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Communication in the

weakly electric brown ghost knifefish,

Apteronotus leptorhynchus

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by

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Communication in the weakly electric brown ghost knifefish, *Apteronotus leptorhynchus*

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The weakly electric brown ghost knifefish emits a sexually dimorphic sinusoidal electric organ discharge (EOD) for electrolocation. High-quality (larger, dominant) males discharge at the highest and females at the lowest EOD frequencies (EODFs), which can be further modified in frequency and amplitude for communication. In this dissertation, I describe several EODF increases: abrupt "chirps" of low (LoC) and high frequency excursion (HiC), and more gradual frequency rises ("GFRs"), which have been hypothesized to function in signalling aggression, courtship and submission, respectively. I investigated the information content of these signals through signalling and approach responses to playbacks of EOD mimics and during agonistic encounters in male dyads. Throughout these analyses, I also examined how an individual's quality affects their responses. I conclude that electrical information is sufficient in conveying reliable information about sex and size, and that individuals have some internal representation of their own quality. Whereas higher EODFs and LoCs appear to correlate positively with measures of size, dominance and threat, the functions of HiCs and GFRs are much less clear. Multiple roles, particularly for GFRs, in signalling submission, courtship and agonistic intent are discussed.

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CHAPTER 1

Overview

Animal communication involves the transmission of information from a sender to a receiver (Bradbury & Vehrencamp 1998). Natural selection should favor the evolution of communication if the transmitted information influences the receiver to give the sender some fitness advantage (Otte 1974). To the extent that both senders and receivers benefit from the transmission of information, this exchange has been termed “true communication” (Marler 1977), and the vehicle of the exchange is called a signal. Although it may not always be in the best interest of a sender to provide honest information (Dawkins & Krebs 1978, Krebs & Dawkins 1984), there is agreement among ethologists and behavioral ecologists that signals at evolutionary equilibrium should be reliable, as some form of cost must constrain misinformation in order for signals to be informative (Zahavi 1975, Johnstone & Grafen 1993).

Mate recognition- wherein species recognition is implied- seems an obvious example of true communication benefiting both sender and receiver, in that attempting to mate with heterospecifics has adverse fitness consequences (Futuyma 1998). Typically, signals that function in mate attraction are sufficiently different from those of closely related species in sympatry, and receivers have a hard-wired peripheral sensory filter or receptor organ fine-tuned to the essential characteristics of the mate-attraction signal (Capranica 1965, Barlow 1992), thus reducing recognition errors.

However, communication often occurs in contexts where there is a conflict of interest between sender and receiver, as in sexual selection. Female mating errors carry a larger cost by virtue of their greater gametic investment, and so female fitness is primarily limited by how well they discriminate among males and

choose those with the highest resource-holding potential (RHP; Trivers 1974). Though there is thus a temptation especially for lower-quality males to cheat by sending signals which inflate their RHP, they are kept honest both by females that discriminate between signals of varying reliability, and by other males that challenge such putatively inaccurate signals by putting them to an often costly physical test (Andersson 1994). At evolutionary equilibrium, cheaters are expected to be kept in check by receivers that discriminate well enough to know better, and thus reliability in signalling quality is maintained by some cost to the sender (Zahavi 1975, Grafen 1990, Maynard-Smith 1994).

Game theoretical models have classified signals according to the source of the costs constraining their reliability. As discriminating receivers will select for the most reliable indicators of a sender's quality, many signals are intimately tied to a male's RHP (size, strength or motivation), which presumably correlates with their ability to outcompete consexuals, and are thus unbluffable. These are called performance or index signals, because weaker individuals are constrained from bluffing by virtue of their physical inability to perform them (Hurd & Enquist 2005, Vehrencamp 2000). Strategic signals are those that all individuals have the option of producing, but there is an associated cost less feasible for relatively lower-quality individuals to carry. These costs can be in the form of either their production (general handicaps) or an increased likelihood of opponent retaliation (vulnerability handicaps and conventional signals). Another way of looking at the differences between performance and strategic signals is that in the former, the costs have been paid prior to their use; in the latter, the cost is accrued as a result of their use. Theoretical and empirical work suggests that among strategic signals, mate attraction signals tend to be of the former (handicap), intrasexual aggressive threat displays of the latter (conventional) variety (Szamado 2003, Vehrencamp 2001).

Much as emitted signals are expected to vary with sender quality in that a higher-quality sender is able to absorb such costs more easily than weaker individuals, it

is conceivable that recognition of signals vary with a receiver's cost-bearing potential (i.e. RHP) as well. For example, a larger male would be expected to recognize a smaller male's aggressive signal as less threatening. There is a paucity of literature on how a receiver's state affects their signal recognition.

The chapters that follow are papers which address the information content of signals of the weakly electric brown ghost knifefish, *Apteronotus leptorhynchus*. Wave-type gymnotiform electric fish produce a quasi-sinusoidal electric organ discharge (EOD) of electrical current at an extremely precise and steady frequency. The frequency of this current discharge (EODF) is determined directly by the autorhythmic oscillation frequency of the medullary "pacemaker nucleus". Using electroreceptors concentrated near the head, fish monitor deflections in this field produced by nearby objects, and thus perceive the electrical properties of their environment (Bullock & Heiligenberg 1986). The electrosensory system is tuned such that individuals perceive their own EODF best and are less sensitive to higher and lower frequencies (Knudsen 1974).

Like many other species of electric fish (Hopkins 1999), brown ghosts feature sexual dimorphisms in morphology, EODF and its modulations (Black-Cleworth 1970, Moller 1995). However, unlike in other "wave" species in which males discharge at lower frequencies than females, brown ghost males have higher EOD frequencies (EODFs) than females (female range ~600-800Hz, male range ~800-1000Hz). Moreover, EODF is positively correlated with size, dominance and 11-ketotestosterone (11-KT) levels in males (Hagedorn & Heiligenberg 1985, Zakon & Dunlap 1999). The relationship between electrical behavior and size or status in females is unclear (but see Tallarovic & Zakon 2003).

Permanent attributes such as species and sex are signalled by the baseline EOD frequency, which is stable over hours or days. Electric fish must also signal motivational state, which may change over seconds or minutes. For this purpose fish use rapid modulations of EOD frequency, which in *A. leptorhynchus* include

four stereotyped rapid and transient amplitude and frequency modulations, described as “chirps” (Bullock 1969), or “pings” (Larimer & MacDonald 1968), Whether spontaneous or evoked in social interactions or by a jamming stimulus, males chirp at higher rates than females (Dye 1987, Zupanc & Maler 1993). By virtue of the contexts in which chirps have been elicited in past studies, many have suggested, but never tested, the hypothesis that short and long chirps function as intrasexual aggressive and courtship displays, respectively. Individuals of both sexes also make more gradual frequency rises whose functions are unclear.

The main objective of this dissertation is to examine empirically some of the previously hypothesized functions of the characteristics of the electric organ discharge and its modulations in brown ghosts. Chapter 2 addresses sex differences in sex and individual recognition by measuring chirp and rise emissions as a function of stimulus frequency, i.e. in response to playbacks of a broad range of EOD mimics that include and exceed the conspecific frequency range. This chapter in part focuses on how focal signal production is influenced by a receiver’s sensitivity. Chapter 3 then examines focal recognition of the EOD modulations described in chapter 2. In both chapters, I also look at the effects of a receiver’s quality (size or EODF) on their signal production/recognition. Finally, chapter 4 examines the information transferred by these modulations during combat between male dyads.

CHAPTER 2

Effects of sex, sensitivity and quality on signal recognition

Abstract. Maintaining a stable social organization necessitates that animals recognize their own dominance status relative to that of other conspecifics. The weakly electric brown ghost knifefish emits a sexually dimorphic sinusoidal electric organ discharge (EOD) for electrolocation. High-quality (larger, dominant) males discharge at the highest and females at the lowest EOD frequencies (EODFs). Each individual is most sensitive to its own EODF, which can be modulated for communication. In order to examine how sensitivity and quality influence an individual's response to mimics of EODs, I recorded electrical signals emitted by ten males and seven females in response to playbacks of sine waves mimicking a wide range of con- and extraspecific EODFs. While all individuals emit small chirps (LoCs) mostly to stimuli around their own EODF, they are more likely to emit rises (gradual non-chirp signals) to frequencies to which they are less sensitive; males similarly emit larger chirps (HiCs) to frequencies more distant from their own, especially to female mimics. Larger males are less likely to emit rises, stimuli in the female range elicit more rises from both sexes, and females emit rises to male EOD mimics. Although low male EOD mimics elicit more LoCs from all, and especially from smaller males, larger males chirp more at progressively higher male EOD mimics than do smaller males. I conclude that a) although much of the variation in an individual's response is attributable to its sensitivity, individuals recognize sexual and size cues and have some internal representation of their own quality, and b) whereas LoCs appear to function in intrasexual aggression, HiCs and rises could be used in both courtship and submissive signalling.

To maintain a stable social organization, animals must recognize their own dominance status relative to that of others in the group or at least be able to discriminate between group members of different status (e.g. Colgan 1983, Hurst et al. 1994). How recognition occurs is not often obvious, and the nature of its cognitive template has been the subject of recent debate (Vauclair 1996, Griffin 2001, Johnston & Bullock 2001). Electric fish are a good model system in which to study this question because they constantly emit and monitor a cue--their

electric organ discharge (EOD)-- which conveys their size and presumably social status. Thus, every animal can instantaneously compare its own EOD with those of conspecifics.

Wave-type gymnotiform electric fish continuously produce a quasi-sinusoidal EOD at an extremely precise and steady frequency. They detect their own EOD and EODs of conspecifics with sensory receptors called electroreceptors. They use the EOD for two functions: electrolocation and electrocommunication. During electrolocation, a fish monitors the deflections in its electric field produced by nearby objects and thus perceives the electrical properties of its environment (for a review see Bullock & Heiligenberg 1986). In addition to its role in electrolocation, it can serve as a cue for species-, sex- and individual recognition. In the brown ghost knifefish, *Apteronotus leptorhynchus*, males have higher EOD frequencies (EODFs) than females (Zakon & Dunlap 1999), and the dominant, spawning male is the largest and has the highest frequency (Hagedorn & Heiligenberg 1985). A dominance hierarchy has not been documented for females, and patterns of female dominance characteristics are unclear. Among nonreproductive females, EODF and size are positively correlated only in some social situations (Tallarovic & Zakon 2002), and although females with higher EODFs are more likely to occupy shelters when availability is limited, they are also much more likely than males to share shelters (Dunlap & Oliveri 2002).

Since a fish continuously emits its EOD, the EOD of another individual necessarily interacts with its own. This creates a beat frequency at the difference between the two EOD frequencies. Thus, this unique sensory system must extract external electrical information, such as conspecific signals, from the beat frequencies created by its own behavioral output. It would probably benefit an individual discharging at 750Hz to distinguish between an approaching dominant male frequency of 900Hz and one equidistant from its own in the female range, i.e. at 600Hz, both of which create the same beat frequency. An additional complexity is that, in order to optimize electrolocation, each fish's

electroreceptors are most sensitive to its own EODF. Behavioral tuning curves for *A. albifrons* show a symmetrical decrease in sensitivity to higher and lower frequencies, at least near the fish's own EODF (Knudsen 1974). Thus, fish may be differentially sensitive to the EODFs of conspecifics depending on the values of their own EODFs.

In order to know whether the fish respond to these cues differentially, I noted their tendency to produce transient modulations of EOD amplitude and frequency, described as "chirps" (Bullock 1969), or "pings" (Larimer & MacDonald 1968) upon presentation of a stimulus. Observations of social groups indicate that fish make short chirps (~15-30 msecs) presumably as an aggressive signal and other signals, such as short and long "rises" to signal submissiveness (Hagedorn & Heiligenberg 1985). Recently, Engler et al. (2000) described four types of spontaneously emitted chirps, SP-1 through 4, distinguishable by their frequency excursions, durations and presence or absence of a baseline frequency undershoot. In this study, I break chirps down into categories of low and high frequency excursions (LoC and HiC, respectively).

I asked whether individuals simply respond more to stimuli to which they are more sensitive (Ryan & Keddy-Hector 1992), or if they distinguish between two frequencies to which they are equally sensitive but that carry different information. I presented males and females with an array of EOD mimics spanning a range of con- and extraspecific frequencies, designed to be able to compare responses to stimuli equidistant from an individual's own EODF. If simple tuning accounts for most of the variation in a receiver's response, I expected symmetrical responses to stimulus frequencies in either direction from the individual's own EODF. If the EOD has communicative value, I predicted a) that the responses to two stimuli to which an individual is equally sensitive should elicit asymmetrical responses, and b) that individuals should show variation in responses to different absolute stimulus frequencies, regardless of the relative distance from their own EODF. Furthermore, since size is correlated with EODF,

which in turn correlates with hormonal state (e.g. Dulka & Maler 1994), I expected a difference in responses from individuals of different sizes, such that larger fish should respond more aggressively (e.g. with more chirps) or less subordinately (e.g. with less rises) to a wider range of stimulus frequencies. To test the latter prediction, I reanalyzed the data by examining the responses of small and large individuals of each sex to the absolute frequency values of the "relative" stimuli previously presented.

I also addressed the related question of how a sender increases its signal's active space with respect to a receiver of the opposite sex, given that individuals are tuned best to EODs of their own sex. I would expect elements of either the EOD or its modulations to correlate with properties of the sensory substrate of the opposite sex (Ryan & Keddy-Hector 1992). To test this prediction, I examined male and female EODs and several randomly selected modulations, quantified the distribution of energy in different spectral components and compared these to conspecific sensitivity ranges.

METHODS

Animals

Fish were purchased from a commercial vendor and kept in group and individual tanks (temperature between 25 and 26.5°C; conductivity between 500 and 1200 μS). *A. leptorhynchus* EODFs are directly dependent on water temperature. Within a temperature range of 25-27°C, typical female EODFs range from 500-750 Hz, those of males from 800-1000 Hz. I haphazardly selected males (N=10) and females (N=7), placed them in a playback arena (see below), and allowed them to acclimate to the tank for at least 24 hrs prior to the playback session. Afterwards, I confirmed the sex of the fish by dissection in ten cases. Weights

were measured for some fish. The experiments complied with all federal, state and local regulations concerning the use of animals.

Setup of the Playback Arena

A playback arena (100 x 55 cm; water level 9 cm) contained a 50W thermostat/heater attached to one side of the tank that kept water temperature at 26 ± 0.4°C (conductivity=600 ± 100μS). The center of the arena contained a PVC tube shelter (18 x 5 cm) with its top cut and replaced with plastic mesh to allow visibility of the fish from above. Two plastic mesh partitions at each end of the tank spanned the arena to create two compartments 10cm wide, behind which 2 pairs of carbon stimulus electrodes (spaced 15 cm apart) were placed through plastic grating attached to the ends of the tank. Prior to the introduction of the fish, I set and confirmed the amplitude of the stimuli at 1.5 ± 0.1 mV/cm by measuring with a pair of Ag electrodes placed in the center and parallel to the stimulus electrodes. Because these fish are most active nocturnally, I obstructed light entry to the arena during the trials with a black felt curtain hanging from the ceiling and attached to the sides of the table holding the tank. I thus ensured that the fish experienced a 12h:12h light:dark regimen and had acclimated to the dark at least one hour prior to the trials.

Stimulus Presentations

I generated 19 stimuli in Cool Edit Pro (Syntrillium) and presented each separately for two minutes with 60 seconds of silence between stimulations. All but one of the stimuli were sine waves and consisted of percent deviations from the fish's own EODF in both directions: ± 1, 5, 10, 15, 20, 25, 30, and 50%. Because I wanted to compare responses to stimuli to which the fish are equally sensitive, I presented frequencies equidistant from the fish's own EODF in a pairwise fashion, so as to minimize treatment order effect contamination of these

most salient comparisons. I randomized the order that each pair were presented, as well as the order of presentation of each stimulus within the pair. The side of the tank at which the stimuli of each pair were presented were also randomized. For example, for a fish discharging at 750 Hz, the +10% stimulus (825 Hz) might have been presented first and from the left side of the tank. The –10% stimulus (675 Hz) would then immediately have followed on the right side. An additional "pair" was made of the second harmonic (200% of the EODF) and a control of pink noise (band limit of 5512.5 Hz) at similar amplitude, which were estimated visually. I was interested in responses to the harmonic, as the beat frequencies created when this frequency interacts with slight deviations of the fish's EODF from baseline are similar to those elicited by playbacks of stimuli within a few Hz of the EODF. Since the latter have been extensively shown to elicit a jamming avoidance response (see e.g. Zupanc & Maler 1993), I expected the second harmonic also to jam the fish's sensory system. To compare with responses to the harmonic stimulus, I also presented a stimulus at the fish's own EODF in some cases. To control for habituation, I presented the same random sequence in reverse order 24 hrs later. For the analysis I averaged the responses to each stimulus from both days. For clarity of presentation, plotted responses to the 95% and 105% stimulus treatments reflect their consolidation with responses to the 99% and 101% treatments, respectively.

EOD Recording and Signal Definitions

EODs were recorded with two perpendicular pairs of carbon electrodes placed across the width and the length of the tank, processed through an A/D converter (Terratech EWS-88MT), digitized at a sampling rate of 11025 Hz and analyzed in Cool Edit Pro. Spectral measurements were made using Fast Fourier Transformation (FFT) at different output sizes, depending on the analysis. Half the sampling rate divided by the output size determines the frequency resolution, the reciprocal of which in turn determines the temporal window over which the FFT is calculated at each cursor position. I generated an FFT (size 2048) of each

individual's EOD and calculated the intensity difference between the fundamental and each of the subsequent two harmonic frequencies (Fig. 2.1a(i)). For one analysis (see below), I then compared the relative intensity of each harmonic in males and females. I did not measure the absolute intensity, because the fish were free-swimming, and the recorded amplitude were thus also a function of the fish's position with respect to the recording electrodes. These amplitude modulation artifacts also limited my ability to conventionally define a signal based on amplitude changes alone (e.g. Zupanc & Maler 1993; but see Fig. 2.1b for amplitude-time plots of chirps from a stationary fish in the absence of a stimulus). To verify the chirp categories determined by Engler et al. (2000), I measured the instantaneous peak frequency every ms on a frequency-time plot (spectrogram) calculated at an FFT size of 2048. Signal onset and offset were determined as deviations of more than 2Hz from and returns to within 2Hz of the baseline EODF, respectively. Chirps were defined as frequency modulations greater than 30Hz and less than or equal to 35ms. Figure 2.2 shows a plot of duration and maximum frequency excursion for 30 randomly selected chirps from two males (N=10 each) and two females (N=5 each). From these data, I comprised the more intuitive categories of low (LoC: 30-90Hz) and high frequency modulations (HiC: >200 Hz), analogous to what Engler et al. (2000) call SP-2 and SP-1 type chirps, respectively. Male and female LoCs differed in their total frequency excursion (males: X $\pm$ SE=61.2 $\pm$ 4.5 Hz; females: 35.8 $\pm$ 5.6 Hz) but not in duration (males: 23.1 $\pm$ 1.4 ms; females: 23.9 $\pm$ 1.7 ms; Fig. 2.2). Male HiCs were slightly longer than LoCs (X $\pm$ SE= 27.4 $\pm$ 1.6 ms) and characterized by an initial increase of 307 $\pm$ 13 Hz and a subsequent undershoot of the baseline EODF of 40 $\pm$ 18 Hz. These agree with more quantitative examinations of chirp structure by Engler et al. (2000) and Bastian et al. (2001).

For the remainder of the analyses, I categorized each modulation by calculating an FFT of size 4096 after placing the spectrogram cursor in or near the middle of the signal (Fig. 2.1b). LoCs have a characteristic single "bulge" of increased energy at higher than baseline frequencies, whereas HiCs have several peaks,

including a substantial lower frequency component (Fig. 2.1a). Four males emitted several chirps that were substantially longer (>200ms) than those plotted in Fig. 2.2. Although these correspond to Engler et al.'s (2000) SP-3 and SP-4 distinctions, I include them in my HiC category by virtue of their HiC-typical frequency excursions. All other signals, usually less than 30 Hz in frequency excursion and often of much longer duration than chirps, were collectively termed "rises" (Hagedorn & Heiligenberg 1985).

Analysis

I counted and compared the numbers of LoCs, HiCs and rises emitted in response to each stimulus by each fish and configured the data as signalling probabilities, i.e. the number of each chirp type and the number of rises as a percentage of the total number of signals. These percentage data were normalized using an arcsine transformation prior to analysis. From within a small portion of the stimulus array corresponding to the range of species-typical EODFs, I established three "conspecific stimulus groups" by averaging individual responses to stimuli in each range: 10-15% below, within 5% of, and 10-15% above the fish's own EODF (Fig. 2.3). I performed either a repeated-measures ANOVA on the three "conspecific stimulus groups", with sex as the between-subject and stimulus frequency as the within-subject variable (Fig. 2.3a & c), or a repeated-measures MANOVA on related variables (LoC and HiC responses), followed by ANOVAs for each individual variable (Fig. 2.3b).

For the analyses presented in Figs. 2.4 and 2.5, individual EODFs were calculated from an average of five haphazardly selected EOD measurements in each trial. To assess responses as a function of an individual's quality, I divided individuals into two size groups per sex (small females: $X \pm SE=1.83 \pm 0.33$g, N=4; large females: $X \pm SE=3.86 \pm 0.21$g, N=3; small males: $X \pm SE=3.56 \pm 0.45$g, N=4; large males: $X \pm SE=7.02 \pm 1.14$g, N=5). I then compared numbers

of chirps and rises, and EODFs using ANOVAs with sex and size as between-subject factors (Fig. 2.4).

To assess how responses are affected by the absolute frequency of stimuli, I reanalyzed the data after distributing the previous stimulus groups across new treatment groups. These "treatments" consisted of species-typical frequency ranges spanning 50 Hz each (seven between 625 and 975 Hz and six between 625 and 925 Hz for females). I then averaged each individual's responses to stimuli falling within each range group. Due to missing data for some individuals and a difference in chirp rate between males and females of nearly one order of magnitude, I performed a regular ANOVA separately for each sex (Fig. 2.5). Figure 2.5c shows the differences between large and small males in group mean LoC emissions to the four stimulus treatment groups in the male range (800, 850, 900 and 950Hz).

I used t-tests when comparing any two groups, such as males and females on variables averaged for each fish across all stimulus treatments. When the above ANOVAs were significant, Sidak and Scheffé tests were used (α=0.05) for multiple comparisons in repeated-measures (Fig. 2.3) and regular ANOVAs (Figs. 2.4 & 2.5), respectively.

RESULTS

Sex Differences in Responses to Stimuli as a Function of Relative Frequency

The control stimulus of pink noise elicited LoC responses in only one male and one female and were thus excluded from all analyses. All individuals produced LoCs at some point in the trials, although males produced many more than females (males: X $\pm$ SE=59 $\pm$ 11.1, females: 8 $\pm$ 2.4; t_{15}=-3.7, P<0.002) and at shorter latencies (males: 23.6 $\pm$ 6.5 s, females: 91 $\pm$ 6.1 s; t_{15}=51.6, P<0.0001).

The patterns of LoC responses to stimuli consolidated into the three “conspecfic stimulus groups” along the tuning curve showed a significant effect of sex (ANOVA: $F_{1,14}$=17.46, P=0.0009), stimulus frequency group (ANOVA: $F_{2,28}$=26.13, P<0.0001) and an interaction (ANOVA: $F_{2,28}$=14.33, P<0.0001, Fig. 2.3a). The Sidak tests revealed that stimuli within 5% of the fish’s own EODFs elicit more LoCs than do more distant stimuli, significantly so for males (Fig. 2.3a). Interestingly, stimuli at 200% of the fish’s own EODF, which corresponds to the second harmonic, also elicited LoCs in both sexes.

Whereas 80% of the males produced HiCs as well as LoCs, only two of the seven females produced HiCs two orders of magnitude less than males, and were thus excluded from analyses on HiC emissions (males: X $\pm$ SE=5 $\pm$ 2.9; females: 0.04 $\pm$ 0.03). Figure 2.3b shows the pattern of male chirps as percentages of total signals across the stimulus trials. An examination of the three conspecific stimulus groups showed significant response differences for the two chirp types (MANOVA $F_{4,36}$=4.09, P=0.008). Males were more likely to emit LoCs to frequencies within 5% of and higher than their own ($F_{2,18}$=4.96, P=0.019, Fig. 2.3b) and HiCs to lower frequencies, in the female range ($F_{2,18}$=12.88, P=0.0003, Fig. 2.3b). The long-duration HiCs (described in Methods: EOD Recording and Signal Definitions) were emitted by four males exclusively in response to stimulus frequencies lower than their own EODFs by at least 15%; these correspond to female EODFs.

Visual inspection of the normalized percentages of rise emissions suggests an inverse trend to LoC emissions. There were significant effects of sex (ANOVA: $F_{1,14}$=17.06, P=0.0009), stimulus frequency group (ANOVA: $F_{2,28}$=11.62, P=0.0002) and an interaction (ANOVA: $F_{2,28}$=4.78, P=0.0157, Fig. 2.3c). There was no significant difference for males but a significantly greater tendency for females to emit rises to frequencies greater than within 5% of their own (Fig. 2.3c).

Sex Differences in Responses to Stimuli as a Function of Absolute Frequency

Figure 2.4 shows differences between small and large males and females in total chirp and rise emissions, and EODFs. Males emitted more chirps than females (ANOVA: $F_{1,12}$=17.77, P=0.0012), and there were no effects of size (ANOVA: $F_{1,12}$=0.042, P=0.842) or an interaction (ANOVA: $F_{1,12}$=0.218, P=0.649, Fig. 2.4a). Overall effects on rise emissions of sex (ANOVA: $F_{1,12}$=0.003, P=0.957) and size (ANOVA: $F_{1,12}$=0.156, P=0.700) were nonsignificant, but there was a significant interaction (ANOVA: $F_{1,12}$=10.19, P=0.0007, Fig. 2.4b), such that larger females and smaller males made the most rises. Males were also higher than females (ANOVA: $F_{1,12}$=38.36, P<0.0001), as were larger individuals in general (ANOVA: $F_{1,12}$=4.51, P=0.0552, Fig. 2.4c).

In the previous section, I examined the effects of stimulus frequencies relative to the individuals' own EODFs. In order to assess the value of absolute frequency to fish of different sexes and EODFs, I reanalyzed the data by grouping responses into new treatment groups consisting of several absolute frequency ranges (see Methods: Analysis). Both males and females emit LoCs in a nonrandom fashion to the range of absolute frequencies (ANOVA: males: $F_{6,54}$=4.64, P=0.0007; females: $F_{5,30}$=5.41, P=0.0012; Fig. 2.5a), and Scheffé tests revealed the significantly greatest responses at 800Hz for males and at 700Hz for females. Although males decreased chirp responses to progressively higher frequencies than 800Hz, this trend was more pronounced for smaller than for larger males. At 800 Hz, smaller males chirped more than larger males, but they tended to chirp less than larger males at progressively higher male stimulus frequencies (R^2=0.86, $F_{1,3}$=12.33, P=0.072, Fig. 2.5c). There were no apparent differences between smaller and larger females.

Rise emission variation across stimulus frequencies was nonsignificant for males (ANOVA: males: $F_{6,54}$=1.84, P=0.108) but significant for females (ANOVA: $F_{5,30}$=2.85, P=0.0319; Fig. 2.5b). Females had one peak response at 650Hz, significantly different from every stimulus frequency group except 850Hz (Fig. 2.5b).

Sex Differences in EOD Harmonic Content

A comparison of the intensity differences between the fundamental frequency and the second harmonic yielded no significant sex difference (males: –16.8 ± 2.4 dB; females –10.9 ± 2.4 dB; t_{10}=-1.7, P=0.06). However, females had significantly more relative energy in their third harmonics than males (males: -18.8 ± 1.3 dB; females: –12.3 ± 1.3 dB; t_{10}=-3.6, P<0.005).

DISCUSSION

Responses to Cue Mimics as a Function of Sensitivity and Quality

Past studies have shown that weakly electric fish chirp when presented with a stimulus close to their own EODF (e.g. Larimer & MacDonald 1968, Dye 1987, Zupanc & Maler 1993, Dunlap et al. 1998). In this study, I extended the stimulus array to cover the entire range of conspecific EODFs to ask whether individuals would discriminate between and respond differently to frequencies to which they were equally sensitive, as one would expect given that these stimuli carry different information about sex and quality or social status. I was further interested in how an individual's own quality might influence its responses.

An individual's sensitivity influences its signal emissions. In my playback trials, males and females responded similarly to frequencies to which they were

presumably most sensitive, i.e. within 5% of their own EODF (Fig. 2.3). Individuals of both sexes emit more LoCs to similar frequencies than to more distant frequencies, consistent with a concurrent study by Bastian et al. (2001). These smaller chirps are likely those other researchers have elicited with playbacks of frequencies which jam the sensory system (e.g. Larimer & MacDonald 1968, Dye 1987, Zupanc & Maler 1993, Dunlap et al. 1998). Interestingly, there is a marked increase in LoC emissions at the second harmonic similar to that seen during the jamming avoidance response. Coupled with the fact that the fish presumably show a sensitivity peak here, this suggests that signals close to the second harmonic also jam the fish's sensory system. In contrast, the tendency for males to emit HiCs increases with stimulus deviation from the fish's own EODF, but significantly so only for lower, female-typical, frequencies. Similarly, the patterns of male and female rise emissions resemble those of male HiCs. Thus, signalling responses in general symmetrically increase or decrease away from an individual's own EODF, depending on the emitted signal type, and are thus significantly a function of an individual's sensitivity.

However, individuals discriminate between EODFs indicating different sexes and quality or social status. Colgan (1983) reviews an abundant body of literature on recognition of individuals and features of their status (see also Swaisgood et al. 2000). In this study, recognition of sex and quality cues is particularly evident a) by a peak male chirp response to low male stimulus frequencies and b) by peak rise responses of both sexes to female stimulus frequencies (Fig. 2.5). Males have higher EODFs than females, and their EODFs are correlated with body size (Zakon & Dunlap 1999, Fig. 2.4c), levels of 11-ketotestosterone (Dunlap 2002) and with dominance and spawning access (Hagedorn & Heiligenberg 1985). In contrast to males, obvious dominance patterns for females have not been shown. Variation in quality and clear, honest indicators thereof may be under weaker selection in females of this polygynous species, although some evidence, including Fig. 2.4c of this study, indicates a similar relationship between EODF and size in females as in males (Dunlap & Oliveri 2002, Tallarovic & Zakon

2002). Nonetheless, EODF is both a good indicator of sex and a “badge of status” in males (Rohwer & Rohwer 1978). Furthermore, EODF is sufficient as an indicator in that recognition does not necessitate other cues or interactive sequential assessment of fighting ability (Simpson 1968, Clutton-Brock & Albon 1979, Caldwell 1987, Waas & Colgan 1994). It would be intriguing to determine to what extent individuals can cheat by controlling these “badges of status” or whether androgens constrain cue honesty (Rohwer & Rohwer 1978).

Interestingly, the results of the current study reveal not only recognition of male dominance cues but also differential responses dependent on the receiver’s quality. The androgen 11-ketotestosterone also increases chirp rate in males (Dulka & Maler 1994, Dunlap et al. 1998, Dunlap 2002). Although larger males did not chirp more overall than smaller males in this study (Fig. 2.4a), they did so at higher male EOD mimics (Fig. 2.5c). Recognition of EODFs thus does not follow a simple, all-encompassing paradigm like, “if higher in frequency than myself, the stimulus likely represents a larger individual, therefore chirp less”, as smaller males adhere to this pattern more than larger males.

Individuals must therefore have some internal representation of their own social quality, a conclusion not meant necessarily to evoke or exclude a conscious process (Griffin 2001). One possibility for such a representation is a recognition template for absolute spectral characteristics (Evans 1993, Elepfandt et al. 2001), a form of “perfect pitch”, that differs between small and large males, such that individuals “know” which end of the frequency spectrum they are on. A simpler, related explanation for the differential chirp responses is that individuals differ in their sensitivities, such that smaller males may be less sensitive to high EODFs than larger males are to low EODFs. Alternatively, the differential responses could be explained by a difference in “confidence”, or motivational state. A phenomenon that exemplifies this is the prior residency effect whereby residents tend to fight harder and win against larger intruders simply because of ownership (Krebs 1982). Such differential motivational predisposition is likely

influenced by gonadal state (Neat et al. 1998) and androgen levels (Rohwer & Rohwer 1978, Wingfield & Marler 1988). Social experience, such as during hierarchy formation, is in turn likely to be an important factor affecting motivational predisposition (e.g. McMann 1993) and androgen levels (Carlson et al 2000).

The results of previous studies suggest that only a small percentage of females chirp during stimulus playback (Dye 1987, Zupanc & Maler 1993, Dunlap et al. 1998, Dunlap 2002). My results confirm that males chirp more often than females, but the discrepancy with the literature in that all females chirped at least once testifies to the importance of several differences in experimental paradigm. In this study, the stimulus array covered the entire range of conspecific EODFs and I extended the length of the playback trials to 120 s per stimulus. As females had an average LoC latency of 91 s, this could explain why past presentations of shorter duration than a minute have failed to elicit chirps from females (Dye 1987, Zupanc & Maler 1993). Also, in past studies stimuli have been presented across the width of a plastic tube which constrained the fish, a paradigm which boasts clarity of EOD recordings at a cost of presenting an unnatural electric field geometry (e.g. Dye 1987, Zupanc & Maler 1993). In my study the relative position of the stimulus electrodes more closely mimics a distant fish, and I allowed fish to move around freely (see also Dunlap 2002). It is possible that confinement decreases chirp propensity in females due to stress effects, and/or that females in this study chirped because they had more space than in other studies to be territorial about.

Communicative Value of the EOD and its Modulations

It is clear from the above discussion that EODF cues are sufficient to represent sex and male quality and elicit different signalling responses, as expected. I had also hypothesized that in order to communicate effectively with the opposite sex, there should be elements of the EOD and/or modulations thereof which

somehow tap into the contrasexual sensory channel (Ryan & Keddy-Hector 1992).

I found that females have more relative energy in their harmonics than do males. Interestingly, this difference is more pronounced at the third harmonic, which for females, is a frequency that approximates the male second harmonic. For example, the third harmonic of a female EOD at 600Hz would have appreciable energy at 1800Hz, which is incidentally also the frequency of the second harmonic of a male discharging at 900Hz. Since males show increased sensitivity at their second harmonic, this implies a potentially interesting coevolved match of female signalling and male tuning. Perhaps females increase their cues' active space by allotting more energy to frequencies in the male range.

Since the frequency excursions of LoCs are restricted to the range of maximal consexual sensitivity, my results are consistent with the implication that the short LoCs are used in intrasexual aggressive interactions (Hagedorn 1986). Compared with LoCs, HiCs have an appreciable portion of their energy at frequencies approaching the female EOD range (Fig. 2.1a), during the terminal undershoot of the HiC. It remains to be tested whether this undershoot is merely a biophysical constraint due to the inactivation of sodium channels in the pacemaker nucleus, or whether it has any functional significance, such as communication with females. If various chirp parameters have evolved to increase the signals' active space, we need to consider that most of the energy of a HiC is at frequencies above the baseline EODF, and these peak frequencies correspond to the stimulus frequencies in the higher direction that are also likely to elicit HiCs. Thus the increased HiC emission probability at both female frequencies and those typical of dominant males suggests that this type of chirp could serve the function of simultaneously signalling courtship and submission. Alternatively, this signal category's greater frequency excursions could merely

serve the function of increasing the active space for aggressive communication with individuals of more distant EODFs.

The functions of rises are also unclear. The suggestion that rises are submissive signals (Hopkins 1974) is substantiated by my finding that smaller males emit more rises than larger males (Fig. 2.4). Interestingly, when examining the responses to absolute frequency, males also tend to show a peak in rise emissions to female frequencies. Although this pattern is stronger for smaller males (not shown), suggesting they could be submissive to larger females, male rises could thus also function in signalling courtship. It has long been suggested that spawning necessitates mitigation of aggression (Bastock 1967). Perhaps signals employed to communicate courtship and submission are at least to some extent borne out of a similar motivational condition. It is however unclear, if rises to some extent signal submission, why larger females emit more rises (Fig. 2.4b) or why low female stimulus frequencies elicit more rises from females (Fig. 2.5b). A concurrent study has revealed that female rises can be broken down into several categories, some or all of which may in turn have different communicative value (Tallarovic & Zakon 2002). Thus it is probable that by consolidating rises into a single category we are missing meaningful differences between rise subtypes, which may be used differentially in intra- and intersexual communication.

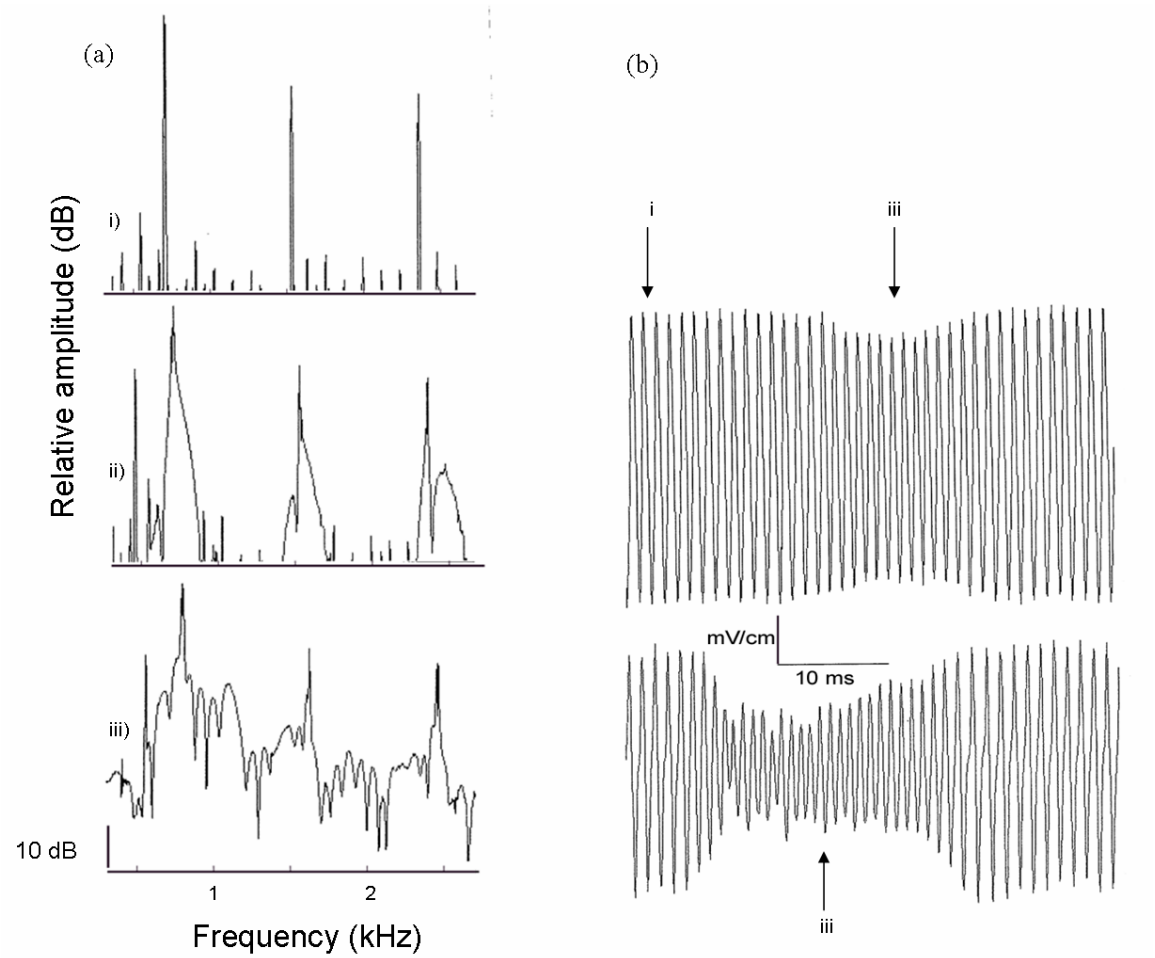

Figure 2.1. Structure of chirps from a male A. leptorhynchus. (a) Spectra (FFT size 4096; frequency resolution 1.3Hz) of a typical male's i) unmodulated EOD, ii) LoC and iii) HiC. (b) Oscillograms of the LoC and HiC. Note the greater amplitude deflection of the HiC. Arrows indicate the approximate positions of the cursor in Cool Edit Pro when generating the FFTs shown in (a).

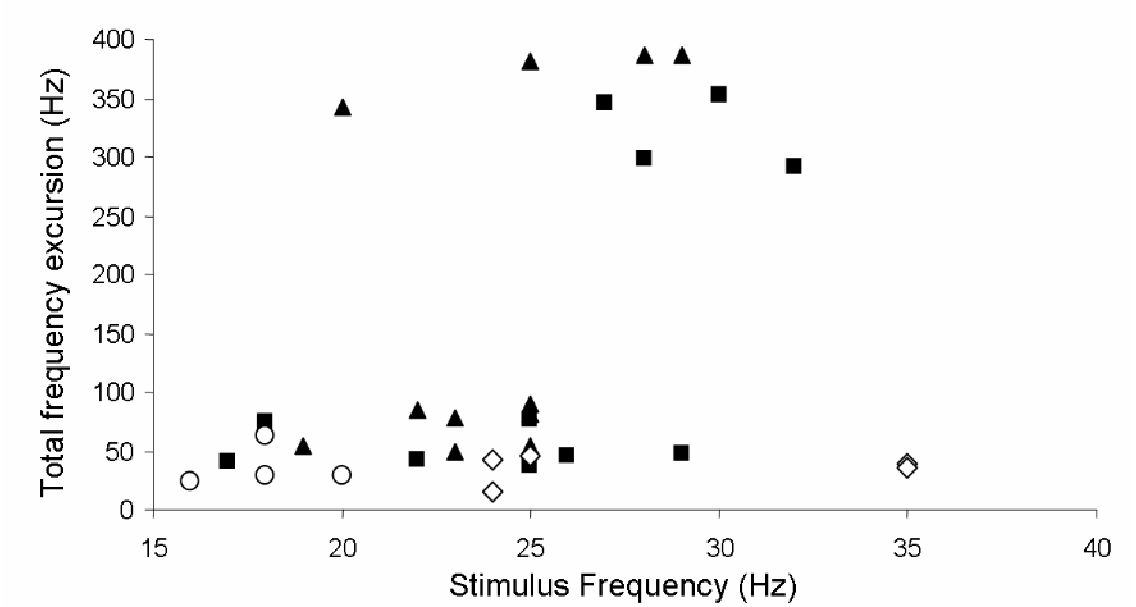

Figure 2.2. Distribution of LoC (bottom) and HiC (top) chirp types based on correlations between duration and frequency excursion for two females (open symbols) and two males (black symbols).

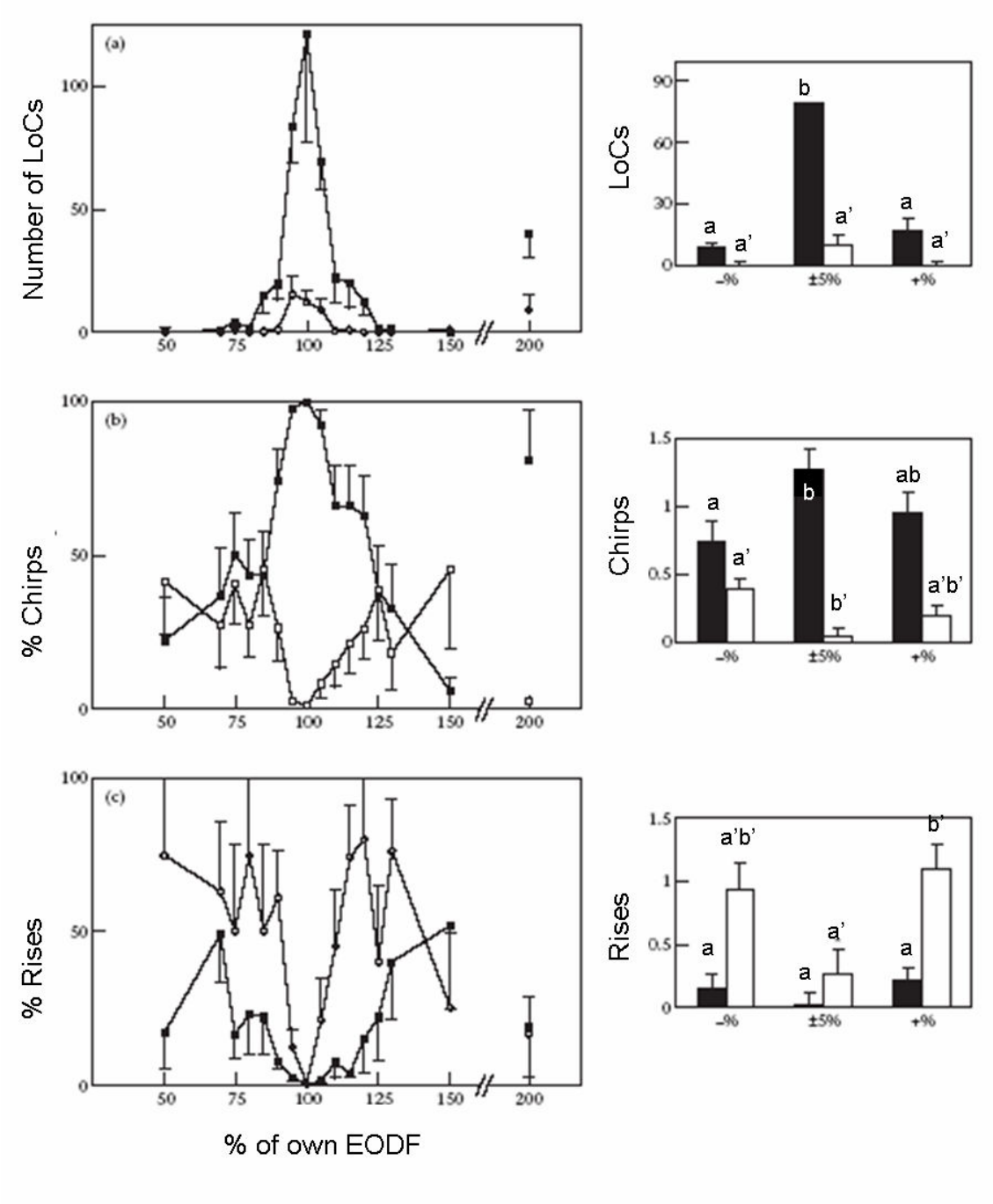

Figure 2.3. Signal emissions as a function of percent difference in stimulus frequency from the fish's own EODF. (a) Numbers of LoCs for females (open circles; N=7) and males (filled squares; N=10). (b) Male LoC and HiC chirps (black and grey squares, respectively), and (c) female (open circles) and male (filled squares) rises as a percentage of total signal emissions. Panels on the right show responses averaged over three ranges of conspecific stimulus presentations (--%: 10-15% below, ± 5%: within 5% of, +%: 10-15% above the fish's own EODF). Groups that were statistically nonsignificant share a common letter; Sidak test performed separately for each factor or variable: $P<0.05$.

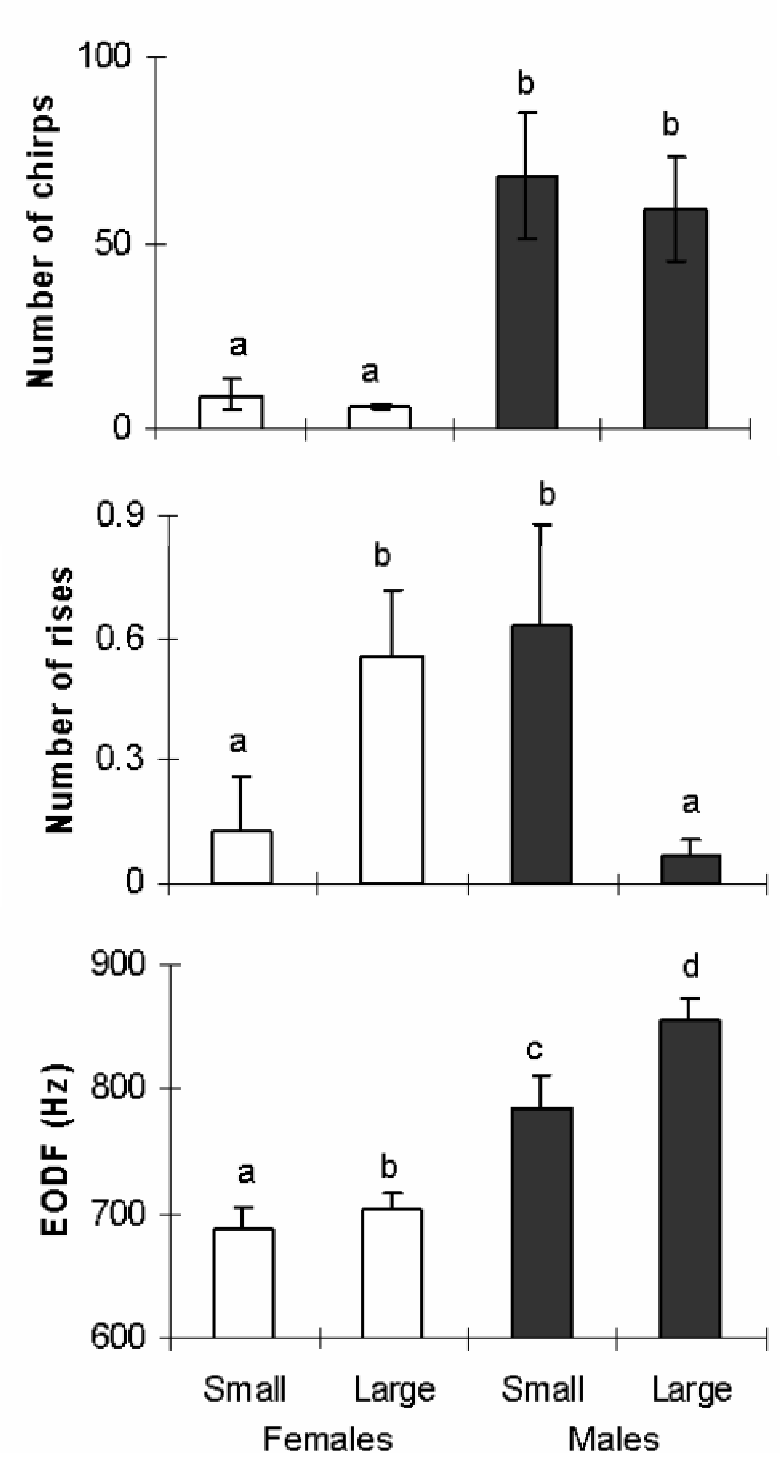

Figure 2.4. Characteristics of small and large females (open, N=4 and 3, respectively) and males (filled, N=4 and 5, respectively): (a) total chirp emission, (b) total rise emission and (c) body length. Groups that were statistically nonsignificant share a common letter (Scheffé: $P<0.06$).

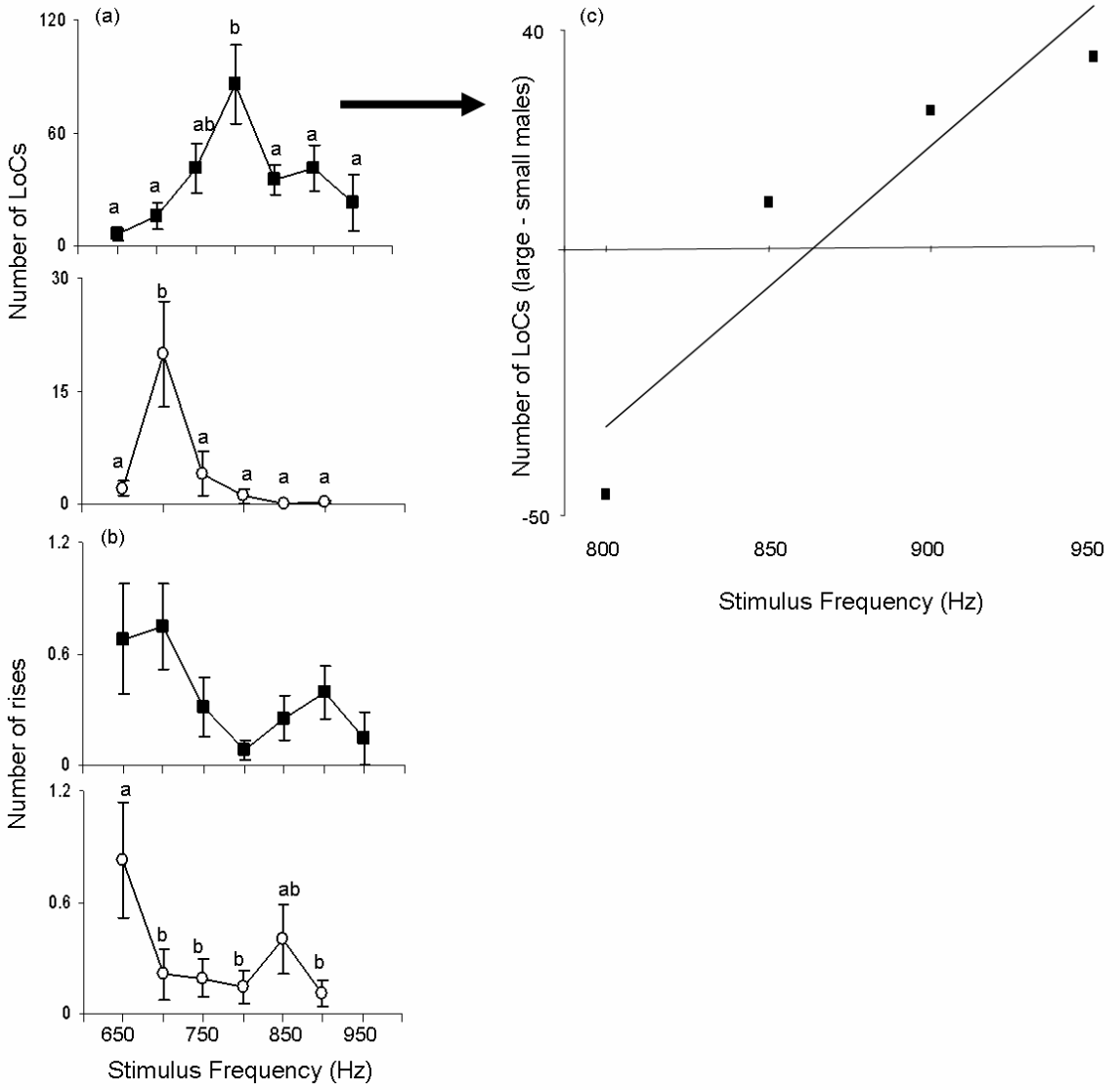

Figure 2.5. Numbers of (a) LoCs and (b) rises as a function of absolute stimulus frequency for females (open circles; N=7) and males (filled squares; N=10). Groups that were statistically nonsignificant share a common letter (Scheffé: $P<0.05$). (c) Differences between small and large males in number of LoCs to absolute frequency groups in the male range.

CHAPTER 3

Effects of chirp playbacks on sender recognition

Abstract. The electric organ discharge frequency (EODF) of brown ghost knifefish is sexually dimorphic, correlated with size in males, and can be modified for communication. Previous studies have described several discrete types of abrupt modulations ("chirps") of the EOD which are differentially elicited with EOD mimics at various stimulus difference frequencies (dFs) from the fish's own EODF: nearby and distant frequencies elicit chirps of low and high frequency excursion and amplitude decrease (LoC and HiC), respectively. Males also emit long-duration HiCs to much lower EOD mimics, in the female range. Here I examined the effect of stimulus carrier frequency and superimposed chirp types on male chirp responses and male and female approaches in a novel arena. I found that a) chirping is increasingly inhibited by chirps of greater frequency excursion and amplitude decrease; b) lower-frequency (smaller male) EOD mimics elicit the most chirping and approach behavior, c) the type of chirp emitted is influenced by the details of the stimulus regime; d) larger males chirp more overall, sustain a higher chirp rate and are less likely to decrease their chirp rate to chirp stimuli, and e) females and males prefer to associate with long duration chirps (HiCs) more than LoCs. I conclude that LoCs are intrasexual aggressive signals, long duration HiCs are attractive to both sexes and elicit the fewest chirp responses, and shorter HiCs may serve a dual function in rival deterrence and mate attraction.

Wave-type gymnotiform electric fish produce a quasi-sinusoidal electric organ discharge (EOD) of electrical current at an extremely precise and steady frequency. The EOD is species-, sex-, and individual-specific: in brown ghost knifefish (*A. leptorhynchus*), males have higher EOD frequencies (EODFs) than females (female range ~600-800Hz, male range ~800-1000Hz). Male EODF is often correlated with body size (Zakon & Dunlap 1999, Triefenbach & Zakon 2003) and social status (Hagedorn & Heiligenberg 1985).

Males (and to a much lesser extent, females) modulate their electric organ discharge (EOD) to emit several discrete types of transient frequency increases

and amplitude decreases of different durations, called chirps. Previous studies have either anecdotally described chirp patterns in social groups (Hagedorn & Heiligenberg 1985) or in dyadic interactions (Dunlap 2002), or have elicited chirps by stimulating fish with EOD mimics, while constraining them in a chirp chamber (Dye 1987) or allowing them to swim freely in a playback arena (Triefenbach & Zakon 2003).

Emitted chirp types vary depending on the difference in stimulus frequency (dF) from the focal's own EODF (Bastian et al. 2001, Triefenbach & Zakon 2003), the amplitude of the signal (Engler & Zupanc 2001) and in different social contexts (Triefenbach & Zakon submitted). Recently, Engler et al. (2000), Engler & Zupanc (2001) and Triefenbach & Zakon (2003) made more quantitative descriptions of chirps emitted spontaneously and when stimulated at various stimulus difference frequencies (dFs) from the fish's own EODF. LoCs (aka type-II chirps) are short (10-15ms) amplitude-modulated (ca. 10%) frequency increases of ca. 60Hz emitted primarily in response to similar stimulus frequencies and at higher stimulus amplitudes. Lower amplitude stimuli and stimuli further away from a fish's EODF elicit HiC-type (aka type-I) chirps which feature much greater frequency increases (>200Hz) and amplitude decreases (ca. 50%) and are longer and more variable in duration. For simplicity, chirps of this frequency excursion are grouped as either HiC-1s (15-25ms), HiC-2s (50-100ms), or HiC-3s (>100ms). All previous quantitative studies have found these signal types to be discrete categories.

By virtue of the ranges of stimulus frequencies that best elicit them, LoCs and HiCs have been hypothesized to function in intra- and intersexual communication, respectively. Also, Hagedorn & Heiligenberg (1985) observed that longer HiC types seem to be used by males to attract females during courtship. Although numerous playback and dyadic interaction studies have measured chirp emissions in response to consexuals or mimics thereof, none of them distinguished between chirp subtypes or attempted to assess their

potentially differential effect on receivers or correlated them with qualities of the sender, like size or EODF (Engler et al. 2001, Bastian et al. 2001, Dunlap et al. 1998, Dunlap 2002, Dunlap & Larkins-Ford 2003).

This is the first study to manipulate different chirp parameters and examine their effect on individual responses under varying conditions. I hypothesized that chirp stimuli should affect chirp responses according to the threat potential of the stimulus and the condition of the receiver. If chirps are indeed aggressive, then a chirping carrier EOD mimic should elicit fewer chirp responses or less approach than a carrier EOD alone, especially from lower-quality individuals who might be less able to afford escalating aggression. Similarly, a higher frequency carrier, which would mimic an EOD characteristic of larger males, should elicit fewer chirp responses and less approach than a lower frequency carrier. To the extent that perceived stimulus amplitude affects the probability of LoC vs. HiC emission, chirps with greater amplitude decreases (HICs) should also elicit more HiCs relative to LoCs.

METHODS

Animals

Fish were obtained from a commercial vendor and kept in individual tanks (dimensions: W23cm x H20.5cm x L49cm, temperature: 25-26.5°C; conductivity 300-700 μS). Weights were measured for some fish.

Stimulus generation and playback

Chirp stimuli were generated with a program in Matlab (co-written with Jee-Hyun Kim, available from the author). The program produced output files of sine wave modulations given the following inputs: a) onset of modulation, b) maximum

frequency excursion, c) interval between onset and maximum excursion, d) duration at maximum frequency, and e) interval between maximum excursion and return to baseline frequency. It also had the option of using a Gaussian smoothing function to generate any desired amplitude decrease at the maximum frequency excursion. Table 3.1 shows the parameters of the four chirp types used in playbacks. In preliminary trials, I found no difference in chirp responses to playbacks of a LoC mimic I generated and playbacks of LoCs of a spontaneously chirping male.

Chirp type	Frequency excursion (Hz)	Duration (ms)	Amplitude decrease (%)
LoC	50	15	90
HiC-1	250	20	50
HiC-2	250	80	50
HiC-3	250	200	50

Table 3.1. Frequency, duration and amplitude characteristics of generated chirp stimuli (LoC, HiC-1, HiC-2 and HiC-3).

In each trial I presented focals with a series of 10s “pulses” of a carrier sine wave (with or without modulations) that faded in and out linearly over two seconds. The 8-second interval at maximum carrier amplitude contained 8 samples of one of the four chirp types (LoC, HiC-1, HiC-2 and HiC-3). The interchirp intervals were randomized with the condition that there was at least 200ms between each chirp. Control stimuli were the carriers without modulations. Carrier frequencies varied, depending on the experiment, but stimulus amplitude was kept at a constant 1mV/cm at the center of the stimulus electrodes.

Experiment 1: Chirp chamber in home tanks

As emitted chirp rate is a function of stimulus amplitude (Engler et al 2001), and as the perceived stimulus amplitude varies with a focal’s position with respect to

the electrodes, playbacks in this experiment were conducted while confining fish in a chirp chamber to control for their movement. Males (N=9) were placed in a plastic mesh tube (2.5cm x 15cm), plugged with rubber stoppers to prevent escape, and allowed to acclimate for at least 15 minutes prior to stimulus playback. The chamber contained a fixed pair of carbon stimulus electrodes across the fish's body and a perpendicular pair of Ag recording electrodes across the length of the tube to monitor electrical activity. EODs were recorded, transduced through a D/A converter (Terratech EWS-88MT) and digitized at a sampling rate of 11025Hz in Cool Edit Pro (Syntrillium). Chirps were visually distinguished on a spectrogram (Triefenbach & Zakon 2003), counted and summed over each trial (see trial definitions below). As longer-duration HiC types were very infrequent, I did not differentiate between emitted HiC types.

Each fish underwent one playback trial of four 60-s stimulus sets played four consecutive times in the same order. Each set consisted of a carrier sine wave (50Hz above the focal's EODF) with or without one of three chirp types superimposed (LoCs, HiC-1s or HiC-3s, Table 3.1) whose presentation order was counterbalanced across subjects. Thus, the four consecutive repetitions of the stimulus sets comprised a total of 16 minutes of stimuli per trial.

Experiment 2: Simulated intruders in home tanks

I placed an "intruder" tube (7.5x25cm) in each focal's hometank (N=6 males). The tube, whose ends were plugged with plastic mesh to prevent entry, encased a pair of carbon playback electrodes (spaced 10cm apart) to simulate a fish in a shelter. This design was intended to circumvent the blatantly unnatural stimulus geometry generated by a naked pair of electrodes used in previous studies (Triefenbach & Zakon 2003, Tallarovic & Zakon in press). I waited at least 15min after tube placement before commencing playback. Electrical responses were measured as in Experiment 1, except that here the Ag recording electrodes were attached to and spanned the length of the sides of the tank.

In these trials, each (15-min) stimulus array was presented on a different day. Stimuli were either a carrier alone or one of the four chirp categories superimposed on the carrier (Table 3.1). To examine the effect of carrier frequency, I presented these stimuli on carriers 50Hz higher and 50Hz lower than the focal's EODF. Stimulus presentation order was pseudo-randomized in that I initially randomized the order, but then adjusted presentations so that each stimulus had the same order number average overall.

Experiment 3: Playbacks in a novel arena

A playback arena (Chapter 2) contained a 50W thermostat/heater attached to one side of the tank that kept the temperature at 26±1°C. Two plastic mesh partitions spanned the arena to create two compartments 10cm wide at each end of the tank. Stimuli were presented through a pair of carbon electrodes (spaced 10cm apart) embedded in a PVC tube to simulate a fish in a shelter. I placed one stimulus tube in each compartment but only ever played back stimuli from one side of the tank.

The center of the arena contained a plastic cubic mesh cage (6cm) with an open top extending above the water level into which fish could be easily placed. A string, attached to the cage across the width of the tank and guided through a pulley system, allowed easy leverage of the cage from outside of the tank, minimizing disturbance upon release of the fish into the arena. Fish were transferred and acclimated in the dark for one minute before being played a stimulus. After one 10-s pulse had elapsed, the cage was lifted and the fish were allowed to move freely around the tank for another two consecutive 10-s stimulus pulses. I analyzed the fish's position with an infrared camera (Sony DCR-TRV120) mounted on the ceiling above the tank that captured one frame every sec. At the end of the trial, the cage was lowered and the light was turned on. After the fish was netted and replaced gently into the cage, the light was turned

off again. Sixty seconds later, the procedure was repeated for a different stimulus trial. At the end of a round of all trials, fish were placed back in their hometanks for at least 24 hours before receiving the same stimulus regime again in reverse order.

Two pieces of black tape, 30 cm from each mesh partition, demarcated three zones. The area on the side closest to the stimulus tube was termed the “approach” zone, the area on the other side the “avoidance” zone, leaving a 20cm “neutral” zone in the middle. The number of frames at least 50% of the fish’s body was located in a given zone is my estimate of time spent in that zone. To assess an individual’s preference for, or the propensity to approach, a stimulus, I subtracted the time spent in the “avoidance” from the time spent in the “approach” zones during the 20s free-swimming phase.

Stimuli for males (N=13) were controls (carriers alone) and three chirp types on two carrier frequencies (focal EODF ± 50Hz). Females (N=7) received a single higher carrier (1000Hz) with or without the three chirp mimics. The order of presentations was randomized with respect to carrier frequency and the stimulus on that carrier, but counterbalanced to minimize order effects. I performed two separate analyses, one comparing male responses to stimuli presented on the higher and lower carriers, the other comparing male and female responses to stimuli on the higher carrier.

Statistical analysis

All analyses were performed using either t-tests or ANOVAs. In analyses of related variables (LoC and HiC responses), MANOVA was initially used and followed by ANOVAs on the individual variables. Graphs show means and standard errors.

In Experiments 1 & 3, I performed repeated-measures ANOVAs, with stimulus type (carrier alone vs. chirps) as the within-subject variable, and with carrier frequency as an additional within-subject and focal sex a between-subject variable in Experiment 3. Simple main effects analysis was performed in the case of an interaction (Fig. 3.4). Significant main effects were further analyzed by pairwise comparisons using the Sidak adjustment for multiple comparisons.

For the analyses of Experiment 3 (Fig. 3.4), I also performed a paired t-test (between approach and avoidance times) for each treatment group to test the null hypothesis that approach scores did not differ significantly from zero. Thus, e.g., I considered individuals to be truly approaching a stimulus only if time spent in the approach zone were significantly greater than time spent in the avoidance zone. I applied a Bonferroni correction on these eight or three comparisons (Analysis 1 & 2, respectively, see Fig. 3.4), disqualifying as significant any P-value greater than $\alpha=0.006$ and $\alpha=0.017$ (Analysis 1 & 2, respectively).

In Experiment 2 (Fig. 3.3), where data points were missing and highly variable, I first normalized individual chirp responses to each stimulus over total chirp responses to all stimuli, and performed either a simple MANOVA and/or ANOVA, followed by a Scheffé test for comparisons either of all pairs (Fig. 3.3b & c) or of all pairs of levels within a factor (Fig. 3.3a), if the ANOVA was significant.

RESULTS

Experiment 1: Chirp chamber

Stimuli were presented to males on a higher frequency carrier only (focal EODF + 50Hz), either alone or containing one of three chirp types (LoC, HiC-1, HiC-3). For these trials, I omitted the HiC type of intermediate duration (HiC-2) to simplify the stimulus regime.

LoC and HiC emissions across the four stimuli on the higher frequency carrier varied significantly (MANOVA: $F_{6,48}$=4.40, P=0.001, Fig. 3.1). Analysis of the individual variables showed that while there were no differences in HiC emissions (ANOVA: $F_{3,24}$=1.37; P=0.28, Fig. 3.1), males emitted significantly more LoCs to the carrier alone and to the carrier with LoCs than to the carrier with HiCs superimposed (ANOVA: $F_{3,24}$=11.34, P<0.001, Fig. 3.1). Thus, chirping was significantly inhibited by chirps of higher frequency excursion and longer duration.

Focal size affected a subject's chirp behavior. For one, larger males made more chirps than smaller males, averaged over all stimuli (y=1.6241x - 3.8592, R^2 =0.55, ANOVA: $F_{1,7}$=8.48, P=0.02, Fig. 3.2a). Also, when presented with the carrier sine wave alone, larger males continued chirping whereas smaller males significantly reduced their chirp rate over time (y = -1.3372x + 23.108, R^2 = 0.5854; ANOVA: $F_{1,6}$=7.06; P=0.045, Fig. 3.2b).

Individuals of different sizes also treated stimuli differently. Two distinct response patterns could be identified that correlated with focal size. Males that made more LoCs to the carrier alone than to the LoC stimulus were significantly smaller than males that made more chirps to the LoC stimulus relative to the carrier alone (t-test: t_7=-4.92, P=0.002, Fig. 3.2c).

Experiment 2: Simulated intruders in home tanks

In this experiment I examined the effect of carrier frequency on chirp responses. I also extended the stimulus regime on each carrier to include three HiC mimics (HiC-1, 2 and 3) that varied in duration only and featured a frequency excursion of 250Hz and a 50% amplitude decrease. Stimuli were presented through a playback tube placed in the hometanks of free-swimming males. Responses to HiC stimuli were consolidated for some analyses.

I performed a two-way ANOVA on total chirp responses to address the hypotheses a) that lower frequency carriers, which would mimic EODs characteristic of smaller males, should elicit more chirps than higher frequency carriers, and b) that chirps should inhibit chirping. Carrier frequency had a significant effect on chirp responses. Males chirped more to stimuli on the lower than the higher frequency carrier (ANOVA: $F_{1,24}$=5.44, P=0.028, Fig. 3.3a), suggesting that stimuli involving the higher frequency carrier were recognized as more threatening. Stimulus type (carrier alone vs. one of the two chirp types) had a marginal effect on chirp responses (ANOVA: $F_{2,24}$=2.57, P=0.097, Fig. 3.3a) and there was no significant interaction between carrier frequency and stimulus type (ANOVA: $F_{2,24}$=1.51, P=0.24, Fig. 3.3a).

The type of chirp males emitted in response to stimuli varied significantly with carrier frequency (MANOVA: $F_{2,17}$=4.40, P=0.0289) and moderately with stimulus type (MANOVA: $F_{2,17}$=3.04, P=0.0743), with no interaction (MANOVA: $F_{2,17}$=1.70, P=0.212). Analysis of the individual chirp variables showed that LoC responses were unaffected by both carrier frequency (ANOVA: $F_{1,18}$=0.38, P=0.547, Fig. 3.3b) and presence/absence of chirps (ANOVA: $F_{1,18}$=0.69, P=0.418, Fig. 3.3b), whereas HiCs were emitted more to lower than higher frequency carriers (ANOVA: $F_{1,18}$=5.08, P=0.0369, Fig. 3.3b), and more to carriers alone than to carriers with chirps superimposed (ANOVA: $F_{1,18}$=6.17, P=0.0230, Fig. 3.3b). There was also a moderate interaction (ANOVA: $F_{1,18}$=3.60, P=0.0741, Fig. 3.3b), in that males made significantly more HiCs to the lower carrier alone than to the other three stimulus groups (Scheffé test for all pairs: P<0.01).

Males clearly discriminated between different chirp types, regardless of carrier frequency. I had hypothesized that relative chirp emissions should change with perceived amplitude. I tested this by comparing responses to the three HiC stimuli which featured a 50% amplitude reduction with varied duration (Table 3.1). The proportion of LoCs to HiCs decreased as a function of HiC stimulus

duration. The percentage of LoC emissions was significantly lower for HiC-3s than HiC-1s, when responses to these HiC subtypes were pooled for both carriers; that is, males tended to make relatively more HiCs to the HiC-3 stimuli, i.e. the chirps with the longest duration of 50% amplitude reduction (ANOVA: $F_{2,9}$=5.32, P=0.03, Fig. 3.3c).

Experiment 3: Playback arena

In Analysis 1 (see Methods), I examined the tendency for males to associate with some of the above stimuli presented on lower and higher frequency carriers. While I found no significant effect on approach scores of carrier frequency (ANOVA: $F_{1,24}$=0.005, P=0.94), there were a marginal stimulus effect (ANOVA: $F_{3,72}$=2.25, P=0.090) and interaction (ANOVA: $F_{3,72}$=2.55, P=0.062, Fig. 3.4). Simple main effects analyses showed significant male approach differences for the lower (ANOVA: $F_{3,36}$=4.07, P=0.0138) but not the higher carrier (ANOVA: $F_{3,36}$=1.14, P=0.346, Fig. 3.4). The Sidak test revealed that, on the lower carrier, males approached HiC-1 stimuli significantly less than the carrier alone. I further asked whether the approach scores reflected true approach or avoidance by performing paired t-tests on approach and avoidance times; thus, true approach was indicated by significantly more time spent in the approach than in the avoidance zones. After Bonferroni correction, the only truly significant approach by males was to the lower carrier without chirps (t_{13}=3.07, P=0.005, Fig. 3.4), consistent with the hypothesis that lower frequencies are less threatening, especially without chirps.

In Analysis 2, I compared male and female responses to the higher carrier stimuli, using a) the above data for males and b) data from females presented with one carrier at 1000Hz with and without the chirp mimics. Comparing approach scores of males and females revealed no sex difference (ANOVA: $F_{1,18}$=0.02, P=0.897, Fig. 3.4) but a marginal stimulus effect (ANOVA: $F_{3,18}$=2.21, P=0.098, Fig. 3.4). When I combined male and female responses and responses

to the two HiC stimuli (indicated by brackets in Fig. 3.4), there was a significant stimulus effect (ANOVA $F_{2,38}$=3.26, P=0.0495), such that individuals tended to approach HiC more than LoC stimuli (Sidak test: P=0.066, Fig. 3.4). Paired t-tests on the three groups revealed that individuals spent significantly more time approaching than avoiding the HiC stimuli only (t_{20}=3.66, P=0.0008, Fig. 3.4).

Comparison of chirp responses in home tanks and novel arena

To determine how familiarity and/or stress might influence chirp emissions, I calculated LoC and HiC rates for male responses in the hometank and the arena experiments. MANOVA shows a nonsignificant trend ($F_{2,24}$=2.08, P=0.147) toward increased HiC emissions in the familiar home tanks than in the novel arena (ANOVA: $F_{1,25}$=4.27, P=0.049, Fig. 3.5), and no difference in LoC emissions between the two settings (ANOVA: $F_{1,25}$=0.001, P=0.979, Fig. 3.5).

DISCUSSION

Inhibition of chirps by chirps

EOD modulations with greater frequency excursion, amplitude decreases and durations elicited fewer chirp responses. Thus, fish consistently chirped less at HiCs than unmodulated carriers, and in Experiment 1, less than LoC stimuli as well. Many studies have shown chirp production to be affected by amplitude and frequency of the unmodulated EOD (Dye 1987, Engler & Zupanc 2001), and evidence exists in a related species, *Eigenmannia virescens*, for response reduction to modulations of EOD frequency and amplitude as well (Hopkins 1974). Recently, Dunlap & Larkins-Ford (2003) showed that when confined in a chirp chamber, brown ghosts chirped less at a chirping than a plain EOD. The authors tentatively attributed their observed response inhibition to chirps vis-à-vis EOD alone to the fish's confinement causing reluctance to respond to the

presumably more aggressive stimuli. Although the current chirp chamber experiment replicates their result, confinement is unlikely to be the only explanation, as I also observed the same pattern when fish were free-swimming in their home tanks. Thus, in conjunction with these past studies, my results suggest that chirps per se inhibit chirping proportional to the extent of their frequency and amplitude modulation, and duration. As these parameters sometimes covaried in this study, which of them, if any, is most important in reducing motor output remains to be determined.

Effects of receiver quality

I predicted that stimulus chirps should elicit aggression (chirps) from focals proportionately to their quality (size), as receiver quality has been shown to affect signal recognition (Triefenbach & Zakon 2003). Indeed, larger males in Experiment 1 chirped more to all stimuli on the higher frequency carrier and reduced their responses less over time (Fig. 3.2a & b). In addition, whereas smaller males reduced their responses to chirping stimuli relative to the carrier alone, larger males often showed an increase to chirps compared with the carrier alone (Fig. 3.2c). This behavior of higher quality males is consistent with their previously demonstrated lack of chirp response mitigation to more threatening stimuli (Triefenbach & Zakon 2003), and by inference supports the idea that chirps are relatively threatening. It remains unclear whether these individual differences in response mitigation reflect a difference in habituation or in propensity to sustain aggression. What is clear is that these are intrinsic differences between males. Dunlap (2002) also found a correlation between body size and chirp rate when live fish were interacting in dyads. In addition, he found major differences in the ability of different stimulus fish to elicit chirps. It was thus not clear to what extent the signal/size correlates were intrinsic to the subjects or a result of interacting with other fish of different quality. Here, every fish received an identical set of non-reciprocating stimuli. Therefore, the fact that chirp rate still

correlated with size indicates an intrinsic difference between males of different sizes in their propensity to chirp, independent of their status relative to others.

Effect of carrier frequency

As EODF correlates positively with size in males, a lower frequency "intruder" stimulus should in turn be recognized as less threatening. To the extent that emitted chirps indicate aggression, lower frequency carriers should therefore elicit more focal chirp responses than higher frequency carriers. Comparing chirp responses to playbacks across all stimuli on each carrier (Fig 3.3a), males chirped more at stimuli on the lower than the higher carrier. This is consistent with other playback studies wherein chirp emission rates, though mostly symmetrical to positive and negative stimulus difference frequencies, tend to be slightly greater toward lower EOD mimics (Bastian et al. 2001, Triefenbach & Zakon 2003). That the lower carrier alone was the only stimulus to significantly elicit approach by males is further evidence indicating that lower carriers are less threatening.

Probability of LoC vs. HiC emissions

Relative emissions of LoCs and HiCs were influenced by carrier frequency, stimulus chirp parameters and the experimental setting. Fish made relatively more HiCs to the lower frequency carrier than the higher frequency carrier alone (Fig. 3.3b). The tuning curves in a study by Bastian et al. (2001), who examined LoC and HiC responses to sine stimuli at various difference frequencies, also indicate a greater HiC probability and greater overall HiC emissions to the lower than the higher stimulus of 48Hz difference from focal EODF.

Whereas fish emitted fewer HiCs at the lower frequency carrier with chirps than without modulations, this was not apparent for the higher frequency carrier stimuli (Fig. 3.3b). One possible explanation for this pattern observes that responses to

chirps are actually quite similar on both carriers, suggesting the possibility that information provided by carrier frequency may be less relevant to the receiver when chirps are added. The presence of chirps thus may serve to normalize the effect of the EOD alone, instead of augmenting it. Focal responses to chirp stimuli may reflect a generalized recognition of an invitation to negotiate dominance, regardless of the quality of the initiating stimulus.

In past studies, the propensity to emit HiCs over LoCs has been shown to be increased by stimuli more distant from an individual's own EODF (Bastian et al. 2001, Triefenbach & Zakon 2003) and by stimuli of lower amplitude (Engler & Zupanc 2001). All HiC stimuli in this study featured a 50% amplitude reduction and 250Hz frequency excursion but varied in duration, with HiC-3s thus featuring much longer periods of these frequency and amplitude changes than HiC-1s. Consistent with past implications of amplitude and frequency modulation effects on focal chirp rate, I found that HiC-3s, i.e. stimuli with longer periods of amplitude decrease and frequency excursion, elicited relatively more HiCs than the shorter duration HiC-1s (Fig. 3.3c).

Focals emitted more LoCs than HiCs in the chirp chamber (Fig. 3.1), and more HiCs when free-swimming in their hometanks (Fig. 3.3b). The fact that LoC production probability increases with perceived stimulus amplitude (Engler & Zupanc 2001) could account for the increased LoC probability in the chirp chamber where focals were invariably relegated to the middle of the stimulus field where amplitude was maximal. Alternatively, the increase in HiC production relative to LoCs in home tanks may have been due to a prior residence effect, absent in the relatively novel environments of the chirp chamber and the playback arena, where fish emitted LoCs almost exclusively (Fig. 3.5). Engler et al. (2000) observed that brown ghosts, when undisturbed for long periods, tend to make more HiCs than LoCs, albeit at a drastically reduced rate compared with responses to stimulation. This corroborates the idea that HiCs are territorial advertisement signals.

Who is the receiver? Aggression vs. attraction

Results from the playback arena indicate that the male carrier is attractive to females only when it has HiCs superimposed, consistent with earlier suggestions that longer duration chirps serve intersexual communication (Hagedorn & Heiligenberg 1985). Males similarly tend to approach HiC-3s on both carriers, indicating that these courtship chirps are perhaps less threatening or even attractive to males or that they trigger an aggressive response, neither scenario being necessarily mutually exclusive. Given the predominantly lower stimulus frequencies previously shown to elicit HiCs (Fig. 3.3b, Triefenbach & Zakon 2003, Engler & Zupanc 2001), a male emitting this chirp type might be inferred by eavesdroppers to be in female company and thus more attractive.

Interestingly, fish avoided HiC-1s (relative to the carrier alone) on the lower but not the higher frequency carrier. HiC-1s may serve a dual function to the aggressive LoC and the courting HiC-3, namely to court females while threatening males (Berglund et al. 1996). In competitive dyadic interactions between males, LoCs are emitted significantly more by winners than by losers (Triefenbach & Zakon in prep), whereas HiC-1s tend to be emitted by males that are of lower EODF, regardless of the outcome of combat, albeit not significantly (unpublished data). One possiblity is that lower frequency males employ HiC-1s in intrasexual encounters to simulate the maximum frequency excursion of a LoC on a higher frequency carrier, and thus appear more threatening. The observation that males treated HiC-1s on the lower frequency carrier somewhat similarly to LoCs on the higher frequency carrier (Fig. 3.4) is consistent with this hypothesis. A higher frequency male emitting HiC-1s might by this logic be recognized as a fish unwarrantedly inflating their motivational display.

Realism of stimuli

In many systems, the presence of other males causes an increase in aggressive signalling. In turn, simple playbacks of some of these signal components are often sufficient in eliciting both aggressive responses from males and approach from females. Evidence from live interactions between brown ghost knifefish also suggests that chirping rivals stimulate chirping (Dunlap & Larkins-Ford 2003, Triefenbach & Zakon submitted). Because fish in their study chirped much more to live stimuli than playbacks, Dunlap & Larkins-Ford (2003) noted that fish may reduce their chirp rate when they recognize that their responses do not elicit a corresponding change in the stimulus. In my study I did not examine the details of the stimulus-receiver interaction, but other studies suggest that lack of stimulus change can stimulate, rather than inhibit responses. Gherardi & Pieraccini (2004) showed for crayfish, using an information theoretical approach, that relative inactivity ("motionless" and "no observable change") had the greatest effect on eliciting a change in an opponent's behavior. Similarly, signal redundancy stimulates aggression in frogs (Wagner 1989).

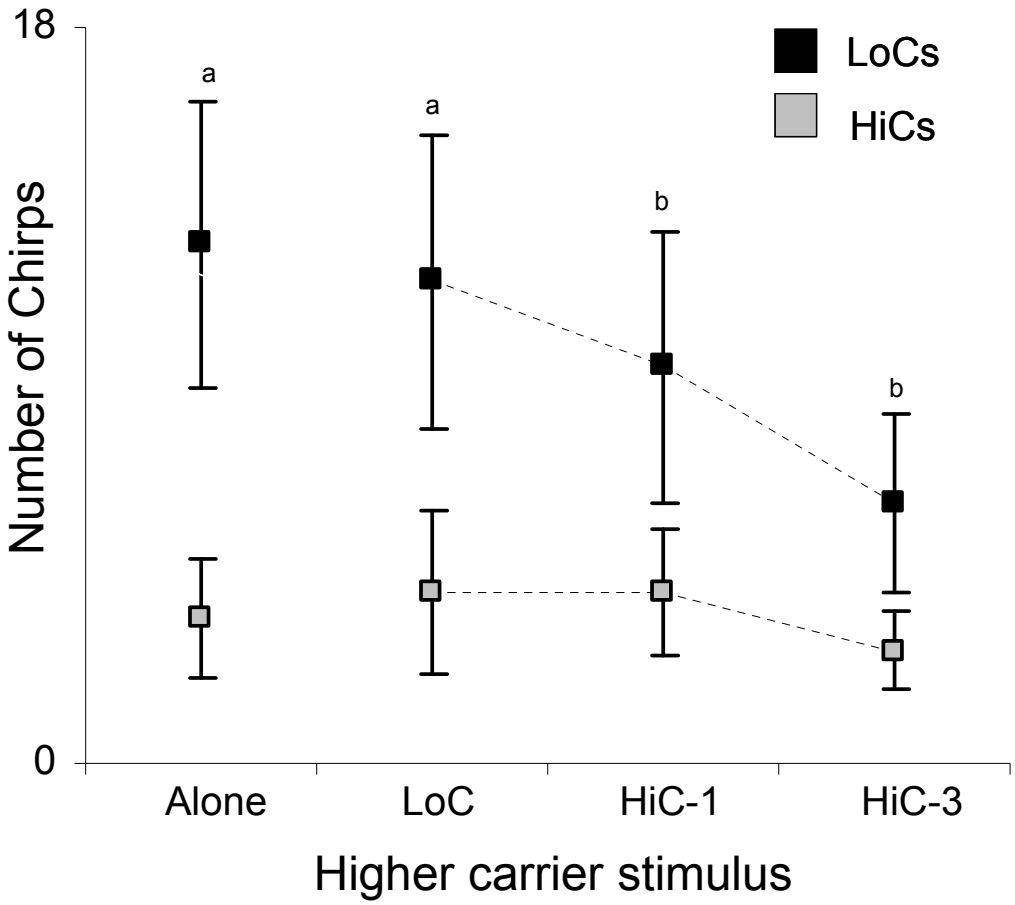

Figure 3.1. Normalized LoC (*dark* □) and HiC (*light* □) emissions to chirp playbacks on a higher frequency carrier for males (N=9) confined in a chirp chamber. Groups that were statistically nonsignificant share a common letter (LoC emissions only, Sidak: $P<0.05$).

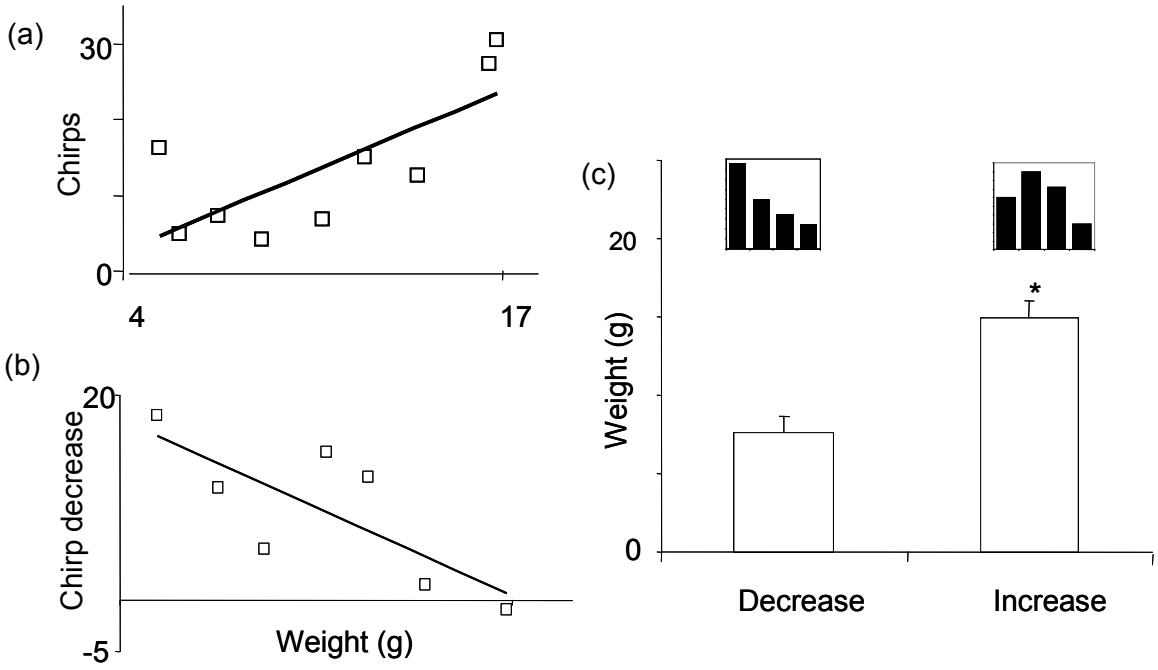

Figure 3.2. Effects of focal weight on chirp responses to the higher frequency carrier in the chirp chamber. (a) Total chirp number vs. weight; $P<0.05$. (b) Decrease in chirp number from the initial to the final two presentations of the carrier alone as a function of focal weight; $P<0.05$. (c) Weight of males that decreased vs. those that increased their LoC responses to LoC stimuli relative to the carrier alone; *$P<0.05$. Each inset shows a typical example of each response pattern across the four stimuli (categories as in Fig. 3.1).

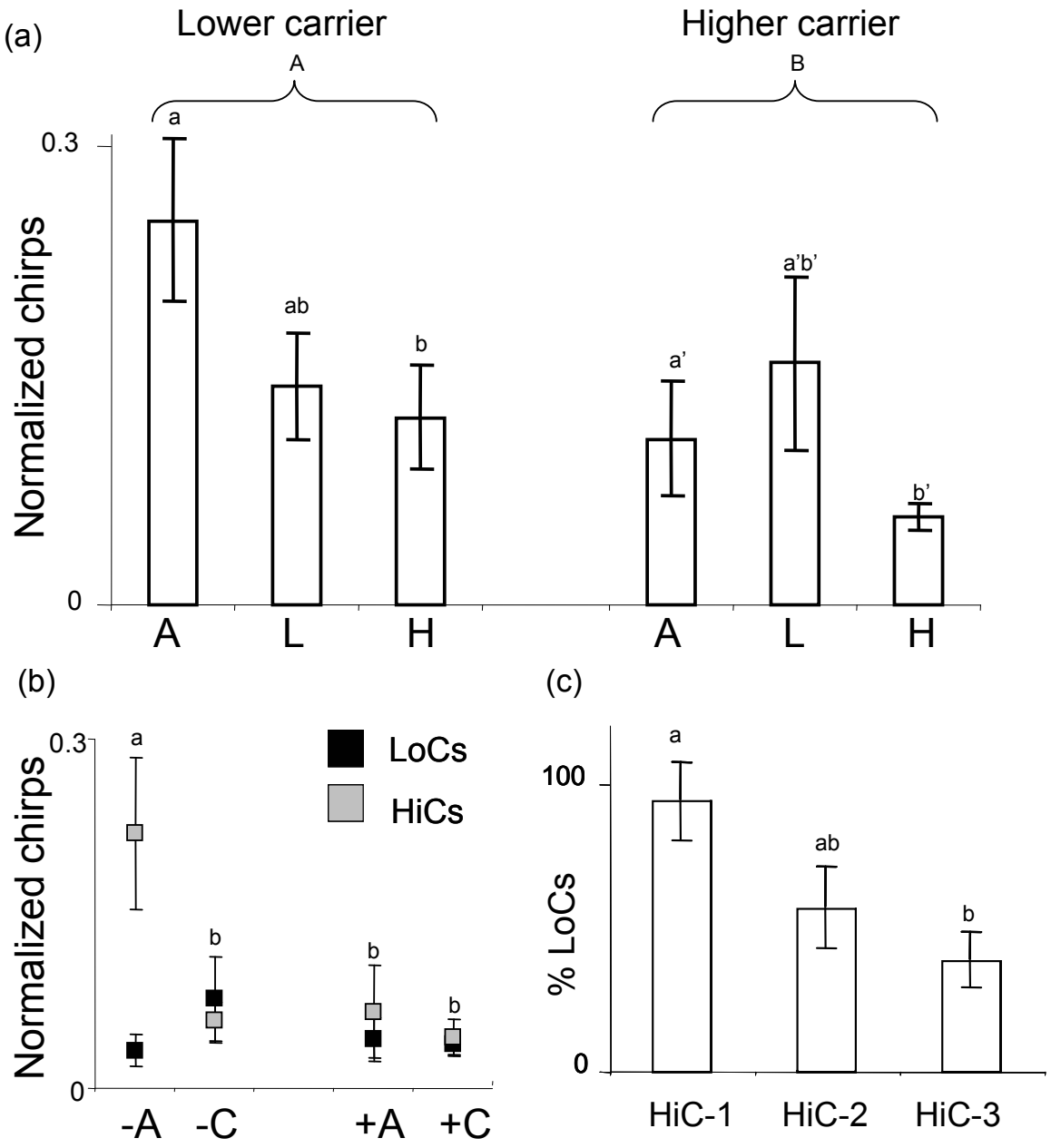

Figure 3.3. Male (N=6) chirp responses to home tank playbacks of simulated intruders (50Hz lower and higher than the focal's EODF). (a) Normalized total chirp responses to carriers alone (A) or carriers containing LoCs (L) or HiCs (H: responses to all HiC stimuli combined). Comparisons were made within each factor (carrier frequency and stimulus type, Scheffé: $P<0.05$). (b) LoC (*dark* □) and HiC (*light* □) emissions to lower (-) and higher (+) carriers alone (A) or with chirps superimposed (C: responses to all chirp stimuli combined; Scheffé: $P<0.05$ for HiC responses only. (c) LoC emissions (as a percentage of total chirps) to the three HiC stimuli, pooled for both carriers. Groups that were statistically nonsignificant share a common letter (Scheffé: $P<0.05$).

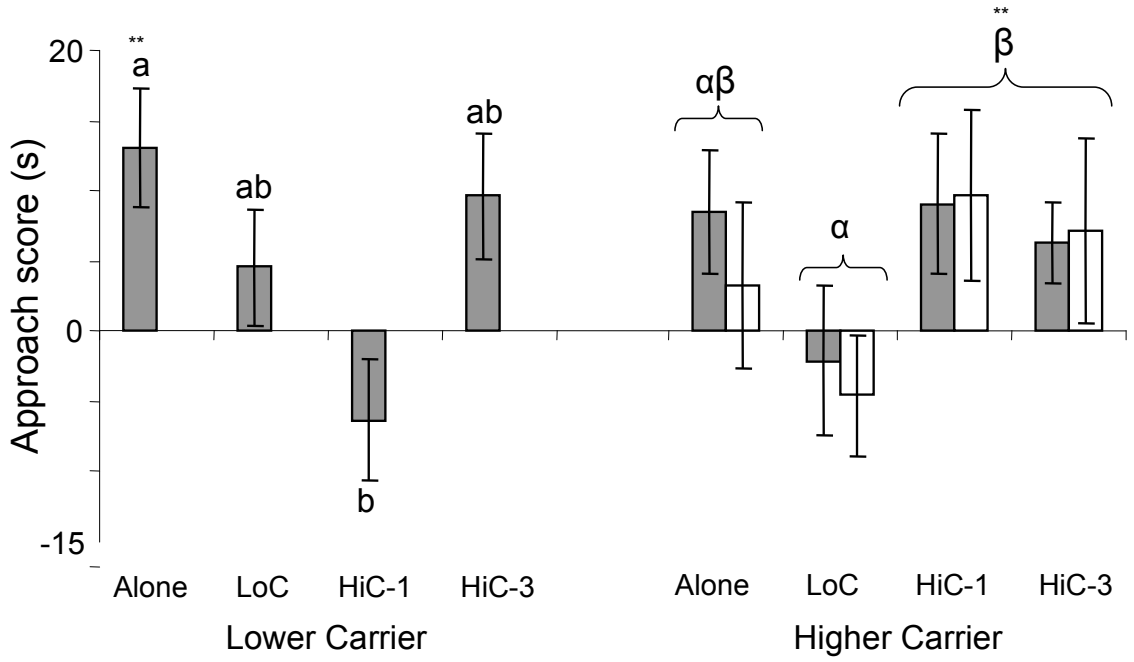

Figure 3.4. Results of playbacks in a novel arena. Approach scores (approach – avoidance times) for the four stimulus treatments on 50Hz higher and lower carriers for males (filled bars, N=13) and higher frequency carriers only (1000Hz) for females (open bars, N=7). Analysis 1 of male responses: only the lower carrier main effect was significant. Groups that were statistically nonsignificant share a common letter (Sidak: $P<0.05$). Analysis 2 of males and females: brackets indicate consolidated variables after nonsignificant ANOVA. Groups that were statistically nonsignificant share a common Greek letter (Sidak: $P<0.07$). Additional analyses on true approach/avoidance: **$P<0.006$ for paired t-tests on approach vs. avoidance times. See text.

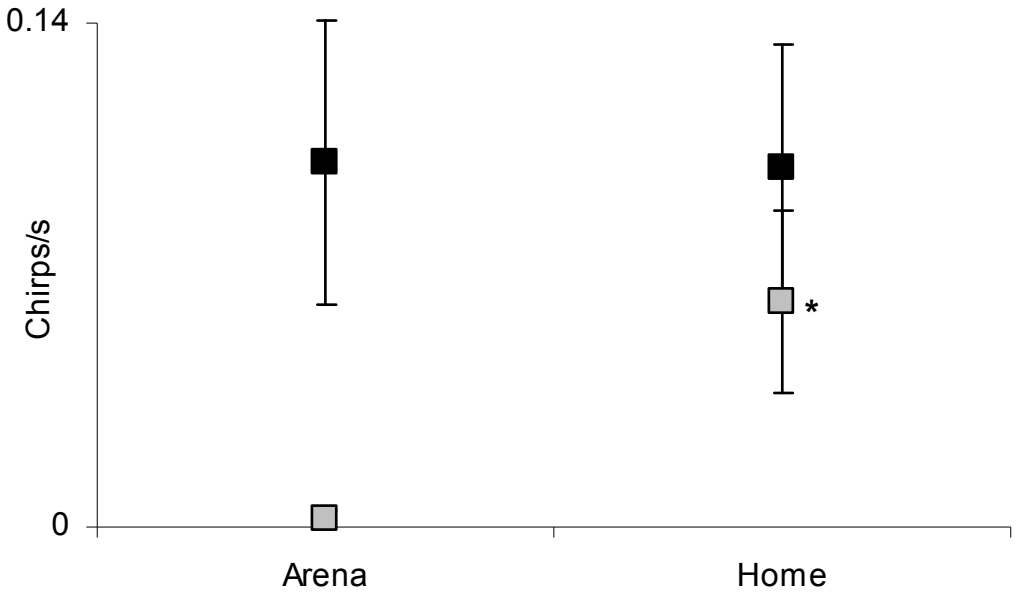

Figure 3.5. Comparison of male LoC (*dark* □) and HiC (*light* □) emissions in the novel playback arena and the familiar home tanks. *P<0.05 for HiC emissions only.

CHAPTER 4

Signals during agonistic interactions

Abstract. Signals emitted preceding and during combat have been proposed to aid in sequential assessment of opponent quality and competitive motivation. In many communication systems, signals that convey aggressive and non-aggressive motivation are antithetical in structure. During intrasexual encounters males of the brown ghost knifefish, *Apteronotus leptorhynchus*, modulate the frequency of their electric organ discharge (EODF). Decades of research have corroborated the hypothesis that abrupt EODF increases ("chirps") signal aggression and dominance, whereas the antithetical, more gradual frequency rises (GFRs) indicate submission or subordination. This closer analysis of modulations during male combat reveals that winners ultimately emit more chirps and less GFRs than losers. However, just prior to and during negotiations involving physical contact, aggressors gradually rise in EODF significantly more than receivers of an attack. I discuss the paradox of an aggressor producing seemingly submissive signals in anticipation of an assault.

Combat embodies the risk of costs in the form of physical injury or even death. This imposes a conflict of interest for rivals competing over a resource. Ideally, rivals would assess each other's body size to determine rightful ownership of the resource without incurring the costs of fighting over it. Game theoretical models of competitive behavior have generated predictions about how much and how long rivals are likely to signal given asymmetries in their resource-holding potential (RHP), i.e. their strength, fighting ability or motivation. These models differ in their assumptions of whether one's decision about how much to invest in a contest is made prior to (war of attrition) or during the interaction (sequential assessment; Maynard-Smith 1982, Enquist & Leimar 1983). To the extent that information accrued during the interaction with another influences subsequent interaction dynamics and contest duration, the sequential assessment model predicts that individuals closer in quality will require more time and escalation than those between whom there are obvious differences in RHP.

Some work has also addressed the form these signals are expected to take (e.g. Morton 1977, Hurd et al. 1995). Darwin was the first to note that signals conveying orthogonal states, such as aggression and submission, often occupy opposite extremes of some variable in signal space, presumably reducing receiver error (Hurd et al. 1995). Thus, aggressive signals which serve to invite conflict by indicating readiness to fight differ unequivocably from submissive signals which indicate to a receiver that the opponent is not inclined to engage in what presumably would be a losing battle.

Previous studies have examined agonistic behaviors in *Gnathonemus petersii* (Bell et al. 1974, Crockett 1986, Moller 1995, Terleph 2004) and in *Gymnotus carapo* (Black-Cleworth 1970). My study is the first on male waveform gymnotiforms to examine how electric signalling changes with escalation during dominance negotiations. During dyadic interactions and when played mimics of conspecific EODFs, fish produce modulations of categorically distinct frequency excursion, duration and onset slope. Signals with relatively rapid on- and offset (and variable frequency excursion and duration) are termed “chirps”. Playback stimuli of similar frequency to an individual’s EODF elicit bursts of ~15ms, ~60Hz “LoC” chirps that are hypothesized to function in intrasexual aggressive communication (aka type II chirps, e.g. Engler et al. 2001, Triefenbach & Zakon 2003). More gradual frequency rises (GFR’s) of much lower frequency excursion but often longer duration have been presumed to function in signalling submission (Hopkins 1974, Serrano-Fernandez 2003, Triefenbach & Zakon 2003). However, recent evidence suggests that GFRs are proactively emitted in aggressive contexts (Tallarovic & Zakon in press).

I tested the hypothesis that chirps and rises signal aggressive and submissive states, respectively, and that the state signalled varies temporally with information transfer over the course of dyadic interactions. I predicted size asymmetry to affect contest duration and signal types to correlate with the

variations in competitive motivation of each combatant at each stage. Thus, I expected increasing polarization in signal emissions for each individual, as status (relative quality) of the combatants becomes increasingly determined through negotiations.

METHODS

Setup

I measured lengths, weights and EODFs of 14 brown ghost males that had been isolated in their home tanks (dimensions: W23cm x H20.5cm x L49cm, temperature: 26±2°C; conductivity ~400 μS) for several months. Randomly selected pairs of fish (N=7) were simultaneously transferred from their home tanks to a neutral arena, a rectangular 70-L aquarium (W30cm x H20cm x L60cm) containing water from their home tank system. Individuals were allowed to interact across a plastic mesh barrier dividing the tank into 2 halves (Tallarovic & Zakon, in press) for 5-10min before the barrier was removed and replaced by an opaque plastic tube, a preferred shelter type (Dunlap & Oliveiri 2002).

Interactions

Five of the seven dyadic interactions were filmed with a Sony PC-9 Mini-DV camera, and electrical activity was recorded at a 22,050Hz sampling rate (16-bit) with a PC (Cool Edit, Syntrillium) through a pair of Ag electrodes spanning the length of the tank. To later synchronize the video and audio tracks, I filmed the monitor screen showing the audio timeline (in 30ms/frame bouts) at the beginning and end of a trial and imported video and audio into Vegas Video (Sonic Foundry). Here I matched the times displayed on the filmed computer screen with the time of the computer-recorded audio track.

I recorded the time opponents spent within 10cm of each other in both parallel and anti-parallel orientation and divided the number of chirps emitted by both (see Signal analysis below) by total time in each orientation to obtain the chirp rates in Fig. 4.2c. I also scored the time more than 50% of any individual's body occupied the tube shelter and defined the winner as the individual who spent at least 10s and an order of magnitude more than the other in and/or within 10cm of the tube, without being approached. Often, during this phase, the winner would chase and viciously attack the loser, at which point I terminated the trial. I describe some of the behaviors observed during the interaction below. For the analyses in Figs. 4.3 & 4.4, four 3-min phases were defined: "Barrier" refers to the interaction right before the mesh barrier was removed; "1" refers to the ensuing 3-min period; phases "2" and "3" are the periods right before and right after a winner was declared (Fig. 4.3d).

Signal analysis

EODs were visualized on a spectrogram. For signal analysis I first created two copies of the original audio file, one for each focal individual. I then bandpass-filtered out all but the range of frequencies encompassed by the focal EOD's 2nd harmonic. Examining the 2nd harmonics made visual inspection easier and, compared with the first harmonic, gave twice the range to filter out around each focal EODF. Occasionally, when one fish jammed the other's EODF, the combatants were so close in frequency that individual identification was impossible, rendering those data points unavailable. Chirps were recognized (see Triefenbach & Zakon 2003) and tallied in 5-s bouts. Estimates of baseline frequency shifts (GFR emissions) were made in three different ways. Figure 4.1 depicts, in 5-s bouts, the number of frequency shifts faster than 1Hz/s. GFRs in Fig. 4.3b were inferred by measuring EODF every 10s and calculating the standard deviation of those data points for each 3-min phase (defined above, Fig. 4.3d). For the quantification of GFRs in Fig. 4.4, I divided the 2-minutes before and after winner determination into 12 20-s bouts and measured the time and

difference between the maximum and minimum frequencies in each bout. If the highest frequency in a bout occurred later than the lowest frequency, the difference was considered a “rise”; conversely, if it occurred earlier, I assigned a negative value to the frequency decline. Fig. 4.4c reflects the number of bouts featuring a net rise.

For the analyses in Figs. 4.5 & 4.6, I only considered combat bouts that were separated from one another by at least 10s. To calculate frequency increase rates, I first measured both combatants’ EODFs at six points: 10s and 5s before and at the point of physical contact, at the point of combat termination, and 5s and 10s thereafter. For “frequency increase rate”, I subtracted the frequency at the end of a particular bout from its initial frequency and divided that difference by the duration of the bout. Thus, e.g., the “-5” bout in Fig. 4.5a is comprised of the mean of time-normalized differences between the frequency at combat onset and the frequency 5s before onset. Each individual’s data for each fight bout were averaged (N=10); Fig. 4.5 reflects the mean of those averages.

Statistical analysis

I used paired t-tests to compare winners and losers on variables of quality (size and EODF). For all analyses of responses over time (i.e. across phases of the interaction) I performed a repeated-measures ANOVA, with phase as the within-subject variable. Winner/loser was the between-subject variable in all cases except for the fine analyses around combat bouts (Fig. 4.5), for which I pooled the data for all individuals. In addition here, although I show the means of all groups, for the purpose of analysis I averaged the data for the 5s and 10s bouts for both phases (before and after contact), reducing the number of levels for the ANOVA to three. If ANOVAs were significant, I followed up either with a Sidak test for comparisons of all pairs (Fig. 4.5), or in the case of Figs. 4.3 & 4.4, with two sets of paired t-tests: each opponent’s response between the “phases” before and after winner determination (all “before” responses pooled), and

between individuals in each of those two “phases”, to comprise a total of four comparisons. Statements of significance for the latter comparisons take into account a Bonferroni correction (α=0.0125).

RESULTS

From the point of introduction to the neutral arena, even before physical contact was possible, all opponents signalled with GFRs and chirps across the barrier. Prior to barrier removal, emission rates of both types of signals did not correlate with any measure of quality (size, EODF or ultimate victory) for either opponent, but were significantly correlated with each other among all males (R^2=0.41, $F_{1,9}$=5.54, P=0.047). Thus, for any given individual, GFR rate was correlated with chirp rate.

Upon barrier removal, all pairs of males displayed and fought for access to the tube shelter. Figure 4.1 shows an ethogram depicting the time course of opponent tube occupancy, physical contact (i.e. when fish were within 1cm of one another), and the numbers of chirps and rises in 5-s bouts (see Methods). Superficial inspection suggests that most chirping occurred while one or both opponents were in the tube and that individuals appear to chirp less and emit more rises during contact. During the interaction, opponents engaged in multiple physical display and contact behaviors, often oriented either parallel or anti-parallel to one another (Fig. 4.2a & b). Opponents spent similar amounts of time in parallel (X±SE: 24±3%) as in anti-parallel orientation (X±SE: 23±3%; t-test: t_8 = -0.24, P=0.81), but chirped more when in anti-parallel orientation, with their tails near each other’s heads (t-test: t_8 = 3.53, P=0.008, Fig. 4.2c). Prior to physical contact in the form of nipping the other’s tail or engaging in a mouth-locking wrestle (Fig. 4.2d2 & e2), opponents often quivered their heads, jaws agape, the rest of their bodies in an otherwise stationary position (“jaw quivering”; Fig. 4.2d1 & e1). Throughout the interaction fish would attempt to clamp the opponent’s

head with their large jaws and wrestle the opponent around the arena ("head clamp", Fig. 4.2f1 & f2). A hitherto undescribed behavior that caught my attention was what I call a "tail sting" (Fig. 4.2g). Here the actor slowly approaches the receiver with its curved, probing tail and, just before contact, rapidly curves the tail even further and "flicks" it upward and away from the receiver.

Fight duration (time from barrier removal to winner declaration) was longer the more similar opponents were in length (R^2=0.81, $F_{1,4}$ =12.02, P=0.04, Fig. 4.3a), consistent with predictions of the sequential assessment model. Not surprisingly, larger males won more fights, and length was the best predictor of victory: the winner was significantly longer in 6/7 fights (X±SE winners: 21.9±0.3cm; losers: 20.4±0.6cm; paired t-test: t_4=2.93, P=0.016), but not significantly heavier (X±SE winners: 23.6±1.3g; losers: 22.0±1.0g; paired t-test: t_4=1.53, P=0.18). Unlike in other studies (Zakon & Dunlap 1999, Triefenbach & Zakon 2003), EODF did not correlate with measures of either length or weight, and winners were higher than losers in only four fights and did not differ in EODF (X±SE winners: 858±18Hz vs. losers: 847±15Hz; paired t-test: t_4=-0.59, P=0.29). However, whereas my range of EODFs (773-859Hz, coefficient of variation=3.5) was comparable to that of Zakon & Dunlap (1999: 900-1030, c.v.=4.4), my range of body sizes (19.7-24.7g, c.v.=9.1) was considerably lower than theirs (5-35g, c.v.=60.2). Considering that males in a given population can differ by more than 100Hz and 30g, the comparatively small differences between winners and losers in EODF and size (mean differences: 22.9±5.7Hz, 3.2±1g; ranges: 6.1-40Hz, 0.6-6.5g) are a likely explanation for the lack of a relationship between EODF and size/victory in my study.

Both individuals emitted GFRs and chirps throughout their interaction, and both types of modulations increased over time for both individuals. Chirp rate differed significantly between winners and losers (ANOVA: $F_{1,8}$=8.50, P=0.019, Fig. 4.3c) and among phases (ANOVA: $F_{3,24}$=6.87, P=0.002, Fig. 4.3c). Whereas in phase 3 winners chirped more than losers, GFR emissions tended to be higher for

losers, consistent with the “antithesis” hypothesis that GFRs are submissive and chirps dominance displays, although differences between individuals (ANOVA: $F_{1,8}$=1.72, P=0.23, Fig. 4.3b) and among phases (ANOVA: $F_{3,24}$=1.78, P=0.17, Fig. 4.3b) were not significant. Using another measure to quantify rise emissions (see Methods), I showed that rises emitted by losers were significantly higher than those of winners (ANOVA: $F_{1,8}$=7.23, P=0.028, Fig. 4.4b). No difference between opponents could be detected in rise frequency excursion during the interaction, i.e. before winner determination. The number of 20-s bouts wherein a net rise was found (i.e. where the maximum frequency occurred after the minimum frequency) was also greater for losers after winner determination (ANOVA: $F_{1,8}$=5.41, P=0.049, Fig. 4.4c).

As suggested above, all interactions eventually escalated to physical combat, defined as the occurrence of head butts and mouth wrestling. Figure 4.5 shows GFR and chirp rates before, during and after bouts of combat. Males continuously rose in EODF before and during combat and reached a peak after the fight that differed significantly from the EODF before fight onset (ANOVA: $F_{2,18}$=3.77, P=0.043, Fig. 4.5a). Chirps were emitted significantly less during than before and after a fight (ANOVA: $F_{2,18}$=4.79,P=0.022, Fig. 4.5b). Thus, chirps appear to be displays made around combat bouts, whereas GFRs were made predominantly during combat. Interestingly, both combatants commenced their frequency rises in anticipation of physical contact.

Winners initiated 68% of all attacks (average percentage from all trials of approaches winners made that lead to an ensuing fight: t_8=-1.93, P=0.09). A closer analysis of pre-contact GFR behavior revealed that aggressors preceded attacks by signalling with GFRs more than receivers. Fish tended to increase their EODF between 10s and 5s before a fight (Fig. 4.5a). I calculated the difference between initiators and receivers in this increase. Initiators showed a greater increase than receivers during that bout than vice versa (X^2_1=3.85, P=0.050, Fig. 4.6). Thus, GFRs significantly predicted aggressive behavior by the

initiator of attack. Initiator EODF was lower in only 46% of attacks (t-test: t_{28}=-0.37, P=0.36), and thus the pre-combat frequency increase differences cannot be be simply accounted for by the aggressor attempting to jam the opponent.

DISCUSSION

To minimize the probability of physical combat when competing over a resource, assessment of opponent RHP features (such as size, fighting ability or motivation) should be optimized. If assessment of such features allows the outcome of fights to be predicted even slightly above chance, display signals emphasizing such features should evolve (Parker 1974). When competitors are similar in RHP, assessment is likely to be less certain, and more signalling and escalation are expected to be required. As predicted from the sequential assessment model, size asymmetry inversely correlated with contest duration. Interestingly, length, not weight asymmetry, was a more predictive assessment variable. The electric organ in *Apteronotus* is comprised of neural electrocytes, arranged in series in the tail, and EOD amplitude is a function of the number of electrocytes in series (Hopkins 1999, Franchina & Stoddard 1998). Since this number is more likely to vary with the length of the fish's tail than with total body weight, this could account for the increase in assessment duration when fish were closer specifically in length. It is thus possible that rivals were using EOD amplitude as a reliable assessment variable.

Throughout the interaction, males engaged in several physical contact behaviors, some of which, like head butting, head clamping and mouth wrestling, presumably lead to a good assessment of strength. As fights escalated to include more bouts of physical contact, electrical activity increased as well. Although chirps were emitted during all behavioral states after barrier removal, fish chirped the most while in antiparallel orientation, when their tails were closest to the opponent's head. As electroreceptor density is highest in the head region (Zakon

1986), signallers thus seem to be maximizing their communication efficacy. Reciprocally, because the tails are the signal sources, increased chirp rate by the receiver is also consistent with previous work demonstrating that increasing playback stimulus amplitude elicits more chirps (e.g. Engler et al. 2000).

Dunlap (2002) did not find any correlation between chirp rates of males in dyads (over a window of 15s). I also did not find any indication that an individual's chirp rate was influenced by the opponent's chirp rate before barrier removal, but as physical interactions escalated, there was a clear increase in the difference between chirp rates of winners and losers, at least at the level of the overall population of dyads (Fig. 4.3c). Once the conflict was resolved, i.e. after winner determination, there was a significant difference between winner and loser chirp rates, corroborating the hypothesis that chirps serve as aggressive/dominance signals (Hagedorn 1985).

Conversely during that same period, especially while winners were chasing losers, losers made more GFRs than winners, consistent with the submissive role proposed for GFRs (Hopkins 1974, Serrano-Fernandez 2003, Triefenbach & Zakon 2003). However, while contestants were negotiating dominance, GFR paralleled chirp emissions in that both types of signals increased with escalation during initial contact for both contestants. Thus, whereas the role of chirps was consistent throughout the trials, I showed here that rise semantics seem to be context-dependent. While they appear to signal submission after a winner has been determined, in that losers make more and greater frequency excursions (Fig. 4.4), they are clearly used in a directly aggressive context as well, when, for example, attack initiators emit them in anticipation of an assault and continue to do so during a fight.

There are several possibilities to explain increased GFR emissions by both opponents during combat. One is that the raising of frequency during contests might function in appearing more dominant. In frogs, where call frequency is

inversely correlated with size, males lower their frequency in the presence of other males (e.g. Wagner 1989). In brown ghosts, where a fish's size is often positively correlated with EODF, raising frequency might similarly serve to inflate a fish's perceived quality.

Gymnotiforms have long been studied for a classic sensorimotor adaptation to prevent signal interference known as the jamming avoidance response (Bullock et al. 1972). When presented with a stimulus frequency close to their own, focals shift their EODF away from that of the stimulus to prevent it from jamming, i.e. interfering with, their electrosensory system (Heiligenberg 1973). After performing a jamming avoidance response, EODF can remain elevated for a period of up to several minutes (long-term frequency elevation, or LTFE: Oestreich & Zakon 2002). Often upon stimulus offset, fish perform "yodels", a type of GFR suggested to represent a "victory cry" or a release from stress (Dye 1987), that features LTFE. The functional relevance of LTFE is unclear, but may reflect a bias to occupy a higher frequency in signal space after a stimulus (such as a fleeing fish) has disappeared. The general tendency to shift to higher frequencies in *Apteronotus* may thus in the long term result in a resetting of the pacemaker/electric organ's oscillation frequency to one more appropriate to a fish's recently negotiated position in a hierarchy.

Tallarovic & Zakon (in press) recently discovered a phenomenon which complicates the scenario of this classic signal interference "avoidance" model. In staged dyadic same-sex interactions, the lower of two contestants often raised their EODF to actively jam the opponent's EOD. Of all the fights I examined, active jamming occurred consistently in only one dyad, wherein fish were less than 10Hz apart. Although I cannot rule out the possibility, it is unlikely that GFRs in my study only reflected attempts of the lower frequency individual to jam the higher one, as attack initiators were lower than receivers less than half of the time. Thus, the occurrence of pre-combat frequency rises cannot be sufficiently explained by jamming attempts.

There is some evidence from playback studies that chirps and GFRs both signal threat. Triefenbach & Zakon (in prep) showed that males approached carriers with chirp mimics less than unmodulated carriers, and Tallarovic & Zakon (in press) noted that fish made GFRs just before attacking a playback electrode. Furthermore, when the authors presented focals with EOD mimics 15Hz higher and lower, with or without a 15Hz rise superimposed, they found that fish attacked electrodes broadcasting the lower carrier alone but showed significantly less aggression to the same stimulus when it contained the (eventually jamming) rise. They found no difference between the amount of aggression toward the higher carrier and toward that carrier with a rise, leading them to conclude that jamming specifically, not rising per se, inhibited focal aggression. However, it is perhaps worth noting that although subjects in their study did not significantly reduce the aggressive responses measured when a GFR were added to the higher carrier, responses to the unmodulated higher carrier were significantly lower than to the unmodulated lower carrier to begin with. It is quite possible that their response measure captured a basal level of aggression to the higher carrier alone, beyond which there was little to diminish. Thus, the possibility remains that GFRs in general, whether they jam the focal or not, have an aggression-inhibiting effect.

Perhaps alternatively, GFRs may function to coordinate fighting behavior. Due to the potentially high costs of combat, the interests that both rivals are expected to share in assessing each other accurately without injury should not be underestimated; thus rules of cooperation are not unexpected. Hurd (1997) showed in a cichlid (*Nannacara*) that specific visual displays preceded unbluffable performance displays (lateral displays and mouth wrestling). He concluded that these anticipatory indicators served to coordinate subsequent direct assessment behaviors, and as such indicated elements of cooperation in combat bouts. Similarly here, GFRs might be used to allow for a more seamless transition to tests of strength, like head butting/clamping and mouth wrestling. As

EOD is used for electrolocation, i.e. orientation, it is possible that GFRs are merely increases in sampling rate required to better approach the target neighbor.

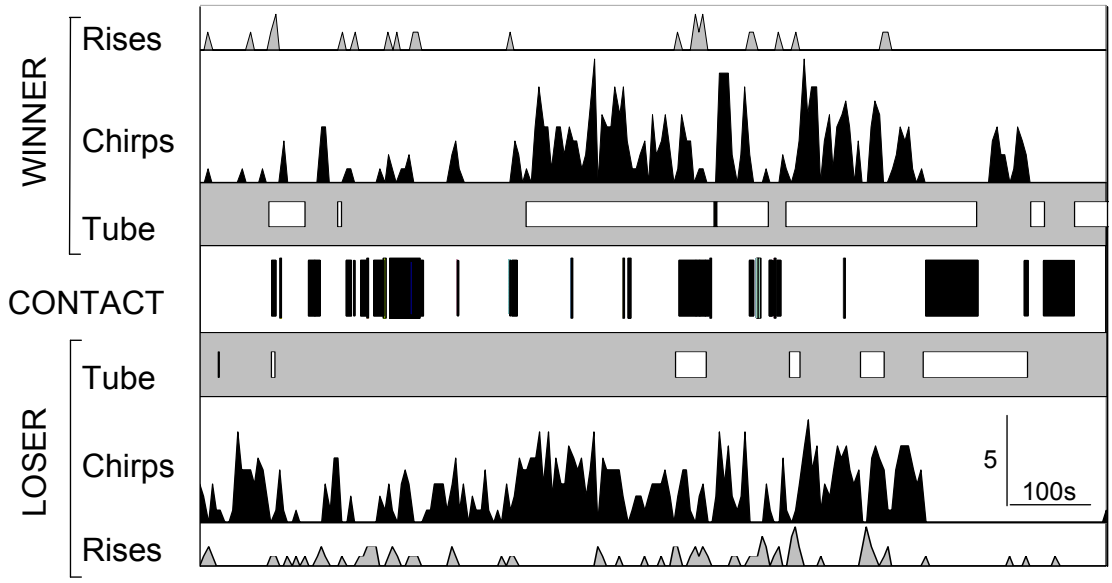

Figure 4.1. Ethogram of a typical male brown ghost interaction after barrier removal, depicting the numbers of chirps and rises in 5-sec bouts, time both opponents spent occupying the tube, and time spent within 1cm of each other (CONTACT).

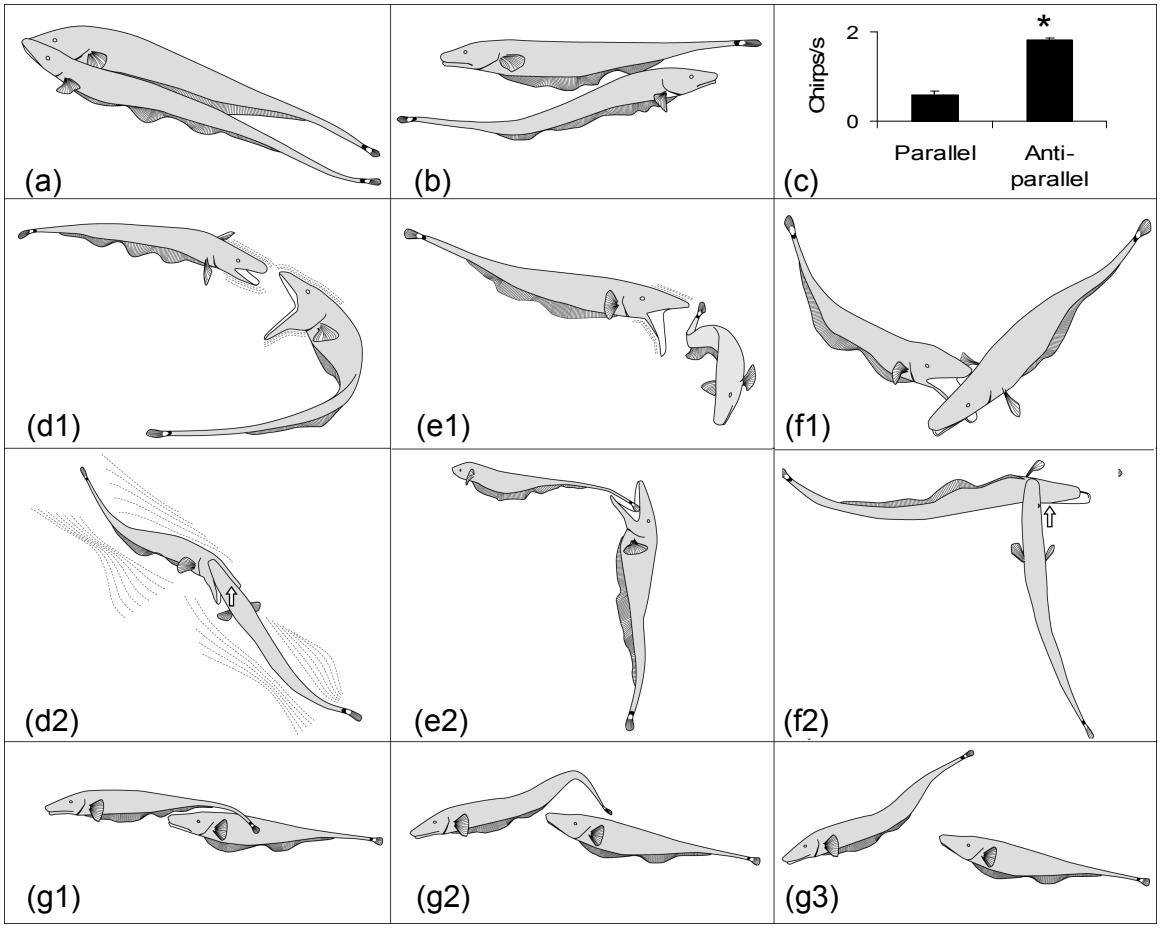

Figure 4.2. Threat and fight behaviors. (a) Parallel and (b) anti-parallel orientation, during which (c) the greatest exchange of chirps occurred. *P<0.05. (d1) & (e1) Jaw quivering in anticipation of (d2) mouth wrestling and (e2) tail nipping. (f1) Head clamp: a fish grasps the opponent's head and (f2) pushes it around. (g) Tail sting: sequence depicting the movement of the aggressor's tail.

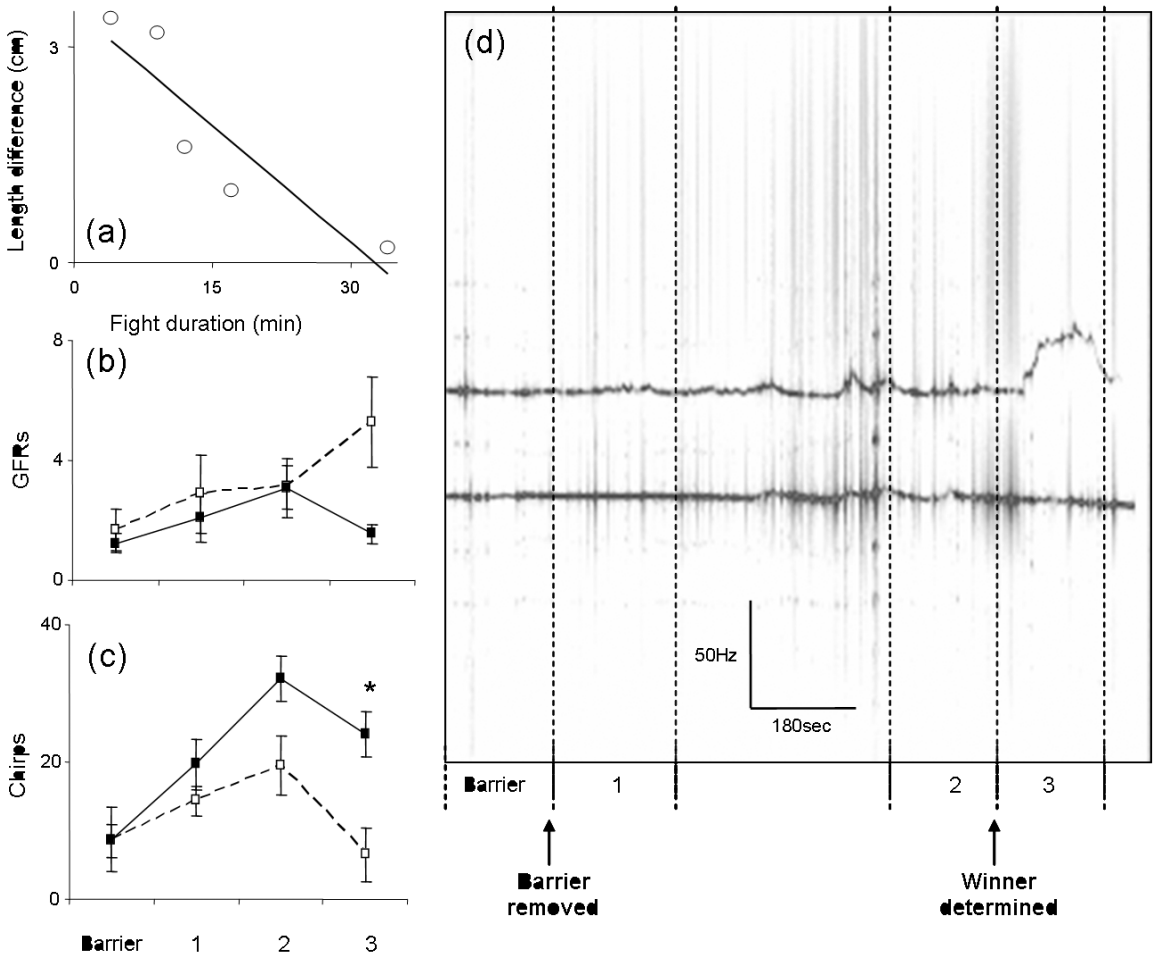

Figure 4.3. (a) Fight duration as a function of opponent size difference (N=5). (b) GFR and (c) chirp emissions for all interactions between eventual winners (filled squares) and losers (open squares) during the four 3-min phases (see Methods). *P<0.0125 between winner and loser. (d) Spectrogram delineating the four phases of an encounter where the higher-frequency male eventually lost. Note the increase in chirp emissions (grey vertical lines) by the winner and the large GFR by the loser in phase 3 of the interaction.

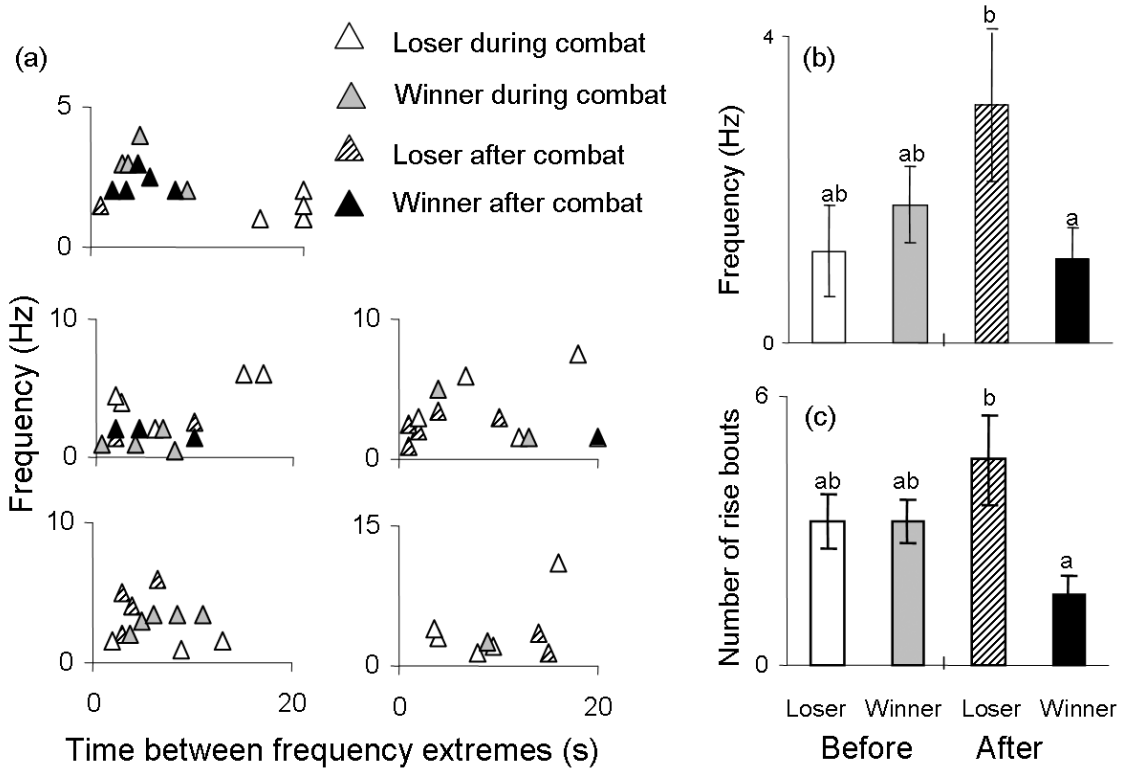

Figure 4.4. Characteristics of GFRs emitted during phases 2 and 3 (right before and right after winner determination). (a) Plots of difference vs. time between maximum and minimum frequencies per 20-s bout for all five dyadic interactions (see Methods). (b) Mean differences between frequency extremes. (c) Numbers of 20-s bouts where the highest frequency occurred after the lowest frequency, for winners and losers before and after winner determination. Groups that were statistically nonsignificant share a common letter.

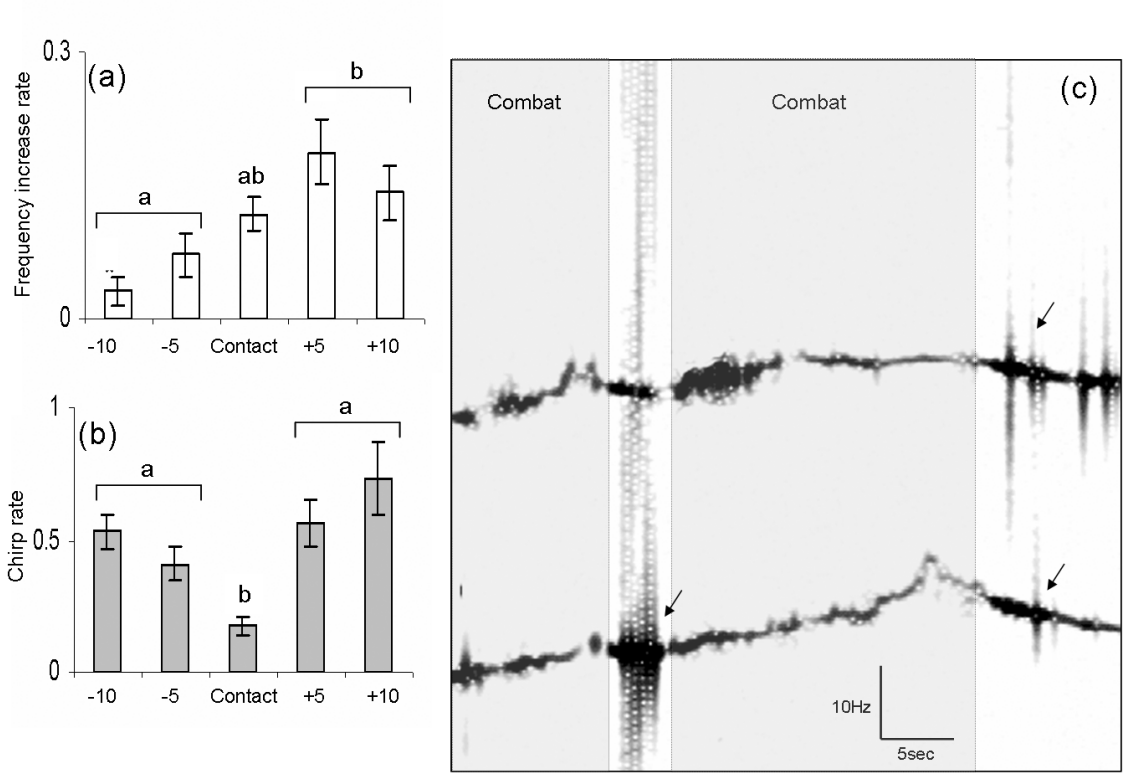

Figure 4.5. EOD modulations before, during and after combat (head butt and mouth wrestle). (a) Frequency increase and (b) chirp rates during and 5 and 10 sec before (-) and after (+) physical contact; (-) and (+) bout pairs were consolidated for analysis (see Methods). Groups that were statistically nonsignificant share a common letter. (c) Spectrogram delineating when fish are and are not in physical contact (grey and plain areas, respectively). Arrows indicate bouts of chirps. Note the frequency increases and absence of chirps during combat bouts. The lower frequency male in this trial initiated both combat bouts and were ultimately the winner.

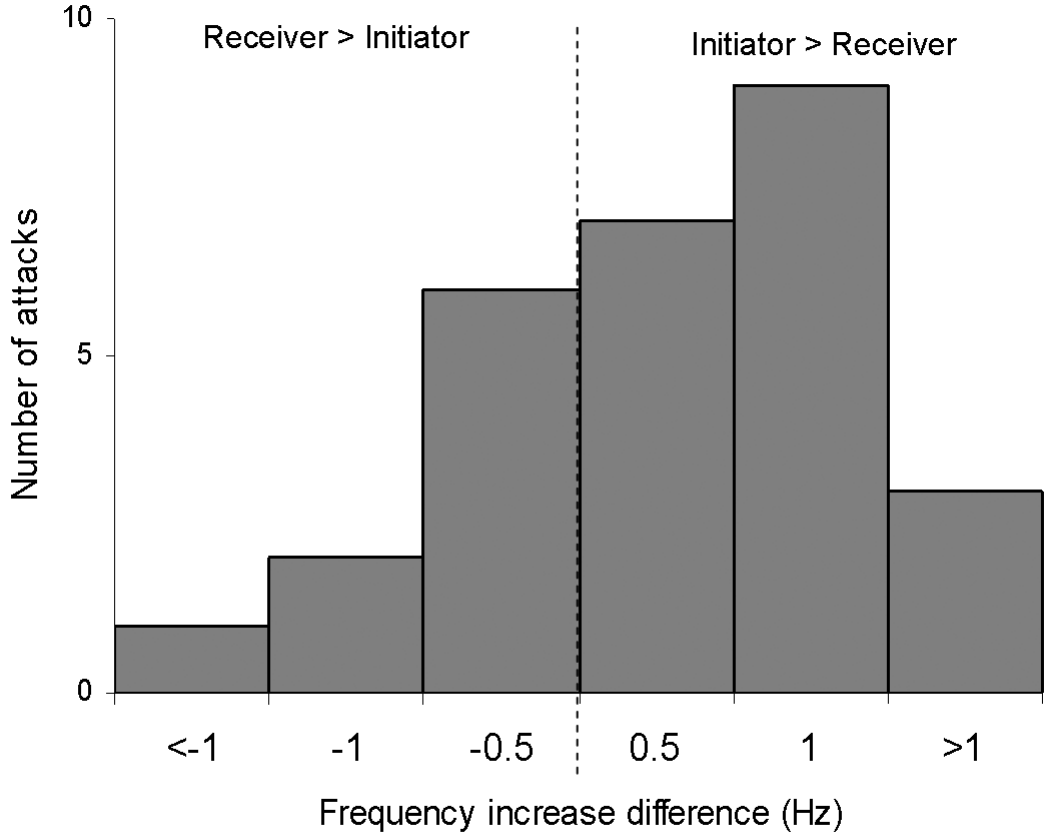

Figure 4.6. Distribution of differences (between the initiator of combat and the receiver of an attack) in frequency increases between 10 and 5 sec before the onset of physical contact. Initiators increased in frequency more than receivers during an approach leading to physical contact.

CHAPTER 5
General discussion

In the preceding chapters I showed that the electric organ discharge and its modulations carry information about sex and quality, and that an individual's own sex and quality influenced their recognition. Judging by receiver responses, EOD signals appear to be reliable indicators serving in sex and individual recognition.

Many examples of signal characteristics suggest coevolved matches between senders and receivers. Males made more LoC-type chirps to stimuli near their own EODF, i.e. in the male range, which is higher than that of females. As LoCs feature energy only at higher than basal frequencies, LoC-eliciting stimuli and the signal's active space are well matched, corroborating the hypothesis that they function in intrasexual communication. In contrast, males made HiCs to more distant stimulus frequencies. HiCs feature considerably more energy at frequencies both above and below the baseline EOD, which (as for LoCs above) corresponds to the stimulus frequencies which best elicit them, namely much higher (dominant male) and lower (female) frequencies. Thus it appears that males adjust their signal active space according to the type of receiver they encounter by altering the emitted chirp type. Female signals also feature characteristics that could increase their probability of detection by males. Their second harmonic has more energy than their fundamental and corresponds to a frequency range to which males show increased sensitivity (Chapter 2).

I also demonstrated that individuals show recognition of not just relative but absolute stimulus quality. Males chirped most at lower frequency (smaller male) mimics. To the extent that chirps are aggressive, the data suggested that individuals recognize lower frequencies as less threatening, and that this recognition is not merely relative to their own frequency, but also reflects an "understanding" of a given focal's place in the "hierarchy" of the male frequency

range. The correlation between chirp propensity and body size in Fig. 3.2 (Chapter 3) corroborates this idea. As every fish received an identical set of non-reciprocating stimuli, the fact that chirp rate still correlated with size indicates an intrinsic difference between males of different sizes in their propensity to chirp, independent of their status relative to others.

Much theoretical and empirical work suggests that in order for signals to reliably indicate quality, they have to be costly in some way, such that weaker individuals cannot cheat and deter rivals unwarrantedly (synthesis by Hurd & Enquist 2005). If low quality individuals could/did make all the same signals and at the same rates as high quality individuals, those signals would quickly be rendered meaningless over evolutionary time. Index (Vehrencamp 2001) or performance signals (Hurd 1997) are those that are directly constrained by an individual's RHP (strength/fighting ability, motivation, aggressiveness) and are thus unbluffable. For example, the lowest frequencies in frog calls are achievable only by larger males, as only larger males have the capacity to harbor the larger vocal cords necessary to create vibrations of sufficiently long wavelengths. In contrast to performance signals, strategic signals are available to all signallers. Variation in signal use is a result of variation in the choice of, rather than the performance of, the signal (Maynard Smith & Harper 1988; Guilford & Dawkins 1995). Among strategic signals, handicaps are constrained by production costs, and conventional signals by increased likelihood of receiver challenge. Signal production costs that constrain their use to higher-quality individuals, i.e. their reliability, include physiological expenditure and risk of predation. In electric fish there is potentially an interesting additional cost unique to this system fish by virtue of the function of EOD in orientation.

Stoddard (1999) proposed that predation costs drove the evolution of higher EODFs in gymnotiforms. All weakly electric fish (and their predators such as electric eels and catfish) are endowed with primitive ampullary electroreceptors used in passive electrolocation which are sensitive to low-frequency DC signals

emitted by prey items. Whereas many pulse-type fish retain DC components in their EODs, wave-type gymnotid EODs are DC neutral. If chirp emissions contained a DC component, this would presumably be beneficial in stimulating female ampullary receptors during courtship (Stoddard 2002). Whether *Apteronotus* chirps contain a DC component is unclear, but recent evidence suggests that they do not (K. Dunlap, pers. comm.).

Very little work has been done on physiological costs of electric signalling, and has focused on pulse-type fish (Stoddard 2002). Hopkins (1999) suggested that whereas the energy of signalling should be proportional to discharge rate in male pulse fish, this is not true for wave fish, for any EODF changes are accompanied by a change in pulse duration in order to keep a more or less constant duty cycle. That is, whatever savings are obtained by discharging at a lower rate are negated by a longer duration of each EOD pulse. Preliminary measurements of pulse duration indeed show an inverse correlation with EODF (unpublished data). However, there is a different potential source of discharge cost. It stands to reason that signal amplitude is diminished at higher frequencies due to the inactivation of more sodium channels, whose availability is required to generate the action potential of the pulse. That is, of two same-sized fish, the one charging at the higher frequency should have a lower amplitude. To increase detection, that fish would have to compensate by adding electrocytes, i.e. increasing in size. Thus, a detectable signal of high frequency is more likely to indicate a large, high-quality male, and reliability of the signal is maintained.

Finally, there is a possibility unique to this autocommunicative (electrolocation) system whereby the production of chirps may be a functional handicap. Fish use the precision of the EOD for electrolocation and they detect objects by miniscule modulations of their own EOD amplitude and phase. During a chirp, EOD amplitude and frequency change so rapidly as to probably make object detection and orientation difficult, perhaps making signallers temporarily more vulnerable to attack. An emitter's own chirps should be more detrimental to electrolocation

than a neighbor's chirps, because its chirps are always louder and perceived all over the body, whereas a neighbor’s chirps influence only two patches of their body (where the current vector enters on one side and leaves on the opposite side). Other parts will be less contaminated by the other fish's EOD. Recent theory and empirical support suggest that threat displays are not expected to be handicaps, whereas courtship displays are (Szamado 2003, Hurd 2004). my results are at least consistent with this hypothesis in that the courting HiC chirps feature an even greater obstruction to the regular EOD cycles (longer, noisier, greater amplitude reduction) than the intrasexual LoC displays. However, perhaps chirps do not even qualify as “threat” displays, in that they do not appear to predict impending escalation. In contrast, GFRs do, and it will be interesting to determine how playbacks in which GFR parameters are varied in the presence or absence of chirps affect the perceived threat content.

APPENDIX: ANOVA TABLES

CHAPTER 2					
FIGURE RESPONSE	EFFECT	DF	MS	F	P
2.3a LoCs	Sex	1	10708	17.46	0.0009
	Subject	14	613		
	Stimulus range	2	7327	26.13	<0.0001
	Sex x Stimulus	2	4018	14.33	<0.0001
	Stimulus x Subject	28	280		
2.3b LoCs	Subject	9	0.315		
	Stimulus range	2	0.715	4.96	0.0192
	Residual	18	0.144		
2.3b HiCs	Subject	9	0.122		
	Stimulus range	2	0.314	12.877	0.0003
	Residual	18	0.024		
2.3c Rises	Sex	1	5.06	17.06	0.0009
	Subject	14	0.30		
	Stimulus range	2	1.25	11.62	0.0002
	Sex x Stimulus	2	0.52	4.78	0.0157
	Stimulus x Subject	28	0.11		
2.4a Chirps	Sex	1	12237	17.77	0.0012
	Size	1	29	0.042	0.842
	Interaction	1	150	0.218	0.649
	Residual	12	689		
2.4b Rises	Sex	1	0.0003	0.003	0.957
	Size	1	0.015	0.156	0.700
	Interaction	1	0.954	10.19	0.0077
	Residual	12	0.094		
2.4c EOD Frequency	Sex	1	60009	38.36	<0.0001
	Size	1	7054	4.51	0.0552
	Interaction	1	2742	1.75	0.210
	Residual	12	1564		
2.5a Male LoCs	Stimulus frequency	6	6052.6	4.64	0.0007
	Residual	54	1303.3		
2.5a Female LoCs	Stimulus frequency	5	343.06	5.41	0.0012
	Residual	30	63.45		
2.5b Male Rises	Stimulus frequency	6	0.58	1.84	0.108
	Residual	54	0.31		
2.5b Female Rises	Stimulus frequency	5	0.46	2.85	0.0319
	Residual	30	0.16		

CHAPTER 3					
FIGURE RESPONSE	EFFECT	DF	MS	F	P
3.1 LoCs	Subject	8	350.63		
	Stimulus type	3	70.68	11.34	<0.001
	Interaction	24	6.22		
3.1 HiCs	Subject	8	75.29		
	Stimulus type	3	4.04	1.37	0.28
	Interaction	24	2.95		
3.3a Total Chirps	Carrier frequency	1	0.047	5.44	0.028
	Stimulus type	2	0.022	2.57	0.097
	Interaction	2	0.013	1.51	0.242
	Residual	24	0.009		
3.3b LoCs	Carrier frequency	1	0.001	0.38	0.547
	Stimulus type	1	0.002	0.69	0.418
	Interaction	1	0.003	1.07	0.315
	Residual	18	0.003		
3.3b HiCs	Carrier frequency	1	0.038	5.08	0.0369
	Stimulus type	1	0.046	6.17	0.0230
	Interaction	1	0.027	3.60	0.0741
	Residual	18	0.007		
3.3c % LoCs	Stimulus (HiC type)	2	3064	5.32	0.030
	Residual	9	576		
3.4.1 Approach score (males)	Carrier frequency	1	1.16	0.005	0.942
	Subject	24	213.45		
	Stimulus	3	597.00	2.25	0.090
	Stimulus x Carrier	3	677.11	2.55	0.062
	Stimulus x Subject	72	265.60		
3.4.1 Approach low carrier (males)	Subject	12	280.69		
	Stimulus	3	929.08	4.07	0.0138
	Interaction	36	228.55		
3.4.1 Approach high carrier (males)	Subject	12	146.22		
	Stimulus	3	344.99	1.14	0.346
	Interaction	36	302.65		
3.4.2 Approach score (males vs. females)	Sex	1	3.70	0.017	0.897
	Subject	18	212.56		
	Stimulus	3	546.98	2.21	0.098
	Stimulus x Sex	3	56.09	0.23	0.878
	Stimulus x Subject	54	247.67		
3.4.2 Approach score (combined levels)	Subject	19	207.07		
	Stimulus	2	676.22	3.26	0.0495
	Interaction	38	207.69		
3.5 LoCs	Setting	1	<0.001	0.001	0.979
	Residual	25	0.017		
3.5 HiCs	Setting	1	0.024	4.27	0.0492
	Residual	25	0.006		

CHAPTER 4					
FIGURE TEST RESPONSE	EFFECT	DF	MS	F	P
4.3b GFRs	Winner/Loser	1	15.68	1.72	0.230
	Subject	8	9.11		
	Phase	3	7.40	1.85	0.165
	Winner/Loser x Phase	3	7.13	1.78	0.177
	Subject*Interaction	24	4.00		
4.3c Chirps	Winner/Loser	1	798.64	8.50	0.019
	Subject	8	93.93		
	Phase	3	505.91	6.87	0.002
	Winner/Loser x Phase	3	149.02	2.03	0.137
	Subject*Interaction	24	93.93		
4.4b Frequency	During/After	1	0.03	0.03	0.862
	Subject	8	0.80		
	Winner/Loser	1	5.05	7.23	0.028
	Winner/Loser x Phase	1	2.53	3.63	0.093
	Subject*Interaction	8	0.70		
4.4c Number of bouts	During/After	1	0.29	0.12	0.738
	Subject	8	2.39		
	Winner/Loser	1	6.96	5.41	0.0485
	Winner/Loser x Phase	1	7.10	5.52	0.0468
	Subject*Interaction	8	1.29		
4.5a EODF increase rate	Subject	9	0.025		
	Phase	2	0.041	3.77	0.0429
	Subject x Phase	18	0.011		
4.5b Chirp rate	Subject	9	0.21		
	Phase	2	0.57	4.79	0.022
	Subject x Phase	18	0.12		

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