed some light on the function of the three burst types in *B. brachyistius*. Scallop-like bursts were defined by highly stereotyped temporal patterns, especially within individuals, whereas accelerations were typified by graded increases in EOD rate. Rasps, like scallops, also contained highly stereotyped temporal patterns in the initial scallop-like phase, but the second acceleration-like phase could be quite variable. These differences suggest fundamentally different roles for these signals, in that stereotyped signals tend to be involved in behaviours such as mate attraction and individual recognition, and signals with graded components tend to be linked to behaviours such as aggression and courtship (Marler 1961; Otte 1974).

Previous studies describing a wide range of acceleration-like bursts have linked these behaviours with overt aggression (Bell et al. 1974; Kramer 1974, 1976, 1979; Kramer & Bauer 1976; Hopkins 1986), and the current

study showed an increase in acceleration production when fish were housed as pairs. Thus, the available evidence suggests that they play a role in agonistic behaviour, and variability in this display may be directly linked to the intensity of aggressive threats. It is likely that many of the different acceleration-like behaviours described by previous authors simply represent variability along a similar continuum, and using the quantitative methods of the current study will allow this question to be addressed both within and between species.

Scallops were first subjectively defined for *B. niger* (Serrier & Moller 1989), and their basic structure appears similar to that of scallops in *B. brachyistius*. In both species, scallops are highly stereotyped within individuals and individuals vary greatly in their rates of scallop production (Moller et al. 1989; Serrier & Moller 1989). We found no direct relationship between scallop production and social housing condition, although in *B. niger*, scallop production actually decreases markedly during overt social interactions, and individuals that frequently produce scallops while resting are least likely to go electrically silent during such interactions (Moller et al. 1989). Electrical silence is considered a submissive behaviour (Carlson 2002a), suggesting that periodic scallop production at rest may serve as an advertisement signal for relatively dominant fish.

The fact that rasps were mostly produced by breeding males suggests that they function as a mate attraction or courtship signal. We therefore adopted the terminology used by Hopkins & Bass (1981) for courtship bursts in male *B. 'VAD'*, although the temporal structure of rasps for the two species appears quite different. Preliminary observations of male–female interactions prior to spawning in *B. brachyistius* indicate that males produce rasps at high rates prior to mating, and it appears that the duration of the second phase may increase as the time of spawning nears (M. B. Krosby, D. M. Boone, B. A. Carlson & C. D. Hopkins, personal observation). Thus, females may use individual, stereotyped differences in characteristics of the first phase of rasps for assessing male quality, whereas variability in the second phase may be used as a means of signalling the escalation of courtship prior to mating. The apparent combination of two different displays into a single display indicates that novel displays may arise through evolution by a combination of pre-existing displays. This represents a fairly simple evolutionary mechanism of creating a signal with both a highly stereotyped component and a graded component that can serve multiple functions (Johnstone 1995).

Sensory Coding and Generation of Stereotyped Temporal Patterns

Recent research has shed light on the mechanisms involved in the temporal coding of EOD waveform in mormyrids (Xu-Friedman & Hopkins 1999), but there has been no research on the sensory coding of the SPI. The results from the current study provide a conceptual framework for tackling this issue. Research on several different systems has demonstrated a diversity of mechanisms for

encoding temporal patterns in communication signals (Pollack 2001), and research into the sensory basis of encoding the SPI should provide fresh insights into the complexity of mechanisms involved in behaviourally relevant temporal coding.

Production of the three burst types would appear to require two distinct mechanisms, one for driving scallops and one for accelerations. When combined, the two mechanisms could generate rasps. The medullary command nucleus that drives EOD production receives its primary input from two distinct sources, the mesencephalic precommand nucleus and the thalamic dorsal posterior nucleus (Carlson 2002b). Recent physiology experiments suggest that the former is responsible for driving scallops, and the latter is responsible for driving accelerations (Carlson 2003; Carlson & Hopkins 2004). Differences between the two display types may result from any combination of differences in intrinsic neuronal properties, network connectivity, neurotransmitters, receptors or synaptic responses. Given the relative simplicity of electrical signalling behaviour in mormyrids, it may prove to be an ideal model system for studying how these various features may be modified by the nervous system for generating different stereotyped temporal patterns. This will complement the increasing body of research on the importance of temporal coding in the nervous system and help to advance basic concepts in neural function and information processing.

Acknowledgments

Thanks to J. Bradbury and B. Land for helpful discussion. M. E. Arnegard, A. H. Bass, K. E. Holekamp, T. D. Seeley and two anonymous referees provided helpful comments on earlier versions of the manuscript. Research support was provided by the National Institutes of Mental Health (MH37972 to C.D.H.). B.A.C. was supported by a National Science Foundation Predoctoral Fellowship.

References

- Alexander, R. 1962. Evolutionary change in cricket acoustical communication. *Evolution*, **16**, 443–467.
- Barlow, G. 1977. Modal action patterns. In: *How Animals Communicate* (Ed. by T. Sebeok), pp. 98–134. Bloomington: Indiana University Press.
- 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. & Russell, C. J. 1974. Electric organ discharge patterns during dominance-related behavioral displays in *Gnathonemus petersii* (Mormyridae). *Journal of Comparative Physiology*, **92**, 201–228.
- Bigorne, R. 1989. The genera *Brienomyrus* and *Isichthys* (Pisces; Mormyridae) from west Africa. *Revue Hydrobiologique Tropicale*, **22**, 317–338.
- Blair, W. 1958. Mating call in the speciation of anuran amphibians. *American Naturalist*, **92**, 27–51.
- Bratton, B. O. & Kramer, B. 1989. Patterns of the electric organ discharge during courtship and spawning in the mormyrid fish, *Pollimyrus isidori*. *Behavioral Ecology and Sociobiology*, **24**, 349–368.

- Brenowitz, E., Margoliash, D. & Nordeen, K.** 1997. An introduction to birdsong and the avian song system. *Journal of Neurobiology*, **5**, 495–500.
- Carlson, B. A.** 2002a. Electric signaling behavior and the mechanisms of electric organ discharge production in mormyrid fish. *Journal of Physiology-Paris*, **96**, 405–419.
- Carlson, B. A.** 2002b. Neuroanatomy of the mormyrid electromotor control system. *Journal of Comparative Neurology*, **454**, 440–455.
- Carlson, B. A.** 2003. Single-unit activity patterns in nuclei that control the electromotor command nucleus during spontaneous electric signal production in the mormyrid *Brienomyrus brachyistius*. *Journal of Neuroscience*, **23**, 10128–10136.
- Carlson, B. A. & Hopkins, C. D.** 2004. Central control of electric signaling behavior in the mormyrid *Brienomyrus brachyistius*: segregation of behavior-specific inputs and the role of modifiable recurrent inhibition. *Journal of Experimental Biology*, **207**, 1073–1084.
- Carlson, B. A., Hopkins, C. D. & Thomas, P.** 2000. Androgen correlates of socially induced changes in the electric organ discharge waveform of a mormyrid fish. *Hormones and Behavior*, **38**, 177–186.
- Evans, C. & Evans, L.** 1999. Chicken food calls are functionally referential. *Animal Behaviour*, **58**, 307–319.
- Friedman, M. A. & Hopkins, C. D.** 1996. Tracking individual mormyrid electric fish in the field using electric organ discharge waveforms. *Animal Behaviour*, **51**, 391–407.
- Gill, T.** 1862. On the West African genus *Hemichromis* and description of new species in the museums of the Academy and Smithsonian Institutions. *Proceedings of the National Academy of Sciences, U.S.A.*, **14**, 134–139.
- Hopkins, C. D.** 1981. On the diversity of electric signals in a community of mormyrid electric fish in west Africa. *American Zoologist*, **21**, 211–222.
- Hopkins, C. D.** 1986. Behavior of Mormyridae. In: *Electroreception* (Ed. by T. H. Bullock & W. Heiligenberg), pp. 527–576. New York: J. Wiley.
- Hopkins, C. D. & Bass, A. H.** 1981. Temporal coding of species recognition signals in an electric fish. *Science*, **212**, 85–87.
- Johnstone, R. A.** 1995. Honest advertisement of multiple qualities using multiple signals. *Journal of Theoretical Biology*, **177**, 87–94.
- Kanwal, J., Matsumura, S., Ohlemiller, K. & Suga, N.** 1994. Analysis of acoustic elements and syntax in communication sounds emitted by mustached bats. *Journal of the Acoustical Society of America*, **96**, 1229–1254.
- Kawasaki, M.** 1993. Temporal hyperacuity in the gymnotiform electric fish, *Eigenmannia*. *American Zoologist*, **33**, 86–93.
- Kihlslinger, R. & Klimley, A.** 2002. Species identity and the temporal characteristics of fish acoustic signals. *Journal of Comparative Psychology*, **116**, 210–214.
- Kirschbaum, F.** 1995. Reproduction and development in mormyrid and gymnotiform fishes. In: *Electric Fishes: History and Behavior* (Ed. by P. Moller), pp. 267–301. London: Chapman & Hall.
- Kramer, B.** 1974. Electric organ discharge interaction during interspecific agonistic behavior in freely swimming mormyrid fish: a method to evaluate two or more simultaneous time series of events with a digital analyzer. *Journal of Comparative Physiology*, **93**, 203–235.
- Kramer, B.** 1976. The attack frequency of *Gnathonemus petersii* towards electrically silent (denervated) and intact conspecifics, and towards another mormyrid (*Brienomyrus niger*). *Behavioral Ecology and Sociobiology*, **1**, 425–446.
- Kramer, B.** 1979. Electric and motor responses of the weakly electric fish, *Gnathonemus petersii* (Mormyridae), to play-back of social signals. *Behavioral Ecology and Sociobiology*, **6**, 67–79.
- Kramer, B. & Bauer, R.** 1976. Agonistic behavior and electric signaling in a mormyrid fish, *Gnathonemus petersii*. *Behavioral Ecology and Sociobiology*, **1**, 45–61.
- Lestienne, R.** 2001. Spike timing, synchronization and information processing on the sensory side of the nervous system. *Progress in Neurobiology*, **65**, 545–591.
- Lloyd, J.** 1977. Bioluminescence and communication. In: *How Animals Communicate* (Ed. by T. Sebeok), pp. 164–183. Bloomington: Indiana University Press.
- Marler, P.** 1961. The logical analysis of animal communication. *Journal of Theoretical Biology*, **1**, 295–317.
- MathWorks** 2000. *Matlab 6.1*. Natick, Massachusetts: MathWorks.
- Moller, P.** 1970. Communication in weakly electric fish, *Gnathonemus niger* (Mormyridae) Part 1. Variation of electric organ discharge frequency elicited by controlled electric stimuli. *Animal Behaviour*, **18**, 768–786.
- Moller, P. & Bauer, R.** 1973. Communication in weakly electric fish, *Gnathonemus petersii* (Mormyridae) Part 2. Interaction of electric organ discharge activities of two fish. *Animal Behaviour*, **21**, 501–512.
- Moller, P., Serrier, J. & Bowling, D.** 1989. Electric organ discharge displays during social encounter in the weakly electric fish *Brienomyrus niger* L. (Mormyridae). *Ethology*, **82**, 177–191.
- Otte, D.** 1974. Effects and functions in the evolution of signaling systems. *Annual Review of Ecology and Systematics*, **5**, 385–417.
- Paulin, M. G.** 1992. Digital filters for firing rate estimation. *Biological Cybernetics*, **66**, 525–531.
- Pimentel, R. A.** 1992. An introduction to ordination, principal components analysis and discriminant analysis. In: *Ordination in the Study of Morphology, Evolution and Systematics of Insects: Application and Quantitative Genetic Rationals* (Ed. by J. T. Sorensen & R. Footit), pp. 11–28. New York: Elsevier.
- Pollack, G. S.** 2001. Analysis of temporal patterns of communication signals. *Current Opinion in Neurobiology*, **11**, 734–738.
- van Rossum, M.** 2001. A novel spike distance. *Neural Computation*, **13**, 751–763.
- Serrier, J. & Moller, P.** 1989. Patterns of electric organ discharge activity in the weakly electric fish *Brienomyrus niger* L. (Mormyridae). *Experimental Biology*, **48**, 235–244.
- StatSoft** 2003. *Statistica 6.1*. Tulsa, Oklahoma: StatSoft.
- Sullivan, J. P., Lavoue, S. & Hopkins, C. D.** 2002. Discovery and phylogenetic analysis of a riverine species flock of African electric fishes (Mormyridae: Teleostei). *Evolution*, **56**, 597–616.
- Szücs, A.** 1998. Applications of the spike density function in analysis of neuronal firing patterns. *Journal of Neuroscience Methods*, **81**, 159–167.
- Taverne, L.** 1972. Ostéologie des genres *Mormyrus* Linné, *Mormyrops* Müller, *Hyperopisus* Gill, *Isichthys* Gill, *Myomyrus* Boulenger, *Stomatorhinus* Boulenger et *Gymnarchus* Cuvier. Considérations générales sur la systématique des poissons de l'ordre des Mormyriiformes. *Annales du Musée Royal de l'Afrique Centrale (Tervuren, Belgique), Séries Octavo Sciences Zoologique*, **200**, 1–194.
- Victor, J. & Purpura, K.** 1997. Metric-space analysis of spike trains: theory, algorithms and application. *Network: Computation in Neural Systems*, **8**, 127–164.
- Xu-Friedman, M. A. & Hopkins, C. D.** 1999. Central mechanisms of temporal analysis in the knollenorgan pathway of mormyrid electric fish. *Journal of Experimental Biology*, **202**, 1311–1318.