Polarity Discrimination and Quantitative Analysis of Agonistic Rasp Signaling in the Weakly Electric Fish, *Brienomyrus brachystius* (Mormyridae)

by

Brian Isett

Dr. Carl Hopkins

May 2009
ABSTRACT

Mormyrid electric fish extract species, gender, dominance, and orientation information from the waveform of their electric organ discharges (EODs). Orientation information was examined in this study by alternating the polarity of 4 different EODs delivered by a dipole playback electrode (PBE). Fish were found to show a strong head-butt and circle response to the head (+) lead of the electrode, confirming previous studies.

A second experiment was performed with the same playback apparatus to quantify the temporal characteristics of electrical responses to agonistic signals called ‘rasps’. Rasps consist of two bursting patterns: a fast burst (FBr) with inter-spike intervals (ISIs) of 8-20ms, followed by a medium burst (MBr) with ISIs of 30-70ms and are performed when males fight for dominance status, as well as during courtship. Experimental stimulus rasp patterns were gathered from dominant and subordinate males in 3 different contexts, and a looped pattern of natural nocturnal swimming discharges was used as a control.

Testing 14 fish, it was found that rasps quickened in response to the experimental stimuli along three different temporal parameters: rasps/second, rasp duration (seconds) and the shortest ISI achieved (ms). Many dominant individuals were observed ‘rasp matching’ the experimental stimuli, while several subordinates went silent during playback. The dominance status of experimental fish was also measured and revealed that dominant males performed rasps at a higher rate and with shorter ISIs than subordinate males. Subordinate males were often observed delivering more medium bursts and continuous discharges than dominant males.
INTRODUCTION

The weakly electric fish, *Brienomyrus brachyistius* belongs to the west African family Mormyridae, and uses its electric sense for agonistic and courtship communication, as well as for navigation in its nocturnal environment (Hopkins 1986). The electric organ discharge (EOD) is a triphasic, pulse-type discharge which can be delivered in several stereotyped sequences of pulse intervals (SPIs) when used for communication (Bell et al. 1974, Carlson 2002, Wong 2006). In normal polarity, the triphasic wave occurs with the head positive phase first (McKibben et al. 1993). This polarity inverts when fish change orientation relative to a receiver, offering an important cue for nearby fish.

It has been shown that a slight preference exists for approaching the ‘head’ (+) end of a dipole during playback experimentation in Gymnotids (Westby 1974, Davis & Hopkins 1987, McKibben et al. 1993, Hopkins et. al 1997) and in Mormyrids (Schluger & Hopkins 1987, Hopkins et al. 1997). All of the mentioned experiments, however, did not use natural EODs during playback, instead approximating EODs with single period sine waves. Using sine waves for polarity discrimination, roughly 60% of approaches were observed towards the head-positive lead of the dipole, and 40% of approaches were observed towards tail-negative (McKibben et al. 1993). Therefore, Experiment 1 of this study examined polarity discrimination in 5 male fish using natural EOD playback stimuli.

In addition to offering orientation cues, the EOD has been quantified in communicating species (Hopkins & Bass 1981), gender (Hopkins 1972, Bass & Hopkins 1985), individual differences (McGregor & Westby 1992) and even male dominance status (Hagedorn & Zelick 1989). The behavioral significance of specific EOD discharge patterns has been less well quantified. From the earliest SPI studies, a higher discharge rate has been observed during
aggressive behavior in Mormyrids, suggesting a possible display of dominance status (Bauer 1972, Bell et al. 1974, Westby 1974, Kramer and Bauer 1976, Kramer 1978, Kramer 1979, Wenmeyer and Kramer 2002). Increased resolution of SPI study has revealed that several distinct electrical behaviors are produced by *B. brachyistius* (reviewed in Carlson 2002). One particular signal, the ‘rasp’ (Hopkins and Bass 1981, Hopkins 1983; Fig. 1), has been implicated in both agonistic and courtship displays performed by male individuals (Carlson & Hopkins 2004, Wong 2006). During agonistic interactions, male *B. brachyistius* perform anti-parallel motor displays, as observed in other Mormyrids (Bell et al. 1974). It is during this anti-parallel display that rasps are most frequently produced.

![Graph showing rasp sequence](image)

Figure 1. A typical rasp sequence of pulse intervals (SPI) shown in instantaneous frequency. Rasp were defined as an SPI with a fast burst (FBr) consisting of 3-15 pulses with intervals of 8-20ms (50-125Hz), followed by a medium burst (MBr) lasting less than 1000ms with 25-70ms intervals (15-40Hz; updated from Wong 2006). Medium bursts were defined in the same way as the MB period of rasps. This rasp is stimulus rasp (b) (Fig. 4).

The definition of a rasp used in this study was adapted from Wong (2006) as a fast burst (FBr) of 4-10 pulses with 10-20ms intervals, followed by a medium burst (MBr; 30-70ms intervals) lasting up to 1000ms. These parameters were slightly altered to fit observed variation (Fig. 1). In previous literature, these features have been called ‘scallops’ and ‘accelerations’ respectively (Carlson 2002, Carlson & Hopkins 2004). Note that the abbreviations ‘FBr’ and
'MBr' will always refer to the fast burst and medium burst segments within a rasp; not to be confused with fast bursts (FB) and medium bursts (MB) produced by themselves.

The discovery of rasps in both courtship and agonistic behaviors implicates the rasp in conveying dominance, aggression, and potentially even fitness information about male individuals. Therefore, temporal characteristics of rasping were examined on three different levels of analysis: 1) The spacing of rasp behaviors (rasps/second), 2) the duration of rasps (seconds), and 3) the spacing of EOD pulses within rasps (ISI in ms). In regards to 3), the FBr region of the rasp is likely the most difficult to produce, and may contribute strongly to the display of dominance. To test these hypotheses and to quantify the behavioral significance of rasps in agonistic interactions, a playback experiment was conducted using natural rasp patterns collected from both dominant fish during agonistic and courtship displays, and subordinate fish during agonistic displays. The electrical responses of the 14 fish tested were then analyzed for rasp and medium burst patterns, as well as the rate these displays were performed throughout stimulation, using custom Matlab software (Matlab 2006b, The Math Works, Inc.). Additionally, relative dominance ranks were assigned to fish based on pair-wise interactions in groups of 3 or 5. Rasp and medium burst responses were examined in light of fish dominance rank and stimulus treatment using two level mixed-mode analyses.

METHODS

The methodology that pertains to both the polarity discrimination playback (Experiment 1) and the rasp signal playback (Experiment 2) is discussed first. Both experiments used the same basic setup (Fig. 2) and aspects of the same stimulus delivery. Where the experiments diverge, the methods are split into relevant sections for each.
Housing Conditions

In Experiment 1, 5 adult males were physically isolated in tanks separated by plastic mesh. This housing arrangement allowed consistent water conditions (26°C± 1°C, 950 ± 25μs/cm conductivity) but also permitted limited electrical interaction between tanks. The tanks each contained PVC hiding tubes with midge larvae supplied ad libitum. All fish experienced a 12L:12D light cycle using artificial lighting and a timer. Their gender was determined by the presence of an anal fin notch (Brown et al. 1996).

For Experiment 2, 14 male fish were physically isolated in tanks separated by plastic mesh with the same tank conditions mentioned above except for water conductivity (275 ±30μs/cm). Four of the fish used in Experiment 2 also participated in Experiment 1, but the experiments were sufficiently far apart (>20 weeks) to assume that no confounding effects would arise from the two unrelated treatments.

Playback Tank System

The playback tank system is largely identical between Experiments 1 and 2, but minor layout differences exist (Fig. 2). The testing tank (87cm x 46cm x 27cm) was maintained at the same temperature and approximate conductivity as the main tanks described above, respective of experiment. All electrodes were custom-built dipoles made from PVC piping and insulated with silicon caulk. In the polarity discrimination experiment, a PVC hide tube was placed parallel to the playback electrode (PBE, carbon leads) to allow maximal stimulus perception, even while in the tube (Fig. 2A). This orientation was necessary because fish would often return to the tube during silent control periods in Experiment 1.
Figure 2. Playback tank arrangements used with *Brienomyrus brachyistius*. (A) Experiment 1 setup for polarity discrimination. The testing tank (87cm x 27cm x 46cm) contained a PVC hide tube (PVC), a carbon lead playback electrode (PBE) and one silver lead recording electrode (RE). (B) Experiment 2 setup for rasp playback. This setup included the components of (A) with the addition of one inactive electrode (IE) for tank symmetry. Both setups used the signal paths displayed in red and black: the red path displays the stimulus delivery pathway; the black path displays the experimental recording pathway. The asterisk along the red path highlights where the stimulus is directly recorded onto the second channel of the camera.

In Experiment 2, the PVC hide tube was oriented perpendicularly to the PBE because the continuous stimulus of this experiment elicited little hiding. The advantage of the perpendicular orientation was that it allowed for better fish observation. The recording electrode (RE) was shifted to the right side of the tank, and an additional inactive electrode (IE) was added to maintain tank symmetry (Fig. 2B). In both experiments, the RE was perpendicular to the PBE in order to minimize noise from the stimulus being delivered. To provide lighting for night video recording, 5 infrared LED lights (Clover Infrared LED, Model IR030) were attached to metal brackets placed above the tank.

During both experiments, all unnecessary pumps and electronic devices were turned off to reduce noise. The signal from the RE was amplified 100x by a differential AC amplifier (A-
M Systems, Model 1700) and band-pass-filtered from 0.1Hz to 10kHz. The signal was recorded
directly to miniDV cassette via Panasonic Digital Camcorder at 48kHz (Model AG-DVC60), and
additionally captured to a computer in real-time via firewire, using STOIK Capturer (v 1.0,
DiVX codec v 6.5.1 2 logical CPUs, uncompressed audio). The digital camera performed A/D
conversion for the video and electrical recording of both experiments. The electrode recordings
were extracted from captured AVI video files using Adobe Audition v1.0 and exported as 48kHz
WAV files for later analysis.

In addition to recording the experimental fish’s electrical responses, the stimulus being
delivered during experimentation was recorded directly onto the second channel of the Panasonic
camera and computer recordings, providing precise temporal alignment of stimulus and response
(Fig. 2, asterisk). The delivered stimuli were generated using custom software written in Matlab
(R2006b, The Mathworks Inc.) and converted to an analog signal using an A/D converter (Edirol
UA-5 at 48 kHz, 16bits). In order to have the characteristics of a dominant fish, the stimulus
amplification (Custom-built DC amplifier) was calibrated to the average EOD amplitude of
several large fish. The stimulus was then separated from ground by an isolation transformer
before reaching the PBE (Fig. 2).

Experiment 1: Polarity Discrimination Playback

In order to examine the behavioral responses of \textit{B. brachyistius} to normal and inverted
EODs, a playback experiment was performed using 4 natural EODs (Fig. 3). The EODs were
extracted from 48kHz recordings made during previous observational studies and normalized to
1V peak-peak amplitude \((n=2\ \text{female}, 2\ \text{male EODs})\). The peak-frequency was measured for
each EOD using g-Prime (Table 1; Lott 2007). Each of the 4 EODs was also inverted, giving 4
normal and 4 inverted polarity EODs (Fig. 3C). These EODs were paired with a random pattern
of inter-spike-intervals (ISIs) generated from an exponential distribution with a mean interval length of 30.8 ± 15.7ms, and a minimum interval of 14.3ms (Fig. 3A, B). This distribution was chosen in order to provide a stimulus that contained no electrical patterns, but that would still provoke high response rates due to the aggressively short ISIs. Additionally, the exponential distribution guaranteed that no exceedingly long ISIs (>200ms) would be generated, so as to maintain high response rates.

The random patterns of inverted and normal EODs were 30s long and filtered with a 1s amplitude envelope at the onset and offset of spiking for natural transitions. Each trial had the order of experimental periods (n=8) randomized and separated by 45s periods of silence (Fig. 3D). 45s periods of silence were found to keep response rates high throughout the 645s trials. Using custom Matlab software, each trial was constructed into a WAV file (48kHz) and delivered using the playback tank setup described above (Fig. 2B, red path).

Figure 3. Stimulus creation for polarity discrimination experiment. (A) An example of the random inter-spike intervals (ISIs) generated using an exponential distribution with a mean of 30.8 ± 15.7ms. Note that only the first 10s are shown of the full 30s period. (B) A histogram of the ISI duration frequencies found within a typical 30s experimental period. (C) The 8 EODs shown are the 4 EODs described in Table 1 with normal and inverted polarity. They are arranged according to the order of their presentation as described in (D). (D) This diagram represents the random order of experimental periods delivered during one trial, each surrounded by 45s periods of silence. Each block represents the EOD found above it, paired with a random ISI pattern such as the one found in (A). The number in each box represents the EOD used during that period (Table 1).
All experiments were performed and video recorded in complete darkness within 2 hours of artificial nightfall. Before experimentation, each fish was allowed to acclimate to the playback tank for 10 minutes. All 5 fish were tested on February 13\textsuperscript{th}, 2008 and again on February 20\textsuperscript{th}, 2008. The resulting videos were scored for head-butts and circling of the PBE on the left or right sides using event recorder software (JWatcher v1.0, Macquarie University and UCLA). Head-butts were scored any time the fish’s head made contact with the left or right tip of the PBE. Circling was scored each time the fish swam a complete circle around the left or right side of the PBE, or made contact with the electrode using a body part other than the head (this often occurred during incomplete circles). These behaviors were assigned to computer keyboard keys and scored as counts. Latency to approach the electrode was measured as the duration of time between stimulus onset and the fish approaching within 3 cm of PBE. Chi-square analysis ($\alpha=0.05$, $n=5$ fish, DF=1) was performed on the heat-butt and circling counts using statistical software (Minitab v15.1.1.0, Minitab Inc.). Additionally, correlations between behavioral responses and the peak frequency of EOD used during the period were examined (Pearson, $\alpha=0.05$, $n=5$ fish).

\textit{Experiment 2: Rasp Signaling Playback}

In order to examine the behavioral responses of \textit{B. brachyistius} to the ‘rasp’ sequence of pulse intervals (SPI), a second playback experiment was performed using a collection of 15 rasp ISIs collected from 5 males during different social interactions. 5 rasps were collected from dominant males during agonistic interactions (B\textsubscript{1}, Fig. 4B\textsubscript{1}, a-e), 5 rasps were collected from dominant males during courtship interactions (B\textsubscript{2}, (Fig. 4B\textsubscript{2}, f-j), and 5 rasps were collected from subordinate males during agonistic interactions (B\textsubscript{3}, (Fig. 4B\textsubscript{3}, k-o). These SPIs were carefully
gathered from previous observational recordings using g-Prime software. Individual separation was achieved through EOD peak frequency and amplitude differences.

Figure 4. Stimulus creation for Experiment 2. (A) The control periods consisted of a slow 15s swimming SPI looped 3 times (mean ISI 112.7 ± 55.1ms). (B) During experimental periods, rasps were repeated randomly 20 times, the first 20s of which is displayed in (B) (A) (B). A total of 15 rasps were organized into 3 groups depending on the context in which they were originally produced. Rasps (a)-(e) were produced by dominant males during agonistic interactions, (f)-(j) were produced by dominant males during courtship, and (k)-(o) were produced by subordinate males during agonistic interactions. Rasps following the labeled rasps are random repeats of those same rasps. The experimental periods were then randomly ordered in each full trial (FT) with the period durations shown. FT duration was 296.3s. These stimulus patterns were paired with EOD 1 (Table 1) and delivered to 14 fish, with 3 replicates (42 trials, n=14 fish).
During experimental periods, each group of 5 rasps was randomly repeated 4 times (20 rasps/experimental period), with 1s of silence in between rasps. Due to rasps of different lengths used in each experimental period, this resulted in three periods of different duration (Fig. 4FT). These differences were controlled for in all statistical tests.

For a control stimulus, 15s of slow nocturnal swimming discharges were captured as ISIs from a medium sized adult male, similar to the resting interval control used in a Kramer agonistic playback experiment (Fig. 4A; Kramer 1979). During experimental periods, this 15s pattern was looped 3 times, creating a 45s control (mean ISI 112.7 ± 55.1ms). Each experimental period (Fig. 4B) was randomly ordered between 4 control periods, resulting in a full playback trial lasting 296.3s (Fig. 4FT). This SPI pattern was then paired with EOD 1 (Table 1), and compiled into a WAV file (48kHz) that could be delivered using the playback tank setup described above (Fig. 2A, red path). All experiments were performed and video recorded in complete darkness within 2 hours of artificial nightfall, and each fish was allowed to acclimate to the playback tank for 10 minutes before testing.

Experiment 2: Scoring Electrical Responses

Electrical behaviors made in response to the playback were scored automatically using custom software. The algorithm eliminated noise caused by the original playback signal by subtracting the playback pattern from the response recording of the experimental fish. The program then isolated FBs, MBs and rasps based on ISI thresholds adapted from Wong 2006 (Fig. 1). FB by itself occurred in too few individuals to be analyzed statistically. Electrical behaviors were considered to occur independently if they were at least 200ms apart ('short cessation', Wong 2006). Once a behavior was found, its position in the trial, interval
characteristics and duration were measured. Additionally, the program accessed information regarding the randomization of experimental periods, allowing the detected responses to be precisely correlated with treatment.

Experiment 2: Scoring individual dominance status

To determine dominance status, the male fish used in this experiment were randomly assigned to three triads and one group of five (n=14 fish). A dominance hierarchy was determined for each group through paired interactions using a method adapted from Hopkins 1974. In this procedure, two fish are forced to compete during the day for one hide tube. After 5-10 minutes, the more dominant fish will have control of the hide tube, and may also chase and attack the subordinate fish. In several instances, interactions were interrupted to prevent harm from occurring to conspicuously subordinate fish. In triads, ranks of 1, 2, or 3 (R1, R2, R3) were assigned in descending dominance order. For the group of five, the top two fish were assigned R1, and the bottom two fish were assigned R3, with the middle fish maintaining R2. Paired interactions of all groups were performed on 2-3 different occasions with high reproducibility: fish switched dominance positions on only 1 occasion (these fish were assigned an averaged rank).

Experiment 2: Statistical Methods

Two-level mixed model analyses with unbounded variance were performed on the electrical responses of experimental fish using the statistical package JMP 7 (SAS Institute Inc.). In each analysis, the individual fish were treated as a random effect while treatment period, dominance rank, and their interaction (treatment • rank) were treated as fixed effects, unless
noted otherwise. The chosen fixed effects were used to explain the variance in rasp rate, shortest rasp interval, and rasp duration, as well as MB rate and MB duration. For every mixed model analysis, post-hoc tests were performed on Least-Square means (LS means) with Student’s t-test ($\alpha=0.05$ for all tests). Results cited are therefore in the format (LS mean ± Standard Error) unless noted otherwise.

To control for duration differences between experimental and control periods, mean response rates (behavior/second) were examined, averaged over 3 trials. When total responses were compared between periods, the control period responses were multiplied by the ratio of total experimental duration to total control duration (163.33s/180s). Residuals were checked for normality in all tests.

RESULTS

*Experiment 1*

Confirming previous experimentation with stimulus polarity, Experiment 1 revealed a strong preference for the head-positive side of the dipole PBE during both normal and inverted EODs. Chi-square analysis shows a significant association between the signal polarity and the placement of the head-butt response ($\chi^2=235.71$, DF=1, $p < 0.0001$, $n=5$ fish), as well as the circling response ($\chi^2=170.7$, DF=1, $p < 0.0001$, $n=5$ fish). In other words, during a normal EOD, activity was focused towards the left (head-positive) side of the PBE, and during an inverted EOD, this activity was focused towards right (head-positive) side of the PBE (Fig. 5).
Figure 5. Polarity playback responses. The chi-square tables shown contain raw counts for head-buts and circles during the experimental periods of the playback experiment. For head-buts, a significant association was seen between signal polarity and the location of attacks ($\chi^2 = 235.71$, DF=1, $p < 0.0001$, $n=5$ fish). Similarly, a significant association was seen between polarity and the location of circles ($\chi^2 = 170.7$, DF=1, $p < 0.0001$, $n=5$ fish). Shown in the same orientation are photos of one fish during 4 different periods of one trial (2 inverted, 2 normal) performing head-buts and circles.

Experiment 2

Experiment 2 playback stimuli elicited a wide range of electrical responses. Three R2 males went silent for all or part of a trial ($n=3$ trials) while two R3 males went silent for all or part of a trial ($n=4$ trials). No silent periods were observed in R1 individuals. Interestingly, on the one occasion when fish switched dominance rank, the individual that increased from R3 to R2 was silent for less of the playback trial than during the other 2 trials as R3. These observations suggest that silence is a signal displayed by subordinate individuals. When R3 individuals did discharge, it was often a continuous SPI without discrete behaviors like MBs or rasps. Conversely, R1 and R2 fish appeared to closely ‘rasp match’ experimental stimuli during many trials (Fig. 6). But, because there were instances where fish appeared to rasp indiscriminately between control and experimental treatments, further analysis was needed.
An additional observation was made regarding an electrical display called ‘pulse-pairing’. Pulse-pairing is when ISIs rapidly alternate between a short interval of around 10-15 ms and a longer interval above 25ms (90ms in Bell et al. 1974). This pattern was observed in one R1, two R2, and one R3 (n=4 fish), and tended to occur during behaviors otherwise classified as either MB or the MBr of raps. Typical examples of pulse-pairing during MBr are shown in two individuals in Fig. 6A and 6D.

![Graph showing control and experimental conditions.](image)

Figure 6. Four individual fish responses (A-D; black) to control and experimental stimulus periods (red). These examples were taken from 4 different trials during the transition of a control period to an experimental period; only 40s of each trial is shown. Raps (as defined in Fig. 1) are traced by black lines. Note that individuals (A) and (D) perform pulse-pairs during the medium burst segment of their raps.

Experiment 2: Medium Burst Analysis

A breakdown of total medium burst responses by rank and treatment (Fig. 7) reveals that R2 males appeared to medium burst more frequently during the control periods than the experimental periods. Conversely, subordinate males (R3) appeared to medium burst more during the experimental period than the control period (Fig. 7). These observations were tested by analyzing all experimental and control periods with medium bursting individuals, averaged
over 3 trials (14 fish, \( n = 97 \) observations). The interaction of rank and period in a two-level mixed model showed that R2 individuals burst at a significantly higher rate during the control period than during the experimental periods (Student’s t-test, \( p = 0.0262 \)), while R1 individuals did not differ in burst rate between the periods (Student’s t-test, \( p = 0.216 \)). In contrast to R2 responses, R3 (subordinate) individuals produced medium bursts at a significantly higher rate during the experimental period than the control period (Student’s t-test, \( p = 0.0012 \); Fig. 8).

![Graph showing medium bursts and rasps](image)

**Figure 7.** Total numbers of medium bursts and rasps observed in Experiment 2, sorted by rank (1, 2 and 3) across both treatment periods.

**Experiment 2: Rasps**

It was hypothesized that a higher rate of rasping would occur in response to the rasp stimulus than to the control stimulus. It was further hypothesized that the experimental periods (Fig. 4B₁, B₂) containing rasps from dominant individuals would provoke a higher rate of response than the experimental period containing rasps from subordinate individuals (Fig. 4B₃). A breakdown of total rasp responses by rank and treatment (Fig. 7) shows that dominant males appeared to rasp more frequently during the experimental periods than control periods, while
subordinate males do not appear to differentiate between the two. These observations were tested by analyzing all experimental and control periods with rasping individuals, averaged over 3 trials (12 fish, n=56 observations). The interaction of rank and period after post-hoc analysis showed that R1 and R2 individuals rasp at a higher rate during the experimental period than during the control periods (p < 0.0001, n=56), while R3 individuals did not differ in rasp rate between periods (p= 0.857, n=56; Fig. 8). Several examples of dominant males 'rasp matching' with the playback stimuli are displayed in Fig. 6.

![Graph showing least-square means with standard error bars for medium burst and rasp rates (averaged over 3 trials). These results represent the post-hoc Student t-tests performed on LS means derived from two-level mixed model analyses. LS means with asterisks indicate statistical significance. These analyses examined the interaction of dominance rank and experimental treatment as fixed effects on behavior rate. For rasp rates, 12 fish with n= 56 observations were analyzed; for medium bursts, 14 fish with n= 97 observations were analyzed. The negative rasp rate returned for R2 individuals indicates a relative difference along a continuous model; this rate can be interpreted as 0 rasps/s.](image_url)
Next, rasp rate was examined in response to the 3 different rasp groups delivered as experimental periods (Fig. 4B1-B3). For these analyses, rasp groups, rank, and (rasp groups • rank) were analyzed as fixed effects, while fish were treated as a random effect. When rasp rate was examined between all 3 different rasp groups delivered, no significant difference was found ($p=0.112$, 12 fish, $n=29$ observations). However, when only experimental periods containing dominant male courtship rasps (B2) were compared to periods containing subordinate male agonistic rasps (B3), rasp response rates were found to differ significantly ($p=0.0424$, 10 fish, $n=18$ observations). The experimental periods containing courtship rasps elicited a LS mean response rate of $0.194 \pm 0.066$ rasps/s, while experimental periods containing subordinate rasps elicited a LS mean response rate of $0.149 \pm 0.066$ rasps/s. The LS mean response rate to dominant agonistic rasps (B1; $0.161 \pm 0.73$ rasps/s) was not found to differ significantly from response rate to subordinate agonistic rasps ($p=0.647$, 12 fish, $n=20$ observations). This result may suggest that rasps emitted by dominant males during courtship elicit a stronger response than rasps emitted by subordinate males during agonistic interactions.

Upon closer examination, all experimental periods contained 20 rasps and all rasps were delivered 1 second apart. Therefore, a group of shorter rasps (such as those found in B2) would be played at a higher rate than a group of longer rasps (B3). This leaves a more likely possibility: the difference in rasp response found above is simply because B2 rasps were played at a rate of 0.60 rasps/s while rasps in B3 were played at a rate of 0.46 rasps/s. This would also explain why rasps emitted during B1 (0.50 rasps/s) did not have a different rate than those emitted during B3 (0.46 rasps/s). This design flaw confounds the possibility of deducing most of the rasp characteristics that contribute to different response rates in these two groups, but may still offer insight into rasp behavior. If rasps are found to be shorter in duration when they are also
delivered at faster rates (as has already been shown in response to the experimental periods; Fig 8) then the shorter duration of rasps in B2 may be a significant characteristic of rasps emitted by males during courtship: the shorter duration rasps may inherently enable a higher rasp rate.

To examine this possibility, rasp duration and two other rasp characteristics were analyzed within the experimental fish responses using the mixed model described previously (12 fish, n= 56 observations). Significance values for all post-hoc tests described in this section are summarized in Table 2. Analysis revealed that rasps emitted by experimental fish were significantly shorter in duration when emitted during the experimental treatments (770 ± 148ms) than during control treatments (1038 ± 146ms; p=0.0042). Logically, variability in the duration of rasps comes from variability in MBr of the rasp (Fig. 1MBr) since the pulses within FBr of the rasp (Fig. 1FBr) occur very closely together in time and make up the minority of rasp EODs. For this reason, analysis was also performed on MB signals. This analysis revealed the same trend: MBs were found to be significantly shorter during experimental periods (557 ± 122ms) than during control periods (769 ± 117 ms; p=0.0313, 14 fish, n=97 observations). The culmination of these results suggests that shorter rasp durations may enable faster rasping rates.

Table 2. Significance values of fixed effects given 4 different signal characteristics as response (Student's t-test, 12 fish, n=56 observation for rasp characteristics, 14 fish, n= 97 observations for MB duration).

<table>
<thead>
<tr>
<th>Response</th>
<th>Treatment</th>
<th>Rank</th>
<th>(Rank * Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shortest FBr ISI</td>
<td>0.02*</td>
<td>0.039*</td>
<td>--</td>
</tr>
<tr>
<td>Number of FBr Pulses</td>
<td>0.596</td>
<td>0.173</td>
<td>0.201</td>
</tr>
<tr>
<td>Rasp Duration</td>
<td>0.0042**</td>
<td>0.256</td>
<td>0.078</td>
</tr>
<tr>
<td>Medium Burst Duration</td>
<td>0.0313*</td>
<td>0.815</td>
<td>0.936</td>
</tr>
</tbody>
</table>

* p<0.05  **p<0.01

The second rasp characteristic examined was the number of EODs emitted within FBr. Variability in this aspect of the rasp signal was relatively low (6 ± 3 pulses, n=976 rasps), but it
was hypothesized that higher numbers of pulses within FBr would be more prevalent in
dominant individuals. Post-hoc analysis revealed no significant differences within treatments,
dominance ranks, or their interaction (Table 2).

The third rasp characteristic examined was the shortest ISI achieved during FBr. The
shortest rasp interval achieved by any fish within the experiment reached an instantaneous
frequency of 125.6Hz (0.00796ms). After controlling for dominance rank, it was found that
shortest ISIs were significantly shorter during experimental treatments (11.5 ±1.8ms) than during
the control (13.0 ± 1.8ms; \( p=0.02 \)). After controlling for treatment, R1 individuals performed
rasps containing shorter intervals (12.3 ± 1.8ms) than R3 individuals (20.6 ± 2.1ms; \( p=0.039 \)).
In other words, rasps were produced with a faster max instantaneous frequency in the
experimental treatment than the control, and dominant fish were performing rasps with shorter
FBr intervals than subordinate fish.

DISCUSSION

The results from Experiment 1 clearly support previous polarity findings, with head-butt
and circling preferences strongly favoring the head (+) lead of the dipole. The preference seen in
this experiment clearly exceeds the 60% ('head') to 40% ('tail') approach ratio seen in previous
studies (McKibben et al. 1993), suggesting that natural stimuli enable polarity discrimination to a
higher degree than single period sine wave approximations. It is also possible that the high
stimulus frequency (~40Hz) used in this study was found to be more threatening and therefore
provoked a higher response rate than the lower (15Hz) rates used in previous studies, or that the
scoring of behaviors instead of approach paths isolated polarity preference in a more robust
manner. In any case, concerns about the weakness of the polarity behavior expressed in McKibben et al. are likely to be ameliorated by the results found here.

The results of Experiment 2 elucidate many of the nuanced signaling characteristics of agonistic rasping in *B. brachyistius*. Through automatic scoring of electrical behaviors, it was possible to isolate increases in the rasping rate of dominant (R1 and R2) individuals to rasp stimuli. This increase in rasps/second may underlie the increased discharge frequencies observed in many early agonistic SPI studies (Bauer 1971, Bell et al. 1974, Kramer 1979). Within rasps themselves, we saw a decrease in shortest FBr interval duration during the playback of rasp stimuli. In other words, not only were whole rasps performed more frequently in response to rasp stimuli, the fastest instantaneous frequency achieved during rasps increased as well. These observations alone suggest that rasping rate and EOD discharge speed play key roles in displaying aggression.

In agreement with rasps displaying aggression, rasp display characteristics also predicted dominance status: R1 individuals rasped faster and with shorter intervals than R3 individuals. It seems likely that subordinate individuals are less willing or less capable of performing rasps, a conclusion that is consistent with the total behavior counts seen in Fig 7. Instead of rasps, R3 individuals performed a large number of MBs during experimental periods (Fig 7, 8). If aggressive SPIs are a continuum, medium bursting seems to be the first aggressive escalation: silence punctuated by regular discharging offers strong signaling contrast to the continuous discharges seen in nonthreatening swimming individuals. Even in dominant individuals, series of rasps were often preceded by MBs. For an example of this, Figure 6B shows a transition from MBs to rasps as the playback transitions from control to rasp stimuli.
From the results of this study, an optimally aggressive signal contains short rasps with short FBrl ISIs delivered at a high rate: in this way, high signaling rates are achieved on all three levels of temporal analysis. Indeed, there may be a strong correlation between signal duration or rate, and aggression level, but due to the fact that rasp rate was not controlled for in this experiment, much remains to be learned about aggressive signal escalation. For example: Do electric fish increase rasp rate by producing shorter displays, as was inadvertently done in the stimuli generated here, or are the parameters controlled separately? Future experimentation would benefit by manipulating rasp rate and rasp duration independently in order to tease apart the significance of these parameters. Since the highest response rates were observed during experimental periods containing the highest rasp rates, it seems likely that careful increases in stimulus rate would lead to reciprocal rate increases by responding males.

Future study of rasping behavior should expand to examine the fitness value in these various temporal characteristics. Although this experiment shows that rasps correlate well with dominance status in agonistic interactions, the rasp is also displayed by males during courtship of females (Wong 2007). Therefore, future female playback experimentation would be ideal for elucidating which levels of temporal analysis are important in mating success. With additional experiments examining the development of rasp production in juvenile fish, the fundamental communicative properties of the rasp could be fully explored.
ACKNOWLEDGEMENTS

I thank Carl Hopkins for his generous support and mentorship throughout this study, Garry Harned for building much of the hardware used within the playback system and providing wonderful fish care, as well as Françoise Vermeylen, without whom the mixed model analyses performed in this study would not have been possible. I also thank Kevin Gardner, Tom Kraft, Bruce Carlson, Jason Gallant, Natalie Trzcinski and Scott Jackson for insightful advice and support throughout experimentation. Part of the funding for this project was generously provided by the Cornell Hughes Scholars Program.
REFERENCES


