The Effects of Social Interaction on Behavior and Electric Organ Discharge in Two Species of Mormyrid Fish: *Gnathonemus petersii* (Günther, 1862) and *Brienomyrus niger* (Günther, 1866), Mormyridae, Teleostei

by

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THE CITY UNIVERSITY OF NEW YORK

Abstract

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Thomas A. Terleph

Advisor: Professor Peter Moller

African weakly discharging electric fish (of the family Mormyridae*)* use their self-generated electric signals and electroreceptive abilities in orientation as well as social communication. This thesis investigated short-term changes in electric organ discharge (EOD) pattern, EOD waveform, and locomotor behavior during the course of dyadic social interactions in subadult fish. These changes were documented for two species of mormyrid fish, *Brienomyrus niger* and *Gnathonemus petersii*, during free and restricted interactions, when fish were prior residents or intruders, dominant or subordinate, and males or females.

During free interactions, EOD duration and EOD phase amplitude ratios increased in dominant fish. Similar patterns of dominance mediated EOD changes occurred in both sexes, and compared favorably with EOD data from 17α-methyltestosterone treated fish. Socially mediated changes reverted to pre-interaction levels when fish were returned to solitary housing conditions. Under restricted conditions, when neighboring fish were prevented from direct physical contact, the EOD parameters changed in both fish in a dominant-like fashion. Prior residence status led to dominant-like changes in *B. niger* in restricted interactions.

During free competitive interactions, fish often exhibit ‘parallel display’. During these displays two fish line up alongside each other and both rapidly discharge. The data suggest that this behavior serves an assessment function, permitting individuals to gauge each other’s body size.

Acknowledgements

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INTRODUCTION

The specific aims of this dissertation were: (1) To explore whether or not subadult mormyrid fish show socially induced changes in their electric organ discharge (EOD) in a manner comparable to that observed in adults, (2) to ascertain whether such changes differ between the sexes, and (3) to investigate the effects of controlled social interaction on the fish's EOD waveform, discharge patterns, and associated social locomotor behaviors.

Background

African weakly discharging electric fish (family: Mormyridae) use their self-generated electric signals and electroreceptive abilities for orientation as well as communication during social interactions. Social interactions may take many adaptive forms: aggressive/territorial behavior, courtship/mating, parental behavior, feeding behavior, schooling, and recognition of members of the same or other related species. Each of these functions has been studied extensively (reviews: Hopkins, 1986; Kramer, 1990, Moller, 1995; von der Emde, 1998). Social interactions involving electric signaling include breeding behavior (Bratton & Kramer, 1989; Crawford, 1991, 1992; Kirschbaum, 1987; Kramer, 1990a, b, 1994), territorial behavior (Hopkins, 1986; Kramer, 1990a; Kramer & Bauer 1976; Moller, 1995; von der Emde, 1998), and schooling behavior (Heiligenberg, 1976, Moller, 1976). There is ample evidence that EOD characteristics communicate sex and species affiliation (Crawford, 1991, 1992; Hopkins 1974a, 1983; Hopkins & Bass, 1981), but it is less clear whether EODs also convey information about individual identity or social status. The current experiments were designed to further investigate the role of the electric organ discharge (EOD) and select locomotor behaviors in two teleost species of the family Mormyridae, *Brienomyrus niger* and *Gnathonemus petersii.*

Electric signaling

Two aspects of the EOD in mormyrids have been studied. One is the waveform of the EOD (amplitude and duration of the individual discharge as well as the associated spectral characteristics of the Fast Fourier spectrum). The second is the rate and pattern of discharge activity referred to here as sequence of pulse intervals (SPI) (Hopkins, 1986). Each of these EOD characteristics is known to serve in communication. Unlike the centrally controlled SPI, the waveform (referred to here as the EOD) does not vary appreciably in the short-term. The EOD's communicative function is an expression of the fish's long-term state, including developmental state (Kirschbaum 1987,1995; Westby & Kirschbaum, 1977, 1978), as well as sex and species identification (Hopkins & Bass, 1981; Hopkins, 1983, 1986,1988). Position in a dominance hierarchy is known to affect the EOD duration (Carlson, Hopkins & Thomas, 2000) although it is not yet clear if neighboring mormyrid fish recognize individuals and their status in a hierarchy based on EOD cues. In the gymnotiform, *Gymnotus carapo,* variations in EOD waveform do in fact serve in individual recognition (McGregor & Westby, 1992).

Field observations of EOD-related sexual dimorphisms have been seen in several species, with males typically emitting longer EOD's and lower associated peak power spectra (PPSF) relative to females (Hopkins, 1980, 1981, 1986; Landsman 1993a, 1993b; Westby & Kirschbaum, 1982). Males of *B. niger* and *G. petersii* show such EOD and PPSF-related sex differences during the breeding season. Many species in the laboratory, however, do not show an EOD-related sex difference. This may in part be due to the fact that these fish showed a stress-related shortening of the EOD duration (Landsman 1991, 1993a, 1993b). Sexually immature juvenile males also have short, female-like discharges. Measurements taken in the laboratory from groups containing such males will therefore show an overlap in EOD duration with females.

Several EOD parameters have been manipulated in long-term laboratory hormone treatment studies. In these studies females or juveniles were administered androgens in order to induce a 'male-like' discharge (Bass & Hopkins, 1985, Bass & Volman, 1987; Bass, 1986; Landsman, Harding, Moller & Thomas, 1990; Landsman & Moller, 1988, review in Landsman, 1995; Herfeld & Moller, 1998). Hormone treatment can induce male-like changes affecting not only the duration and PPSF but also the relative amplitudes of the different phases of the EOD waveform (Bass, 1986).

Breeding studies have shown that a sustained conductivity change (decrease) over several days, when co-occurring with the additional zeitgebers of raised water levels and acoustical stimulation of rainfall on the water's surface, will serve as a zeitgeber for breeding behavior, triggering gonadal development (Kirschbaum 1987, 1995). The increased androgen levels associated with gonadal activity thicken and increase the surface area of electrocytes of the electric organ, which in turn leads to an increase in the EOD duration, as well as amplitude changes. Such a change over a period of several days to weeks can be attributed to endocrine activity. A good estimate of the normal time course for hormonally induced EOD changes is provided by hormone manipulation studies. Herfeld and Moller (1998) have treated subadult *B. niger* with 17α-methyltestosterone (17-MT) and found male-like changes in the EOD after a few days, with a maximum effect achieved by the end of the second week of treatment.

Environmental influences on the EOD

The EOD is susceptible to changes in environmental conditions. The mormyrid EOD varies following manipulation of certain external parameters, such as temperature (Serrier & Graff, 1985, as cited in Moller, 1995) and water conductivity (Squire, 1981; Squire & Moller, 1982). Discharge duration decreases with increased water temperature (Serrier & Graff, 1985, as cited in Moller, 1995; Herfeld, 1998). This is likely a metabolic affect, not surprising for poikilothermic organisms. It is possible that temperature fluctuations alter electrosensory receptivity of the receiver in a fashion that is concomitant with electromotor changes for the sender. Alternatively, the signal variability resulting from a fluctuation of temperature may be too small to affect significant behavioral changes in the receiver.

Temperature does not appear to fluctuate in the wild as much as has been observed under artificial conditions in small laboratory aquaria. Daily riverine temperature fluctuations have been recorded in the field (Moller, Serrier, Belbenoit, & Push, 1979, in Nigeria), showing a maximal variation at the surface of 4.1° C (range: 26.2-30.3° C), and a maximum daily temperature difference of 0.3° C below 2.5 m. Such minor fluctuations have a negligible effect on EOD duration. In the experiments reported here, EODs were adjusted to a conventional standard of 25 °C.

Signal function of the EOD sequence of pulse intervals (SPI)

Most of the information regarding electrocommunication in mormyrids has come from studies that assess SPIs. SPIs can reflect species, sex, and individual identities (reviews: Hopkins, 1986; Kramer, 1990b; Moller, 1995; von der Emde, 1998). Serrier & Moller (1989) found individual differences in the resting SPIs of isolated *B. niger*, which could potentially broadcast individual identity to neighbors. Kramer (1990b) pointed out that inter-individual differences in SPIs may not enable individual recognition, however, as discharge rates are constantly changing in response to many types of external stimulation (both social and environmental). SPIs may not transmit individual identity simply because SPI variability within individuals is potentially large and constantly varying.

Several studies (Hopkins & Bass, 1981, Kramer, 1974, 1976a, 1976b, 1978, 1979, Kramer & Bauer, 1976, Moller 1970; also see reviews in Hopkins, 1986-Table 2, and Moller, 1995) have documented the large range of social contexts in which specific patterns of SPIs commonly occur. More than half of the display patterns documented in these studies are known to occur in the context of aggressive territorial interactions. These agonistic electrical displays coincide with stereotypical locomotor displays and fighting behavior (Crockett, 1986). Such stereotypical SPI patterns may serve as useful indicators of territorial aggression (to both the experimenter and other fish). Some playback experiments (Kramer 1978, 1979) have in fact relied upon the attack-related SPI activity of subjects as a dependent measure.

Social behavior in electric fish during agonistic interactions

There have been only a few field studies assessing dominance hierarchies in electric fish, mainly because of the difficulties associated with observing these fish in the wild. Most observations have been on South American gymnotiforms. An excellent long-term study of gymnotiform social behavior was conducted in a semi-naturalistic laboratory setting by Cleworth (1969). The breeding season establishment of permanent (likely) mating territories has been established in the gymnotiform *Sternopygus macrurus* (Hopkins, 1974b). Many gymnotiforms prefer shallow water, and remain inter-individually spaced at least 1 m apart during the breeding season (Westby, 1988).

Mormyrid territorial behavior is known primarily from laboratory studies. Prior residence is an important factor contributing to intra-sexual, male aggression in mormyrids. A time course for the establishment of residence in *B. niger* has been established (Moller & Mack, reported in Moller, 1995). The time course of resident responses to naïve intruders indicates that residents establish a territory within three days of introduction to a tank. Residents show more aggressive locomotor behavior and increased discharge activity relative to intruders (Crockett, 1986; Kramer, 1974,1976a, b). In addition, EOD discharge cessation can serve as a means of hiding and/or appeasement for an intruding fish (Moller, Serrier & Bowling, 1989). When an intruder does discharge, it is often immediately attacked (Moller & Mack, in Moller 1995).

The number of fish present and the individual's familiarity with the environment also affect group interactions. Moller (1976) investigated the social interactions of *M. cyprinoïdes* as a function of these variables. The social behaviors measured were either typical of aggressive, territorial behavior (pursuit and aggressive physical contact) or of schooling behaviors. Aggression diminished following adaptation to the novel environment. In a novel situation aggression measures peaked with three fish in a group and decreased as more fish were added. As group size increased, so did schooling measures (group movement and swimming in single file). A third factor, the presence or absence of electric signals, showed that in electrically silent fish social behaviors were reduced. This reduction demonstrated that these behaviors are electrically mediated.

Group size also affects social spacing in *B. niger*. The number of fish present in a group affects the average distance to the nearest neighbor (Squire, 1991). Smaller distances are tolerated in groups of five to nine fish, as compared with groups of three or four individuals. Familiarity with neighbors has also been shown to reduce aggression and increase group cohesion (in *G. petersii*, Serrier &

Moller, 1981).

Parallel display is one of the aggressive behaviors that *G. petersii* engages in during agonistic interactions. During such behavioral interactions, combatants line-up head to head or head to tail (anti-parallel) and engage in a rapid volley of EOD discharging (Bell, Myers & Russell, 1974; Crockett, 1986). During bouts of parallel displays, individuals are often physically touching each other, potentially allowing for both tactile and electrical assessment. Previous studies have assessed the possible aggressive communicative functions of EODs associated with parallel display. To date, the possibility that parallel display serves an assessment function has not been addressed. This possibility will be explored in this study.

Hypotheses and general experimental overview

Experiment 1 observed EOD changes over the course of several days in interacting mixed and same-sex dyads of *G. petersii*, as well as the locomotor behaviors and discharge patterns during the first minutes of interactions. It was hypothesized that social dominance would be associated with increased EOD duration changes over time. Manipulations within the same-sex dyads included the type of interaction (free versus restricted interactions) and the residency status of fish (intruders versus prior residents). It was predicted that free interactions would produce the most robust changes in EOD, with the EOD in dominant individuals increasing in duration relative to subordinates. Prior residents were expected to show dominance relative to intruders. In experiment

2, EOD signals were manipulated by treating subjects with 17α-methyltestosterone (17-MT). A different species that generates a similar EOD waveform, *Brienomyrus niger*, was assessed in this and all subsequent experiments. The effects of 17-MT manipulation on EOD duration and amplitude were compared with EOD changes resulting from social interaction. It was hypothesized that 17-MT would lead to EOD changes analogous to those observed in dominant fish.

In experiment 3, the effect of free interaction on EOD activity was assessed in *B. niger*. In experiment 4, this species was also tested in restricted interactions. Two factors were assessed in experiment 4: the number of neighbors present and prior residence. It was predicted that both of these social manipulations would affect the EOD and locomotor behavior. Prior residents were expected to show a larger increase in EOD duration relative to intruders.

PART A: *GNATHONEMUS PETERSII*

1.0 Experiment 1

Socially mediated changes to the EOD and behavior of *G. petersii*

This experiment was designed to demonstrate that subadult *G. petersii* show socially mediated EOD changes outside the breeding season. The presence of such changes would suggest that the EOD is involved in communication in the context of territorial/dominance interactions and is not restricted to mate attraction. Socially mediated EOD alterations have not been reported previously in subadult mormyrids or outside of breeding conditions. In order to explore possible sex differences, interacting male-male, female-female and male-female dyads were observed.

As EOD length is associated with increased androgen hormone titers, it was hypothesized that dominant individuals in male-male dyads would show longer EODs and larger amplitude ratios than those in male-female and female-female dyads. A greater number of agonistic behaviors and an associated increase in discharge rates were also expected in male-male dyads.

The effect of specific social manipulations on the EOD and on short-term locomotor and SPI rates and patterns were also explored. Dominant individuals were predicted to exhibit a greater number of aggressive locomotor behaviors and associated EOD discharge patterns. The manipulations included the type of interaction and the residence status of interacting individuals.

Social interaction types varied in three ways: (1) fish were allowed to interact freely, (2) fish were restricted in their interaction by a mesh partition, or (3) fish were housed alone. It was hypothesized that EOD changes would be largest in dominant, freely interacting individuals. It was not known if the establishment of dominance was possible in restricted pairs. It was hypothesized that either no EOD elongation would occur in these fish (suggesting that only free interaction leads to EOD changes and social dominance) or that both individuals in a given pair would show EOD elongation (suggesting that socially-induced EOD duration increases can occur when restricted, but are likely suppressed in freely interacting subordinates).

In addition to the nature of the fish's interaction (free or restricted), the effect of residence status was investigated. Fish were either: (1) both simultaneously introduced ("intruded") into a novel tank, or (2) an intruder was paired with a fish that had established residence in the test tank for three days prior to social pairing. It was hypothesized that social dominance and therefore increased EOD duration and amplitude ratio changes would occur in prior resident subjects. In order to follow the fate of dominance-mediated EOD changes, measures were taken from all fish following removal from interaction.

1.1 Material and Methods

1.1.1 Subjects

Subjects were subadult *G. petersii* obtained through the aquarium trade (Aquarium Glaser, Frankfurt, Germany). Females measured 137 ± 2.2 mm (mean standard length), and males measured 140 ± 3.3 mm. Standard length was obtained by restraining each fish in a net along the side of its tank and measuring from the anterior tip of the skull to the posterior end of the body, adjacent to the caudal fin. Mean female weight was 22.1 ± 1.1 g. Mean male weight was 19.7 ± 1.6 g (Acculab V-1200 electronic scale, readability: 0.1 g).

Sex was determined from radiographs. Adult and subadult males show bone expansion at the base of their anal fin rays, as well as an indentation of the posterior ventral body wall adjacent to the anal fin (Brown, Benveniste & Moller, 1996; Pezzanite & Moller, 1998). This characteristic is lacking in females. In addition to the radiographic information, sex was confirmed post-mortem for a number of subjects by gonadal inspection.

1.1.2 Apparatus

Fish were singly housed in 19 L aquaria. The stimulus (interaction) tanks were 57 L aquaria. The tanks were filled to a depth of 25 cm (tank length: 60 cm, width: 30 cm). In all stimulus tanks, access to the filter was blocked by a partition that cordoned off a 10 cm long compartment at the end of the tank (Figure 1). The remaining area of the tank was open to fish in the freely interacting condition. To restrict fish in their interactions, a mesh divider was inserted into the tank, separating the interaction area into two equal 25 cm long compartments.

The plastic partition, which divided the compartments, was 0.75 cm thick. Its mesh squares were 1.5 by 1.5 cm. The partition thus allowed for free water flow between the compartments. The mesh allowed for visual, electric and limited tactile interactions between subjects. The only body part small enough to pass through the partition openings was the (rostral) mental appendage, generally used by these fish in probing for food. The tanks used for freely interacting and restricted conditions are illustrated in Figure 1.

On the day fish were introduced into interaction trials, video recordings of the initial 15 minutes of interaction were made. Gross locomotor behavior of the fish was recorded (Panasonic digital 5000 camera, 10.5-126 mm TV zoom lens - in the full wide position, Panasonic Omnivision II VHS recorder, model NV-8200). All video recordings were made during the fish's early subjective day.

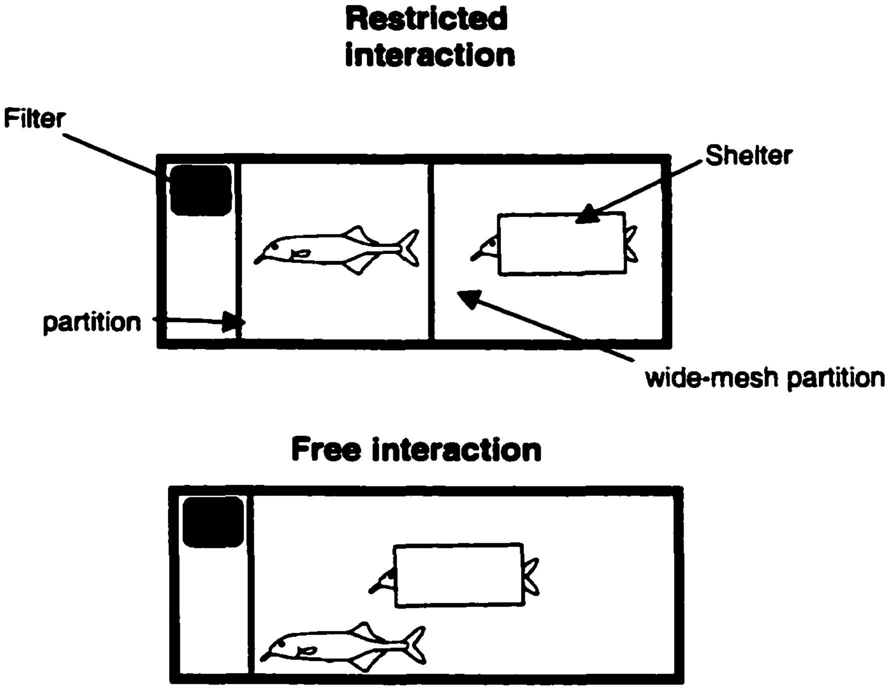


Figure 1

Test tanks illustrating interaction conditions: Restricted (upper panel) and free (lower panel). Partitions consisted of plastic mesh, 0.75 cm wide with 1.5 x 1.5 cm openings allowing for free water flow.

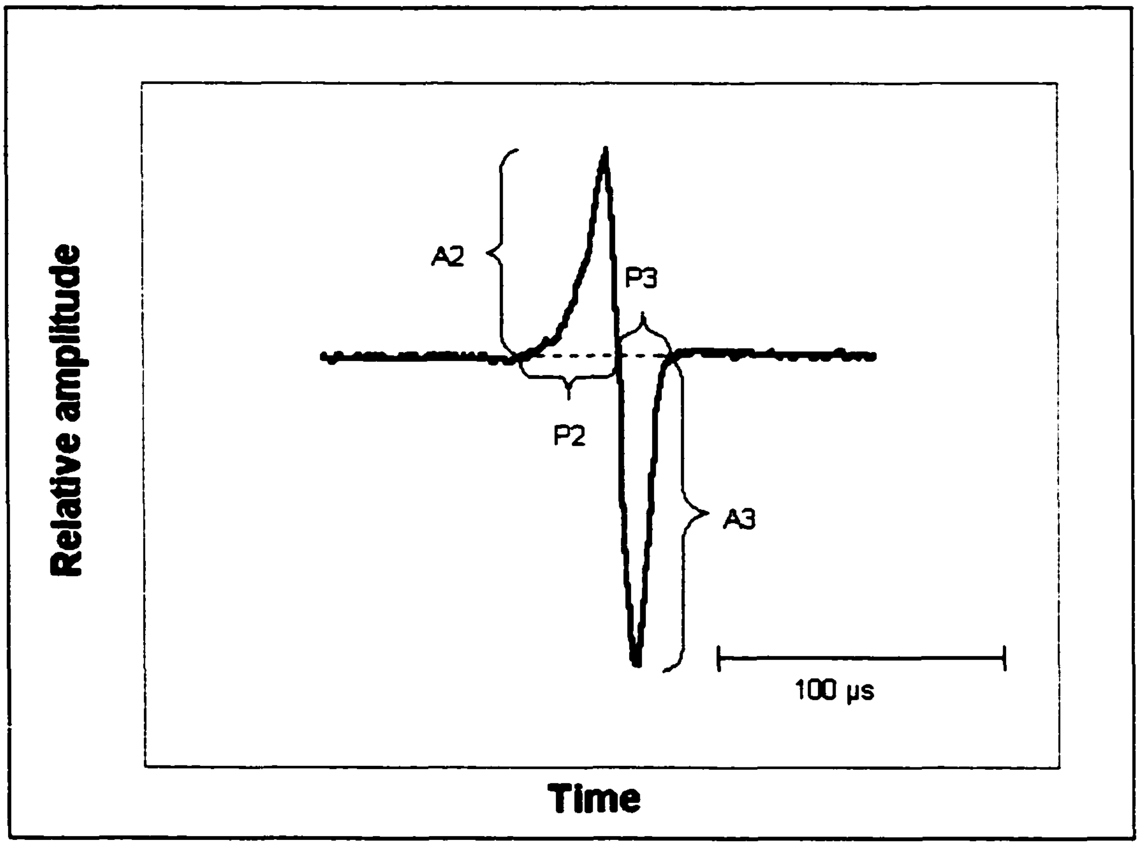
1.1.3 EOD measurements

EODs were monitored with a pair of Ag/AgCl recording electrodes that were placed parallel to the long axis of the fish's body. EODs were pre-amplified (custom-modified AD625) and displayed for measurement on an oscilloscope (Hitachi digital storage, model VC-6023). P1 is the initial, head negative phase of the EOD. This phase was not measured as its amplitude was too small relative to those of P2 and P3. P2 is the major, head-positive phase and P3 is the major head-negative phase. P2 duration was measured from the initial ascending deviation from ground baseline to the intersection of the descending P2 wave with the baseline. P3 duration was measured from the end point of P2 to the intersection of the ascending P3 wave with the baseline. Dependent measures in this experiment included the combined duration of phases P2 and P3, hereafter referred to as EOD duration. Peak amplitude measures were taken for P2 and P3. The amplitude ratio A2/A3 served as a derived variable. Figure 2 illustrates the typical subadult *G. petersii* EOD. Together with EOD measures, the associated Fast Fourier transform was obtained (Hewlett Packard spectrum analyzer, model 3582A, range 0-25 kHz, resolution 100 Hz). Figure 3 shows a typical peak power spectrum frequency (PPSF) from a subadult *G. petersii.*

EOD amplitude, phase duration, and PPSF were taken daily during the fish's early subjective day. Phase duration and PPSF measures were averaged and adjusted to 25°C to control for the effect of temperature (see below).

Taped EOD activity (input from recording electrodes to a Panasonic Omnivision II VHS recorder) was transformed into sequences of standardized 5 V square pulses (pulse duration: 1 ms). Using a Tektronix oscilloscope (type 453, model 2086) as a Schmitt trigger, data were input into a PC via a data acquisition board (Daqboard 2000 series, Iotech, Inc. Cleveland, Ohio). Inter-EOD intervals were determined with Counter/timer software (Daqview 7.7.37 data acquisition system, Iotech, Inc.; timing resolution: 1 ms). Data were subsequently imported into Excel spreadsheets for further analysis. This system measured the interval

between successive EOD pulses regardless of the sender (in recordings from dyads, the data logger was triggered by both fish's EODs).



<u>Figure 2</u>

A typical electric organ discharge from a subadult *Gnathonemus petersii*. Amplitude and duration of phases 2 and 3 are indicated as A2, A3, P2, and P3, respectively. Phases 1 and 4 were omitted from the analysis

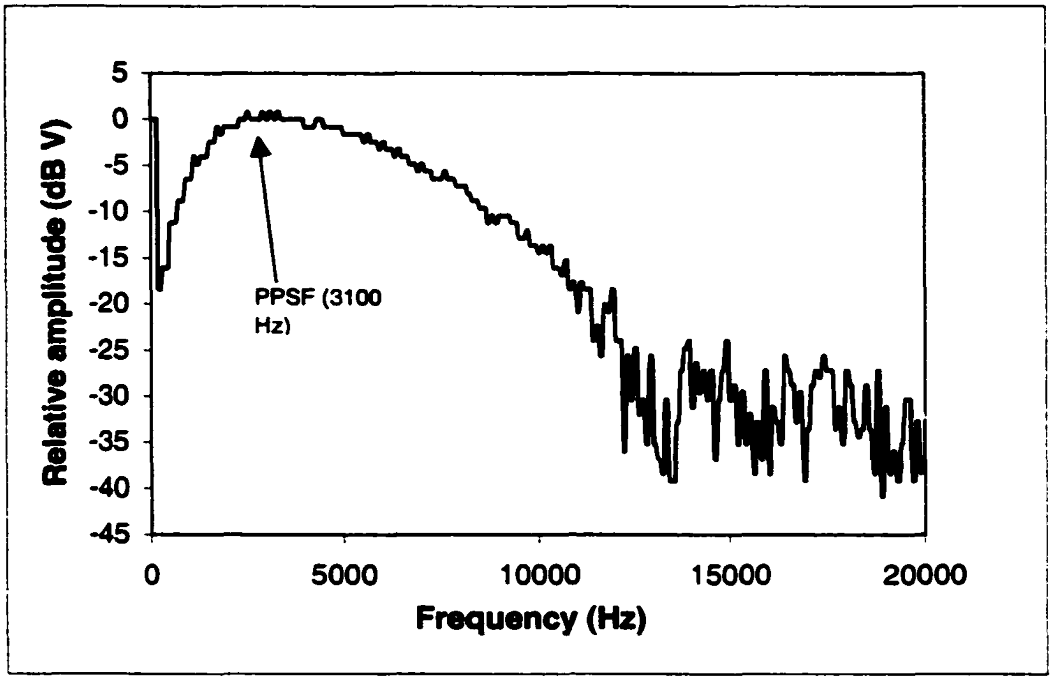
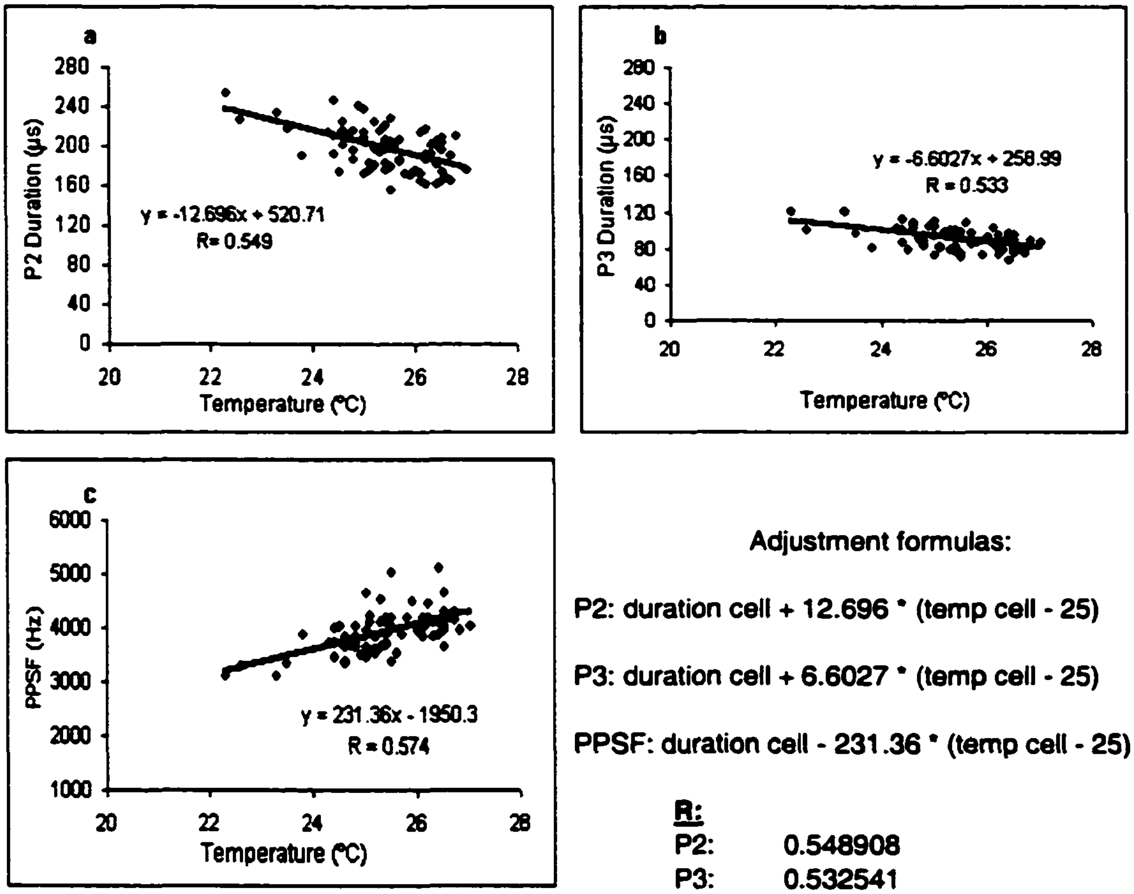


Figure 3

Fast Fourier Transform of a typical subadult *Gnathonemus petersii* EOD. Arrow: Peak power spectrum frequency

1.1.4 Temperature adjustment

In order to control for the effect of temperature on EOD duration and PPSF, measures were adjusted to the conventional standard of 25°C. Adjustments were based upon regression lines calculated for P2, P3, and PPSF from a large pool of measures taken from 75 fish measured over several weeks. Temperature range: 22.3 -27°C (Figure 4). The slopes, derived from the regression equations for each of the measurement parameters, were: P2 = -12.7, P3 = -6.6, PPSF = 231.4. Measurements were adjusted using the following formula: M - S *(T - 25). M is the EOD measure, S the slope, and T the temperature at the time of recording. These slopes were used to adjust all phase duration measures. As the slopes for P2 and P3 differed, to adjust the total duration (P2 + P3) measures, the individual phase durations were adjusted prior to summing.



Adjustment formulas:

P2: duration cell + 12.696 * (temp cell - 25)

P3: duration cell + 6.6027 * (temp cell - 25)

PPSF: duration cell - 231.36 * (temp cell - 25)

<u>R:</u>	
P2:	0.548908
P3:	0.532541
PPSF:	0.574369

<u>Figure 4</u>

Scatter plots of temperature data for three EOD measurements, P2, P3 and PPSF. Regression lines, equations, and coefficients of determination are indicated.

1.1.5 Interaction conditions

Fish were initially housed singly for four days. They were then placed into a tank with a neighbor for a four-day interaction period. Following this period, they were returned to their home tanks for another four days. The social interaction varied in three different ways: fish were allowed to interact freely, were restricted in their interactions by a mesh partition, or were kept solitary.

(1) Free interactions (5 pairs male-male, 5 pairs female-female, 5 pairs male-female). Under this condition, individuals were housed in the same tank as their neighbor. Each tank contained only one ceramic shelter tube. There was no other possible ‘hiding’ place. Dominance status was assessed daily during the interaction period. Dominant individuals excluded subordinates from access to the sole shelter. In addition, dominant fish initiated every agonistic interaction.

(2) Restricted interactions (5 pairs male-male, 5 pairs female-female). Under this condition, fish were separated by a plastic mesh partition. One fish in each pair was randomly assigned access to a shelter. The second subject, isolated in the neighboring compartment, had no shelter access.

(3) Solitary control fish (5 males, 5 females). Fish under this condition were singly housed in the same type of tank used for the other two conditions. They were placed either in a small compartment of the type used for restricted interactions, or in a tank without a partition. Control subjects were randomly assigned to having either access to a shelter during the four-day sampling period or to having no shelter access.

For all dyads described above, both fish were introduced at the same time into the test tank on day 4. These conditions are referred hereafter to as the intruder conditions. An additional group of mixed-sex dyads was tested. These male-female dyads (5 pairs) were all freely interacting intruder pairs. They were randomly chosen from a pool of previously tested fish.

1.1.6 Residency conditions

A second independent variable in this experiment was the residency status of the fish. For the residency manipulation, hereafter referred to as the prior resident condition, one subject was a prior resident in the test tank, and its neighbor an intruder. Subjects were matched by weight and randomly assigned to either prior resident or intruder status.

Following the initial four-day sampling period, prior resident fish were introduced into the test tank for 3-days to establish residency. During this time, prior residents were recorded alone in the stimulus tank, and intruder fish continued to be measured from their home tanks. On day 7, intruders were introduced into the test tank for the four-day interaction period. On day 11, all fish were returned to their home tanks for a four-day post-interaction period.

The same number of subjects was used in the prior resident conditions as had been used in the intruder conditions (5 pairs freely interacting, 5 pairs restricted, and 5 solitary controls, replicated for each sex, i.e. total n = 50 fish). Table 1 summarizes manipulations and subject assignments.

FEMALES		INTERACTION LEVEL		
		Free Interaction	Restricted	Solitary
RESIDENCY STATUS	Intruder	*n*=10	*n*=10	*n*=5
	Prior resident	*n*=10	*n*=10	*n*=5
MALES		INTERACTION LEVEL		
		Free Interaction	Restricted	Solitary
RESIDENCY STATUS	Intruder	*n*=10	*n*=10	*n*=5
	Prior resident	*n*=10	*n*=10	*n*=5
			SEX	
MALES + FEMALES		Male	Female	
(Free Interaction)	Intruder	*n*=5	*n*=5	

Table 1

Experimental manipulations and subject assignments

1.1.7 Time course of conditions and replications

(a) Female intruder pairs were run first. All interaction levels in this group (freely interacting, restricted, and solitary fish) were run simultaneously. Replications of this condition were run over several sessions. For these trials a between-subjects design was employed (all intruder females were naive). (b) The female prior resident condition was run next (again with interacting, restricted, and solitary fish tested simultaneously, and with replications run over several sessions). (c) Intruder males were then tested. Due to a limited supply of identified males, subjects were used again for the different interaction manipulations (interacting, restricted, and solitary fish). The same five pairs used for free interactions were also paired for restricted interactions. A dyad used in a free interaction trial, for example, was later paired for a restricted trial. This re-running of pairs was counterbalanced (some pairs were run initially as restricted fish, and then in free interactions, other pairs were run in the reverse order). (d) Males were then run in the prior resident conditions. Again, the same subject pool was used, with counterbalanced re-running of subject pairs. Although most of the males were re-used for prior resident conditions, the individual pairings used in these conditions were different from those used for the intruder conditions.

1.1.8 Statistical procedures

Between-subjects ANOVAs were run with repeated measures within subjects (mixed model ANOVAs). ANOVAs were performed using *Statistica for Windows* (Statsoft, inc., 1995). In all cases the significance level was set at $p < 0.05$. The dependent measures for different ANOVAs were the EOD (duration, amplitude, and PPSF). The independent variables in different ANOVAs (depending on the group being tested) were interaction level, dominance status, residency condition and sex. The ANOVAs and the independent variables tested for each group are listed in the results sections below (Tables 2-5). Where applicable, Tukey's honestly significant difference (HSD) post-hoc tests were performed. Separate ANOVAs were run for male and female pairs in both intruder and prior resident conditions. In addition, ANOVAs were run comparing each of the mixed-sex group's EOD parameters (the independent variables being sex, dominance status, and interaction period).

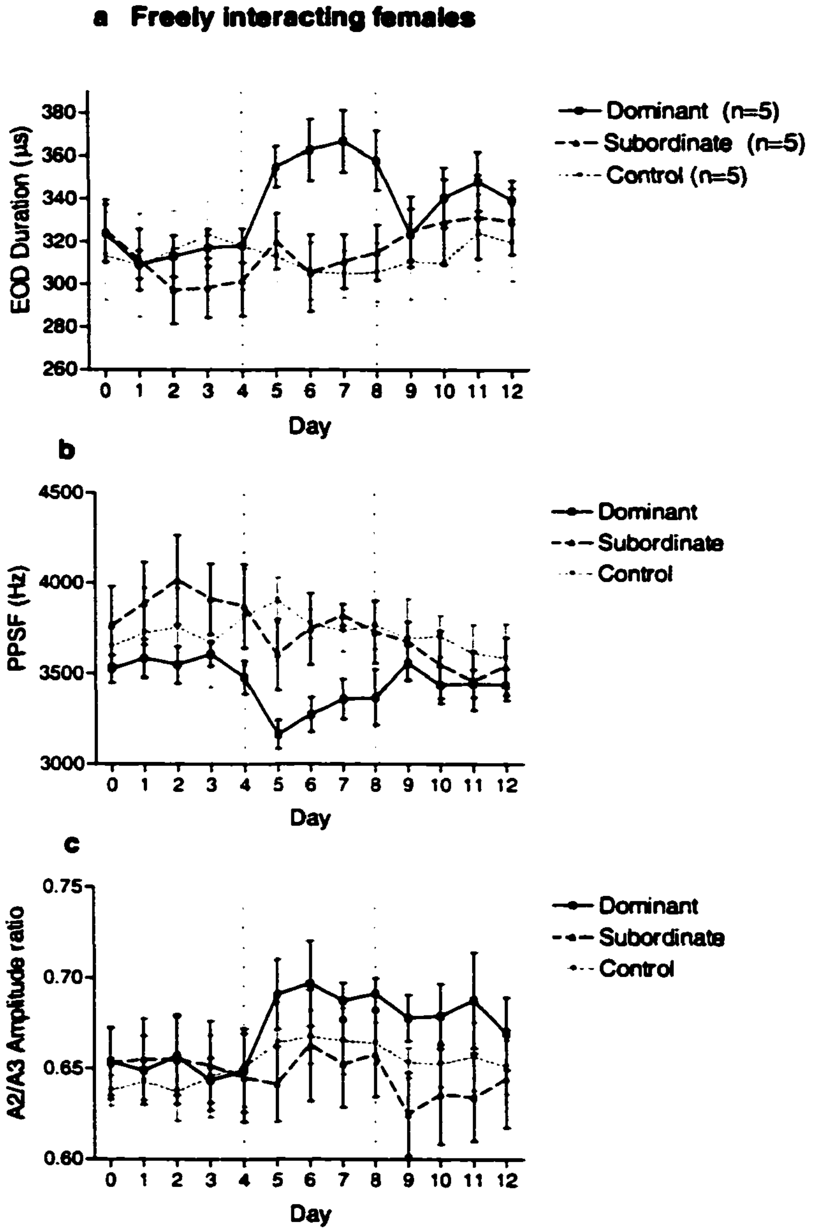
The means of individual time periods (the repeated measures) were calculated as follows: a given time period's measure (PPSF of the pre-interaction period, for example) was the average of all daily measures of a given waveform parameter (PPSF in this example) throughout the duration of that time period (days 1-4, in this example). Three time periods were compared for the intruder conditions: the pre-interaction period (mean of days 1-4), the interaction period (mean of days 5-8), and the post-interaction period (mean of days 9-12). Four time periods were compared for the prior resident conditions: the pre-interaction period (mean of days 1-4), the residency establishment period (mean of days 5-7), the interaction period (mean of days 8-11), and the post-interaction period (mean of days 12-15).

Additional measures included the occurrence of specific social locomotor behaviors and associated discharge patterns during the first ten minutes of interaction. The specific procedures will be introduced in the locomotor behavior and discharge pattern sections below.

1.2 EOD measures

1.2.1 Results: Dominance and Residency Status

Figure 5 illustrates the effect of dominance status on freely interacting females in the intruder condition (fish were introduced at the same time). EOD duration increased in freely interacting dominant fish, followed by a sudden decrease at the beginning of the post-interaction period (day 9). Subordinates did



a Freely interacting females

Figure 5

Effect of dominance status in freely interacting female *G. petersii* under intruder conditions on (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between vertical dashed lines. Error bars: ± 1SEM not show such a decrease.

Mirroring the EOD elongation, PPSFs of dominant individuals were lower than those of subordinates during interaction. During interaction, amplitude ratio increased in dominant fish relative to subordinates, and surprisingly, did not revert to pre-interaction levels (Tables 2, 3: ANOVAs #1-3).

Mean EOD duration of females in the intruder condition (Tables 2, 3: ANOVA #1) showed a significant difference between within-subjects measures (manipulation). Post-hoc analyses of these differences revealed significant differences between the pre-interaction and interaction period ($p = 0.025$), as well as the pre-interaction and post-interaction period ($p = 0.018$). There were no significant differences between the interaction and post-interaction periods. The mean pre-interaction duration for the intruder females was 310.9 μs. The mean interaction and post-interaction durations were 326.8 μs and 327.6 μs, respectively. EOD duration increased during the interaction period for all subjects, and these increases persisted into the post-interaction period.

Post-hoc comparisons of the interaction effect (manipulation x dominance status) were not possible, however. This was due to the fact that there was no clear choice of an appropriate error term for comparisons that involve between- and within-group interactions (Statistica program manual, 1995). Thus, in all subsequent ANOVAs, post-hoc analyses were not performed for any of the observed interaction effects.

PPSFs of freely interacting dominant and subordinate intruder females (along with solitary controls) were compared (ANOVA #2). There was a significant difference between the within-subjects measures (manipulation). A post-hoc analysis of this difference revealed a significant difference between the pre-interaction and post-interaction periods (*p* = 0.028). The mean pre-interaction PPSF for the intruder females was 3739 Hz. The mean interaction and post-interaction PPSFs for the intruder females were 3592 Hz and 3559 Hz, respectively. PPSF decreased significantly following the interactions.

The amplitude ratios of freely interacting dominant and subordinate intruder females (along with solitary controls) were compared (ANOVA #3). There was a significant difference between the within-subjects measures (manipulation). A post-hoc analysis of this difference revealed a significant difference between the pre-interaction and interaction periods (*p* = 0.035). The mean pre-interaction amplitude ratio was 0.649. The mean interaction and post-interaction ratios were 0.67 and 0.656, respectively. The mean amplitude ratio increased significantly during the interaction.

Figure 6 illustrates the effect of dominance status on freely interacting intruder males. EOD duration increased in dominant males during the interaction period while that in subordinate males decreased. During the post-interaction period the subordinate durations rebounded. As expected, PPSFs of dominant fish were lower than those of subordinates during the interaction period. The amplitude ratios of the dominant males increased during interaction, and continued to stay elevated into the post-interaction period (Tables 2, 3: ANOVAs # 4-6).

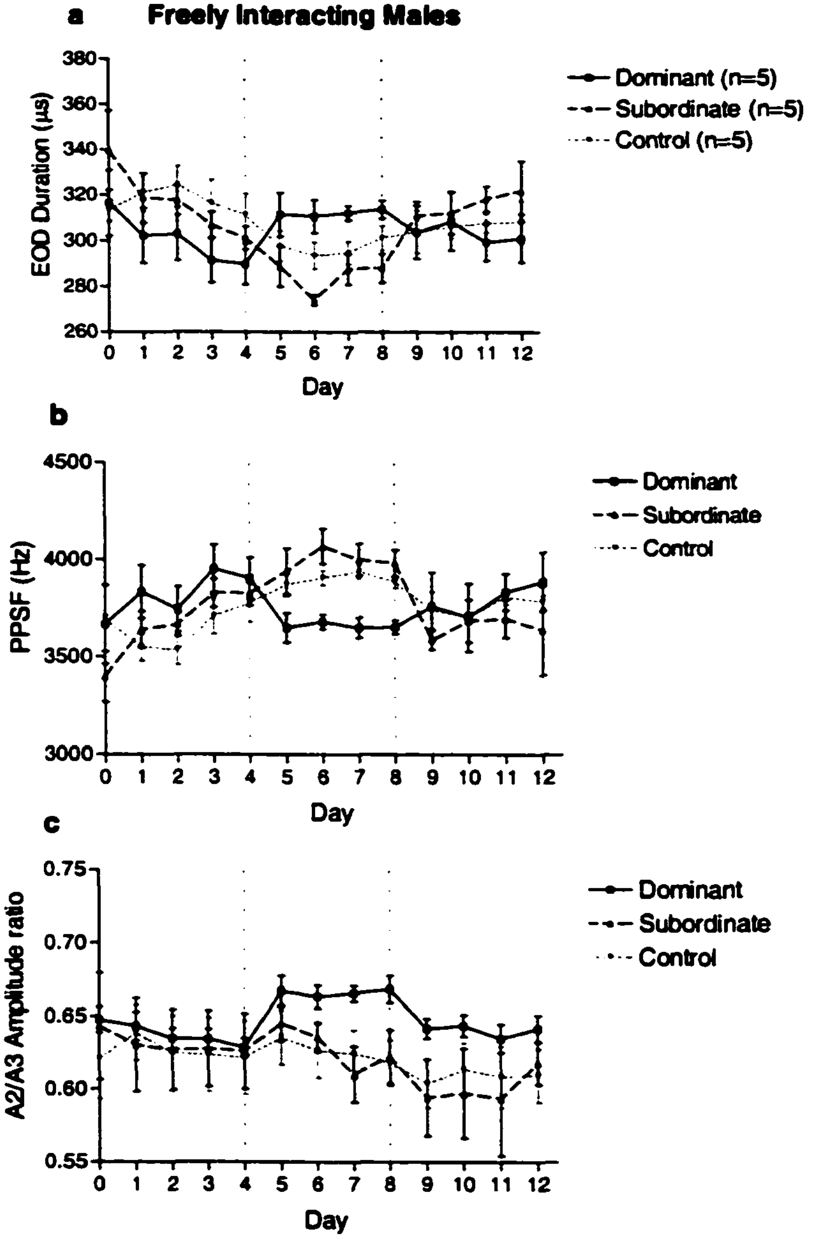


Figure 6

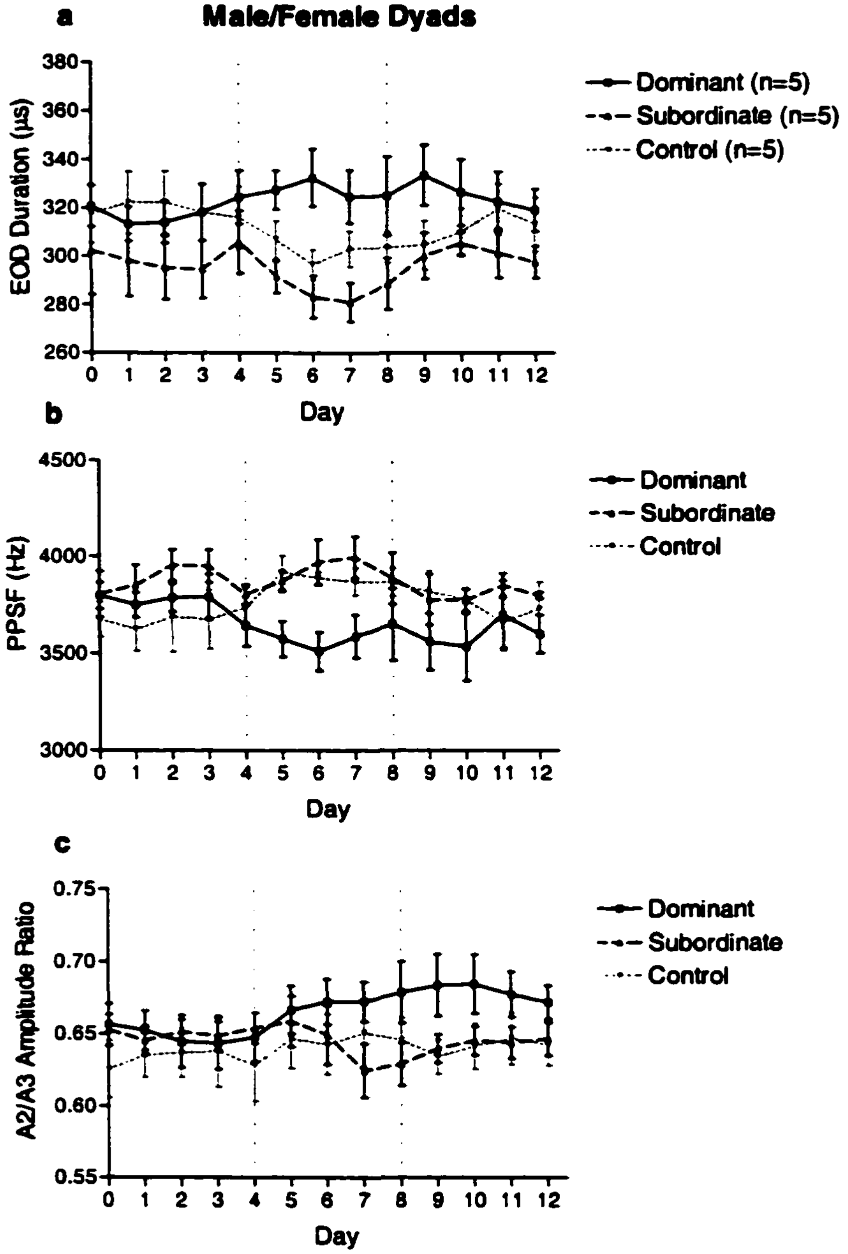
Effect of dominance status in freely interacting male *G. petersii* under intruder conditions on (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the vertical dashed lines. Error bars: ± 1 SEM

EOD duration of intruder males was assessed as a function of dominance status (ANOVA # 4, Figure 6a). The results showed a significant difference between the within-subjects measures (manipulation). A post-hoc analysis revealed significant differences between the interaction period (297.9 μs) and both the pre-interaction (308.8 μs) and post-interaction (308.6 μs) periods (p = 0.013 and 0.015, respectively). EOD duration decreased during the interaction period in all freely interacting males, a change in the opposite direction to that observed in females. Mean EOD duration returned to pre-interaction levels following the interaction, a trend that did not occur in females.

The PPSF of these males was also compared by dominance status (ANOVA #5). The results showed a significant difference between the within-subjects measures (manipulation periods). Post-hoc analysis revealed no differences, although the PPSF mean during the interaction period, when compared with the post-interaction PPSF, approached significance (interaction: 3850 Hz, post-interaction: 3734 Hz, $p = 0.1$). Again, these differences were in the opposite direction to those observed in females.

The amplitude ratios of freely interacting dominant and subordinate males were compared (ANOVA #6). A significant difference was found between the within-subjects measures (manipulation period). Post-hoc analysis of this result revealed a significant difference between the interaction (0.64) and post-interaction (0.617) amplitude ratio ($p = 0.038$). The amplitude ratio decreased following the interactions.

Figure 7 illustrates dominance in male/female dyads. Sex was not a



<u>**Figure 7**</u>

Effect of dominance in mixed-sex *G. petersii* dyads (freely interacting, intruder conditions) on (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the vertical dashed lines. Error bars: ±1 SEM predictor of dominance. Of the five mixed-sex pairs, three possessed dominant males and two dominant females. These pairs did show dominance-associated EOD differences similar to those in the same-sex dyads (Tables 2 and 3, ANOVAs #7-9).

EOD duration was compared in the mixed-sex group as a function of both sex and dominance status (ANOVA #7). A significant status x manipulation period interaction was found. The effect of manipulation period (pre-interaction/interaction/post-interaction) approached significance ($p < 0.103$); there was no significant difference between the sexes. The PPSF of the mixed-sex group also showed a significant status x manipulation interaction (ANOVA #8). There was no between-sex PPSF difference. There was no significant amplitude ratio difference, although the status x manipulation period approached significance ($p = 0.11$, ANOVA #9).

EOD parameters in dominance-based pairings of the prior resident groups (an intruder interacting with a prior resident) are illustrated in Figure 8. As the data from both male and female pairs showed a similar trend, data were combined in figures 8 and 9. Dominance-mediated effects were less pronounced in the prior resident interactions as compared with the intruder groups (as illustrated in Figures 5, 6 and 7). The same freely interacting prior resident pairs are shown in Figure 9, grouped by residency status (intruder versus prior resident) rather than dominance.

The trends in Figures 8 and 9 are similar, with EODs of the subordinate group (Figure 8) closely resembling those of prior residents (Figure 9),

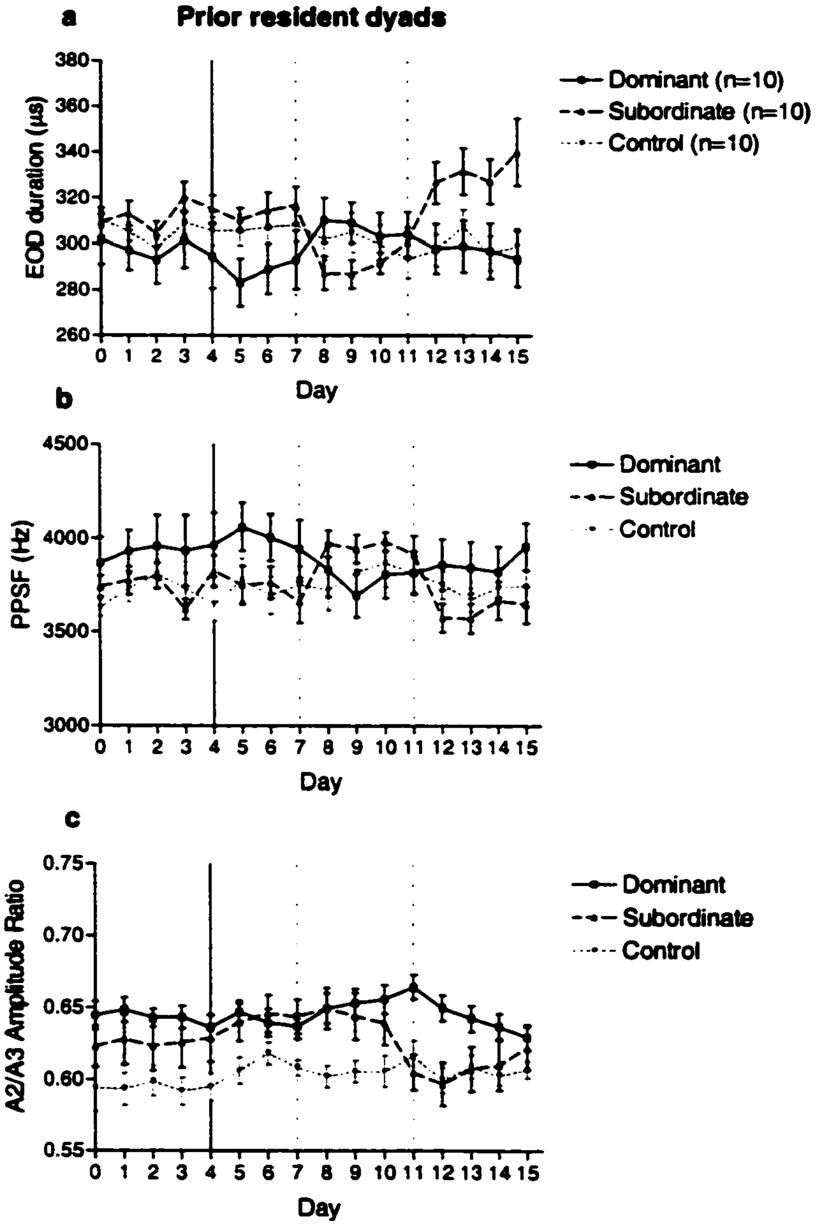


Figure 8

Effect of dominance in prior resident pairs (male-male and female-female dyads combined) on (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the vertical dashed lines. The solid vertical line indicates onset of the residency establishment period. Error bars: ± 1 SEM

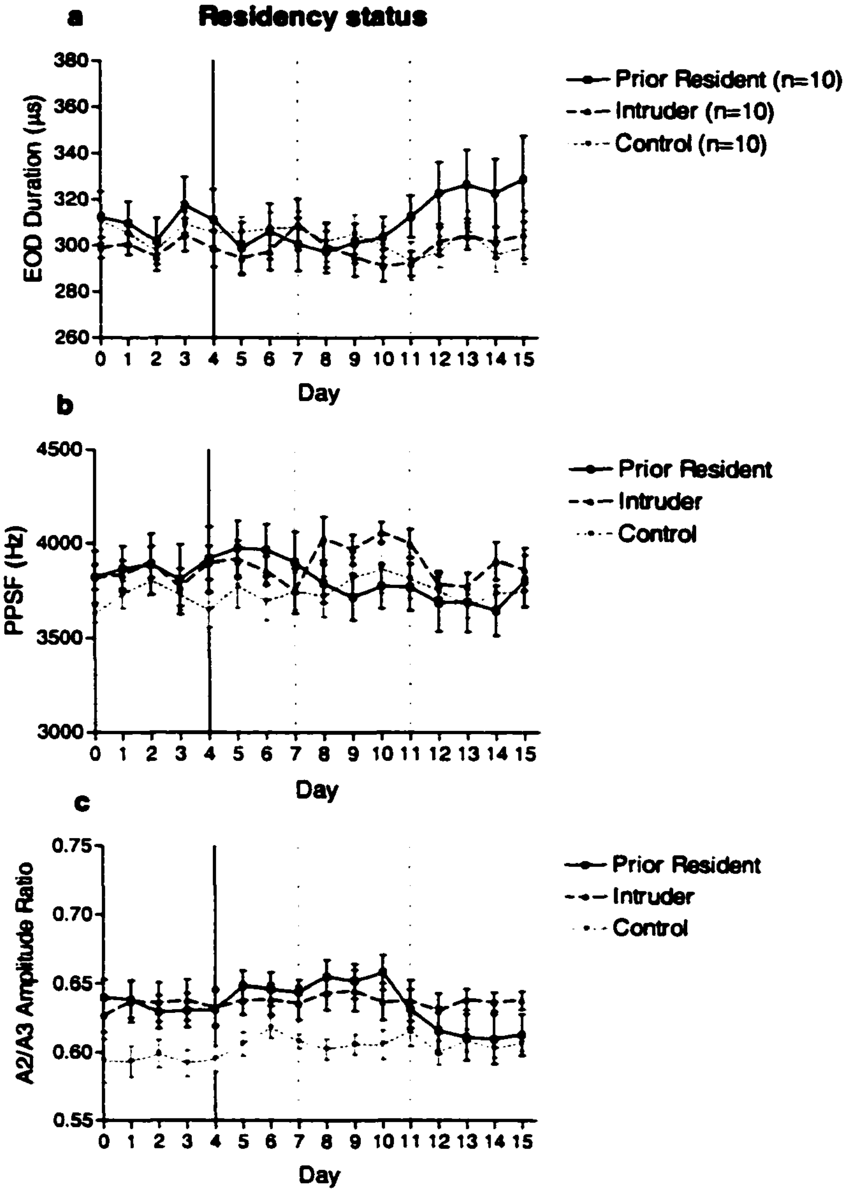


Figure 9

Effect of residency status in prior resident pairs (male-male and female-female dyads combined) on (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the vertical dashed lines. The solid vertical line indicates onset of the residency establishment period. Error bars: ± 1 SEM particularly in regards to a marked duration increase during the post-interaction period. Prior residence did not predict subordinate status, however. Only four of the ten subordinate fish were also prior residents (three of five females and one of five males).

The EOD parameters of prior resident free interactions were analyzed in ANOVAs #10-15. The independent variables in these ANOVAs were residency status (intruder vs. prior resident) and dominance status (dominant/subordinate/ solitary control). The repeated measures (manipulation periods) in these ANOVAs included the same time periods that were compared previously (pre-interaction, interaction and post-interaction periods). In addition, they included the residency establishment period (days 5-7). During this period prior resident fish were housed in the experimental tanks, while their intruder 'neighbors-to-be' remained in their home tanks.

A significant dominance status x manipulation interaction was found for the EOD duration of prior resident female dyads (ANOVA #10). The dominance status variable approached significance ($p = 0.12$). As expected, a significant dominance status x manipulation interaction was found for PPSF means (ANOVA #11). There were no significant differences between amplitude ratio means of female prior residents (ANOVA #12).

The EOD duration of male prior residents showed a significant difference between the within-subjects (manipulation) measures (ANOVA #13). Post-hoc analysis revealed significant differences between the period of interaction and all other time periods (pre-interaction, territory establishment, and post-interaction periods, *p* = 0.004, 0.04, and 0.0007, respectively). The EOD duration during interaction (290.7 μs) was significantly shorter than during all other periods (pre-interaction: 302.1 μs, territory establishment: 299 μs, post-interaction: 304.2 μs). In addition, ANOVA #13 revealed a dominance status x manipulation interaction and a residence status (intruder/prior resident) x manipulation interaction.

The PPSF means of the male prior resident dyads also showed a significant difference between manipulation measures (ANOVA #14). A post-hoc analysis revealed a significant difference between the interaction and the post-interaction periods (*p* = 0.004). The interaction period PPSF (3890 Hz) was significantly higher than the post-interaction period (3725 Hz). ANOVA #14 also showed a dominance status x manipulation interaction and a residence status x manipulation interaction.

Significant differences between the manipulation periods were found in comparisons of the amplitude ratios of male prior resident dyads (ANOVA #15). Post-hoc analysis revealed a significant difference between the pre-interaction (0.618) and residency establishment periods (0.636) (*p* = 0.002). In addition, significant differences existed between the post-interaction ratio (0.612) and both the residency establishment (0.636, *p* = 0.0002) and interaction periods (0.627, *p* = 0.01). ANOVA #15 also shows both a dominance status x manipulation interaction and a residence status x manipulation interaction.

ANOVA #	**Dependent Variable**	**Independent Variables**			**Repeated Measures (manipulation periods)**
		Sex	Residency Condition	Dominance Status	
1	Duration	F	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
2	PPSF	F	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
3	Amplitude	F	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
4	Duration	M	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
5	PPSF	M	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
6	Amplitude	M	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
7	Duration	**M+F**	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
8	PPSF	**M+F**	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
9	Amplitude	**M+F**	Intruder	**Dom/Sub/Alone**	**Pre, Interaction, Post**
10	Duration	F	**Prior resident**	**Dom/Sub/Alone**	**Pre, Residency, Interaction, Post**
11	PPSF	F	**Prior resident**	**Dom/Sub/Alone**	**Pre, Residency, Interaction, Post**
12	Amplitude	F	**Prior resident**	**Dom/Sub/Alone**	**Pre, Residency, Interaction, Post**
13	Duration	M	**Prior resident**	**Dom/Sub/Alone**	**Pre, Residency, Interaction, Post**
14	PPSF	M	**Prior resident**	**Dom/Sub/Alone**	**Pre, Residency, Interaction, Post**
15	Amplitude	M	**Prior resident**	**Dom/Sub/Alone**	**Pre, Residency, Interaction, Post**

Table 2

ANOVAs assessing dominance status. Other independent variables include sex (in mixed-sex pairs only) and residency status (in prior resident groups). All independent variables are indicated in bold. The dependent variables' 'Amplitude' category - amplitude ratio (A2/A3). Dominance status categories: Dom – dominant; Sub – subordinate; Alone - solitary. Repeated measures categories: Pre - pre-interaction period; Post - post-interaction period.

Table 3: ANOVA Results: Interacting Fish

ANOVA #	Significant Effects	Post-hoc Tests (Tukey's HSD)
1	manipulation (pre, interaction, post): $F(2, 24) = 5.58; p < 0.05$	Interaction & post both longer than pre
	Status (dom/sub/alone) x manipulation: $F(4, 24) = 5.02; p < 0.01$	Not applicable
2	manipulation (pre, interaction, post): $F(2, 24) = 4.36; p < 0.05$	Pre is longer than post
3	manipulation (pre, interaction, post): $F(2, 24) = 3.68; p < 0.05$	Pre is lower than interaction
4	manipulation (pre, interaction, post): $F(2, 24) = 6.32; p < 0.01$	Interaction is shorter than pre & post
	Status (dom/sub/alone) x manipulation: $F(4, 24) = 9.13; p < 0.0001$	Not applicable
5	manipulation (pre, interaction, post): $F(2, 24) = 3.41; p < 0.05$	Not significant
	Status (dom/sub/alone) x manipulation: $F(4, 24) = 6.58; p < 0.001$	Not applicable
6	manipulation (pre, interaction, post): $F(2, 24) = 3.48; p < 0.05$	Interaction is higher than post
7	Status (Dom/Sub/alone) x manipulation: $F(4,18) = 3.29; p < 0.05$	Not applicable
8	Status (Dom/Sub/alone) x manipulation: $F(4,18) = 4.21; p < 0.05$	Not applicable
9	Not Significant	Not applicable
10	Status (dom/sub/alone) x manipulation: $F(6, 27) = 6.75; p < 0.001$	Not applicable

11	Status (dom/sub/alone) x manipulation: $F(6, 27) = 2.78$; $p < 0.05$	Not applicable
12	Not Significant	Not applicable
13	manipulation (pre, res, interaction, post): $F(3, 27) = 5.75$; $p < 0.01$	Manip is shorter than all periods
	Status (dom/sub/alone) x manipulation: $F(6, 27) = 6.27$; $p < 0.001$	Not applicable
	Residency status (Int/PR) x manipulation: $F(3, 27) = 3.56$; $p < 0.05$	Not applicable
14	manipulation (pre, res, interaction, post): $F(3, 27) = 3.52$; $p < 0.05$	Interaction is higher than post
	Status (dom/sub/alone) x manipulation: $F(6, 27) = 4.33$; $p < 0.01$	Not applicable
	Residency status (PR) x manipulation: $F(3, 27) = 3.44$; $p < 0.05$	Not applicable
15	manipulation (pre, res, interaction, post): $F(3, 27) = 8.00$; $p < 0.001$	Pre is lower than res Post-interaction is lower than both res & interaction periods
	Status (dom/sub/alone) x manipulation: $F(6, 27) = 3.41$; $p < 0.05$	Not applicable
	Residency status (PR) x manipulation: $F(3, 27) = 14.56$; $p < 0.001$	Not applicable

Table 3

Results of ANOVAs listed in Table 2 that showed significance. Manipulation - the time periods; Res - residency establishment period; PR - prior residency group

1.2.2 Discussion: Dominance and Residency Status

There were four main findings: (1) Dominance-mediated EOD changes occurred in both sexes. This finding was hypothesized for males, but not females. (2) These socially mediated changes occurred in subadults. (3) They occurred outside of the fish's breeding season. (4) EOD duration increases often occurred in subordinate fish following removal from social interaction. These post-interaction increases were not expected.

A previous study (Carlson et. al, 2000) found that only males in mixed-sex groups of the mormyrid *Brienomyrus brachyistius* expressed dominance-mediated EOD lengthening. In their study, however, the EOD changes occurred during a period of decreasing water conductivity that mimicked breeding season conditions. The zeitgebers needed to induce breeding behavior (in *Pollimyrus isidori* and *Mormyrus rume proboscirostris*) include decreasing water conductivity, increasing water level, and acoustic stimuli, all three of which are produced under natural conditions by rainfall (Crawford, 1992; Kirschbaum 1987, 1992, 1995; Kirschbaum & Schugardt, 1995; Schugardt, 1997).

It is possible that dominance signaling by elongated EOD occurs in female *G. petersii* only prior to sexual maturity, only during non-breeding conditions, or at both of these times. Many studies have demonstrated that female subadult mormyrids do respond to androgen administration with male-like changes in the EOD waveform (Bass & Hopkins, 1985, Herfeld & Moller, 1998; Landsman, 1995; Landsman & Moller, 1988). Such laboratory tests have always been conducted under carefully controlled environmental conditions including relatively invariant water conductivity. Such conditions do not provide the zeitgebers necessary to induce gonadal maturation.

Defending a territory is likely to be equally important for subadults of both sexes, particularly during the dry season as territories dry up and food supplies diminish. During breeding season, however, territorial males must attract mates and repel rivals. Under these conditions a female may be at a reproductive advantage if her EOD is less male-like. It remains to be seen if females that show dominance-mediated EOD changes outside of breeding season conditions would fail to do so if such conditions were imposed.

Estradiol has been found to cause an increase in the PPSF of adult, but not juvenile *G. petersii* (Landsman, et al., 1990). An increase in PPSF is a change in the opposite direction to that caused by social dominance and androgens. Landsman et al. (1990) found that plasma estradiol levels were about fourfold higher in adults that had received estradiol implants, as compared with juveniles that had been given the same dose of estradiol. It is possible that estradiol suppresses male-like changes to the EOD of sexually mature female *G. petersii*, facilitating mate attraction by maintaining an EOD that resembles that of a subordinate.

As breeding male mormyrids defend territories, receptive females may require a shortened EOD appeasement signal in order to approach an aggressive male's territory. Both field and laboratory studies have shown that male electric fish defend territories during the breeding season. In their natural habitat and during the breeding season male gymnotiforms with an EOD sex difference *(Sternopygus* *macrurus)* establish permanent (likely) mating territories (Hopkins, 1974b). Under these conditions both gymnotiforms (Westby, 1988) and mormyrids remain inter-individually spaced at least 1 m apart. Such close-spaced territories are likely to lead to intense competition. In addition, only breeding male *Pollimyrus isidori* build and defend nests of plant material (Bratton & Kramer, 1989; Crawford, 1992, 1995; Kirschbaum, 1992, 1995).

Female appeasement signals are well documented in the animal behavior literature, and are so effective at permitting access to a defended territory that in some species 'sneaker' males have evolved that mimic the female so as to gain access to a dominant male's territory for copulation (for an example, the bluegill sunfish, see Gross, 1982).

A single playback study has determined that broadcast mormyrid EODs are received by conspecifics in the context of mate attraction (Hopkins & Bass, 1981). In this experiment male courtship signals (discharge burst 'rasps') in response to female-like versus male-like EOD waveforms were assessed. The authors demonstrated that males responded to female-like EODs with courtship behavior. No playback studies have supported the hypothesis that females similarly respond to male-like EODs. The electric fish literature has extensively documented androgen effects on the EOD, but there is currently no evidence that the elongated EODs of males are assessed in the context of female choice. It is possible that rather than females choosing amongst males based upon elongated EOD, males in fact choose females based upon shortened EOD. Male choice and other sex-role reversals are more common in externally fertilizing fish when the male invests substantially in parenting (Gross & Shine, 1981). It has been reported that some breeding mormyrids may not engage in parental care (Carlson et al., 2000), although male nest building is a substantial investment, and likely leads to further paternal investment. Rather than inter-sexual choice, elongated EODs may serve to communicate in the context of same-sex dominance interactions.

Many researchers view androgens solely as sex steroids, neglecting their other functions. Androgens are also known to serve in the context of competitive interactions, as they prepare individuals for social challenge. Simply observing competitive interactions of other fish is in fact sufficient to induce an androgen response (Oliveira, Lopes, Carneiro, & Canário, 2001).

Much research has demonstrated the effect of androgens on the EOD-producing electrocytes (Bass, 1986; Bass & Hopkins, 1985; Bass & Volman, 1987; Herfeld & Moller, 1998; Landsman, 1995). The electric organ contains columns of stacked, disk-shaped electrocytes. Each electrocyte has a stalk, which is innervated by spinal electromotor neurons. The stalk and electrocyte faces produce distinct action potentials (Bennett and Grundfest, 1961). Depolarizations of the stalk, posterior, and anterior faces lead to P1, P2, and P3, respectively (Bennett, 1971). Androgens alter the waveform by thickening the electrocyte (increasing the width of cells) and by increasing surface area of the anterior face (increasing its number of membrane infoldings). Higher membrane capacitance and therefore longer EOD duration results (Bass & Hopkins, 1985). In addition, the number and/or distribution of different classes of ion channels in the electrocyte membrane may contribute to active membrane properties (Bass & Hopkins, 1985).

The principal fish androgens are testosterone and 11-ketotestosterone, both of which have been shown to influence the EOD of female and juvenile mormyrids when artificially administered under laboratory conditions (Bass, 1986; Bass & Hopkins, 1985; Landsman, 1995). Of the naturally occurring androgens, the EOD is most sensitive to11-ketotestosterone, which is generally considered to be a male-specific hormone in fish. Very small amounts have been assayed in females of some fish species, however (Lokman, Harris, Kusakabel, Kime, Schutz, Adachi & Young, 2000). It is not known if 11-ketotestosterone occurs in female mormyrids in amounts large enough to induce the socially mediated changes described above.

In socially interacting male gymnotiform electric fish (*Apteronotus leptorhynchus*) there is a correlation between levels of 11-ketotestosterone, amount of electric signaling and body weight (Dunlap, 2002). Males show higher rates of electric discharging when interacting with another male, as compared with interactions with females. Such interactions are thought to be influenced by endogenous levels of 11-ketotestosterone. If socially induced changes to the EOD reflect the anabolic effects of androgens, then they have the potential to communicate the fish's physiological condition to a rival.

In the current study, dominance-mediated effects upon the EOD occurred in both the intruder and prior resident dyads. The subordinate prior resident fish, however, showed EOD changes that differed from all other groups. The post-interaction period shows marked increases in EOD durations (with associated lower PPSF) for the subordinate prior residents. Such EOD elongations are usually associated with increased amplitude ratios. In this group, however, the ratios decreased as the duration increased. The amplitude ratio of these fish was reduced throughout the post-interaction period, yet the EOD duration increased markedly. These results suggest that increases in EOD duration do not invariably lead to predictable changes in the amplitude ratio. A possible communicative function of relative EOD amplitudes is discussed below (Experiment 2, discussion).

EOD duration increases were not expected to occur in subordinate fish. Such increases did occur, however, following removal from social interaction. The EODs of subordinate fish during post-interaction were generally longer than during both pre-interaction and interaction. Post-interaction changes were often similar in magnitude to those of dominant fish during interaction. Such changes demonstrate that subordinates are capable of an elongated EOD. Such elongations may be suppressed, however, during free interaction with a dominant fish.

Prior residence status did not predict dominance or the EOD changes characteristic of social dominance. Two of the five prior resident females became dominant and four of five prior resident males became dominant. The number of subjects was too small to draw any conclusions about possible sex differences, although the direction of the findings suggest that prior residence may influence the establishment of dominance in male-male interactions. Observations of the mating and territoriality patterns in several mormyrid species suggest that territory establishment is important for a male's reproductive success (Bratton & Kramer, 1989; Crawford, 1992, 1995; Kirschbaum, 1992, 1995).

Dominance status alone did not significantly differ between all groups. This may be due in part to the pre-interaction values being included in the analyses. Pre-interaction measures are not expected to significantly differ between groups. Including these values in the inter-group analysis therefore reduced inter-group differences. The significant manipulation x status interactions observed support the hypothesis that the inter-group differences were a result of the experimental manipulations.

The subadult male/female dyads showed less socially mediated EOD changes than the same-sex pairs. It is possible that mixed-sex dyads induce less agonistic interaction, and therefore less change to the EOD of either sex. The male/female dyads were investigated last and had been previously run under similar conditions with same-sex neighbors. These factors also could have been in part responsible for the comparatively smaller socially mediated EOD alterations in same-sex pairs.

1.2.3 Results: Interaction Type

The following section compares the changes in EOD parameters as a function of interaction type (freely interacting, restricted interactions, and solitary control). It was not known if dominance could be established in restricted pairs. It was hypothesized that either no EOD elongation would occur in these fish (suggesting that only free interaction leads to social dominance and associated

EOD changes), or that both individuals in a restricted pair would show EOD elongation (suggesting that socially-induced EOD duration increases can occur in most individuals when restricted, but are likely suppressed in subordinate freely interacting fish).

The EODs of restricted, freely interacting and solitary control fish are shown in Figures 10-14. The ANOVAs assessing interaction type (Tables 4 and 5 below) used the same within-subjects measures (interaction periods) that were assessed for dominance status ANOVAs (Tables 2 and 3). In addition, residency status (intruder/prior resident) was also assessed as a function of interaction type (Tables 4 and 5, ANOVAs # 7-12). As both interaction period and residency status were analyzed and discussed above, they will not be discussed in this section (although ANOVA results are presented in Tables 4 and 5). Figure 10 compares the EOD measures of intruder females as a function of their interaction type.

EOD duration increased during the interaction period for both the freely interacting and restricted groups but not the solitary controls. This increase in duration was maintained following interaction. The PPSF followed a similar trend, which was less apparent in the amplitude ratio. The female EOD showed a significant interaction between the manipulation period and interaction type (ANOVA # 1). Although post-hoc tests could not be performed on the interaction results, Figure 10 shows that the solitary group differed most from the freely interacting and restricted groups during the interaction period. Mirroring the EOD duration measure, a significant interaction between the manipulation period and

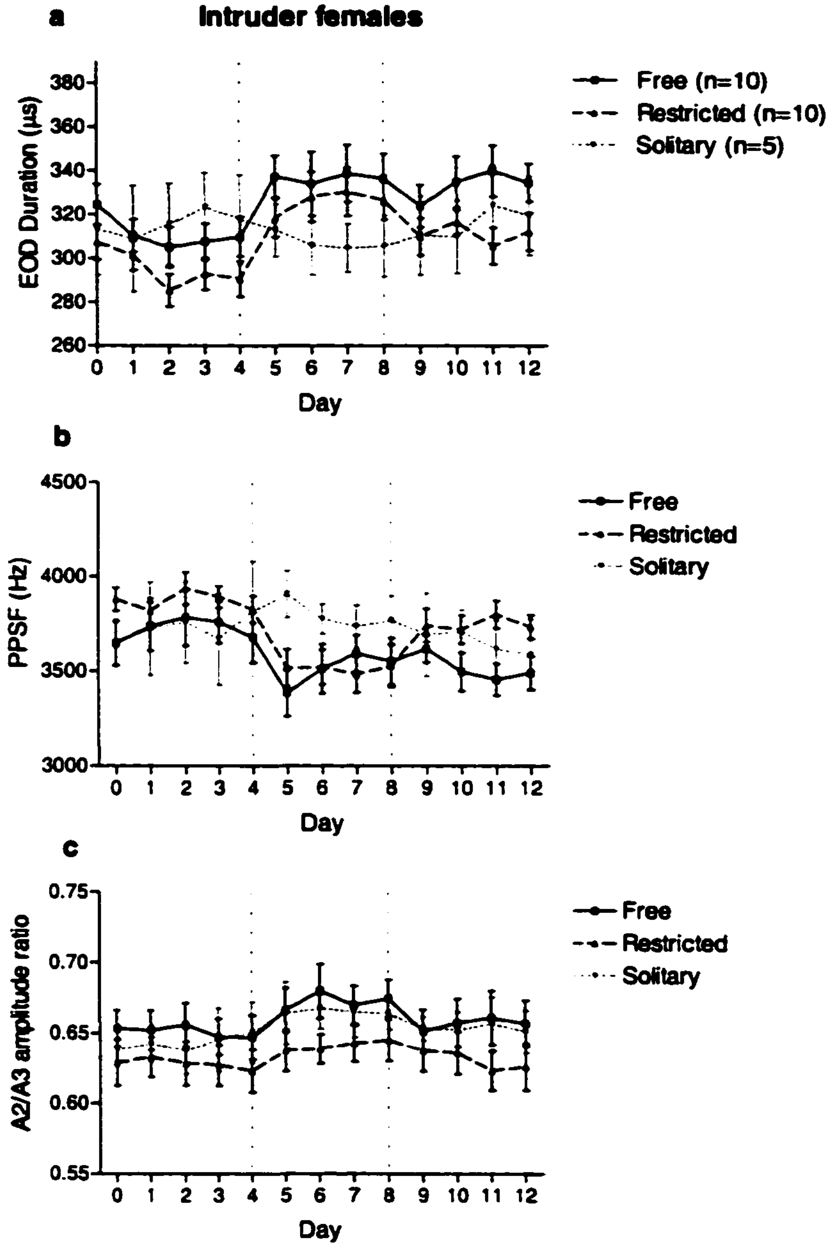


Figure 10

Effect of interaction type on intruder *G. petersii* females (freely interacting, restricted interaction and solitary control). (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the vertical dashed lines. Error bars: ± 1 SEM interaction type was found in the PPSF (ANOVA # 2). Amplitude ratios of intruder females did not differ as a function of interaction type (ANOVA # 3).

Figure 11 compares EOD duration, PPSF, and amplitude ratios of intruder males as a function of their interaction type. There were no significant differences in any EOD parameter between freely interacting, restricted and solitary groups (ANOVAs # 4-6). In contrast to female interaction types (Figure 10), males did not show an increasing trend in EOD duration.

Interaction type was also assessed (freely interacting, restricted interactions and solitary controls) in the prior resident groups (ANOVAs # 7-12). In restricted interactions, it was predicted that prior resident individuals would show greater EOD elongation than intruders, as the establishment of residence was predicted to induce territorial aggression, a possible correlate of increased circulating androgens. Although prior residence did not predict dominance, and associated EOD changes in the freely interacting groups (Tables 2 and 3, ANOVAs # 10-15), it was predicted that restricted interaction would induce greater EOD changes in prior residents, as their territorial aggression could not be directly challenged by free interaction with a neighbor.

The repeated measures (manipulation periods) of prior resident pairs also included a residency establishment period (days 4-7), when prior resident fish were introduced into the experimental tanks while intruder fish remained in their home tanks.

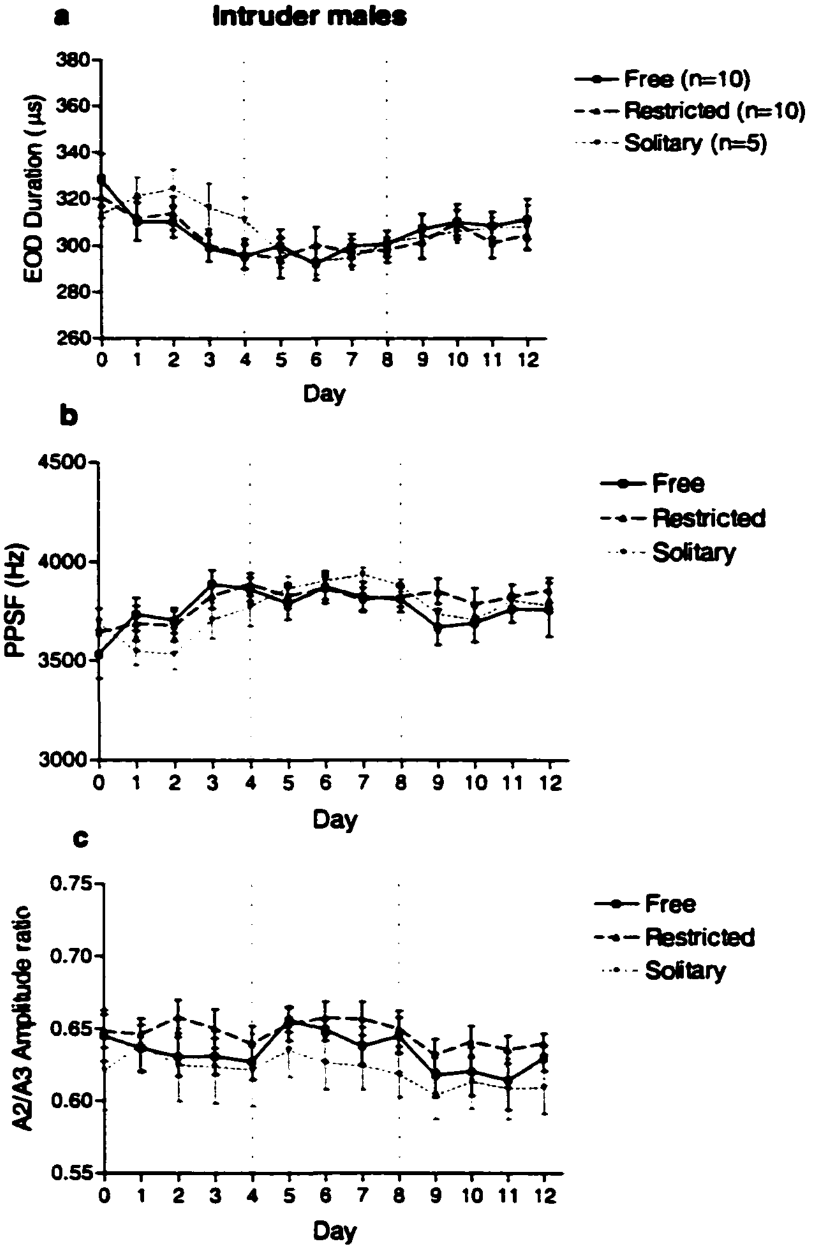


Figure 11

Effect of interaction type on intruder *G. petersii* males (freely interacting, restricted interaction and solitary control). (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the vertical dashed lines. Error bars: ± 1 SEM

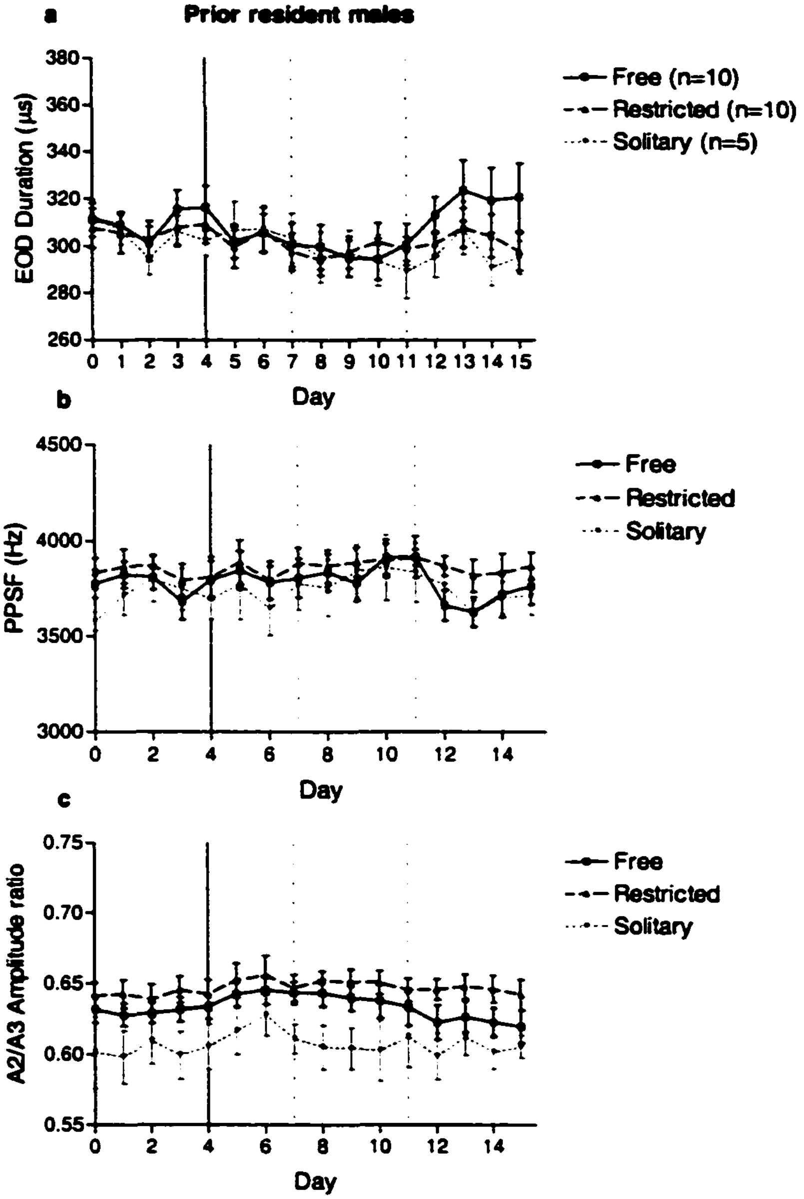


Figure 12

Effect of interaction type on prior resident *G. petersii* males (Measures from both prior resident and intruder individuals are combined). (a) EOD Duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the vertical dashed lines. Fish established residency between days 4 and 7. Error bars: ± 1 SEM

Figure 12 shows the effect of interaction type in prior resident males (EOD duration, PPSF and amplitude ratios). EOD duration of the freely interacting fish increased markedly during the post-interaction period with an associated lowered PPSF.

EOD duration, PPSF and amplitude ratios were compared in prior resident females and males as a function of both interaction type and residency status (ANOVAs # 7-12). No differences were found between the prior resident groups as a function of interaction type, with the exception of ANOVA # 12, which revealed a significant difference between the amplitude ratios of the different interaction types. A post-hoc analysis revealed a difference between the solitary control (0.606) and restricted interaction (0.647) groups. The amplitude ratio in the freely interacting group was 0.634. An interaction level x manipulation period interaction was also found to be significant. Tables 4 and 5 summarize the statistical analyses.

Table 4: Interaction Level ANOVA Assignments

All independent variables compared in a given ANOVA are indicated in **bold font**

ANOVA #	Dependent Variable	Independent Variables			Repeated Measures
		Sex	Residency Condition	Interaction Type	(Manipulation)
1	duration	F	Intruder	**free/restricted/ solitary**	**Pre, Int, Post**
2	PPSF	F	Intruder	**free/restricted/ solitary**	**Pre, Int, Post**
3	amplitude	F	Intruder	**free/restricted/ solitary**	**Pre, Int, Post**
4	duration	M	Intruder	**free/restricted/ solitary**	**Pre, Int, Post**
5	PPSF	M	Intruder	**free/restricted/ solitary**	**Pre, Int, Post**
6	amplitude	M	Intruder	**free/restricted/ solitary**	**Pre, Int, Post**
7	duration	F	**Prior resident**	**free/restricted/ solitary**	**Pre, Res, Int, Post**
8	PPSF	F	**Prior resident**	**free/restricted/ solitary**	**Pre, Res, Int, Post**
9	amplitude	F	**Prior resident**	**free/restricted/ solitary**	**Pre, Res, Int, Post**
10	duration	M	**Prior resident**	**free/restricted/ solitary**	**Pre, Res, Int, Post**
11	PPSF	M	**Prior resident**	**free/restricted/ solitary**	**Pre, Res, Int, Post**
12	amplitude	M	**Prior resident**	**Tog/Sep/Alone**	**Pre, Res, Int, Post**

Table 4

Interaction level ANOVA assignments. Codes for variables: Free - freely interacting group. Manipulation periods: Pre - pre-interaction; Res - residency establishment period; Int - interaction period; Post - post-interaction period.

Table 5: ANOVA Results: Interaction Level Comparisons

ANOVA #	Significant Effects	Post-hoc Tests (Tukey's HSD)
1	Manipulation (pre, interaction, post): $F(2,44) = 8.25$; $p < 0.001$	Interaction & post both longer than pre
	Interaction (free/restricted/solitary) x manipulation: $F(4,44) = 3.73$; $p < 0.05$	Not applicable
2	Manipulation (pre, interaction, post): $F(2,44) = 7.5$; $p < 0.005$	Interaction & post both lower than pre
	Interaction (free/restricted/solitary) x manipulation: $F(4,44) = 3.49$; $p < 0.05$	Not applicable
3	Manipulation (pre, interaction, post): $F(2,44) = 3.66$; $p < 0.05$	Interaction higher than pre
4	Manipulation (pre, interaction, post): $F(2,44) = 5.47$; $p < 0.01$	Interaction shorter than pre & post
5	Manipulation (pre, interaction, post): $F(2,44) = 3.43$; $p < 0.05$	Interaction higher than pre
6	Manipulation (pre, interaction, post): $F(2,44) = 5.78$; $p < 0.01$	Interaction higher than post
7	Not significant	Not applicable
8	Not significant	Not applicable
9	Not significant	Not applicable

10	Manipulation (pre, residency, interaction, post): $F(3,57) = 3.82$; $p < 0.05$	Pre longer than interaction
11	Not Significant	Not applicable
12	Manipulation (pre, residency, interaction, post): $F(3,57) = 4.62$; $p < 0.01$	Residency higher than pre and post
	Interaction (free/restricted/solitary): $F(2,19) = 3.81$; $p < 0.05$	solitary lower than restricted
	Interaction (free/restricted/solitary) x Manipulation: $F(3,57 = 11.16$; $p < 0.0001$	Not applicable

Table 5

ANOVA results, interaction level comparisons. Results in the repeated measures (manipulation) category mirror those of the ANOVAs presented above (Tables 2 and 3). Codes for variables: Free - the freely interacting group. Manipulation periods: Pre - pre-interaction period, Post - post-interaction period.

1.2.4 Discussion: Interaction Type

More significant differences existed as a function of interaction type (free, restricted or solitary) in the intruder groups than in the prior resident groups. Under the intruder condition, freely interacting and restricted groups showed similar trends, both of which differed from those of the solitary controls. The restricted interaction group was affected by the manipulations even though individuals were not in physical contact. These findings suggest that fighting (physical contact) is not necessary to elicit EOD changes that are characteristic of free interactions. It is likely that restricted social interaction is sufficient to induce the EOD responses characteristic of dominant fish (see Figure 10) as such changes occurred in female dyads.

It may not even be necessary for a fish to be involved in 'indirect' interactions in order to induce EOD changes. In the cichlid, *Oreochromis mossambicus*, a bystander observing agonistic interactions (through a one-way glass) has been shown to undergo a surge in both testosterone and 11-ketotestosterone (Oliveira et al., 2001). A similar experiment using mormyrid bystanders (restricted to either electric or visual modality access to competing neighbors) could help to elucidate the cues that induce EOD changes and their hormonal correlates in these fish.

Unlike females, prior resident males did show significant differences in the interaction type ANOVAs, most markedly in the amplitude ratios. Post-hoc analysis of amplitude ratio differences, however, revealed that the significantly differing groups were the solitary control group and restricted interaction group. No significant differences existed between these groups and the freely interacting group. It is likely that these findings were due in large part to between-group differences that existed prior to the manipulations (see Figure 12c).

During restricted interactions the intruder males and prior resident pairs of both sexes did not show EOD changes like those of intruder females. This might have been due in part to the time that males and prior residents had spent in captivity. The effect of time in captivity is discussed below for the freely interacting groups (Section 1.2.7).

1.2.5 Results: Comparisons of combined dominance and interaction level

This section explores the trends that vary as a function of both dominance and interaction level. Although dominance status was not assessed in the restricted interactions, this section presents data from restricted pairs (intruder males) that had also been paired in free interactions. In addition, restricted female pairs were separated into pseudo-dominant and pseudo-subordinate categories based upon EOD changes resulting from restricted interaction. These categories are compared with the data from freely interacting pairs.

The same intruder male pairs that were used in free interactions were also used as restricted pairs in a counterbalanced fashion. As a result of this design, it was possible to compare dominant and subordinate males not only under freely interacting conditions (where behavioral dominance was readily observable) but also during restricted conditions (Figure 13).

There were no significant differences between restricted males based upon dominance status. PPSF and amplitude ratios changed as a function of manipulation: $F(2,24) = 5.68$; $p < 0.01$ and $F(2,24) = 5.26$; $p < 0.05$, respectively. Post-hoc analysis showed that baseline PPSFs were significantly lower than post-interaction PPSFs ($p = 0.0075$) and that amplitude ratios recorded during interaction were significantly higher than during post-interaction ($p = 0.014$). The effect of manipulation on EOD duration measures was not

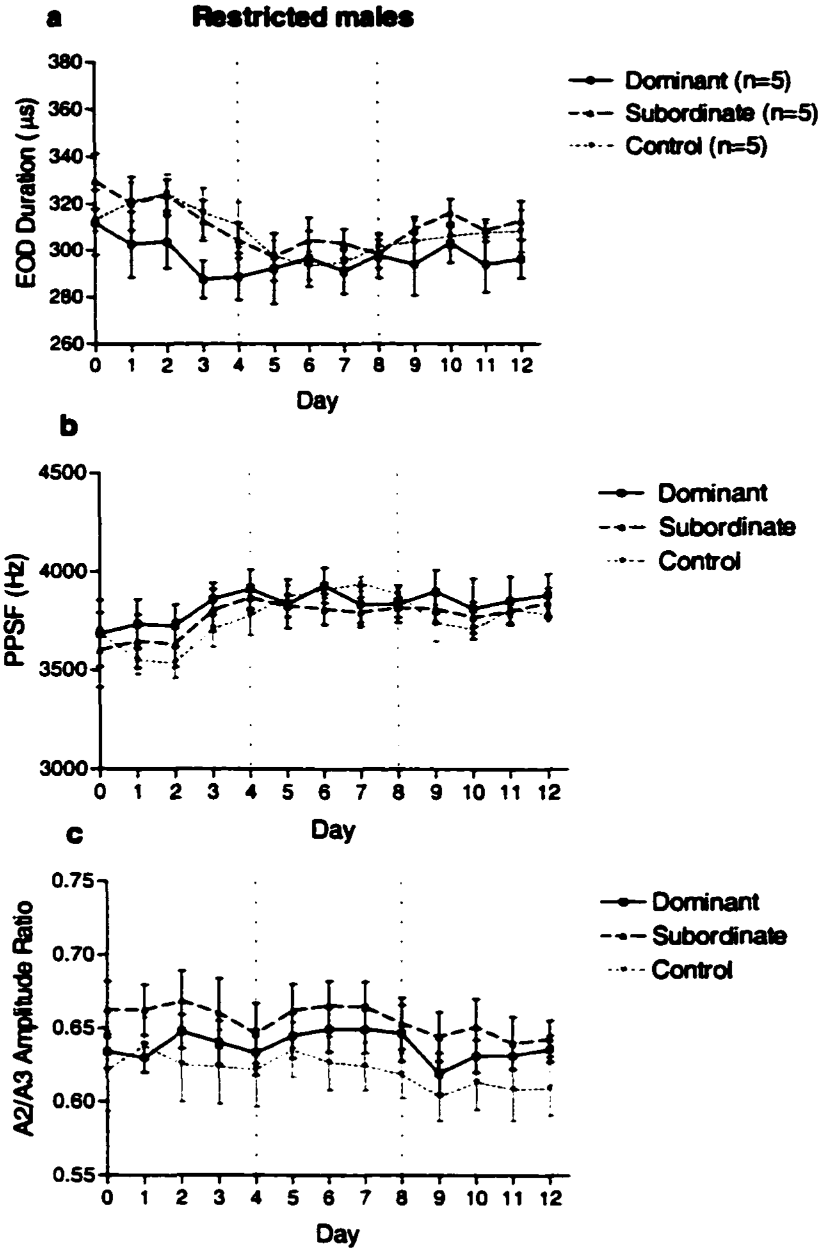


Figure 13

Male-male restricted interactions. Subjects are categorized by dominance status attained during free interaction. (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the dashed lines. Error bars: ± 1 SEM significant: $F(2,24) = 2.78$; $p = 0.0819$. The EODs of these fish did not show pronounced socially induced changes. A decreasing duration trend (in both groups) did cease, however, at the onset of interaction (Figure 13).

Because different pairs of females were tested in freely interacting and restricted conditions, it was not possible to assign dominance status to fish in the restricted female pairs. Females under restricted conditions showed considerable inter-individual variability in EOD changes. These differences were used as a means to designate members of any given restricted female dyad to one of two groups, pseudo-dominant or pseudo-subordinate.

Restricted females were assigned to either pseudo-dominant or pseudo-subordinate status by the following criteria: pseudo-dominant individuals showed a larger increase in their EOD duration relative to their neighbor following the onset of social interaction. The neighbors of these pseudo-dominant individuals were assigned pseudo-subordinate status. Without exception, freely interacting dominant females (see Figure 5), like pseudo-dominant females, showed increases in EOD duration relative to their neighbors.

Figure 14 illustrates the EOD parameters of restricted intruder females following the pseudo-dominant/pseudo-subordinate classification. Note: It was not possible to perform parametric statistical analyses based on the pseudo-dominant/pseudo-subordinate classification of females, as these groups were chosen based on the differences observed in their EODs. The possibility that pseudo-dominant restricted fish would also show dominant status in free interaction was not tested in females.

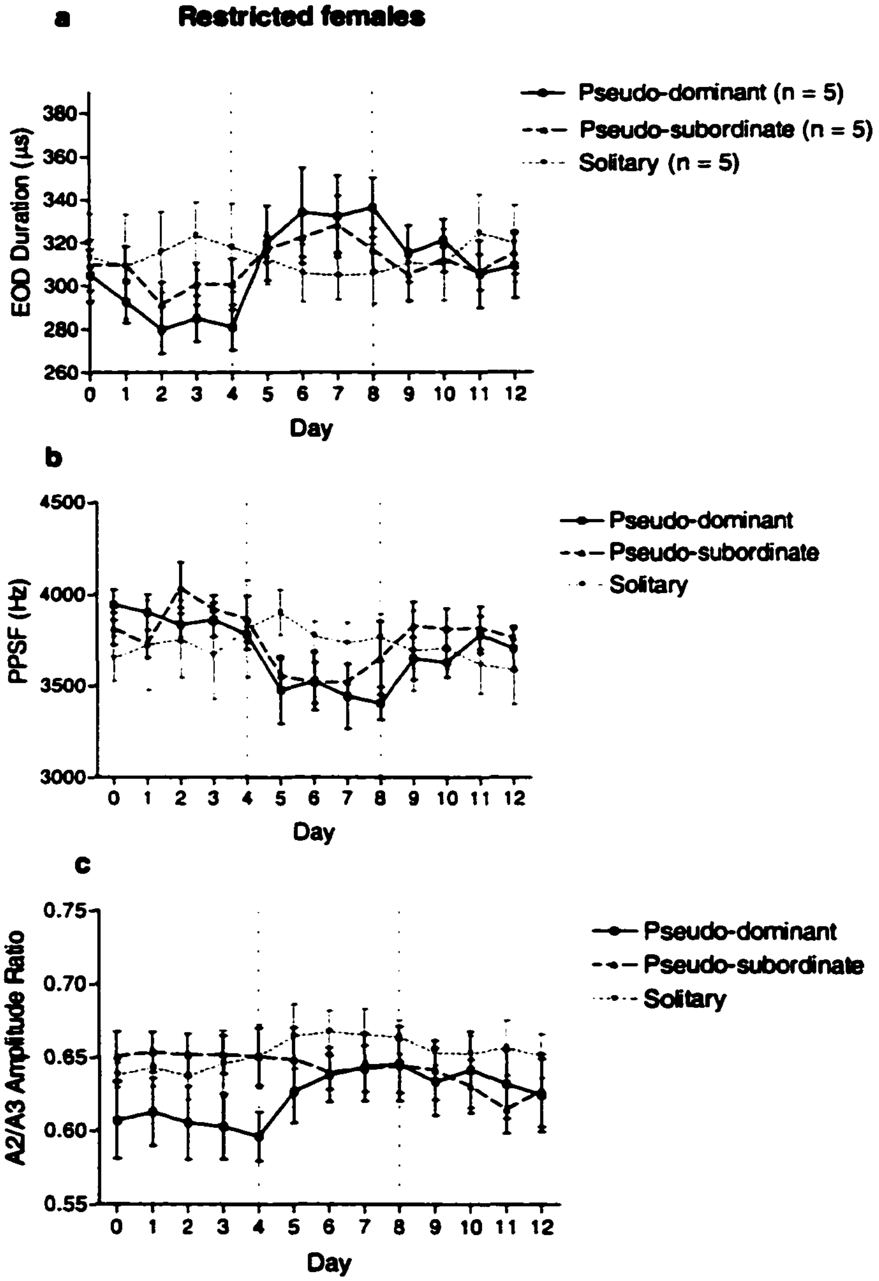


Figure 14

Female-female restricted interactions. Subjects are categorized by pseudo-dominance (see text). (a) EOD duration, (b) PPSF, and (c) amplitude ratio. The interaction period lies between the dashed lines. Error bars: ± 1 SEM

Both pseudo-dominant and pseudo-subordinate groups showed increased EOD duration (and associated PPSF decreases) during the manipulation period. Such a trend occurred only with dominant fish in the freely interacting female dyads (Figure 5a). The pseudo-subordinate group did not show an increase in amplitude ratio following the manipulation (although its amplitude ratio did remain higher than or comparable to that of the pseudo-dominant group throughout the recording period).

ANOVAs comparing freely interacting male-male and female-female intruder groups (along with their respective solitary controls) as a function of dominance status revealed no significant sex difference in EOD duration and PPSF. The sex difference in EOD duration did approach significance: $F(1,24) = 3.87$; $p = 0.0609$. A significant amplitude ratio sex difference was found: $F(1,24) = 4.72$; $p < 0.05$. Post-hoc analysis of these data showed that females (0.658) had a significantly higher amplitude ratio than males (0.629), $p = 0.04$. There were no significant interactions between sex and dominance status.

Freely interacting intruder male and female pairs expressed similar dominance mediated changes to the EOD (compare Figures 5 and 6). During interaction, EODs in dominant fish were longer and PPSFs lower than those in subordinates. The EOD duration of dominant females increased while that in subordinate fish remained largely unchanged. In the males, however, the EOD duration of dominant fish increased slightly, while that in subordinates decreased. Figure 15 illustrates these trends. In order to emphasize the interaction-mediated EOD changes within each group, the figure eliminates pre-existing between-group differences by presenting interaction means that are a percentage of pre-interaction baseline.

Figure 16 illustrates the comparisons of pairs that were restricted in their interactions. Pseudo-dominant and pseudo-subordinate females were grouped according to amount of inter-individual EOD change resulting from social interaction (see above). Dominant and subordinate males, on the other hand, were assigned their respective roles in these restricted trials according to the status they showed in free interactions.

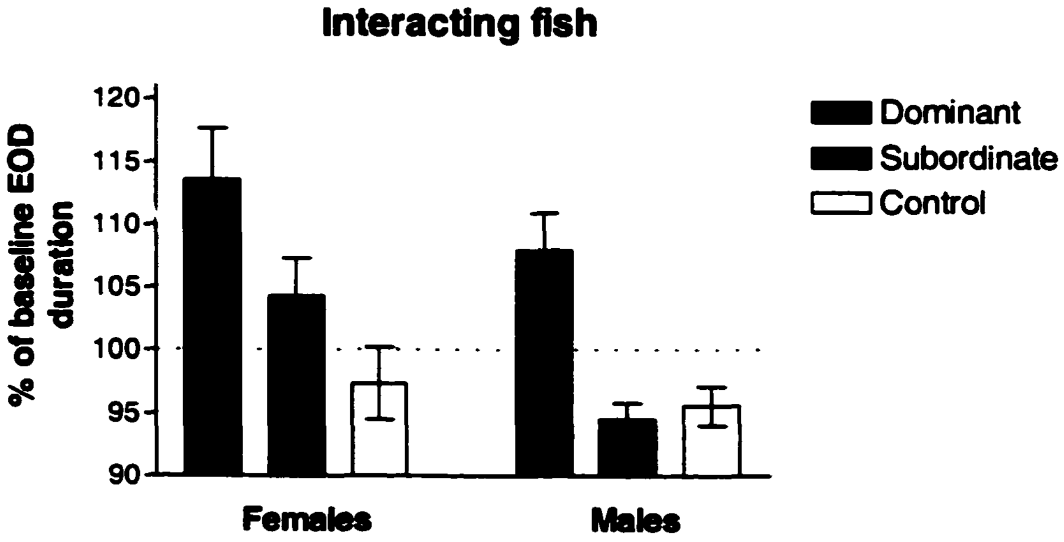


Figure 15

EOD duration as a function of dominance status in freely interacting male-male and female-female pairs. Each bar measure is a percentage of the pre-interaction baseline (mean EOD one day prior to manipulation). Error bars: ± 1 SEM

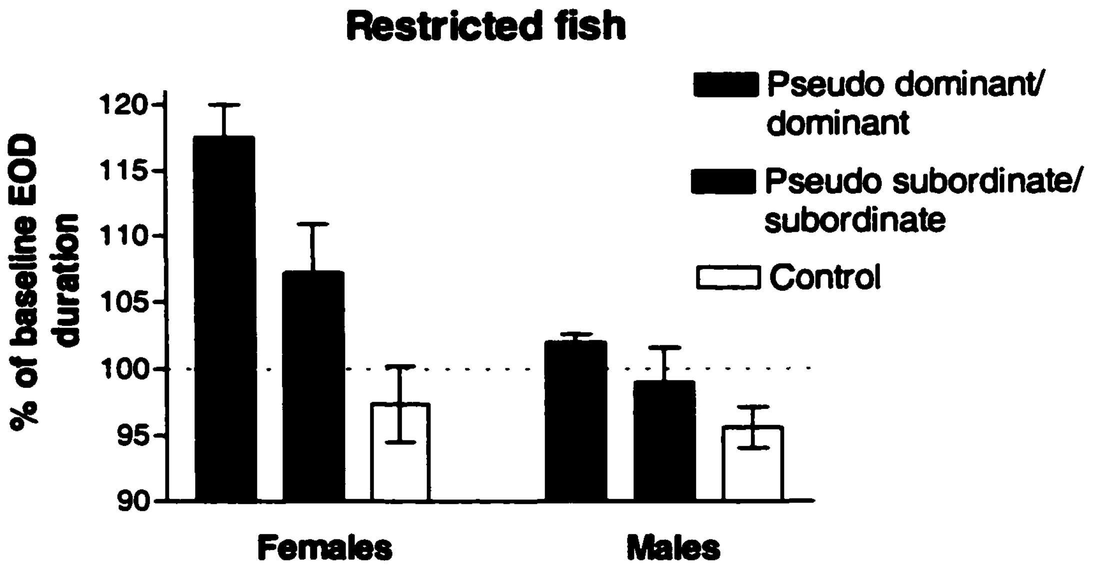


Figure 16

EOD duration as a function of dominance status (male pairs) and pseudo-dominance status (females; for definition, see text) under conditions of restricted interaction. Each bar measure is a percentage of a pre-interaction baseline (baseline: mean EOD one day prior to manipulation). Error bars: ± 1 SEM

Freely interacting and restricted females (Figures 15 and 16) exhibited similar dominance-mediated effects. As individuals in the restricted group were chosen based on differences in EOD duration, it was not surprising that there was a difference in EOD duration between pseudo-dominant and pseudo-subordinate fish. EOD duration in females under restricted conditions showed an increase in both pseudo-dominant and pseudo-subordinate individuals. Subordinate males in restricted interactions exhibited a decrease in EOD duration. This decrease was, however, smaller than that observed during free interaction. These results suggest that in both males and females the presence of a barrier reduced the degree to which shortening of EOD was socially induced.

1.2.6 Discussion: Comparisons of combined dominance and interaction levels

The presence of a neighbor induced larger dominant-like EOD changes in both pseudo-dominant and pseudo-subordinate females as compared with freely interacting pairs. It is likely that socially mediated EOD changes were not suppressed under restricted conditions, and being dominated in free interaction (Figure 5) did suppress such changes.

Restricted dominant and subordinate males (and prior resident groups) showed little changes in EOD parameters. It is possible that the socially mediated EOD changes in males would have been more robust if they had been tested at an earlier date (all female pairs were tested prior to males; for further discussion of the effects of time in captivity see below).

The EOD changes resulting from social interaction may serve to differentiate individual status in groups. Differences in inter-individual EOD duration may serve as badges of current status, reducing unnecessary conflict. Communication using status badges is well documented in birds (Harris sparrows, *Zonotrichia querula*) where black feathers correlate with both testosterone and social dominance (Rohwer, 1975; 1982; Rohwer & Rohwer, 1978). The mormyrid EOD might serve as an electric badge, as EOD duration correlates with both testosterone and social dominance in these fish.

If this were the case, EOD duration would likely be assessed by conspecifics in conjunction with both discharge patterns and locomotor behavior. Discharge cessation is an appeasement behavior, yet electrically silenced *G. petersii* (who cannot discharge and thus mimic this behavior) often continue to engage in locomotor behaviors that are inconsistent with subordinate status. Silenced fish are attacked more often than intact fish (Crockett, 1986). This inconsistency of signaling (both dominant and subordinate signals presented simultaneously) was the cause attributed to increased attack from neighbors (Crockett, 1986). Similar badge manipulations also induced increased attacks in the Harris sparrow (Rohwer & Rohwer, 1978).

If social dominance leads to increased androgens (and associated EOD elongation), it follows that an artificially treated fish that has not attained dominance may receive more attacks from conspecifics, as it will present inconsistent signals. Alternatively, if androgen treatment results in increased aggression from the treated fish, treatment should induce social dominance. Such an experiment would help to determine if increased androgens are the cause or effect of dominance, and if the EOD duration serves as a status badge.

The findings presented here suggest that testosterone levels increase in individuals that have interacted with others. Such individuals did not need to be dominant in a free interaction in order to show such increases, but losing a fight did prevent an increase in EOD duration. In many interactions, the subordinate initially possessed a longer EOD than the dominant fish (these EOD differences reversed during interaction). Many species of fish use coloration and markings to signal dominance, with high-ranking individuals taking on coloration (associated with hormonal condition) only as a result of social dominance (Magurran, 1986).

The EOD duration of dominant males and females (Figures 5 & 6) and both pseudo-dominant and pseudo-subordinate females under restricted conditions (Figure 14) reverted towards shorter pre-treatment levels following interaction. These 'rebounds' suggested that dominance-mediated EOD increases, the androgen increases associated with them, or both may be costly to maintain.

Testosterone is in fact known to adversely impact the immune systems of many animals, as well as reduce survivorship by increasing metabolic costs and/or predation risk (Folstad & Carter 1992; Grossman, 1985; Marler & Moore, 1988, 1989). A lower peak power spectral frequency, associated with longer EOD, is also thought to increase risk of detection by predators (Stoddard, 1999). In addition, longer EODs are less efficient at precision time marking. If patterns of discharging and inter-pulse intervals encode information in time intervals, short-duration EODs will result in more accurate signaling (Kramer, 1990b). As maintaining elevated testosterone levels and elongated EOD are likely to be costly, it is not surprising that such changes did not persist following interaction. Such rebounds did not occur in freely interacting subordinates, as they did not exhibit an increase in EOD duration during interaction. In some of these subordinates, however, a post-treatment rebound in the opposite direction to that of dominants occurred (Figures 6 & 8, for example). The EOD duration in these fish increased following interaction. It is possible that the social interaction initiated anabolic hormone changes, and that these changes affected the electric organ (and therefore the EOD) following social interaction.

Organisms, having become familiar with their environment, will switch from producing energetically costly signals to relying on less costly ones (review: Moller, 1995). For example, *G. petersii* familiarizes itself with a novel environment by using active, costly electrolocation (generating EODs), but will subsequently substitute the active electric sense with hydrostatic pressure cues (Cain, 1995; Cain, Gerin & Moller, 1994).

1.2.7 <u>Results and Discussion: Effects of Captivity</u>

Captivity affects the EOD waveform of *G. petersii* (Landsman, 1993b, 1995). In the current experiment, the period of time fish spent in captivity significantly affected EOD plasticity (the degree to which EOD duration changed as a function of social interaction) ($F(7,42) = 2.75$; $p < 0.05$). It is possible that some of the observed differences between groups were affected by the amount of time fish had spent in captivity, a period of 16-181 days (Figure 17). On day 16 (the earliest day of testing), the socially mediated change in EOD was significantly greater than that of only two of the eight testing periods (the 4th and 7th periods, $p = 0.036$ and 0.017, respectively). It is likely that the results presented above would have been even more robust if all fish were freshly imported. Only the first and second days of measurement (days 16 and 70) showed overall mean increases in EOD duration as a function of interaction.

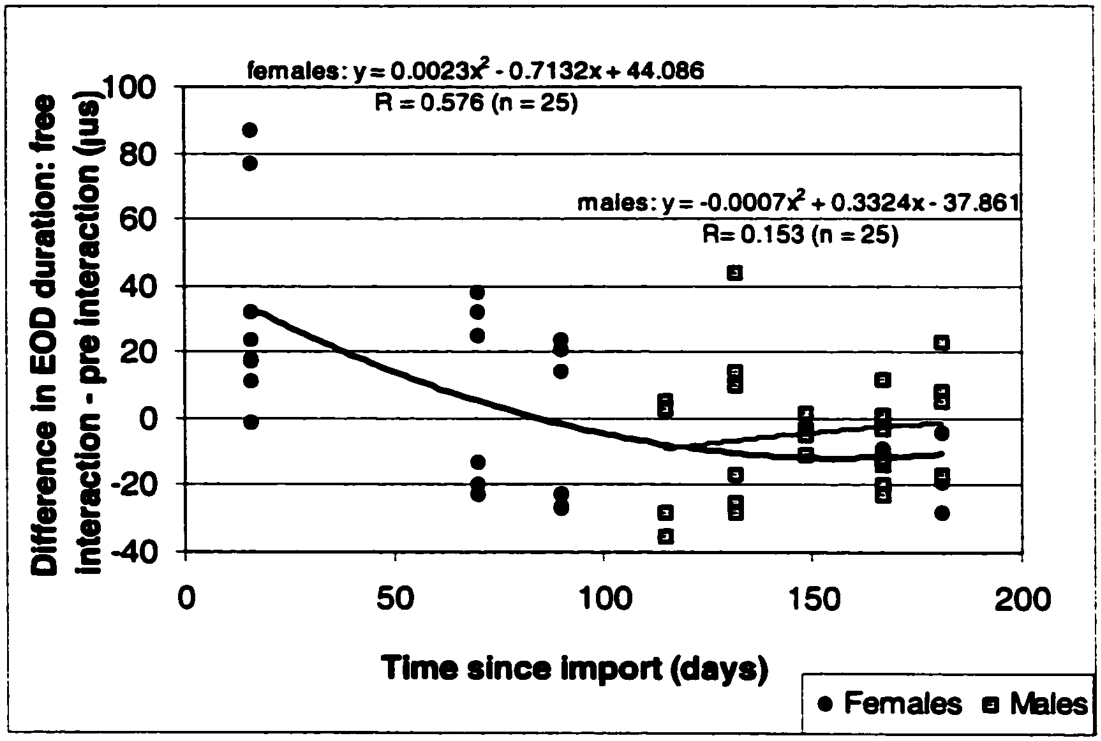


Figure 17

Effects of captivity on socially mediated EOD changes (interaction duration minus pre-interaction duration) as a function of time in captivity. Black circles: females; gray squares: males. Polynomial regression lines are drawn for each sex with corresponding functions.

1.3 Locomotor Behaviors

1.3.1 Introduction: Assessment and Dominance Signaling

Signaling plays a major role in dominance and/or territorial interactions, as it reduces the need for costly fighting, especially once a hierarchy or territories have been established. For a dominance hierarchy to persist, individuals must be capable of recognizing each other, or at least responding to cues that are correlated with dominance. Several studies provide evidence of individual recognition in the dominance hierarchies of teleost fish (Magurran, 1986). Individual recognition in fish can be based on cues from behavior, morphology, and even on olfactory cues (as in the yellow bullhead, *Ictalurus natalis*) (Todd, Atema & Bardach, 1968).

Signaling during agonistic interactions can facilitate the mutual assessment of opponents' fighting abilities (or resource-holding potential). Accurate assessment allows individuals to 'decide' whether or not it is worth incurring the cost of a fight in order to benefit from controlling a resource (Bradbury & Vehrencamp, 1998). If an opponent's resource holding potential is determined to be greater than one's own, it is advantageous to cut one's losses and retreat.

In recent years, a number of game theory models have been developed in order to characterize the types of conflict-resolution strategies that competing individuals may employ (Bradbury & Vehrencamp, 1998). These theories make specific predictions about the behavior of a combatant during an agonistic interaction as a function of its resource holding potential relative to that of its opponent.

Assessment displays are signals that reveal reasonably accurate ('honest') information about one's resource holding potential (such as body size, strength or motivational state). These types of displays are predicted to settle conflicts, thus preventing their escalation, even if such displays only predict the outcome of a fight slightly above chance level (Maynard Smith & Parker, 1976). Relative body size often determines the winner of a fight. A commonly cited example that draws attention to body size is the visual display of red deer (parallel walking) (Clutton-Brock , Guinness & Albon, 1982). The vocal displays of toads also indicate body size (Davies & Halliday, 1978).

Assessment has been implicated as an important factor in game theory models of competitive behavior. A well-known model of competitive behavior is the war-of-attrition game. The asymmetric version of the war-of-attrition game (Parker & Rubenstein, 1981, as cited in Bradbury & Vehrencamp, 1998) predicts that persistence in a combative interaction will depend upon one's assessment accuracy in determining the cost of persisting in the interaction, as well as the perceived value of winning. Some of the predictions made by this game theory model include the following: 1) the behavior must be costly, either energetically, or with a risk of injury, or both 2) As the difference in the body size of opponents decreases (thus making assessment less certain), average contest duration should increase 3) As resource value increases, contest duration should increase.

The sequential-assessment game (Enquist & Leimar, 1990) describes combatants as continually updating their estimates of each other's fighting ability throughout an interaction. This theory assumes that during the course of an interaction combatants will continue to accrue information about their fighting ability relative to the other combatant. The theory makes similar predictions to those of the war-of-attrition: fights are predicted to be longer when combatants are similar in size and when the resource is perceived to have a higher value. In addition, the sequential-assessment game predicts that a narrower range of variation will occur in contest durations of fights involving two intruders (each individual values the resource similarly), as compared with fights involving an intruder and a prior resident.

G. petersii exhibits characteristic locomotor displays during agonistic interactions. This section will explore two of these displays, parallel/anti-parallel display and head butt, and the possibility that the former serves in mutual assessment.

1.3.2 Locomotor Behaviors: Material and Methods

1.3.2.1 Parallel display and head butt

These behaviors were analyzed from video recordings of each freely interacting dyad (see general material and methods section) during the first 10 min of interaction. Both the number of occurrences and the time of occurrence were measured.

Parallel display occurs in one of two types: parallel and anti-parallel. In the current analysis, the occurrence of both were pooled and collectively referred to as parallel display. This display occurs when two fish orient in parallel in a head-to-head (parallel) or in a head-to-tail (anti-parallel) position. During a display, each fish bends its tail inward towards the other fish. Interacting fish will often engage in bouts of displaying that can last for several seconds. During a display, a rapid volley of EODs accompanies head and tail bending, rarely lasting longer than 1 s. Interacting fish usually straighten head and tail, but may remain in the line-up orientation.

The occurrence of parallel display, as defined here, required three of the following criteria to be met: (1) line-up (either parallel or anti-parallel), (2) head/tail bending towards the other fish, and (3) the occurrence of a discharge volley. As all three events occurred simultaneously for very short durations, each parallel display was recorded as an instantaneous event. Fish often engaged in sequences of repeated parallel display. During such a sequence, interacting fish often remained lined up for several seconds, periodically bending towards each other with head and tail while generating EOD volleys.

Parallel display was characterized by mutual participation and was clearly agonistic. This display did not occur with one fish actively displaying while the other remained passive. Mutual tail lashing and body blows often accompanied parallel display. During the inward head bending it appeared as if one or both individuals were attempting to bite the other. The lined-up body position of each combatant prevented such biting, however.

Head butt describes a behavior that occurs when "a fish, approaching an opponent at high speed, delivers blows, or approaching more slowly, lateral strokes to its body (directed towards head, trunk or tail)" (Moller, 1995, p. 245). Rapid EOD volleys (emitted by the butting fish) usually accompany head butt. Individual instances of head butt were recorded whenever the butting fish's head contacted the body of its neighbor.

Head butt is also agonistic, but does not appear to be a mutual display. Once dominance has been established in a dyad, head butts are delivered by dominant fish only. Subordinates attempt to avoid butting, usually by swimming away from the dominant fish as it approaches. (Some fish attempted to jump out of the tank, apparently to escape the butts of a neighbor.)

1.3.2.2 Hypotheses

To date, the behavioral function of parallel display in *G. petersii* has not been adequately explained. It is hypothesized here that parallel display serves an assessment function. A number of predictions were made: (1) parallel display should occur more frequently than head butt at the beginning of an interaction, when opponents assess one other. (2) The establishment of dominance should result in a decrease in parallel display and increase in head butt. (3) More parallel display should occur in pairs of similar-sized fish. (4) Parallel display and head butt are predicted to occur at a higher rate in prior resident pairs. An established territory is assumed to be of more value to its resident than it is to an intruder.

1.3.3 Locomotor Behaviors: Results

Figure 18 illustrates the occurrence of parallel display and head butt during six consecutive 100-s sampling intervals for the freely interacting dyads (data have been pooled from all groups). There was a trend of decreased parallel display and increased head butt suggesting that parallel display serves in mutual assessment and head butt as an expression of dominance. In addition, at the start of interaction more parallel display occurred than head butt. By the second 100-s time bin (and every time bin thereafter) more head butt occurred.

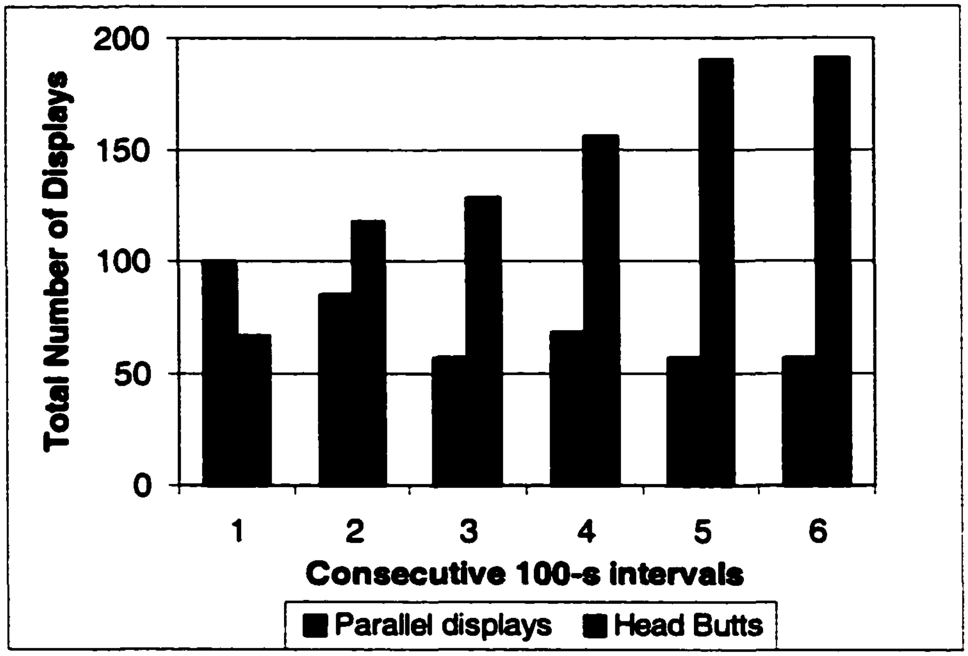


Figure 18

Total number of parallel display and head butt for all dyads (*N* = 26 pairs) during six consecutive 100-s sampling intervals (first ten minutes of interaction).

A significant correlation was found between the mean number of parallel display and time, i.e. consecutive 100-s time bins ($r = 0.754$, $p < 0.05$) as well as between the mean number of head butt and time ($r = 0.95$, $p < 0.01$). As predicted, over the first ten minutes of interaction, there was a trend of decreased parallel display and increased head butt. There were no significant differences in the total number of either head butt or parallel display between groups based on sex (male-male versus female-female dyads) and residency status (intruder versus prior resident dyads).

Figure 19 illustrates the total number of parallel display and head butt for intruder and prior resident dyads. There was a significant correlation between the mean number of parallel display and time, i.e. the consecutive 100-s time bins in prior residents ($r = 0.847$, $p < 0.05$) as well as in the mean number of head butt and time ($r = 0.913$, $p < 0.01$). Over the first ten minutes of interaction, a trend of decreased parallel display and increased head butt was observed in prior resident dyads.

In the intruder dyads, however, there was no correlation between the mean number of parallel display and time ($r = 0.097$), but a significant correlation between head butt and time was seen in prior residents ($r = 0.81$, $p < 0.05$). The first ten minutes of interaction was characterized by a trend of increased head butt.

It was predicted that assessment behavior would increase in frequency if combatants were similar in regards to the attribute being assessed. If parallel display in *G. petersii* is a length assessment behavior, it should be more frequent in dyads with a small between-fish length disparity. Figure 20 supports this hypothesis. A significant negative correlation ($r = -0.488$) existed between the number of parallel display (first 100-s of interaction) and the length difference between interacting fish ($F(1,24) = 7.5$; $p < 0.05$).

Of 26 pairs only four possessed a subordinate fish with a body length greater than its dominant neighbor (in two pairs the fish were equal in length). On average, dominant fish were 9.4 ± 1.7 mm longer than subordinates. In the four pairs with longer subordinates, the average length difference was 3.25 ± 1.7 mm.

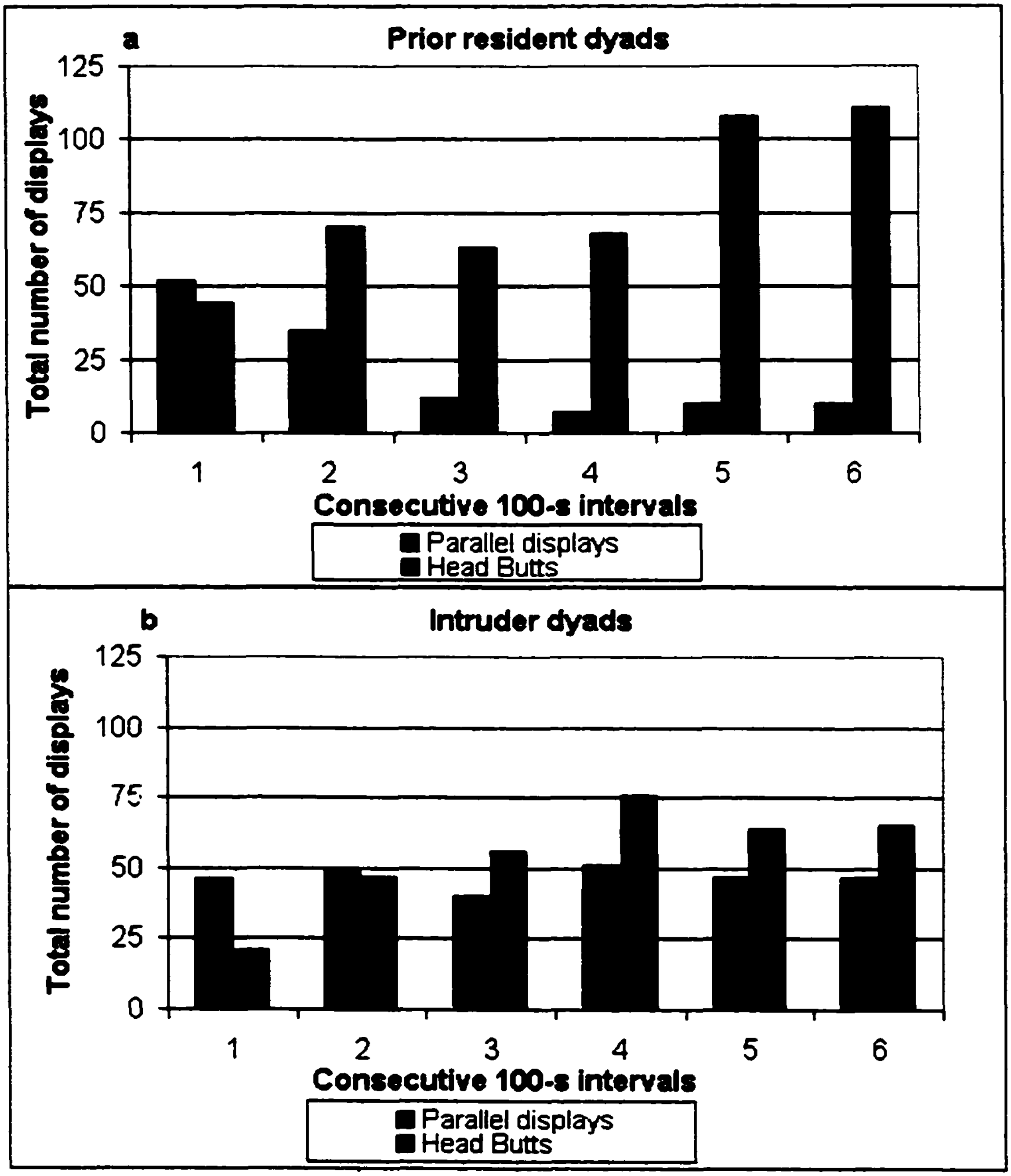


Figure 19

Total number of parallel display and head butt during six consecutive 100-s sampling intervals (first ten minutes of interaction). (a) Prior resident dyads, (b) intruder dyads

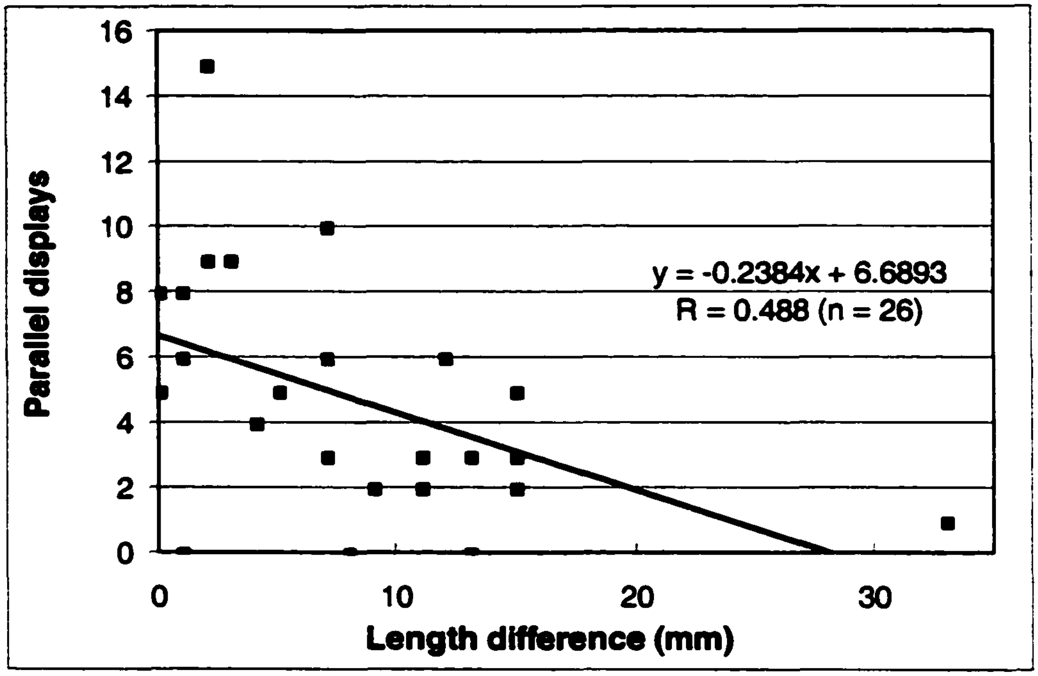


Figure 20

Amount of parallel display in all dyads (*N* = 26) during the first 100 s of interaction as a function of the difference in standard lengths between interacting fish. A binomial regression is plotted.

1.3.4 Locomotor Behaviors: Discussion

Parallel display in *G. petersii* may function as a means of assessment as it is a mutually combative display, and likely permits assessment of size, strength and/or motivation of interacting individuals. The nature of the close parallel line-up positioning in *G. petersii* appeared to prevent combatants from actually biting each other. This behavior may have therefore allowed for assessment of an opponent without a high risk of injury. This is important, as bite wounds are a common cause of death in these fish. Parallel orientation displays are part of the behavioral repertoire in many other teleosts. For example, the South American cichlid *Nannacara anomala* displays a sequence of assessment behaviors, the first of which is a parallel display (Jakobsson, Radesäter & Järvi, 1979). As this display is the first in a characteristic behavioral sequence, the authors presume that it is least costly in terms of the risk of injury to the combatants.

If parallel display serves in assessment during dominance interactions, fish should assess an overt character that is predictive of the opponent's resource holding potential. One such candidate might be EOD duration, as it is affected by hormonal condition (as reviewed above). The current results (Part A) as well as other studies (Carlson et al., 2000) indicate that EOD duration increases following the establishment of dominance but does not predict dominance.

In a number of teleosts, size is often more important than prior residency in determining dominance (Frey & Miller, 1972; Bell, Myers & Russell, 1974). The results of this experiment are in accord with these findings, as prior residency did not predict subsequent dominance.

Parallel displays occurring during the determination of dominance have also been documented in other teleosts. Visual broadside threat displays have been observed in *Betta splendens* and *Macropodus opercularis* (Southwick & Ward, 1968). Such displays are also common in weakly electric fish and are likely mediated by both electric signaling and electrolocation. Both parallel and antiparallel displays occur in gymnotiform electric fish (Cleworth, 1969) and in a number of mormyrids during both inter and intraspecific interactions (Bell, Myers &

Russell, 1974). Parallel displays also occur in electric catfish, *Malapterurus electricus*, in intra- but not interspecific encounters (Rankin & Moller, 1986; 1992).

To date, it is not known if mormyrids use the electrosensory modality to assess body size during parallel display. Although the parallel displays of other animals are often broadcast as visual signals, they need not be restricted to this communication channel. Thrips (an insect, *Elaphrothrips tuberculatus*) use their tactile sense to assess each other's body size during parallel body alignment displaying (Crespi, 1986).

Social dominance in the present study was usually established within a few minutes of introduction into the social manipulation condition. The occurrence of parallel display showed a decreasing trend over the course of interaction, as dominance was established. This supports the hypothesis that the display was used for mutual assessment. Similarly, the occurrence of head butt showed an increasing trend over the first few minutes of dominance, as subordinate fish were unable to escape from the territory that the dominant individual had 'won'.

A decreasing trend in parallel display was only observed in prior resident pairs. This assessment behavior may have been more important in the context of defending a territory that was already established, in contrast to a location that was novel for both fish. As parallel display requires mutual participation, it is possible that the decrease in parallel display in prior resident dyads was due to a decreased participation in displaying by the prior resident individual. Territorial value is expected to be greater for a prior resident relative to an intruder, and game theory models predict that as resource value increases, so will the tenacity in interactions (Parker & Rubenstein, 1981). If the repertoire of agonistic behaviors displayed by *G. petersii* follows an escalating sequence, prior resident fish may have initiated a rapid switch from parallel display to higher risk behavior such as head butts. The cichlid *Nannacara anomala*'s initial parallel orientation display is usually followed by higher risk tail beating, frontal orientation with biting, and mouth wrestling (Jakobsson et al., 1979). Prior resident dyads engaged in more head butt over time than intruders, but it is not known which individual in each prior resident pair (the prior resident or intruder fish) initiated the majority of head butts.

Under natural conditions, assessing a prior resident's resource holding potential may be in the interest of an intruder, as such an assessment (assuming the behavior does not create high injury risk) may result in usurping the territory. The prior resident, on the other hand, has nothing to gain but might lose its territory by permitting assessment. This may also explain why parallel display decreased rapidly over the first minutes of prior resident interactions.

Assessment behavior will increase in frequency if combatants are similar in regards to the attribute being assessed (Bradbury & Vehrencamp, 1998). This is the case in thrips (discussed above). In thrips the number of bouts of parallel assessment display and likelihood of contest escalation are determined in part by inter-individual body size differences. Contests are settled quickly and with few bouts of parallel display in dyads that show a large body size disparity (Crespi, 1986). An earlier study on *G. petersii* (using a smaller sample, Bell, Myers &

Russell, 1974) failed to find a clear relation between parallel display and the length (size) difference of interacting fish. The data presented above, however, support the hypothesis that parallel display was used to assess a neighbor's body length. Individuals of similar length engaged in more parallel display, and body length almost always determined dominance. Body length is a reliable indicator of age and sexual maturity in these fish (Moller, personal communication), which may correlate with fighting ability. Similar to the results presented here, in male-male aggressive interactions of doily spiders (*Frontinella pyramitela*: Austad, 1983) combatants grappled with each other for longer and more variable time periods when they were similar in size. In addition, a residency manipulation that increased resource value to the prior resident doily spider (using a territory containing a female that had not yet been inseminated) led to increased persistence in the combative interaction. There was no difference in the amount of parallel display between prior resident and intruder pairs in this experiment, although the pattern of decreasing displays over time was not observed in the intruders.

1.4 Electric Organ Discharge Patterns

The nature of parallel display, namely the body orientation and physical contact along the body axis, suggests that interacting fish are assessing each other by means of electrical and/or tactile modalities. The next section will investigate the EOD discharge volleys that occur in the context of both parallel display and head butt, as well as EOD discharge patterns emitted under the different residency conditions.

1.4.1 Electric Organ Discharge Patterns: Results

1.4.1.1 Dyad inter pulse intervals (IPIs) at the onset of interaction

The EOD discharge patterns were analyzed as intervals between successive EODs regardless of the sender. The current design did not permit inter-pulse interval (IPI) discrimination of individuals in dyads, due to the proximity and orientation of the interacting fish. All inter-discharge duration measures obtained from fish pairs therefore represent the discharge activity of two fish and are referred to as dyad IPIs. It was possible, however, to discern if one or two fish were discharging at any given moment, and which fish was discharging. This was done by monitoring locomotor activity on the videotape while simultaneously listening to discharge patterns amplified from the audio channel and viewing triggered EODs on an oscilloscope.

The EOD patterns of freely interacting dyads (and solitary control fish) were recorded for the first 60-s of social interaction. Figure 21 illustrates an example of a dyad IPI record, along with the associated sequential interval histogram from an intruder pair of males. The dyad IPI record shows a number of long-duration dyad IPIs (300-500 ms). These long-duration dyad IPIs are likely to represent examples of one or both fish engaging in discharge cessation or social silence (Moller, 1970; Moller, Serrier & Bowling, 1989). Such cessations were not as common in the records of solitary controls (Figure 22).

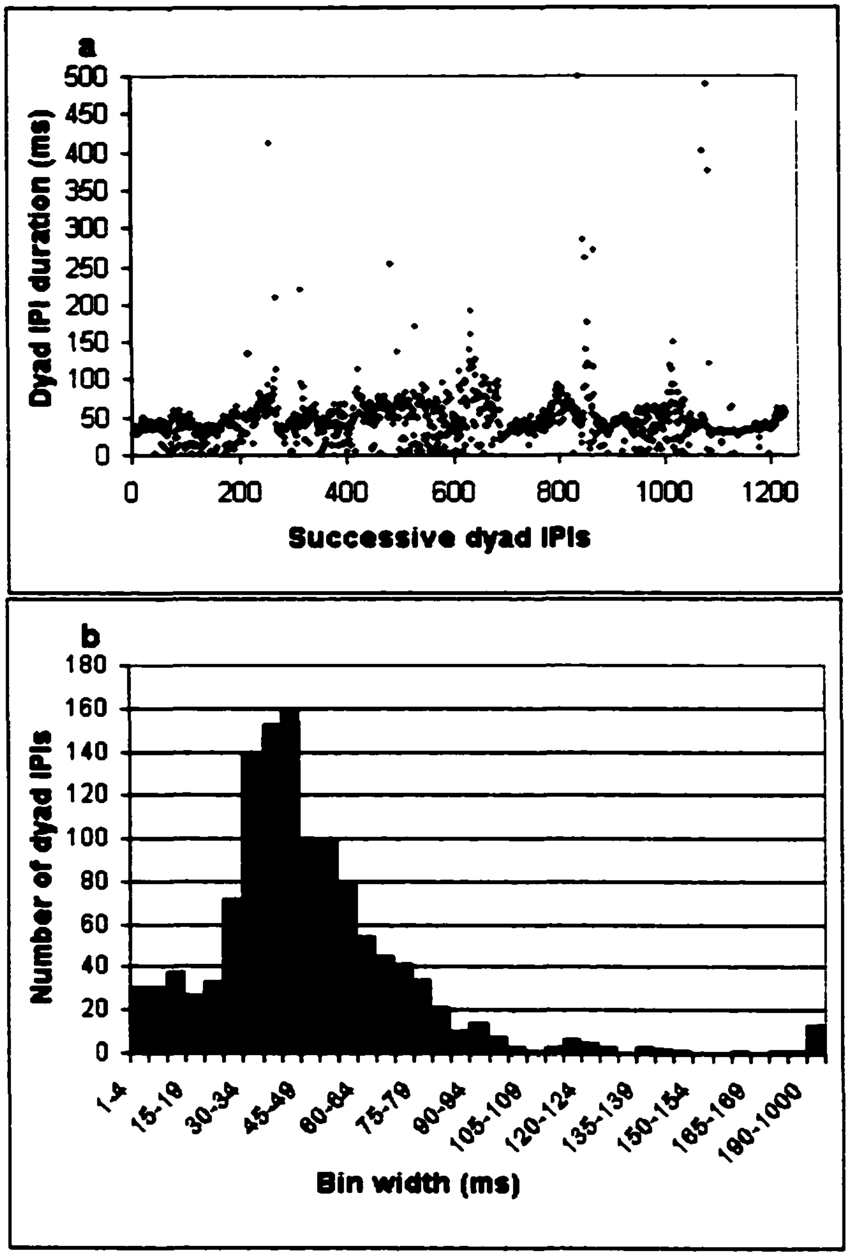


Figure 21

Dyad IPI record (a) and associated dyad IPI histogram (b). Data were recorded during first 60-s of interaction from a freely interacting pair. All dyad IPI measures represent the discharge activity from two fish. The X-axis of 21a denotes successive dyad IPI occurrences; the Y-axis shows duration of each occurrence.

A record from a solitary control fish with its associated sequential interval histogram is shown in Figure 22. Figure 22b illustrates a commonly observed characteristic of the SPI distributions from solitary fish. They were often bimodal or trimodal. These distributions are associated with discharge bouts that contained different mean IPI durations. Figure 22a shows separate bouts with IPIs of approximately 100 ms, and with bouts alternating between 100 ms and 200-300 ms. As these data represent the EOD activity of a single subject, most IPIs are longer than those observed in dyad IPIs. Compared with the dyad IPIs, however, there are fewer IPIs exceeding 350 ms, as the solitary fish does not show social silence.

Regularized rapid discharge bouts often occurred during locomotor behavior, and are usually associated with electrolocation (Toerring & Belbenoit, 1979; Toerring & Moller, 1984). Intruders were observed actively swimming in the test tank (prior residents generally remained in their shelters), and direct behavioral observations confirmed that the high, regularized discharge rates were associated with swimming and probing locomotor behaviors.

Figure 23 illustrates the modal IPIs of solitary fish and modal dyad IPIs of pairs. Modal dyad IPIs were compared by interaction type, residency status, and sex. A significant difference was found between the interaction types (freely interacting vs. restricted interactions): $F(1,35) = 33.07$; $p < 0.00001$. The modal dyad IPI of freely interacting pairs was 15.3 ± 1.6 ms. The modal dyad IPI of restricted pairs was 30.4 ± 2.3 ms.

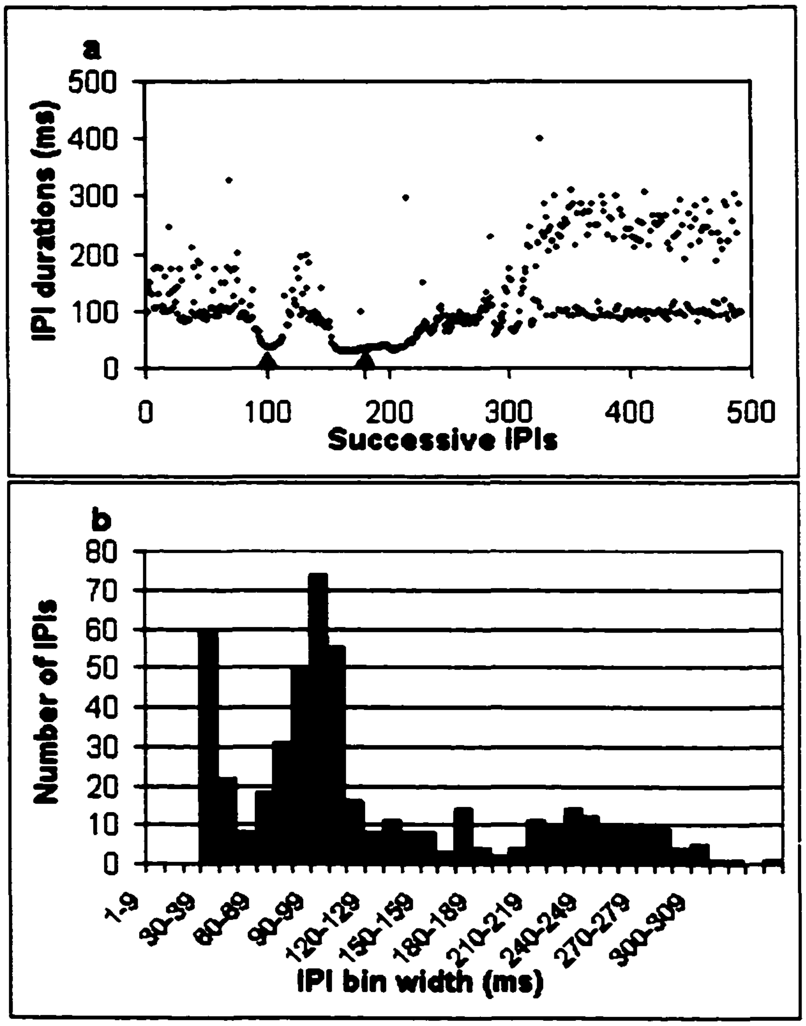


Figure 22

IPI record (a) and associated IPI histogram (b). Data were recorded for 60 s from a solitary control fish (prior resident female). Arrows on the x-axis (a) represent the short IPIs associated with swimming behavior.

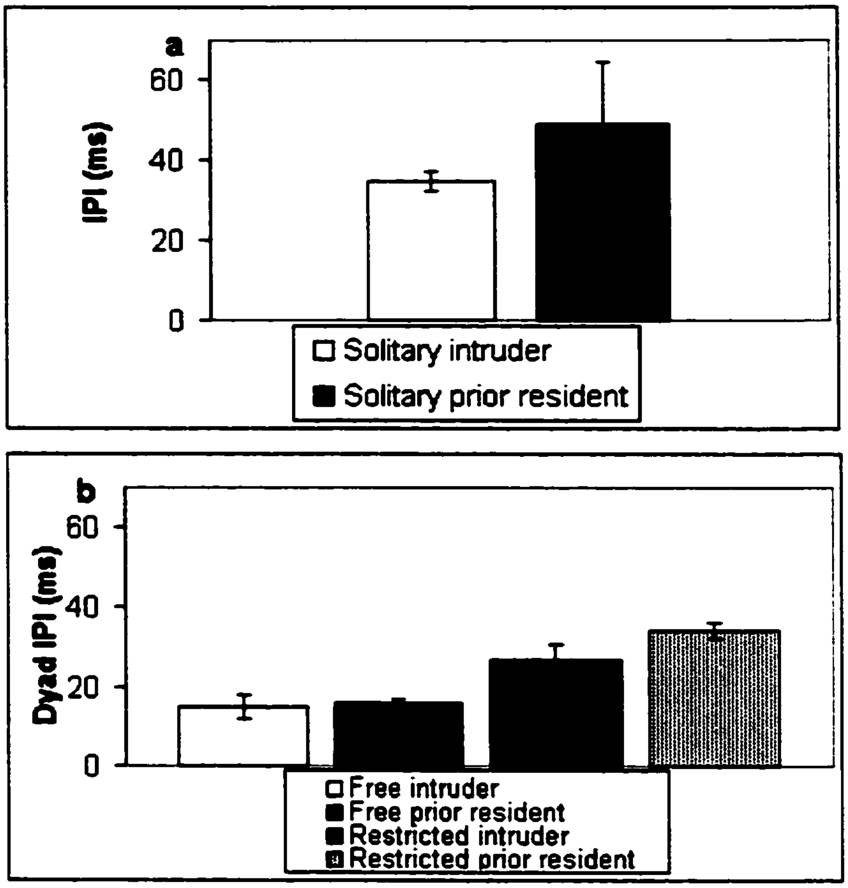


Figure 23

(a) Modal IPIs from solitary fish. Intruders: fish introduced into a novel tank, Prior residents: fish that had been in the test tank for three days. (b) Modal dyad IPIs, fish pairs by interaction type (free, restricted) and residency status (intruder, prior resident. Error bars: ± 1 SEM

There was no significant difference between the modal IPIs of solitary control fish as a function of sex or residency status (fish introduced into a novel tank versus fish that had been in the test tank for three days). The modal IPI of intruder fish was 34.7 ± 2.4 ms. and that of prior residents was 49 ± 14.1 ms.

It is likely that dyad IPIs of freely interacting pairs were significantly shorter than those of restricted pairs because free interaction permitted a greater number of social locomotor behaviors such as parallel display and head butting. Such behaviors were associated with rapid discharging.

1.4.1.2 Dyad IPIs during specific social behaviors

Dyad IPIs were recorded from eight pairs when fish displayed head butt and parallel display. Ten seconds of dyad IPIs associated with each of these behaviors were obtained from 10-minute sampling periods.

Figure 24 shows dyad IPI records associated with parallel display. Figure 25 shows dyad IPI histograms that correspond with the records depicted in Figure 24. Dyad IPIs during parallel display sometimes showed a pattern of alternation between two extremely regular interval durations (see the latter portions of Figure 24a and e). These bouts appear as separate ‘lines’ in Figure 24 and are responsible for some of the bimodal distributions in Figure 25. Such a pattern reflects ‘echo’ or ‘preferred latency’ responding (PLR) in pairs of fish (Russell, Myers & Bell, 1974; Kramer, 1974). During echo/PLR discharging, one fish displays the same interval durations in a fixed phase relationship to those of its neighbor, preventing coincident discharges (‘jamming’). [continued on p. 91].

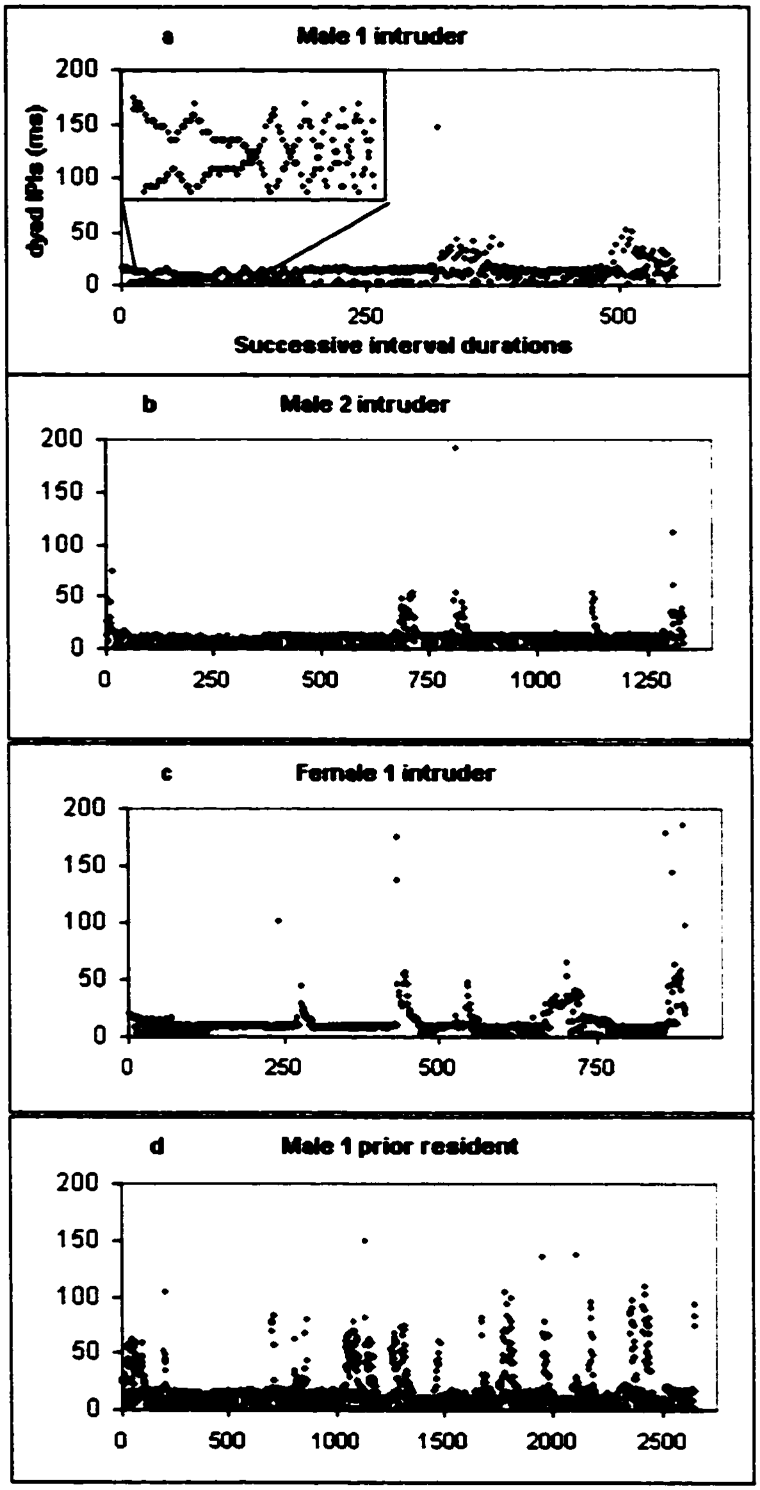

a
Male 1 intruder
dyad IPIs (ms)
200
150
100
50
0
0
250
500
Successive interval durations
b
Male 2 intruder
200
150
100
50
0
0
250
500
750
1000
1250
c
Female 1 intruder
200
150
100
50
0
0
250
500
750
d
Male 1 prior resident
200
150
100
50
0
0
500
1000
1500
2000
2500

Figure 24

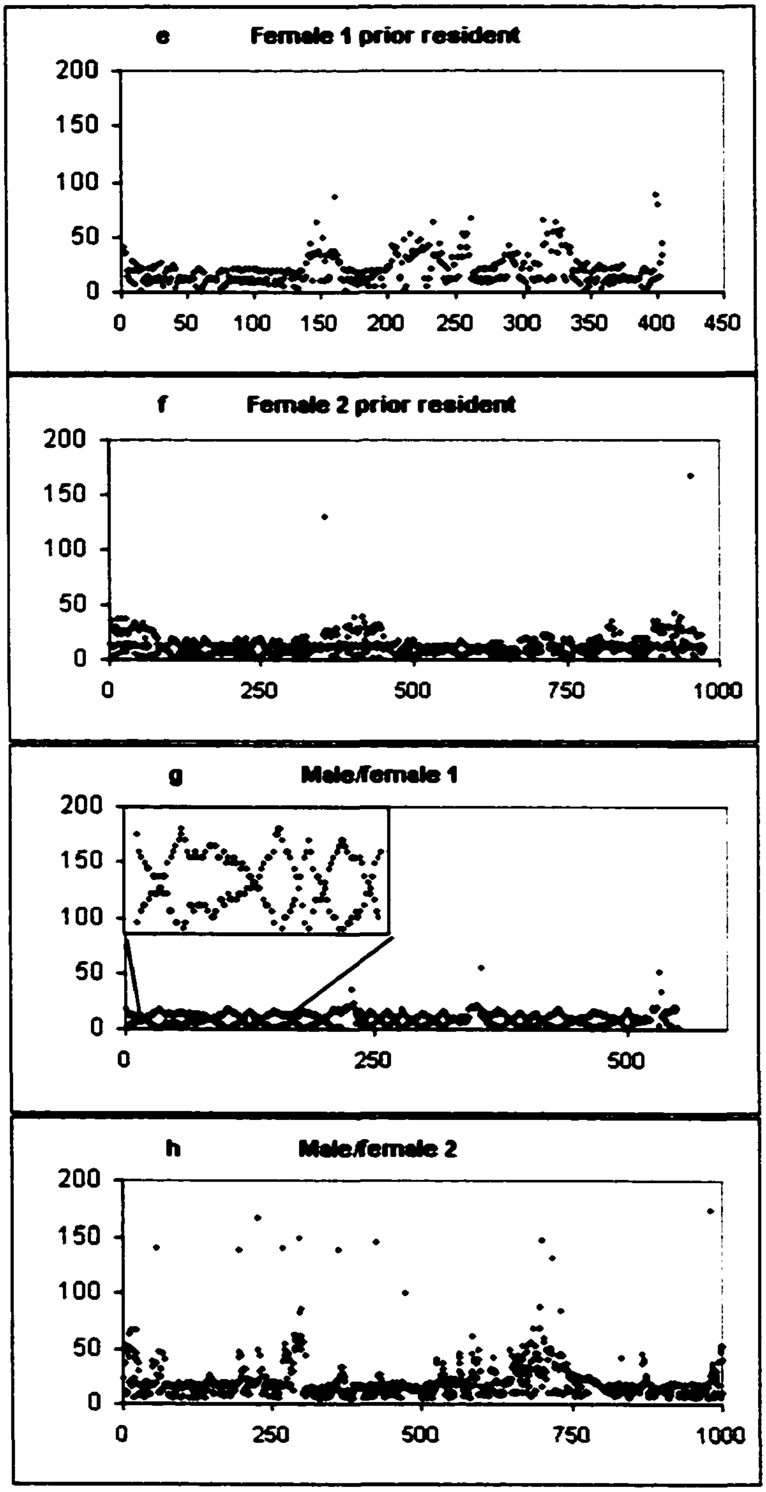


Figure 24 (continued)

Figure 24

Eight dyad IPI records during 10 s of parallel display. As the number of dyad IPIs occurring over 10 s differed between pairs, X-axis scales differ. The upper left corner of a and g are details of the first 175 dyad IPIs, plotted on a smaller scale.

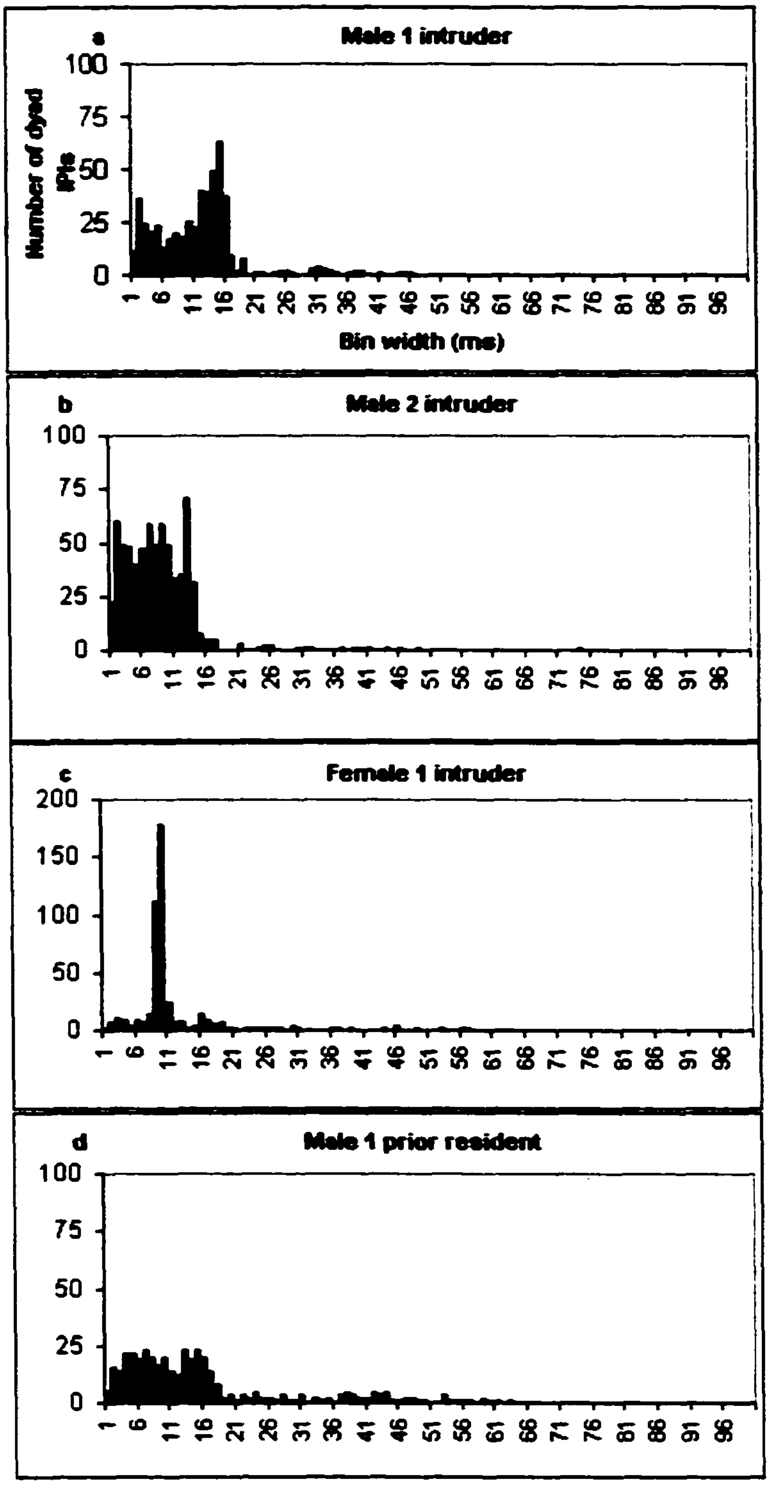

a
Male 1 intruder
Number of dyad IPIs
100
75
50
25
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96
Bin width (ms)
b
Male 2 intruder
100
75
50
25
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96
c
Female 1 intruder
200
150
100
50
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96
d
Male 1 prior resident
100
75
50
25
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81 86 91 96

Figure 25

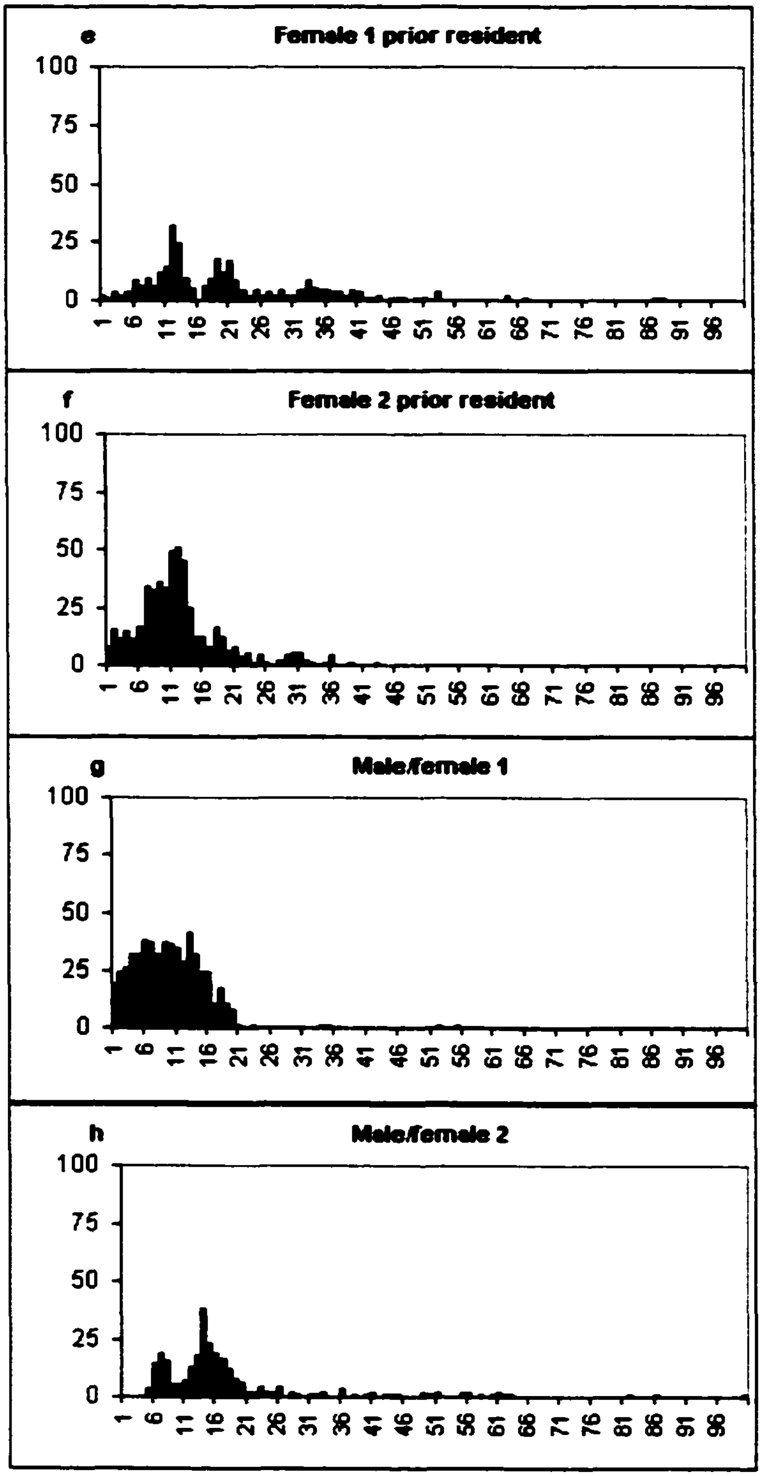


Figure 25 (continued)

Figure 25

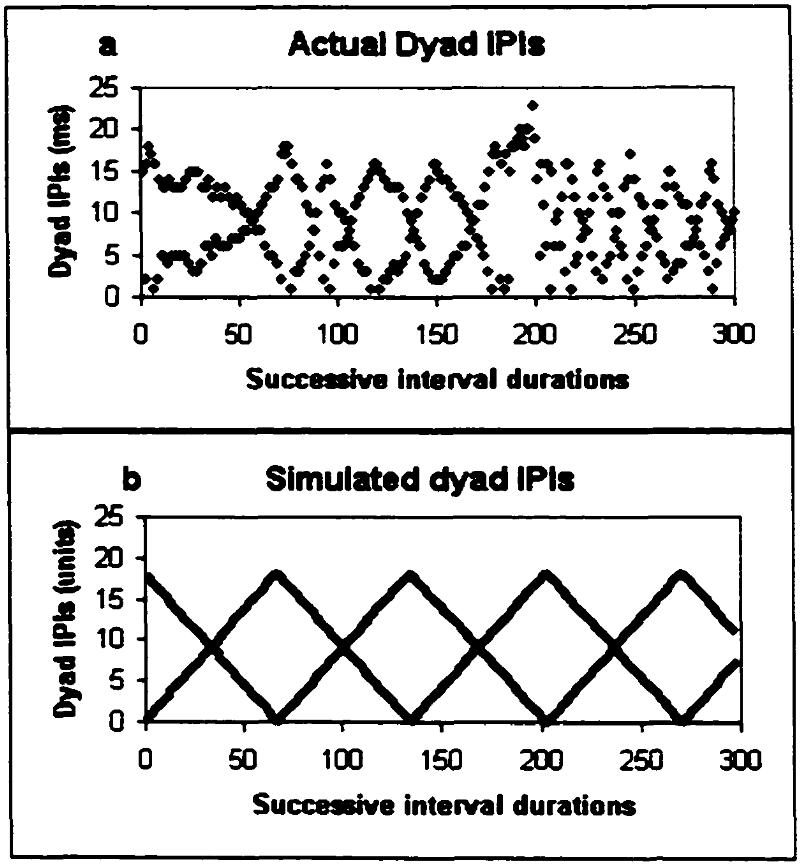
Eight dyad IPI histograms of discharge patterns during parallel display. The scale of the Y-axis in histogram c is double that of the others. Dyad IPIs are separated into 5 ms time bins.

This behavior allows fish to electrolocate at close range without each fish's discharge coinciding with and jamming that of the other. During the echo response, the discharge of one fish usually follows that of the other by a 10-14 ms delay. This response occurs less often during parallel display when both fish are discharging at a very high rate (Bell, Myers & Russell, 1974).

A more common discharge pattern observed in this study during parallel display was rapid, independent (non-echoing) discharging of each fish, with each fish generating a highly regular IPI duration that differed from that of its neighbor. When plotted, the dyad IPIs showing this trend have a mirrored 'saw tooth' appearance (Figure 24 a, g; inserts). The high degree of IPI regularization during parallel display appears on the record as two 'lines' of alternating dyad IPIs. As regularized IPI duration differed between fish, however, the dyad IPI durations waxed and waned (creating the saw tooth pattern in the record).

Figure 26 shows a detail of one such saw-tooth pattern, along with an artificial pattern that was created by estimating the mean IPI duration of each discharging fish (in the actual record) and chronologically ordering the estimated average IPIs. This model assumes that each fish's IPIs occured independently of that of its neighbor.

Several dyad IPI records (Figure 24b, c, d, h) do not show the regularly alternating dyad IPI duration characteristic of either the echo/PLR or the saw-toothed patterns. These records instead appear to primarily consist of a single dyad IPI duration, often more variable than the saw-toothed or echo/PLR records. Plotting such records on a smaller duration scale, however, revealed



<u>Figure 26</u>

(a) Portion of an EOD saw-tooth pattern associated with parallel display. (b) An artificial saw-tooth pattern created by ordering independently occurring 'IPIs'. The two IPIs used for b were 18.02 and 18.56 'ms'. These simulated IPIs were obtained by averaging added adjacent pairs of dyad IPIs from a portion of the actual record.

that most of them also show a saw-tooth pattern. Figure 27 presents a sample of successive dyad IPIs from one such record (Figure 24d). On a smaller scale (and with a line connecting each successive dyad IPI) the saw tooth pattern becomes apparent. This pattern suggests that each fish is independently discharging at a constant frequency, and that the EOD frequencies of the two fish differ.

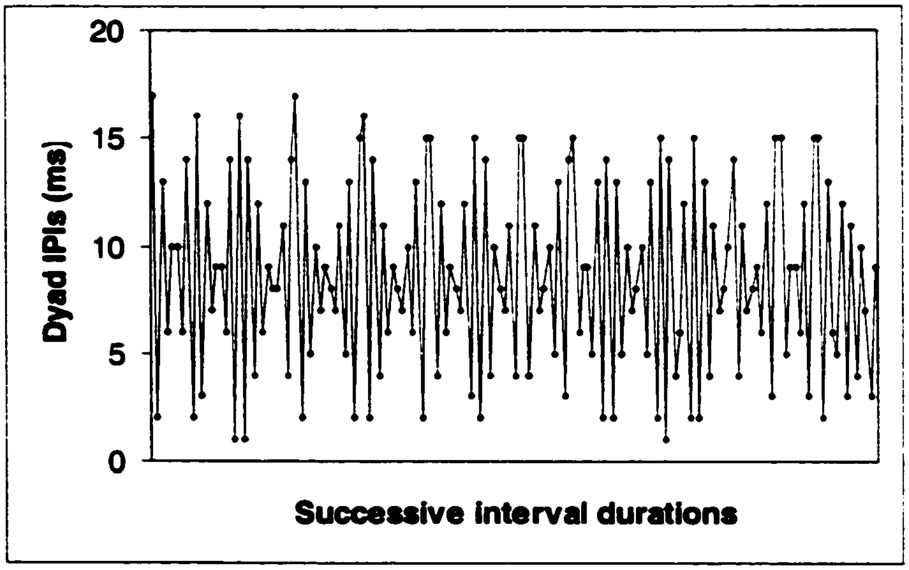
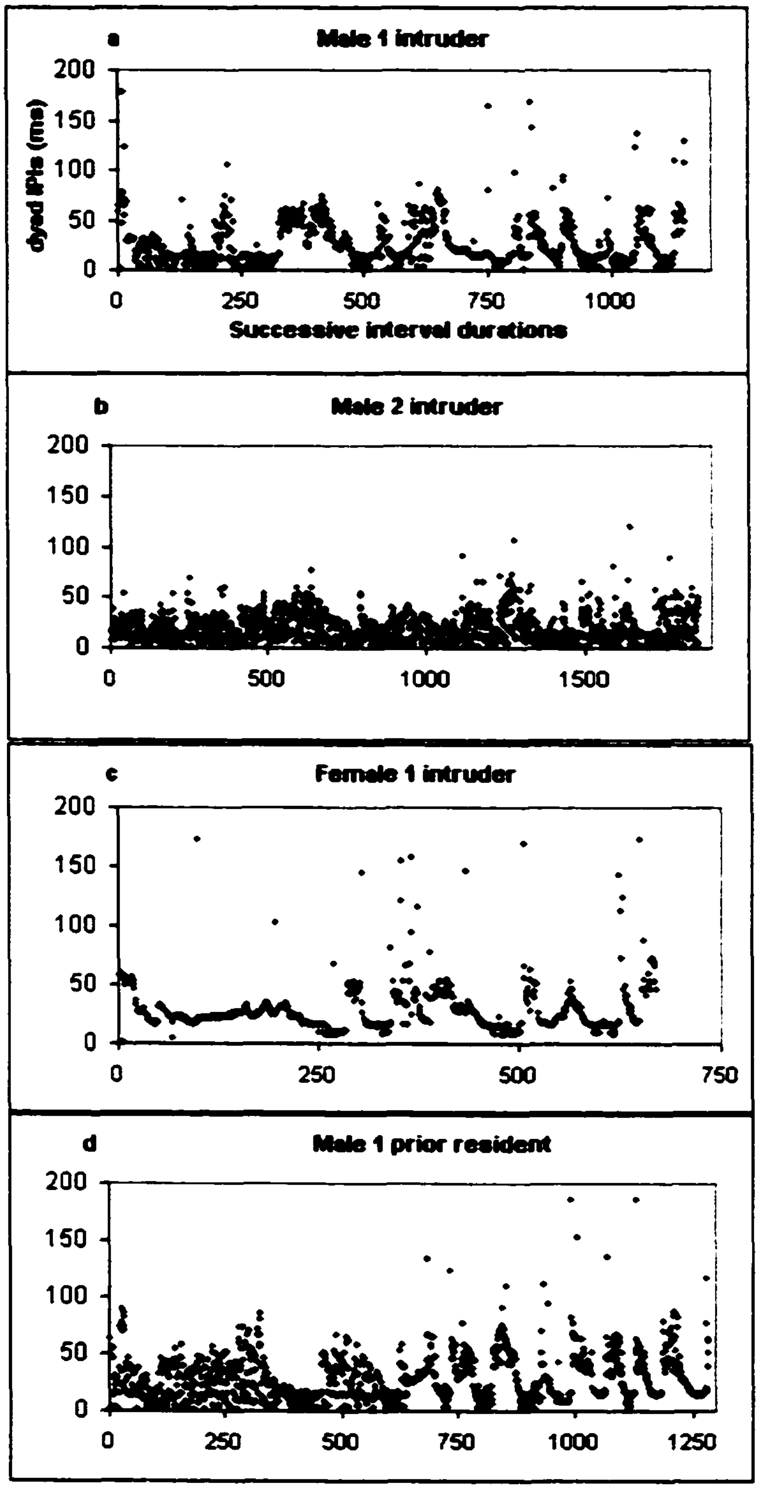


Figure 27

A portion of the dyad IPI record from Figure 24d, plotted on a smaller scale (150 successive interval durations).

Figure 28 shows the dyad IPI records obtained during head butting and Figure 29 shows their associated histograms. Figure 30 also represents the smoothly accelerating discharge volleys that were characteristic of head butts. During the shortest IPI portions of these volleys, when the aggressor was discharging most rapidly and physically butting [continued on p. 100].


a Male 1 intruder

b Male 2 intruder

c Female 1 intruder

d Male 1 prior resident

Figure 28

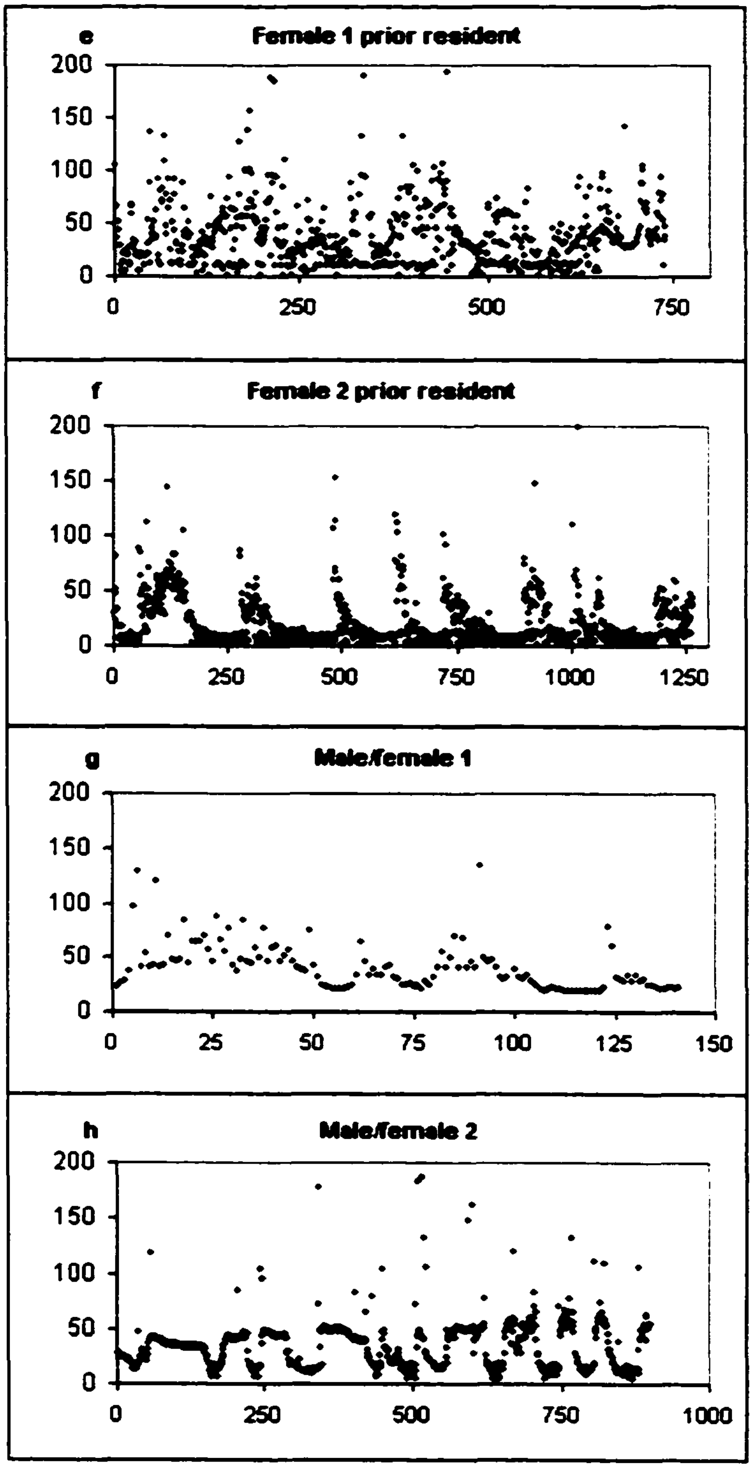


Figure 28 (continued)

Figure 28

Eight dyad IPI records associated with 10 s of head butt behavior. As the number of dyad IPIs occurring over 10 s differed between pairs x-axis scales differ.

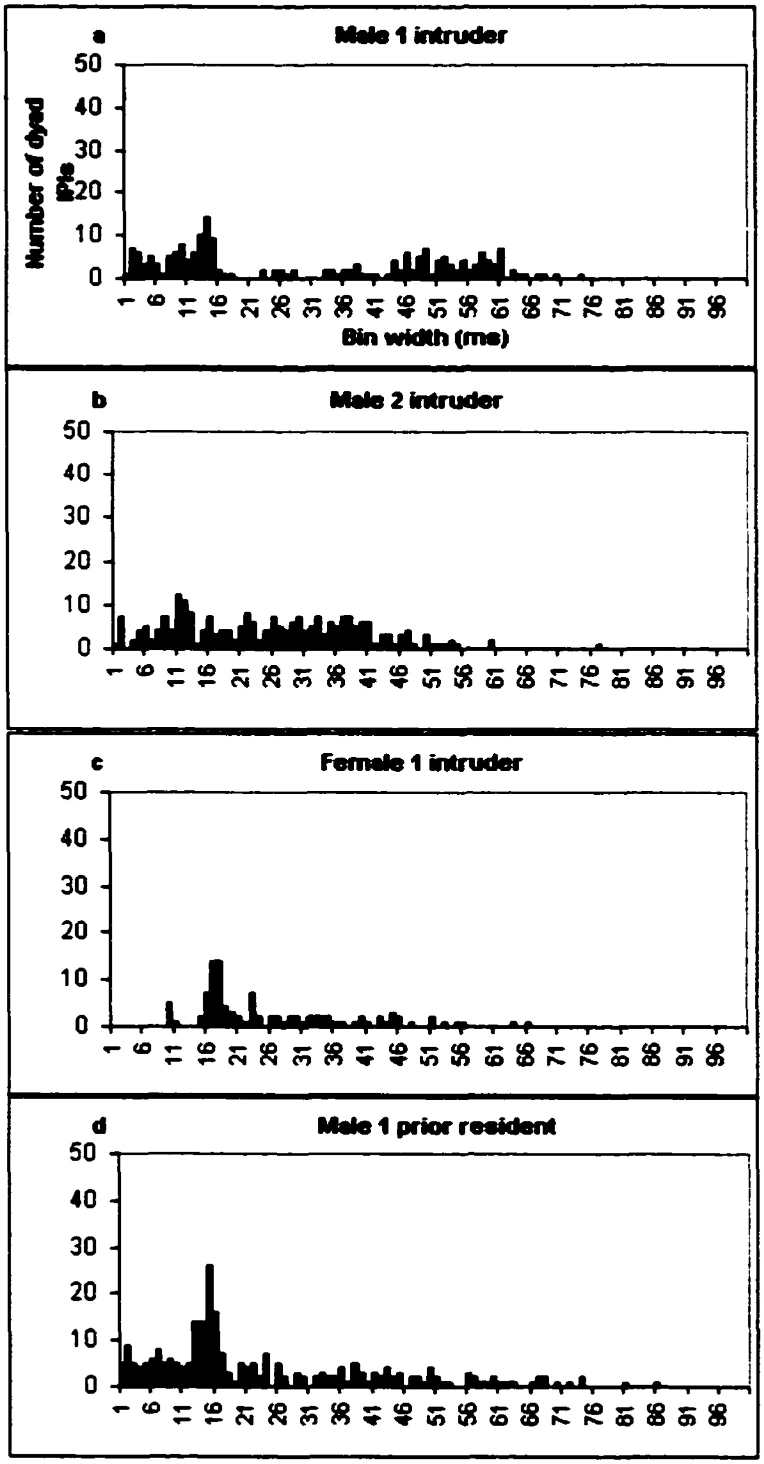

a
Male 1 intruder
Number of dyad IPIs
50
40
30
20
10
0
1
6
11
16
21
26
31
36
41
46
51
56
61
66
71
76
81
86
91
96
Bin width (ms)
b
Male 2 intruder
c
Female 1 intruder
d
Male 1 prior resident

Figure 29

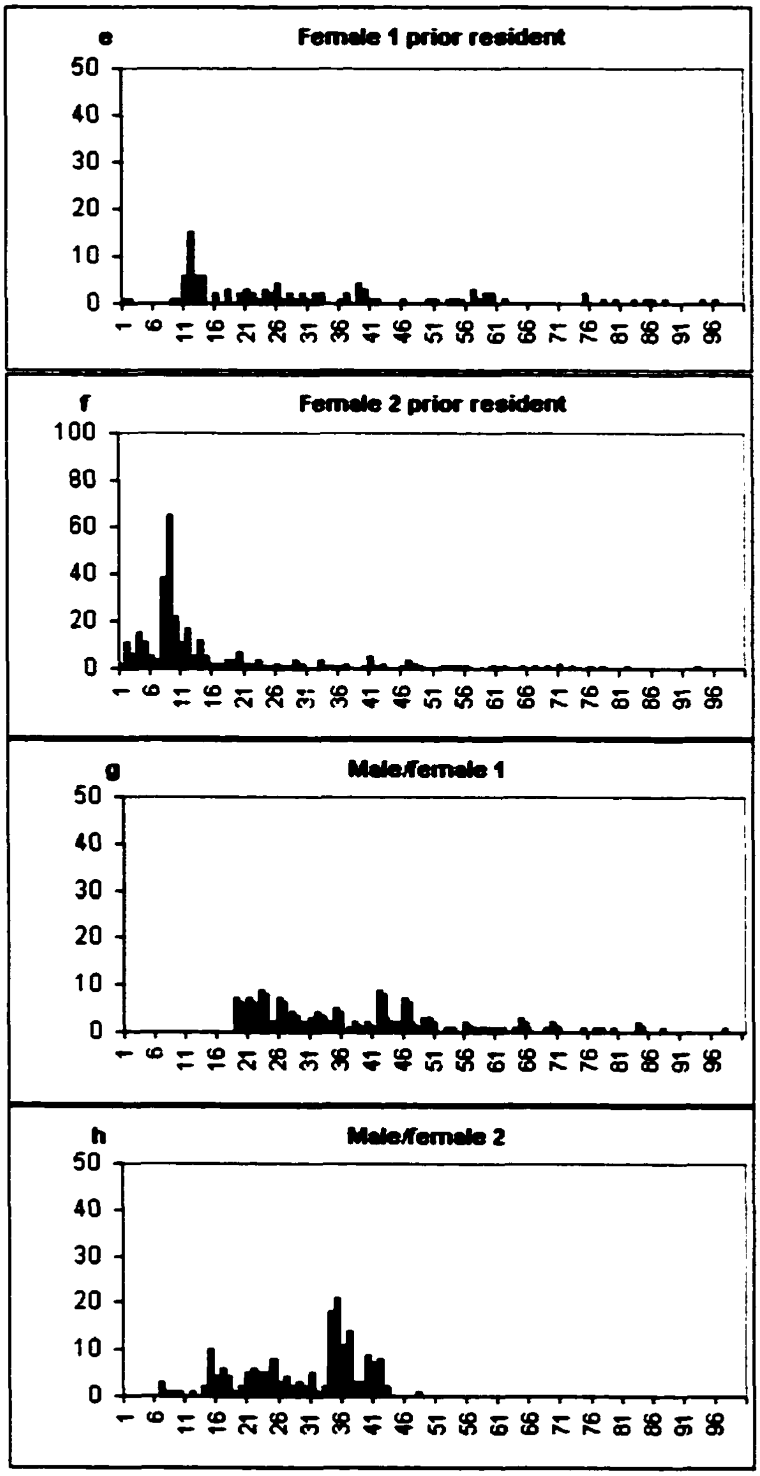


Figure 29 (continued)

Figure 29

Eight dyad IPI histograms of discharge patterns associated with head butt behavior. The Y-axis scale of histogram f is double that of the other histograms. Dyad IPIs are separated into 5 ms time bins.

its neighbor, two 'lines' of dyad IPIs appear on the record (indicated by arrows along the x axis). The brief occurrences of these dyad IPI patterns corresponded with the onset and offset of rapid discharging by the subordinate fish. The subordinate briefly discharged as it swam to escape a head butt. This short burst of high frequency discharging was always associated with swimming behavior, and likely served an electrolocation function for the subordinate fish. During most other times, subordinates avoided discharging, as discharging often led to attacks from the dominant fish. The record shows smooth accelerations of the

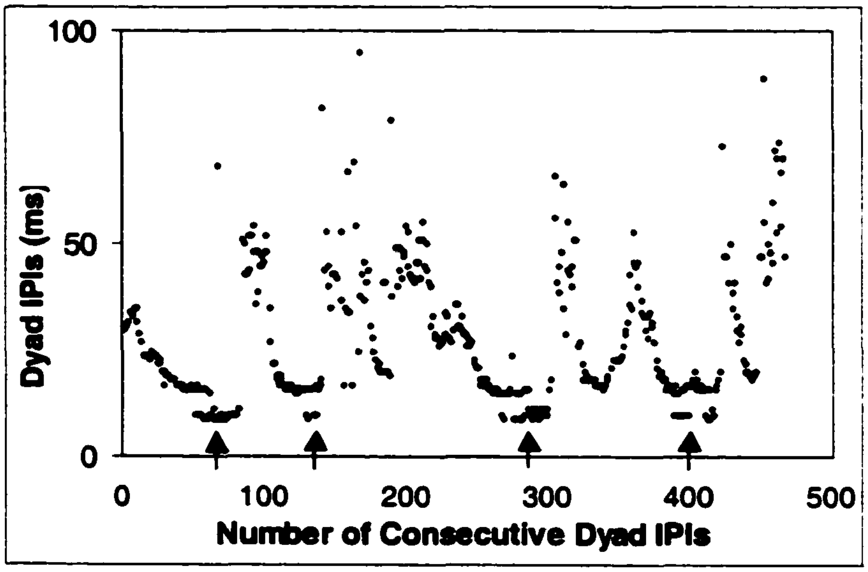


Figure 30

Dyad IPI associated with head butt. The butting fish emitted accelerating discharge volleys. Arrows indicate instances of rapid discharging by the subordinate as it swam away from the dominant fish.

discharge activity in dominant fish. The EOD activity in subordinate fish did not follow this pattern, but was characterized by sudden on- and offset. The echo/PLR response occurred during the head butts, preventing coincident discharging.

Dyad IPI distributions associated with parallel display and head butt were often bimodal and (rarely) trimodal (Figures 25, 29). In order to compare these distributions, the occurrence of primary and secondary modes associated with both behaviors was recorded (Figure 31).

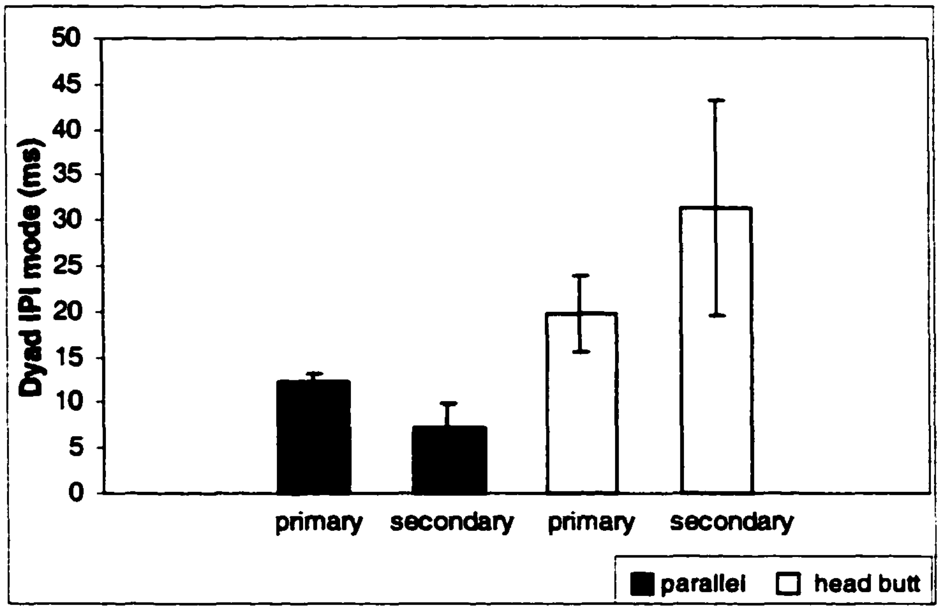


Figure 31

Primary and secondary dyad IPI modes associated with parallel display (n = 9 and 5, respectively) and head butt (n = 9 and 6, respectively). Definition of secondary mode: occurrence at least 50% as often as the primary mode; difference in duration from primary mode by at least 5 ms.

IPIs were also recorded from solitary control fish (n = 11) during 10 s of orientation behavior (both swimming and chin-probing behaviors). These behaviors are associated with high discharge rates, characteristic of active electrolocation. The modal IPI associated with nonsocial orientation was 32.3 ± 3.2 ms. Modal dyad IPI (reflecting the discharge activity of two fish) during parallel display was 12 ± 0.94 ms and during head butt 17 ± 3.2 ms.

1.4.2 Electric Organ Discharge Patterns: Discussion

One would expect that the barrier used to restrict physical interaction would be more of an impediment to the assessment of body size than it would to communication through EOD patterns. The results of this experiment showed that restricted pairs did discharge less than freely interacting pairs (Figure 23). It is likely that restricted dyads discharged less because they were engaging in less social locomotor behavior. There were some instances, however, when pairs engaged in parallel behavior, even though separated by the meshed screen.

Another possibility is that more nonsocial locomotor discharging occurred in freely interacting pairs, as restricted fish had access to only half of the tank, a smaller area to explore than was available to freely interacting fish. This is unlikely to be the case, however, as some solitary control fish were tested in the same tanks used for restricted interactions and others in tanks with no barrier. These solitary control fish did not differ from each other in their discharge rate.

Prior residents and intruders did not significantly differ in their discharge rate although the trend in all groups (free, restricted, and solitary) suggested that discharge rates tended to be higher in intruder pairs, where each fish was exploring a novel environment. The modal rates of solitary prior residents were highly variable. The overall trend (Figure 23) suggested that prior residence resulted in less discharging due to decreased exploratory locomotion, but only in the solitary fish. Both intruders and prior residents that were paired with a neighbor discharged frequently, suggesting that most discharging was associated with social interaction rather than nonsocial behavior. This was confirmed by direct observation of the interacting fish (locomotor activity on the videotape was monitored while simultaneously listening to discharge patterns amplified from the audio channel).

The behavioral evidence reported above supports the hypothesis that discharging associated with parallel behavior serves an electrolocation function, permitting the assessment of body size. A fish often alters its own discharge rate in response to the discharge of a rival, but it may do so primarily to facilitate its own electrolocation (i.e. better detect the rival) rather than communicate with it. The echo/PLR, usually seen in the context of parallel display, likely functions to mitigate the disrupting effect of sensory input arising from the EODs of a nearby fish (Heiligenberg, 1976).

Kramer (1990b) remarks that one unusual aspect of parallel behavior is that it is associated with high rates of discharging, yet is a relatively inactive state of motor behavior. This is presumably considered unusual because motor behaviors such as swimming are associated with electrolocation, requiring rapid discharging. Kramer argues that rapid rates of discharging may serve as "threat" signals. An additional possibility however, is that even though the fish are not swimming, rapid discharging in the context of parallel display does in fact serve an electrolocation function, namely that of assessing a neighbor's size. The physical proximity of fish during parallel display suggests that associated EODs serve an electrolocation function.

During parallel display, each fish's head is aligned with either the head or tail end of its rival. The head region possesses the highest density of electroreceptors (Harder, Schief & Uhlemann, 1967; cited in Kramer, 1990b). The chin appendage of *G. petersii* carries only mormyromast electroreceptors, which are associated with electrolocation rather than communication (Bell, 1986) (knollenorgans are associated with communication). Mormyromasts have a higher response threshold than knollenorgan receptors, and therefore only function at close range (such as the range characteristic of two fish engaging in parallel displays or head butts). The echo/PLR is also mediated by mormyromast receptors (Russell, Myers & Bell, 1974) and strength of the echo/PLR depends upon stimulus amplitude, which declines with distance. Outside the fish's electrolocation range (beyond 30 cm) the echo/PLR vanishes (Serrier & Moller, 1995).

When a mormyrid emits an EOD, an electric organ corollary discharge (EOCD) of the motor command inhibits the knollenorgan system, allowing the fish to attend to feedback from its own EOD via the less sensitive mormyromasts. Similarly, mormyromast electrosensory input is controlled by an EOCD so that only afferent responses that immediately follow the fish's own motor command are effective (Bell, 1979; 1982; 1986; Meyer & Bell, 1983). This is obviously an adaptation, like the echo/PLR, to prevent 'jamming' of the fish's electrolocation by a neighbor. An interesting aspect of parallel display is the high discharge rate associated with it. Although adaptations like the Echo/PRL prevent it, jamming inevitably occurs. This is because combatants do not always echo, and discharge rates are extremely high.

This experiment clearly showed that fish in dyads often failed to exhibit the echo/PLR altogether, or in some cases only showed it for a portion of parallel display bouts. The EOD of *G. petersii* is short, so independent discharging (with no echo/PLR) results in a majority of non-coincident discharges. Instances of coincidence and near-coincidence can still occur quite often. 28% of the dyad IPIs shown in Figure 27, for example, are of 5 ms or less. The degree to which coincident discharging affects an individual's electrolocation ability is not clear at present.

There are a number of reasons why the fish in this experiment often did not exhibit the Echo/PRL. Both parallel (head-to-head) and anti-parallel (head-to-tail) behaviors were examined collectively. Other investigations have assessed only head-to-tail (antiparallel) bouts (Bell et al., 1974), and head negative stimuli have been found to be more effective at eliciting the echo/PLR response than head positive stimuli (Russell et al., 1974). The fish used in this experiment were also subadults. Fish of this age may not yet possess fully developed echo/PLR capability. Lücker and Kramer (1981) found that juvenile mormyrids (*Pollimyrus* *isidori*) of up to 6 months of age failed to show the echo/PLR.

These data point to an interesting fact: As the echo/PLR is not always employed, why do combatants discharge so rapidly, as doing so will jam the electrolocation ability of each individual? The mean discharge rate of *G. petersii* during agonistic behavior can be as high as twice the rate of an isolated swimming fish (Kramer, 1990b). Kramer reports the rate of an isolated swimming fish to be about 17 Hz. The modal rates presented here (Figure 23) suggest even higher IPI rates. A comparison of dyad IPIs of pairs during parallel display and head butt behavior with IPIs of solitary fish during locomotion (Figures 23 and 31) supports Kramer's assertion that a rate difference exists between these behaviors: discharge rates were fastest during parallel display, slower during head butt, and slowest during locomotion. If parallel display facilitates the assessment of body size, one would expect lower rates, comparable to those seen in other electrolocation behaviors (such as locomotion or chin probing). Higher EOD rates increase the probability of coincident discharges and likely necessitate the evolution of a jamming avoidance strategy.

One way to resolve the apparent paradox of higher discharge rates interfering with assessment is the hypothesis that individuals are attempting to jam each other during agonistic interaction. Much attention has been paid to the mechanisms that serve to prevent coincident discharging. If, however, individuals are attempting to jam each other, they may do so in order to glean additional assessment information about an opponent. If one fish is able to discharge faster than its opponent, it will jam more of its opponent's signals and its opponent will not be able to jam as many of its signals. It will therefore be able to more readily gauge opponent size and prevent an opponent from doing the same. The high rates of discharging that occur during parallel behavior may thus be the result of an arms race of escalating frequency. High frequency, regularized bouts of EOD discharge activity have been artificially induced (in *B. niger*) by clamping the stimulus to the EOD of the fish (Moller, 1970). When a clamp occurred over several hundred EODs, the fish initiated a number of discharge bouts of ever increasing rates, each of which had highly regularized inter-discharge intervals. For an interacting pair, the strategy of increasing discharge rate should result in the most rapidly discharging fish of being jammed the least.

The rapid independent EOD activity in fish engaged in parallel display may allow each fish to gauge its neighbor's discharge rate relative to its own. The saw-tooth patterns presented above show periodic occurrences of coincident discharging. The rate of occurrence of these coincident discharges is a function of the difference between one fish's inter-discharge intervals and those of its neighbor. The fish will detect each coincidence as a jammed signal (its own EOD superimposed with that of its neighbor). Hopkins and Westby (1986) proposed a similar mechanism in a pulse-type gymnotiform (*Brachyhypopomus beebei*). Coincident discharges of fish during interaction would be detected by each receiver as an EOD amplitude modulation. They postulated that analysis of coincident discharges would allow a receiver to build a representation of the sender's EODs.

Unlike the knollenorgans, the mormyromasts are amplitude sensitive.

Relative phase amplitudes are related to dominance, body size, and hormonal condition (as discussed above). It is not known, however, if a fish would be able to glean useful information about a neighbor's EOD amplitudes when within the range of mormyromast detection.

It is unlikely that knollenorgans are involved in the detection of body size. The knollenorgan pathway functions in the rapid time coding of a neighbors' EOD patterns, and possibly EOD duration, which need not correspond with body size. Bell et al. (1974) analyzed discharge patterns associated with dominance determination interactions and found "no single feature of the discharge patterns of individual fish or the temporal relationships between them that could inform [the authors] (and presumably the fish) as to which animal was in the process of winning the encounter". If the discharge of a neighbor facilitates size assessment, then one would not expect the discharge pattern itself to be an indicator of who is winning. It would simply serve as a measurement tool, to obtain rather than convey information.

PART B: *BRIENOMYRUS NIGER*

In a series of three additional experiments (experiments 2-4) the effect of social interaction on EOD and locomotor behavior was further explored in a related species, *Brienomyrus niger*. This species is behaviorally similar to *G. petersii*, although somewhat less active during the daytime. Its triphasic EOD is similar to that of *G. petersii*, but with a more prominent first negative phase (P1) that was included in the analysis of experiments 2 and 3. The behavior and EOD of several *Brienomyrus* species have been studied in considerable detail (Bass, 1986; Carlson et. al, 2000; Herfeld & Moller, 1998; Hopkins, 1980, 1981,1986; Moller, Serrier & Bowling, 1989). While it would have been desirable to conduct all experiments on one species, logistics and subject availability (disruption of supply from Nigeria) necessitated the use of a second species.

2.0 Experiment 2: Effects of androgen treatment on the EOD of male *Brienomyrus niger*

Experiment 2 was designed to artificially induce changes in the EOD by treating males with an artificial androgen, 17α-methyltestosterone (17-MT). The rationale for treating these fish was in part to observe specific hormone-induced changes to the EOD, and to see if these changes were analogous to those induced by social interaction. The effects of 17-MT on phase duration and associated PPSF have been reported for female subadult mormyrids (Bass & Hopkins, 1985; Landsman, 1995; Landsman & Moller, 1988), including *B. niger* (Herfeld & Moller, 1998). The current experiment was designed to replicate those results in male subadults. Amplitude measures were also obtained in order to assess the effects of 17-MT on the relative amplitude ratios of the EOD (A1/A2, A1/A3 and A2/A3). Experiment 1 demonstrated that social interaction affected both duration and amplitude ratio components of the EOD in *G. petersii*. It was hypothesized that these changes may have been due to the effects of endogenous androgens. If androgens were responsible for the socially induced

EOD changes observed 17-MT should induce similar changes to both phase duration and phase amplitudes. Further, the time course of changes to the EOD following removal of the androgen was predicted to be comparable to that observed after separating the fish following social interactions.

2.1 Material and Methods

2.1.1 Subjects

Ten subadult males (*B. niger*) served as subjects (standard length: 86.3 ± 2.4 mm, range: 75-94 mm, weight: 9.64 ± 0.75 g, range: 6.4-12 g). Sex of the fish was determined as described in experiment 1. All fish were imported from Nigeria, purchased from a local tropical fish dealer (Quality Tropicals, Inc. Wallington, NJ), and maintained on the same feeding/light schedule used in the *G. petersii* experiment (temperature: 24.8°C, range: 21-30.7°C; conductivity: 101.2 μS/cm; range: 83-147 μS/cm). Fish were individually housed in 13-L aquaria throughout the experiment. Five fish were treated with 17-MT and five fish served as non-treated controls. The two groups were matched for weight prior to treatment.

2.1.2 Apparatus

Equipment discussed in this section was described in experiment 1. Fish were provided with a porous ceramic shelter tube at all times. Conductivity and temperature were taken together with EOD measures during the early subjective day.

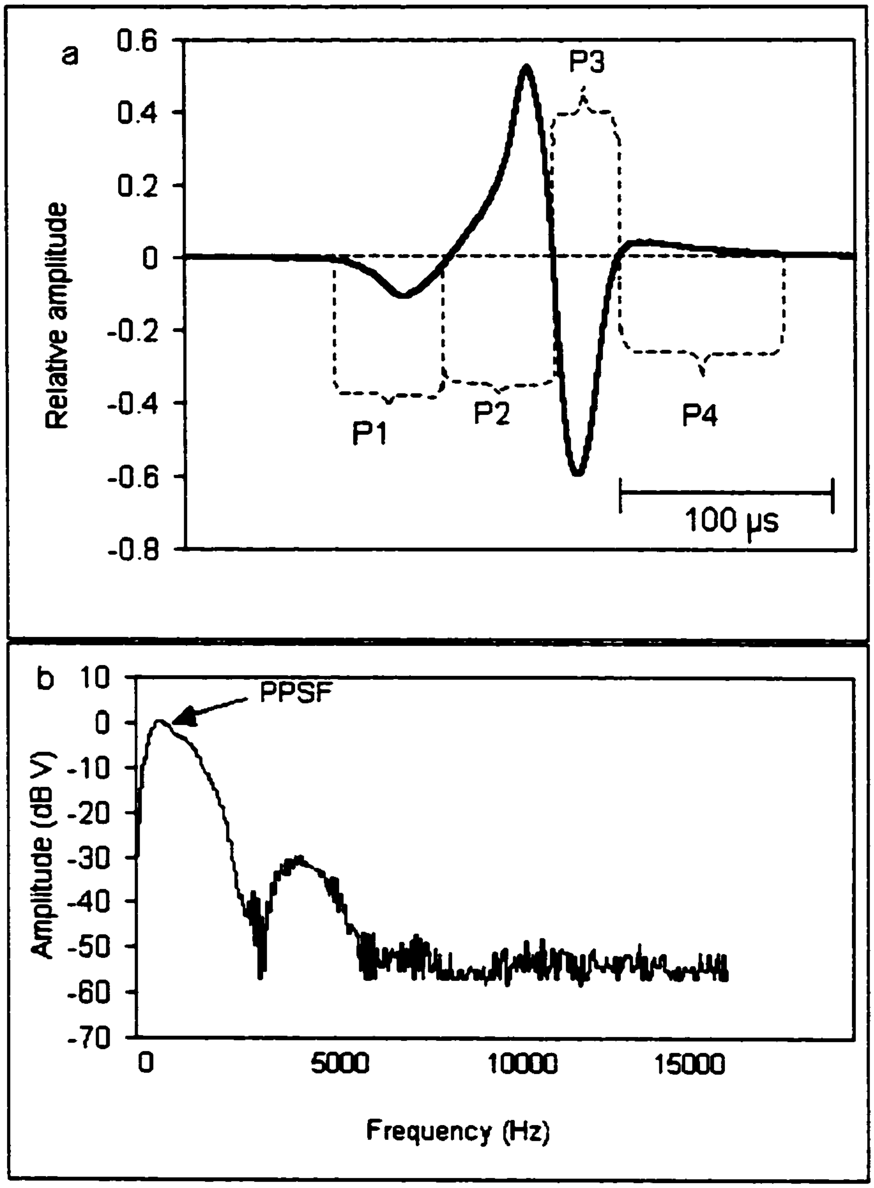


Figure 32

The EOD waveform of *B. niger* (a) and associated PPSF (b). The EOD consists of four phases: P1, P2, P3, and P4. P4 was not analyzed. Each phase is further characterized by its amplitude: A1, A2, A3, and A4).

2.1.3 EOD measures

Figure 32 is represents a typical subadult *B. niger* EOD waveform along with the associated PPSF. Individual EOD phase duration (P1, P2, and P3) were obtained from each fish. Phase 4 was not analyzed. In addition, peak amplitude measures (A1, A2 and A3) were taken from P1, P2, and P3, respectively. Amplitude measures were converted to provide the following derived ratio measures: A1/A2, A1/A3 and A2/A3. The EOD was characterized by its associated fast Fourier transform yielding a peak power spectrum frequency (PPSF) measure.

EOD measures were obtained from all subjects three times during the fish's early subjective day, averaged and adjusted for temperature (adjusted to 25°c, for procedure see below).

2.1.4 Temperature adjustments

Water temperature varied throughout the study: 21-30.7°C (mean = 24.8°C ±1.7). In order to control for EOD duration changes associated with temperature fluctuations, EOD measures (phase durations and PPSF) were adjusted to a conventional standard of 25°C (the procedures were similar to those performed in experiment 1). A pool of measures was obtained from 30 fish (six measures per fish for each parameter, total: 180 measures/parameter, distributed over a 9.7°C temperature range). The slopes, derived from the regression equations for each of the measurement parameters, were as follows:

P1: –5.088, P2: -3.897, P3: -3.7282, PPSF: 208.79. These data demonstrate that the EOD of *B. niger* is more resistant to temperature fluctuation than that of *G. petersii* (Figure 4). Figure 33 shows regression functions from which the adjustment factors were derived.

2.1.5 Treatment conditions

On the first day of treatment 2 mg/L of 17-MT was added to the home-tank (13-L) of each singly housed fish. Once a week, for five consecutive weeks thereafter, 17-MT was added in conjunction with a weekly water change (4.3 L were replaced with fresh water during each change, to which 2 mg/L of 17-MT was added). Non-treated control fish were housed in similar conditions during this period (with the same water changes). Following the treatment period, all fish were placed into a new (uncontaminated) tank containing fresh water, and their EODs were measured for an additional five weeks.

2.1.6 Statistical Procedures

Between-subjects ANOVAs were performed with repeated measures within subjects (mixed model ANOVAs). In all cases, the significance level was set as $p < 0.05$. Measures included EOD duration (P1 + P2 + P3), PPSF, and amplitude ratios (A1/A2, A1/A3, A2/A3). The independent variable was treatment (17-MT and control).

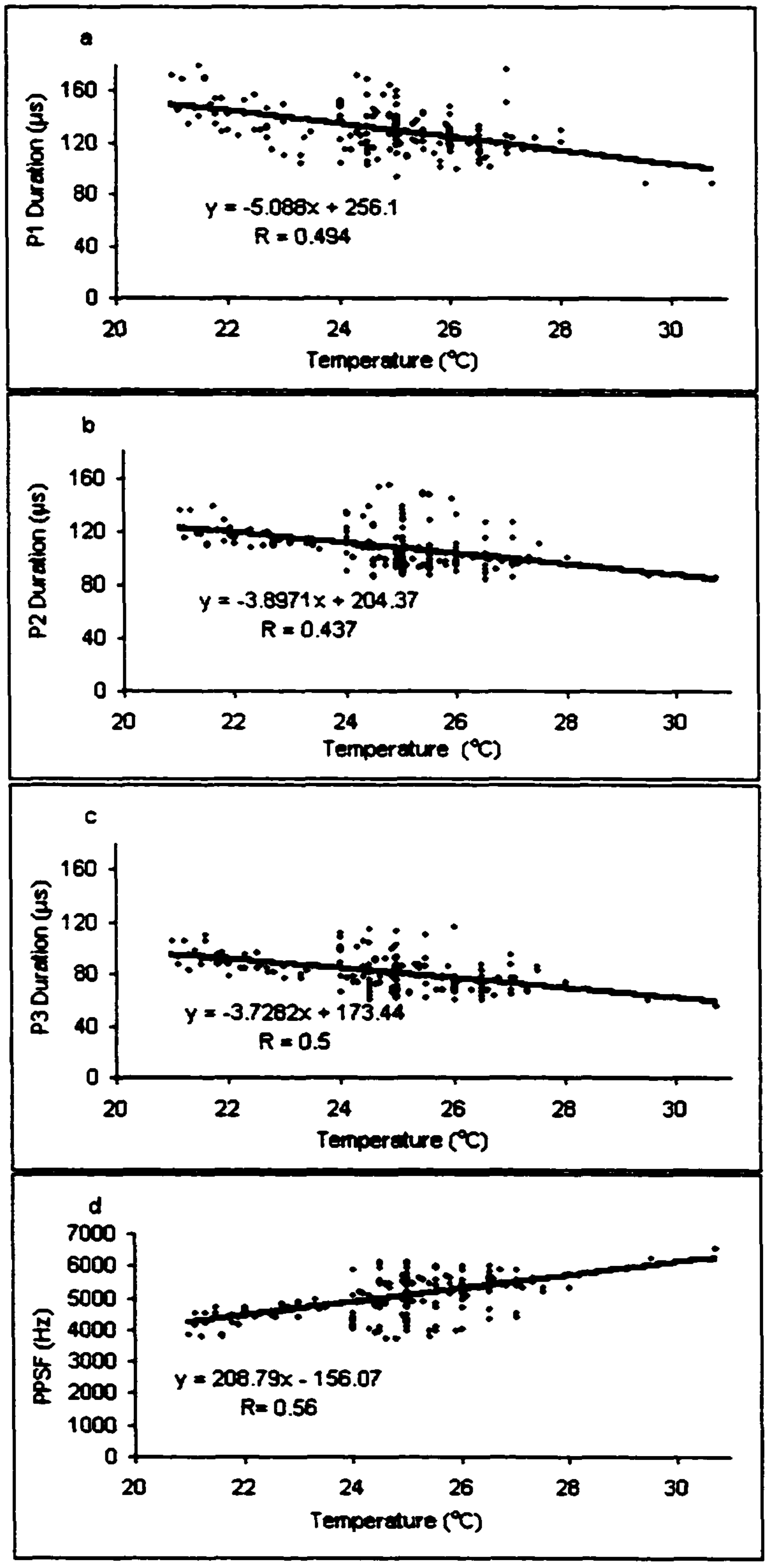

a
P1 Duration (µs)
160
120
80
40
0
y = -5.088x + 256.1
R = 0.494
20
22
24
26
28
30
Temperature (°C)
b
P2 Duration (µs)
160
120
80
40
0
y = -3.8971x + 204.37
R = 0.437
20
22
24
26
28
30
Temperature (°C)
c
P3 Duration (µs)
160
120
80
40
0
y = -3.7282x + 173.44
R = 0.5
20
22
24
26
28
30
Temperature (°C)
d
PPSF (Hz)
7000
6000
5000
4000
3000
2000
1000
0
y = 208.79x - 156.07
R= 0.56
20
22
24
26
28
30
Temperature (°C)

Figure 33

Figure 33

Scatter plots of temperature adjustment data for each of the measurement parameters: P1, P2, P3 and PPSF. Regression lines and coefficients of determination are indicated.

As large between-group EOD differences existed prior to the manipulations (subjects were matched by weight rather than EOD parameters), EOD measures were converted to a percent of day 0 baseline, which served as dependent measures for each ANOVA. Phase durations (P1 + P2 + P3) were summed for analysis prior to conversion into a percent of total EOD duration on day 0. The PPSF and all amplitude ratio measures (A1/A2, A1/A3 and A2/A3) were separately converted to baseline percentages for analysis.

The different treatment times analyzed were: day 14 (2 weeks into treatment), day 28 (4 weeks into treatment), day 42 (1 week after removal from treatment), and day 56 (3 weeks post-treatment). These are hereafter referred to as treatment periods. Each ANOVA, and the independent variables tested for each group are indicated in the results sections below (Table 6). Where applicable Tukey's honestly significant difference (HSD) post-hoc tests were performed.

2.2 Results

Figure 34 shows the EOD parameters (P1, P2, P3, and PPSF) for both the treated and non-treated fish during the treatment and post-treatment periods. The EOD duration during treatment periods (days 14, 28, 42 and 56) differed significantly (ANOVA #1, Table 6). Post-hoc analysis revealed that early post-treatment EODs (day 42, 112.3% of baseline) were shorter than those of both early treatment (day 14, 128.5%; $p = 0.04$) and late treatment (day 28,

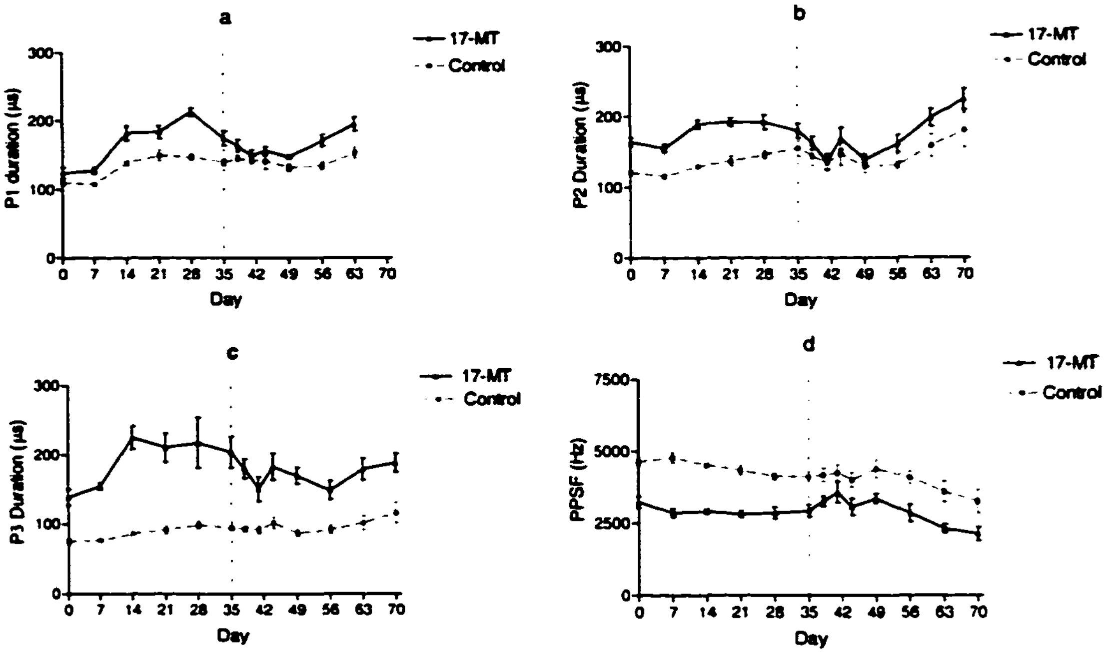


Figure 34

Mean adjusted EOD phase durations (P1, P2 and P3) and PPSFs. Treatment ended on day 35 (indicated by the vertical dashed line). Error bars: ± 1 SEM

137.2%; *p* = 0.001). Figure 34 reveals that most of the duration increase and post-treatment decrease occurred in the 17-MT group. A treatment time period x treatment condition interaction was also significant (ANOVA #1, Table 6). Following removal from the treatment tanks, the 17-MT treated fish rebounded towards pre-treatment levels. Over the 10-week period, both groups showed a trend of increased EOD duration.

PPSF comparisons did not reveal significant differences between groups, although both the time period and period x treatment condition comparisons approached significance ($p < 0.09$ and 0.12, respectively).

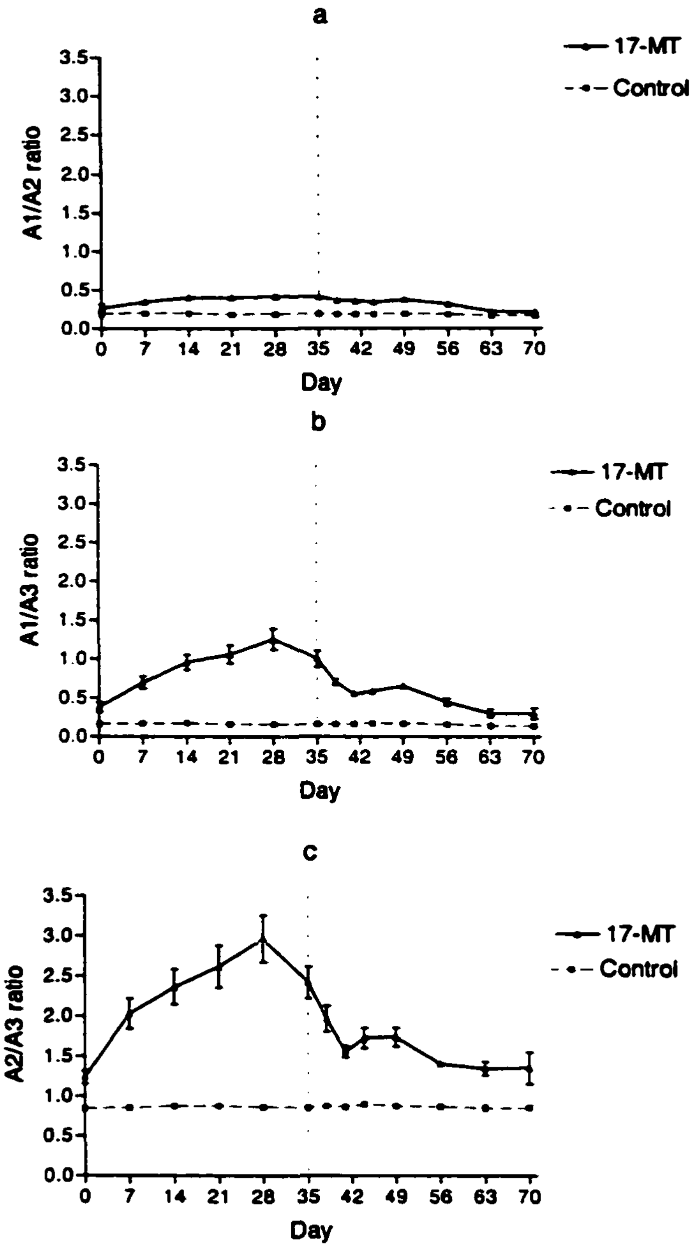


Figure 35

Mean adjusted EOD amplitude ratios (A1/A2, A1/A3 and A2/A3) throughout and following 17-MT treatment. Treatment ended on day 35. Error bars: ± 1 SEM

All three amplitude ratio measures significantly differed as a function of treatment and over the treatment periods (Figure 35). A treatment time period x treatment condition interaction was significant for each amplitude ratio (ANOVAs # 3,4 and 5, Table 6). Post-hoc analysis of the A1/A2 time period differences showed that the early treatment period ratio (day 14, 137.7% of baseline) was higher than both of the post-treatment periods: day 42 (123.0%) and day 56 (114.4%), $p = 0.02$ and 0.0004, respectively.

Changes in amplitude ratio were most pronounced in the A1/A3 and A2/A3 ratio measures. Post-hoc analysis of the A1/A3 time period differences revealed that the amplitude ratio during early treatment (day 14, 199.9% of baseline) was higher than that of late post-treatment: day 56 (112.9%), $p = 0.04$. The ratio during late treatment (day 28, 245% of baseline) was higher than that during both post-treatment periods: day 42 (131.4%) and day 56, $p = 0.005$ and 0.001, respectively.

Post-hoc analysis of the A2/A3 time period differences indicated that both the ratio during early treatment (day 14, 149.3% of baseline) and the ratio during late treatment (day 28, 175.9%) were higher than each post-treatment period: day 42 (115.9%) and day 56 (109.7%), $p = 0.02$ and 0.005 and $p = 0.0002$ and 0.0002, respectively.

Both EOD duration and amplitude ratio measures were affected by 17-MT (Figure 36). Phase durations elongated and amplitude ratios increased as a result of treatment, resembling the changes resulting from social dominance (experiment 1). Following hormone removal, the EOD reverted to its pre-

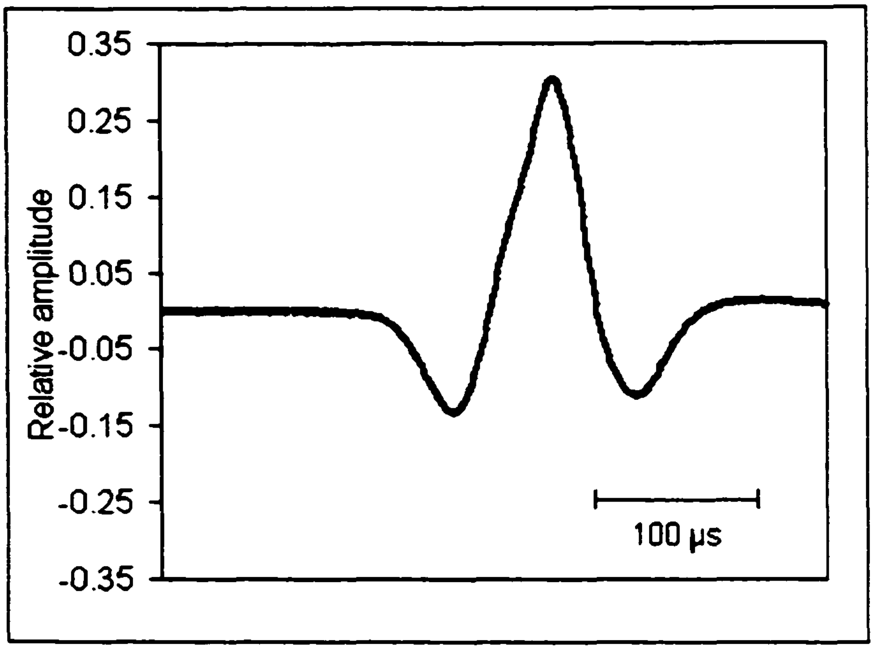


Figure 36

EOD waveform of *B. niger* following 17-MT treatment

treatment waveform. The amplitude change, especially that of A1 relative to A2 and A3, is apparent if one compares the waveform in Figure 36 with that of an untreated fish (Figure 32a). In the treated group, both A1 and A2 increased relative to A3. A1 also increased relative to A2. Prior to treatment, A1 was much lower than the other two phases (26.4% of A2 and 38.3% of A3). Following four weeks of treatment, A1 was 42.3% of A2 and 125.4% of A3. Four weeks after treatment it had dropped to levels below those observed prior to treatment (22% of A2 and 30.3% of A3).

Table 6: ANOVA Results: 17-MT Comparisons

ANOVA #	Significant Effects	Post-hoc Tests
#1 Duration	Time period: $F(3,24) = 8.29$; $p < 0.001$	Day 14 longer than 42
	Time period x Treatment: $F(3,24) = 5.53$; $p < 0.01$	Day 28 longer than 42 and 56
#2 PPSF	Not significant	Not applicable
#3 A1/A2	Treatment: $F(1,8) = 7.47$; $p < 0.05$	Not applicable
	Time period: $F(3,24) = 12.29$; $p < 0.0001$	Day 14 longer than 42 and 56. Day 28 longer than 42 and 56
	Time period x Treatment: $F(3,24) = 7.31$; $p < 0.005$	Not applicable
#4 A1/A3	Treatment: $F(1,8) = 5.5$; $p < 0.05$	Not applicable
	Time period: $F(3,24) = 8.34$; $p < 0.001$	Day 14 higher than 56 Day 28 higher than 42, 56
	Time period x Treatment: $F(3,24) = 7.83$; $p < 0.001$	Not applicable
#5 A2/A3	Treatment: $F(1,8) = 14.04$; $p < 0.01$	Not applicable
	Time period: $F(3,24) = 16.88$; $p < 0.0001$	Day 14 longer than 42 and 56. Day 28 longer than 42 and 56
	Time period x Treatment: $F(3,24) = 16.74$; $p < 0.0001$	Not applicable

Table 6

ANOVAs comparing 17-MT and control *B. niger* at 4 times: early treatment (day 14), late treatment (day 28), early post-treatment (day 42), and late post-treatment (day 56).

2.3 Discussion

The 17-MT manipulation resulted in an elongation of the EOD, which reverted to pre-treatment levels following removal from the treatment condition. Herfeld and Moller (1998) found a similar duration change in subadult female *B. niger* following 17-MT treatment, with a significant PPSF decrease over the course of treatment. The time course of the hormone's effect was similar to that shown here, with most change occurring over the first 14 days of treatment. Changes to the associated PPSF were not significant in this small sample, as compared with Herfeld and Moller's results (they treated 18 fish).

A large discrepancy existed between the EODs of these subadults and those obtained by Herfeld and Moller. The subadults in Herfeld and Moller's experiment (both females and male control fish) had substantially shorter EOD durations (with associated higher PPSFs) than the treated male subadults reported here. The total pre-treatment EOD duration estimated from Herfeld and Moller's subject data (P1 + P2 + P3) was 239 μs (PPSF: 7800 Hz). The pre-treatment EODs reported here were 427 ± 27 μs (PPSF: 3236 ± 212 Hz). Following 5 weeks of treatment, the EOD duration reported by Herfeld and Moller was 420 μs (PPSF: 4100 Hz) and that reported here was 557 ± 43 μs (PPSF: 2921 ± 228 Hz).

This discrepancy was probably not due to age differences as both groups were of similar weight and body length. It is possible that the subjects used in this experiment were at a later stage of gonadal maturation than those used by Herfeld and Moller, regardless of age. Sex of these males was identified by indentation of the ventral, posterior body wall, a characteristic that is more pronounced in sexually mature fish. Regardless of this difference, the EODs of subadult males and females were affected by 17-MT in a similar manner. The hormone induced an increase in EOD duration in each sex.

Analysis of mormyrid responsiveness to androgen treatment has mostly focused on EOD duration and the associated PPSF. The current experiment has shown androgen sensitivity of amplitude ratios as well. The EOD amplitude might serve as a cue to conspecifics about a neighbor's hormonal condition. Experiment 1 demonstrated that EOD amplitudes, like EOD duration, were affected by social interaction. Experiment 3 (below) showed similar socially mediated changes in the EOD of *B. niger.*

As the knollenorgan receptor pathway (mediating communication) is sensitive to temporal rather than amplitude differences (Bass & Hopkins, 1985; Hopkins, 1983, 1988, 1995; Hopkins and Bass, 1981), any possible signal function of the mormyrid EOD amplitude has not been adequately addressed. Although changes in amplitude ratio usually correlate with an elongated EOD (as seen here, and in experiment 1), it is presumed that information is transmitted via EOD duration, and amplitude differences are an undetected side-effect.

In a biphasic pulse-type South American gymnotiform electric fish (*Hypopomus occidentalis*), however, a correlation was established between maintenance of dominance status and display of the largest amplitude EOD (and lowest PPSF, Hagedorn and Zelick, 1989). Bass (1986) has found that both 17-MT and estradiol treatment increased A1 and decreased A3 in *Brienomyrus* (age and sex were not specified). The amplitude ratio data presented here are in accord with this finding. This experiment showed that the ratio of different EOD phase amplitudes, like EOD duration, differ as a function of circulating androgen levels; they therefore could serve as cues in the communication of dominant or reproductive status.

The possibility of amplitude assessment occurring (via mormyromasts) in the context of parallel display was discussed above (Experiment 1). It is also possible that inter-individual differences in the relative amplitudes of EOD phases are transmitted to neighboring conspecifics via their knollenorgan pathway. As a conspecific swims into a receiver's EOD detection range, only the phase with the strongest amplitude (from the sender's EOD) will initially be detected by a receiver. At such a distance, the phases of smaller amplitudes should be indistinguishable from noise. Even though knollenorgans do not code for amplitude differences, the presence of an EOD, manifested by at least one of its phases, will be detected at differing distances, depending upon the phase amplitude. If social dominance and/or sexual maturity results in increases in the amplitude of one or more EOD phases, then the signal of dominant/mature fish will be broadcast over a greater area than subordinate/immature fish, thus increasing the potential communication range. A positive-going transient from an EOD (the onset of P2, for example) will produce a single spike from the knollenorgan receptors on one side of the body (P3, of opposite polarity, will produce a single spike from knollenorgan receptors on the opposite side of the body) (Hopkins & Bass, 1981; Bell, 1986). In sexually immature *B. niger* A1 is much smaller than both A2 and A3, both of which are of comparable amplitude (Figure 32). At a distance, just inside the signal's active space, a conspecific should detect the presence of an immature sender as a biphasic discharge (phases 2 and 3).

The waveform characteristic of a socially dominant or sexually mature male, on the other hand, generates P1 and P3 of similar amplitude, and a much higher amplitude P2 (Figure 35). At the electroreceptive periphery (at the detection boundary of the signal's active space), only a monophasic pulse (P2) should be detectable. When closer, P1 and P3 may also be detected, separated by P2 (which is of opposite polarity).

Knollenorgans have a refractory period, however, which may prevent spiking in response to P3. Refractory unresponsiveness to the second of two polarity changes (of like sign) has been found in *B. brachyistius triphasic* (Hopkins & Bass, 1981) when female EODs were presented to knollenorgan receptors. The EODs of females of this species are short, however, so that these two polarity changes of like sign would have been less than 100 μs apart. The EOD duration of B. niger is substantially longer, and is longest following androgen treatment (Figures 34 and 36). If P1 and P3 of *B. niger* can be discriminated by a receiver fish, both are likely to be detected, as each of these phases are of similar amplitude in treated fish.

At the boundary of the electro-communication range, where fish can first detect a potential mate or rival, the EODs broadcast by sexually mature and immature/subordinate individuals should differ in a simple quantitative way. A receiver needs only to distinguish between the number of knollenorgan spikes generated by a neighbor's EODs. If fish distinguish EODs in this way, it is expected that a dominant/sexually mature fish will initially be detected by a single spike. A subordinate/immature fish, on the other hand, should initially be detected by two spikes. When a dominant/sexually mature fish is closer, its discharge may be detected as triphasic. The phases from a single EOD would occur within a time period on the order of 200-500 μs and inter-discharge intervals would be separated by several ms. There is evidence that mormyrids can distinguish inter-phase durations within a discharge (Hopkins, 1995; Hopkins & Bass, 1981). The 17-MT mediated amplitude ratio changes reported here, and the dominance-mediated amplitude changes reported in experiment 1 suggest the possibility that amplitude ratio differences could also signal individual or sex status. This hypothesis could be tested in future playback studies.

Mormyrids show a substantial range of waveforms, from mono- to multiphasic (Hopkins, 1981, 1995). If these fish are capable of communicating dominant and/or reproductive status via relative phase amplitudes, one would predict that hormone-dependent phase amplitude changes would be most pronounced in multiphasic species like *G. petersii* or *B. niger*. EOD duration differs between species over two orders of magnitude, ranging from 100 μs to over 10 ms (Hopkins, 1988). The EODs of *G. petersii* and *B. niger* are at the shorter end of this continuum. With short discharge durations, discrimination of individual phase duration differences may be difficult. The additional cue of discrete detectable phases may aid in the determination of dominance/sexual receptivity.

3.0 Experiment 3: *Brienomyrus niger* in free interactions

Experiment 3 assesses socially induced EOD duration and amplitude ratio changes in *B. niger.* Freely interacting dyads were tested in a paradigm similar to that used in the *G. petersii* experiment. It was predicted that *B. niger* would show socially mediated changes similar to those observed in *G. petersii* (experiment 1) and to those induced by 17-MT in experiment 2.

3.1 Material and Methods

3.1.1 Subjects

Six subadult fish (*B. niger,* standard length: 85.2 ± 3.1 mm, range: 74-90 mm, weight: 7.9 ± 0.9 g, range: 5.7-10.8 g) were paired as freely interacting dyads (pairs were matched for weight). Ten subjects were initially paired, but two pairs were later excluded from the analysis. One pair was excluded because a subject died and a second pair had to be separated within 15 minutes as the dominant fish was attacking and biting the subordinate.

3.1.2 Apparatus

All equipment used was described in experiment 1. Pairs interacted in 57 L aquaria containing a single porous ceramic shelter (usually occupied by the dominant fish). EODs were recorded with a pair of Ag/AgCl electrodes. Conductivity and temperature were taken together with EOD measures during the early subjective day. Prior to and following social interaction, each fish occupied a 13 L tank with a PVC shelter tube.

3.1.3 Conditions

EOD parameters from solitary subjects were initially recorded daily for five consecutive days. Subject pairs were then placed into a tank containing a single shelter for six days. Following the interaction period, subjects were returned to solitary housing for an additional week of daily EOD recording. Measures were also taken on a single day, two weeks after removal from the interaction. Dominance during the interaction period was defined in the same way as it was for *G. petersii* (Experiment 1).

3.1.4 Statistical Procedures

Dependent measures included EOD duration (P1 + P2 + P3), PPSF and amplitude ratios (A1/A2, A1/A3, A2/A3). The independent variable was dominance status (as defined in Experiment 1). The within-subject measures were interaction periods (interaction and post-interaction).

Between-subjects ANOVAs were performed with repeated measures within subjects (mixed model ANOVAs). In all cases significance was set a $p < 0.05$. Between-group EOD and PPSF differences existed prior to the manipulations (see t-test results, below). As a result of these differences, EOD and PPSF measures were each converted to a percent of baseline (Baseline: the mean of day 0-4 measures). Prior to interaction, the baseline amplitude ratios (A1/A2, A1/A3 and A2/A3) of dominant and subordinate groups did not significantly differ. Amplitude ratio measures (interaction and post-interaction) are compared in Table 7.

3.2 Results

Figure 36 shows EOD duration and PPSF as a function of dominance status. The dominant group exhibited longer lasting discharges than the subordinates prior to social interaction (dominant: 554 ± 36 μs, subordinate: 415 ± 12 μs; $t = 3.12$, $p = 0.03$). The EOD duration in both groups showed an increasing trend during social interaction. Following return to their home tanks, EOD duration decreased significantly (Table 7, ANOVA #1). This trend occurred in both dominant and subordinate groups. Approximately three days following the return to their home tanks, the EOD of fish in the dominant group began to elongate again, rebounding towards pre-interaction levels.

Similar trends occurred in the PPSF (Figure 36b), although these trends were not significant (Table 7, ANOVA #2). The dominant group exhibited lower PPSFs than subordinates prior to social interaction (dominant: 2586 ±158 Hz, subordinate: 3509 ± 88 Hz; $t = -4.43$, $p = 0.01$). Both groups' PPSFs showed a trend of lowering frequency during social interaction, which rebounded in the post-interaction period.

Amplitude ratios in the dominant and subordinate group did not significantly differ during the pre-interaction period. The A1/A2 and A1/A3 ratios in both groups significantly decreased following social interaction (Table 7, ANOVAs # 3 and 4). A2/A3 ratio in the dominant group showed a wide inter-individual variability (Figure 38). Both the A1/A3 and A2/A3 ratios of the dominant fish showed an increasing trend just prior to social interaction accompanied by

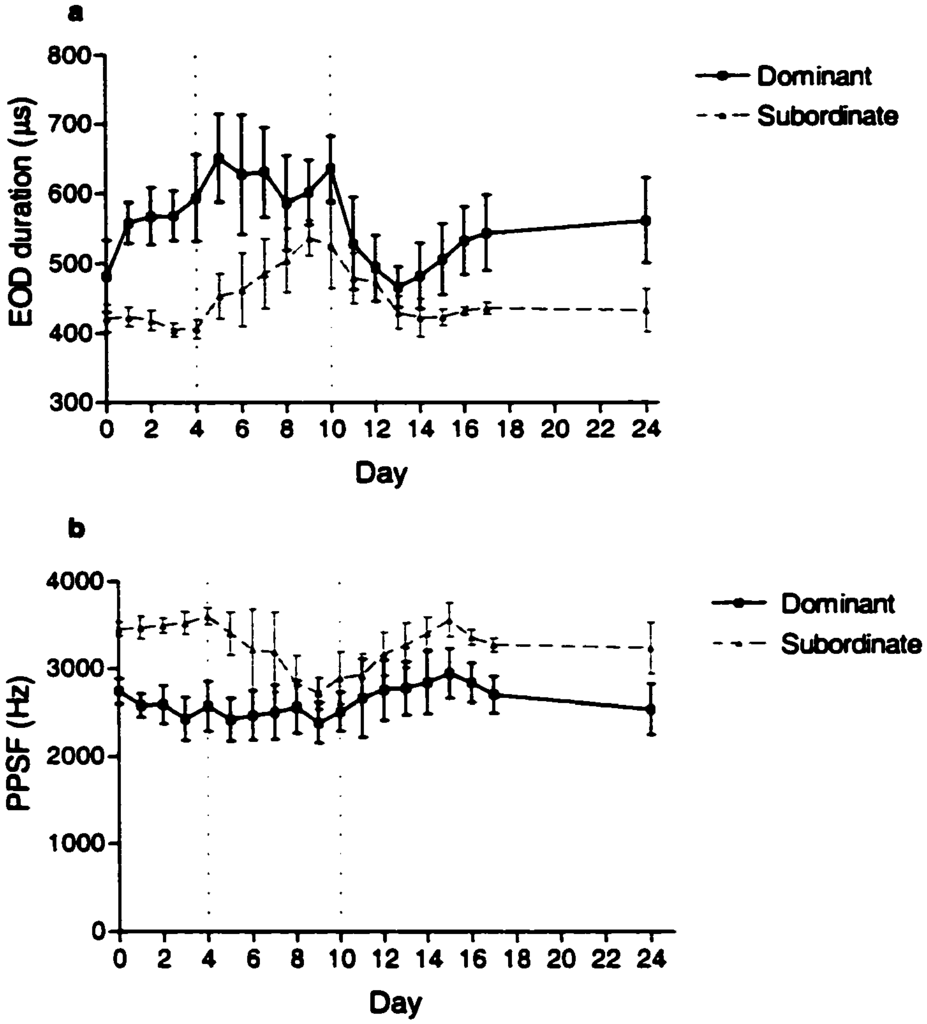


Figure 37

(a) EOD duration and (b) PPSF as a function of dominance (n = 3 per group). The interaction period lies between vertical dashed lines. Error bars: ± 1 SEM

increased variability. During the interaction, however, this trend reversed. This trend in dominant fish was greatly reduced by the end of the social interaction period. ANOVAs showed no significant differences in any EOD parameter as a

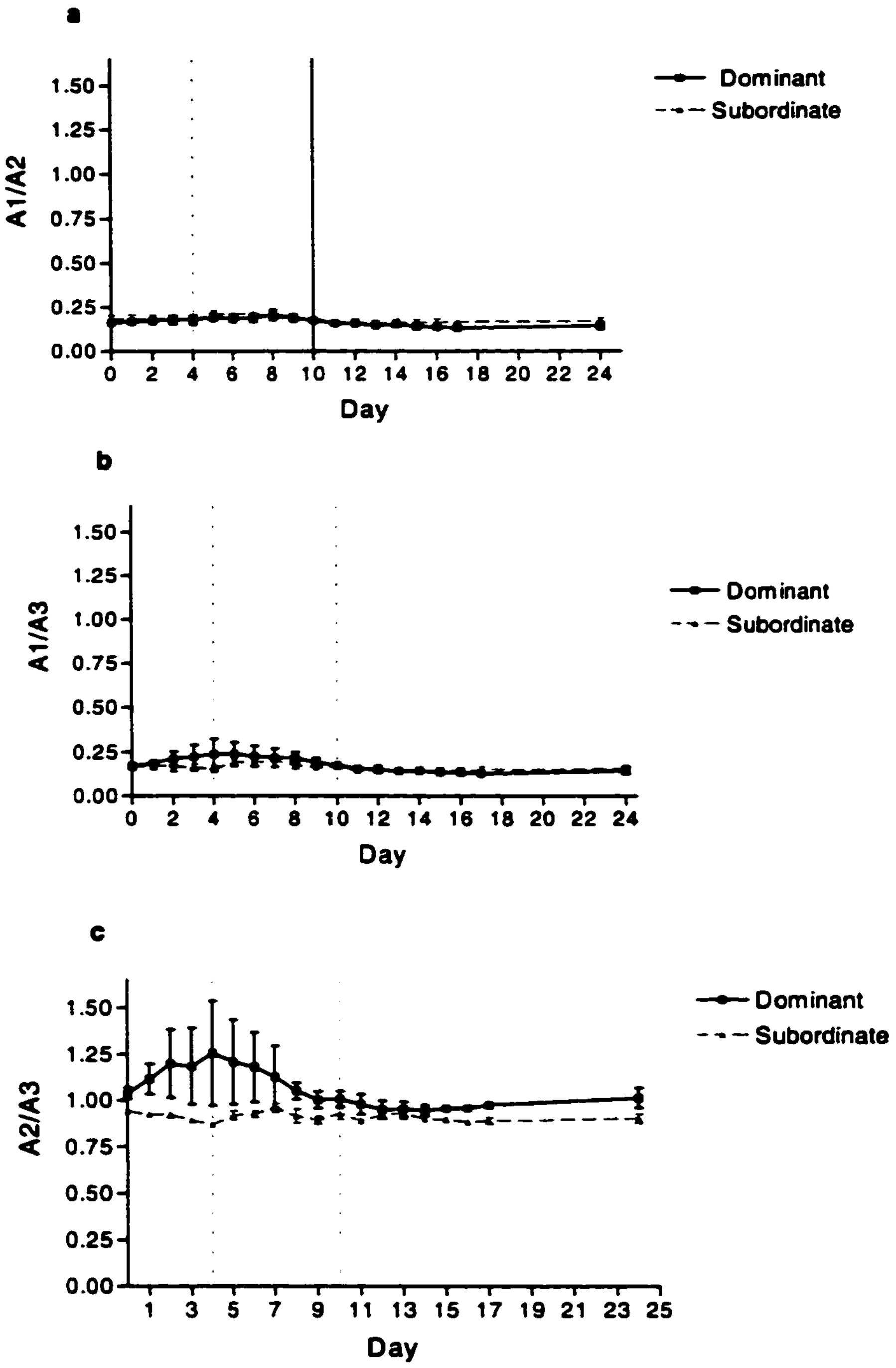


Figure 38

Amplitude ratios as a function of social interaction, dominant versus subordinate fish (n = 3 per group). The interaction period lies between the dotted lines. Error bars: ± 1 SEM function of dominance status, although the pre-treatment between-group duration EOD and PPSF differences were in the same direction as those seen in 17-MT treated fish (Experiment 2).

Table 7: ANOVA Results, *Brienomyrus niger* free interactions

ANOVA #	**Significant Effects**	**Description**
#1) Percent of baseline duration	Time period: $F(1, 4) = 23.2$; $p < 0.01$	Interaction longer than post
#2) Percent of baseline PPSF	Not significant	Not applicable
#3 A1/A2	Time period: $F(1, 4) = 40.54$; $p < 0.01$	Interaction higher than post
#4 A1/A3	Time period: $F(1, 4) = 13.65$; $p < 0.05$	Interaction higher than post
#5 A2/A3	Not significant	Not applicable

Table 7

ANOVA results. *Brienomyrus niger* under conditions of free interaction. Interaction period - mean of days 5-10; post-interaction period - mean of days 11-15

3.3 Discussion

Because of between-group variability in EOD durations, the percent change relative to baseline was analyzed rather than absolute measures. The same percentage of change in two individuals therefore reflected different amounts of absolute change, as individuals' baseline EODs differed. This method of measurement therefore underestimated the amount of absolute change in fish with longer EODs (the dominant group) relative to those with shorter EODs (the subordinates). This may account for the fact that dominant and subordinate fish did not differ in socially mediated EOD change.

If dominant fish communicate status by emitting longer EODs than neighbors, the EODs of dominant individuals in this experiment would not need to increase, as they were already longer at the onset of the social interaction. There was nevertheless a moderate increase in EOD duration in dominant fish during the interaction.

The trend of increasing EOD duration in subordinates may have been due in part to the fact that dominant status in one of the three dyads was more difficult to assess (by the experimental criteria, and possibly by the fish themselves) than it had been in any of the other dyads investigated in experiment 1. Both subjects of the ambiguous pair in this experiment 'acted' dominant at different times, so dominant status was assigned to the individual that excluded its neighbor from the sole shelter for a longer period of time. In this particular pair, all inter-individual EOD changes were in the opposite direction to that usually attributed to dominant and subordinate fish. In addition, the data from only 3 pairs were analyzed in experiment 3.

The trends in increased duration and amplitude ratios in dominant fish (Figures 37 and 38) were in accord with those of the 17-MT treated fish (Figures 34 and 35). The dominant subjects in this experiment also showed pronounced inter-individual amplitude ratio variability similar to that seen in the 17-MT treated fish. These results suggest that socially mediated dominance is associated with androgens and that socially induced EOD changes show large inter-individual variability.

4.0 Experiment 4: *Brienomyrus niger* in restricted interactions

In this experiment the social conditions of male *B. niger* were manipulated in order to assess the way in which social residency status affected EOD waveform during restricted interactions. The independent variables in this experiment were (1) the number of neighbors a given subject was exposed to (ranging from zero to four), and (2) the residency status of the subjects (either intruders or prior residents). It was hypothesized that social interactions would elicit similar changes in EOD signal parameters as those observed in *G. petersii* under restricted conditions. In addition to waveform changes, the amount of time that subjects spent outside of their shelters during the social interactions was recorded. This was considered to be a measure of social behavior, as fish often departed their shelters to initiate social interaction and returned to them for refuge.

The range of 1-4 neighbors used in this experiment is that in which the greatest amount of aggressive territorial behaviors have previously been observed (Moller, 1976; Squire, 1981). It is likely that under natural conditions a large number of neighbors decrease a fish's likelihood of acquiring dominance. If this were the case, a larger number of neighbors present would result in little change to the EOD. It is also possible that social stimulation by a number of neighbors (separated from the subject by a partition) would lead to an increase in EOD duration similar to that seen in *G. petersii* (experiment 1) during restricted interactions.

4.1 Material and Methods

4.1.1 Experimental animals

A distinction was made between subjects and stimulus fish. Measures were obtained only from subject fish, whereas stimulus fish served to elicit changes to behavior and the EOD of subjects. Subjects were exposed to the stimulus fish in restricted interactions in order to elicit these changes. All fish were provided with a porous ceramic shelter tube at all times, and were maintained on the same feeding/light schedule as described for experiment 1.

4.1.1.1 Subjects

Thirty subadult fish served as subjects (standard length: 91.4 mm, range: 78-116 mm, weight: 10.0 g, range: 6.9 - 16.6 g). Subjects were naïve to social interactions (individually housed in 20-L aquaria) for at least 14 days prior to the experimental manipulations.

4.1.1.2 Stimulus fish

Seven males served as stimulus fish (standard length: 88.6 mm, range: 87-93 mm, weight: 10.6 g, range: 8-16 g). Stimulus fish were presented to each subject (except for solitary controls). Stimulus fish were used on a rotating schedule so that all were used in approximately the same number of trials.

4.1.2 Apparatus

Equipment used in this experiment was the same as described for experiment 1. Conductivity, temperature, and EOD measures were taken together with light level during both subjective day and night throughout the 7-day measurement periods. Light level was recorded directly above the water surface with a Lutron LX-101 lux meter (accuracy ± 5%). Behavioral transcription software (Etholog 1.1, Ottoni, 1996) was employed to record social behavior (time spent outside of the shelter).

Social interactions were observed in a 50 cm long x 26 cm wide x 30 cm high tank. The tank was divided into subsections by 0.75 cm thick plastic mesh partitions. All fish were separated in every trial, subjects in an area 15 cm long, 26 cm wide and 30 cm high, at one end of the tank, and stimulus fish divided evenly in the remaining portions of the tank (Figure 39). The subject's compartment faced the compartments of all stimulus fish.

4.1.3 EOD measures

In this experiment the duration of P2 and P3 and EOD-associated PPSFs were obtained. EOD measures were taken from all subjects three times during

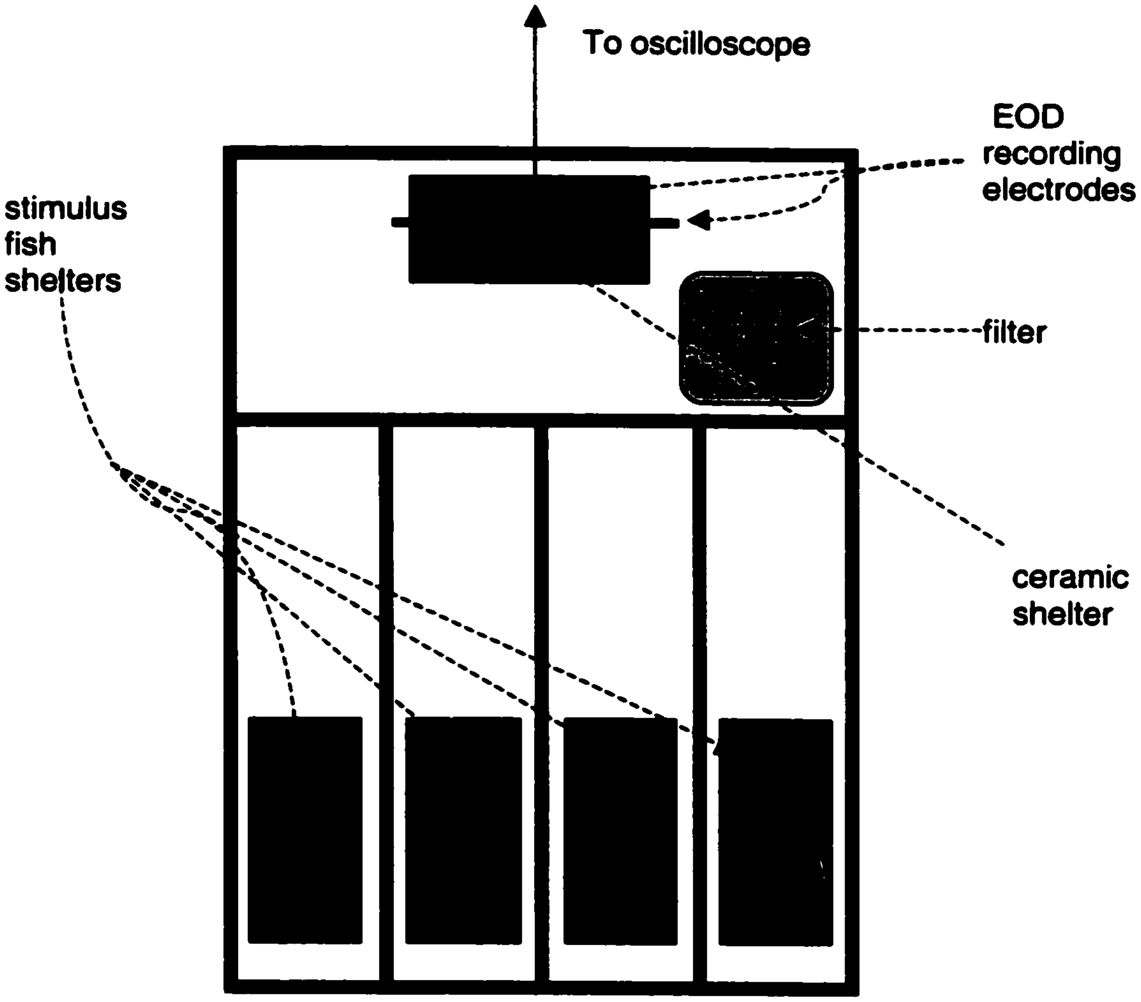


Figure 39

Experimental set-up, *B. niger* in restricted interactions the fish's subjective day and three times during its subjective night on days 1-3 and 5-7. Means from the daytime and nighttime measures were averaged and adjusted to 25°c (for procedure, see experiment 2). On day 4, the first day of social interactions, EOD and PPSF measures were also taken every 20 min for three hours in the restricted interaction trials.

4.1.4 Intruder condition

On day 1 intruder fish were placed into a tank containing no other fish. On day 4, they were moved into the test tank. The test tank contained stimulus fish resident(s) that had been in the tank establishing residence for 3 days prior to the introduction of the subject.

4.1.5 Prior resident condition

On day 1, prior residents were placed in the test tank where they remained for seven days. Intruder stimulus fish were introduced into their respective compartments during the early subjective day on day 4. As intruders had been transferred into a novel tank, movement of prior residents also occurred, in order to simulate the disturbance associated with movement. Less than 10s prior to the onset of social interaction the resident was removed from the tank with a net for approximately one second and then returned to the home tank.

4.1.6 Time course of conditions and replications

Subjects were tested in the following order: (1) intruder and prior resident subjects were tested in alternating order. (2) The separate alternating intruder/prior resident subjects were tested under each of the five possible number-of-neighbor conditions (the order of different numbers of neighbors was randomized). (3) Once a single replication of all the possible neighbor interactions was complete, a new set was begun (3 replications, 30 subjects total).

4.1.7 Statistical procedures

A between-subjects design was employed. Number of neighbors (5) x 2 residency status levels x 3 replicates per condition = 30 subjects. In order to minimize the affects of variability across subjects (a small number of subjects were used for each condition), absolute EOD and PPSF measures were transformed to percent of change relative to day 1 baseline.

Two-way, between subjects ANOVAs were performed, with repeated measures within subjects (mixed model ANOVAs). These repeated measures were taken over the last three days of each trial (approximately 24, 48 and 72 hours into social interaction). Where applicable, Tukey's HSD post-hoc tests were performed. Correlations of locomotor behavior (time spent outside of the shelter) over time and as a function of number of neighbors were calculated for both intruder and resident groups.

4.2 Results

4.2.1 EOD duration

On the day of interaction (day 4), EOD measures were taken every 20 minutes for a period of three hours during interaction. There were no significant changes in EOD duration, PPSF or amplitude ratio during this time period. Throughout the 7-day trials, EOD measures were recorded during both the fish's subjective day and subjective night. There were no significant differences in EODs taken during daytime (mean light intensity: 44.9 lux, range: 21-63) and nighttime (intensity: approximately 1 lux).

As there were no significant differences in EOD measures between groups based on the number of stimulus neighbors present, data from all intruders ($n = 15$) and residents ($n = 15$) were pooled. Figures 40, 41 and 42 show the percent change relative to baseline in P2s, P3s, and PPSFs, respectively for all intruders compared to prior residents throughout the seven-day trials.

The relative change in P2 duration significantly changed as a function of status (i.e. intruder vs. prior resident: $F(1,20) = 5.08$; $p < 0.05$). P2 increased significantly more in prior residents than in intruders (Figure 40). P2 also differed between interaction days: $F(2, 40) = 4.78$; $p < .05$. A post-hoc analysis of these differences showed a significant difference between days 5 (94.6%) and 7 (97.7%) of interaction.

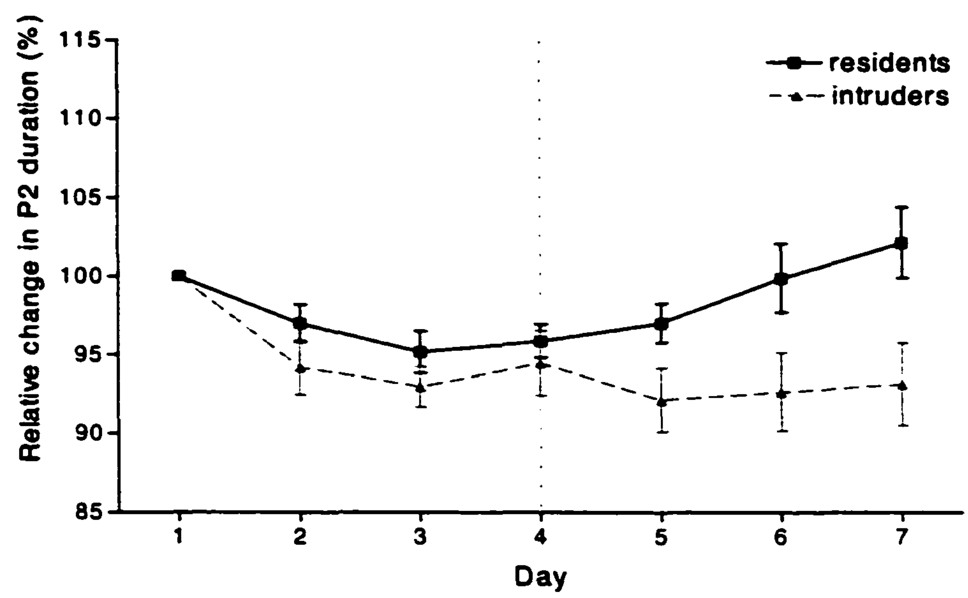


Figure 40

Percent change in P2 (relative to day 1 baseline) as a function of residence status (*n* = 15 per group). The vertical dashed line represents onset of restricted social interactions. Error bars: ± 1 SEM

The relative change in P3 duration also showed significant differences ($F(2,40) = 4.84$; $p < 0.05$). A post-hoc analysis of these data revealed a significant difference between the relative P3 duration on day 5 (91.5%) and day 7 (94.7%). Relative P3 duration increased over time, but there was no significant difference between groups.

The relative change in PPSF was also significant: $F(2,40) = 5.38$; $p < 0.01$. A post-hoc revealed a significant difference ($p = 0.006$) between day 5 (108.2%) and day 7 (105.4%) of interaction. PPSF decreased during the social

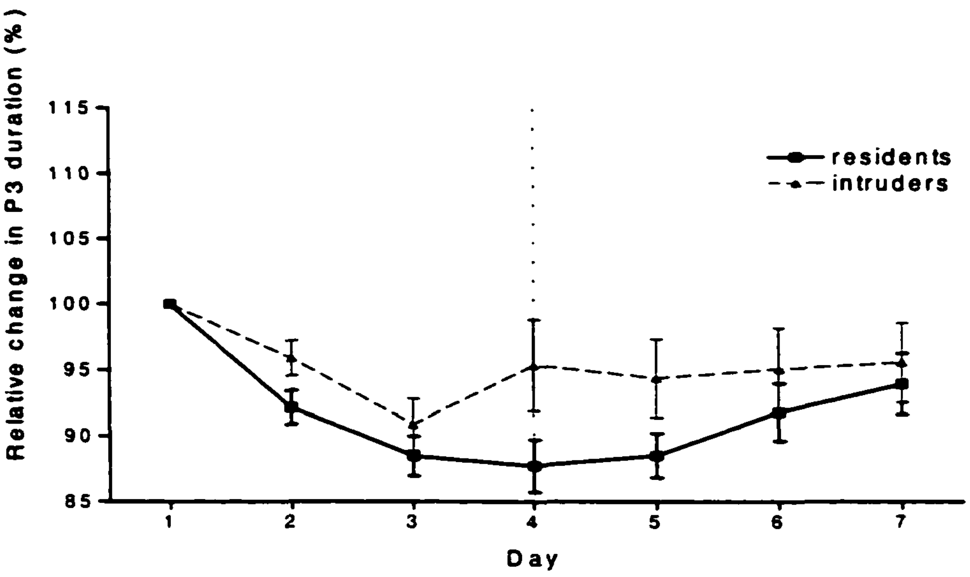


Figure 41

Percent change in P3 (relative to day 1 baseline) as a function of residence status (*n* = 15 per group). The vertical dashed line represents onset of restricted social interactions. Error bars: ± 1 SEM

interaction (Figure 42), and this decrease showed an interaction with residency status: $F(2,40) = 6.99$, $p < 0.005$. Post-hoc comparisons of the interaction effect were not possible, however, as no clear choice of an appropriate error term exists for between- and within-group interactions (Statistica program manual, 1995).

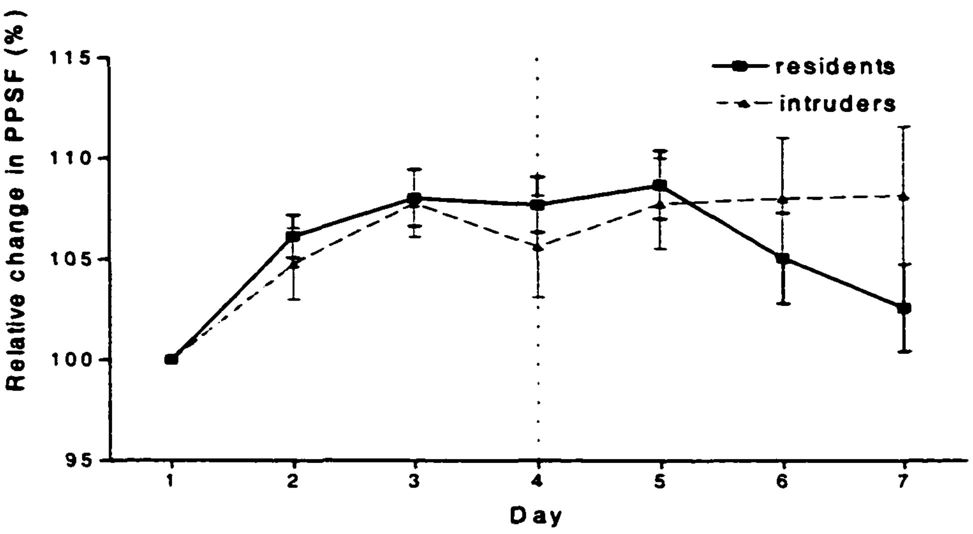


Figure 42

Percent change in PPSF (relative to day 1 baseline) as a function of residence status (*n* = 15 per group). The vertical dashed line represents onset of restricted social interactions. Error bars: ± 1 SEM

4.2.2 Locomotor behavior

Video recordings made during the first 30 minutes of social interaction were analyzed for the total amount of time that each subject spent outside of its shelter, a measure of social behavior. Being outside was defined as a minimum of the fish's entire head or the entire caudal peduncle being exposed (depending on whether the fish was swimming forwards or backwards, respectively). During much of this time, however, subjects were entirely outside the shelter and socially interacting with the stimulus fish.

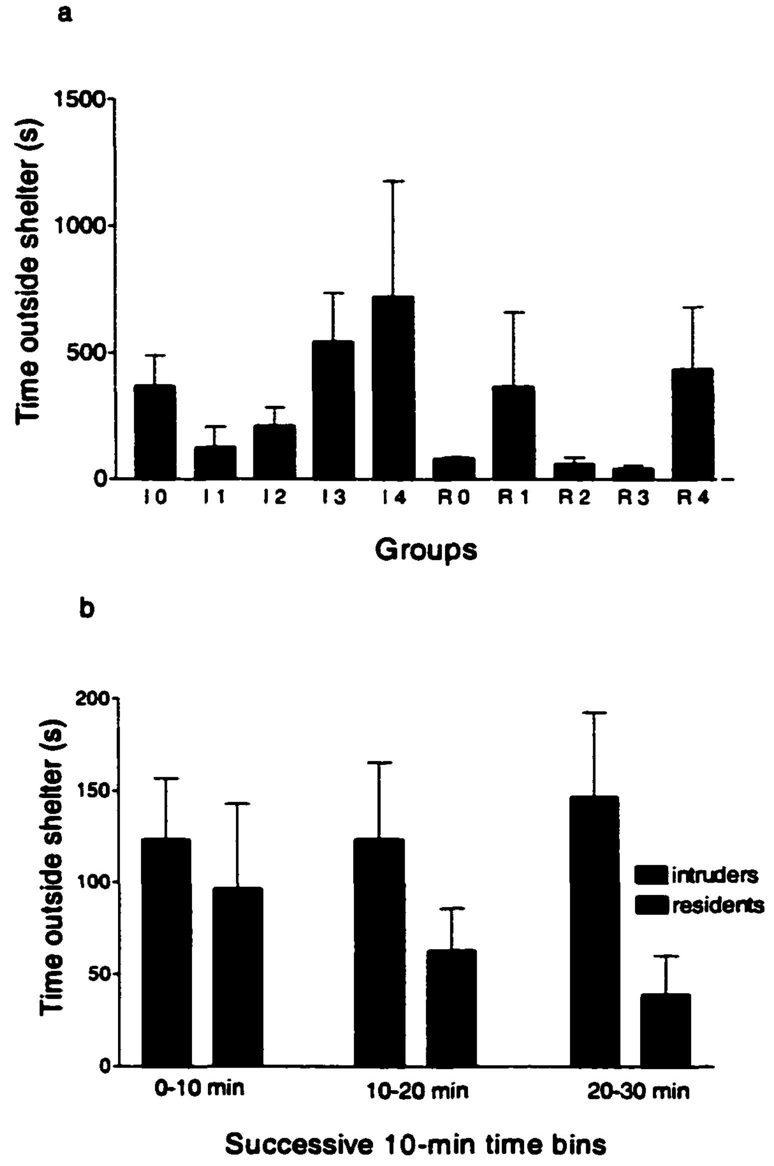


Figure 43

(a) Time fish spent outside shelter during first 30 minutes of social interaction. I: Intruder group, R: Resident group. 0, 1, 2, 3 & 4: number of neighbors present (*n* = 3 subjects per group). (b) Time spent outside shelter (all residents vs. intruders) during first 30 minutes of interaction (10 min increments). Error bars: ±1 SEM

There was no correlation between the total amounts of time that either intruders or prior residents spent outside their shelters as a function of the number of neighbors present (Figure 43a). The correlation coefficient determined for the intruder group (time outside shelter as a function of number of neighbors) did approach significance, however ($n = 5$): $r = .797$, $p < 0.10$.

Initially, prior residents spent a comparable amount of time outside their shelters relative to intruders. The time spent outside the shelter showed a decreasing trend in prior residents relative to intruders (Figure 43b). There was a significant correlation between the total amount of time that prior residents spent outside there shelter and the three successive 10 min periods: $r = 0.995$, $p < 0.01$ ($n = 3$). No correlation existed between the total amount of time that intruders spent outside their shelters and the successive 10 min periods ($n = 3$): $r = 0.87$. Several fish did not spend any time outside their shelter during one or more of the 10 min periods.

4.3 Discussion

Experiment 4 explored the relationship between residence status and number of neighbors on the EOD, PPSF, and locomotor behavior of fish in restricted interactions. Similar to *G. petersii*, changes in EOD parameters occurred during social interaction. Unlike *G. petersii*, *B. niger* showed differences between groups as a function of residency status, with P2 in prior residents elongating during the interaction period.

These results may be due in part to the fact that more subjects were compared in this experiment than were in the *G. petersii* intruder-prior resident groups. In addition, most of the *B. niger* subjects were exposed to more than one neighbor. A greater number of neighbors, each separated from the subject by a barrier, may have elicited a larger territorial response in prior residents than intruders, with associated androgen changes reflected in the EOD.

There is a possibility, however, that a confounding variable caused the between-group difference. This factor may have been the effect of intruders being introduced into a new tank on day 4. All subjects were introduced into a new tank on day 1; therefore all subjects experienced the same conditions from day 1 to day 3. The trend seen for all subjects during these days is a drop in P2 and P3 duration, with an associated increase in PPSF. Only intruder subjects were introduced into a new tank on day 4, a manipulation that produced a similar drop in P2 over days 4-7 as was seen for all subjects on days 1-3. The effect of being moved was controlled in part, however, as residents were also removed from their home tanks briefly on day 4, but then immediately returned to that tank. This may have also been an adequate control for the effect of movement into a novel environment.

Initially prior residents spent a comparable amount of time outside their shelters as compared to intruders. This was likely due to the fact that just prior to interaction, resident subjects were disturbed (moved briefly in order to control for the effect of being moved). Shortly after a resident was returned to the familiar environment, however, it spent less time outside of its shelter relative to intruders that were exploring a novel environment.

5.0 General Discussion

G. petersii and *B. niger* showed similar socially mediated EOD changes. These results demonstrated that changes in the EOD duration and relative phase amplitudes may serve to communicate social status in subadults of either sex, and that such changes are not restricted to the breeding season. The fact that socially mediated EOD changes occurred in non-breeding fish suggests that the EOD may serve a communicative function in agonistic interactions, and that the hormonal correlates of these EOD changes may relate predominantly to dominance signaling rather than mate attraction.

The results from fish observed during restricted interaction demonstrated that direct physical contact is not required to induce the EOD changes that are characteristic of social dominance. It remains to be demonstrated what cues induced such changes. Under such restricted conditions, one could eliminate one or more of the sensory stimulus modalities provided by a neighbor and thus tease apart the relevant cues. Such cues might include not only the electric input from a neighbor's EODs, but also mechanical and/or visual cues.

The behavioral and EOD effects resulting from input to these modalities are likely to be influenced by the specific behaviors and physical traits of neighboring fish. These include not only a neighbor's waveform and/or pattern of discharging, but body size and specific social locomotor behaviors.

The communicative behaviors of animals are often emancipated from the original function that they served (often to the extent that it is difficult to elucidate that function). The EOD, however, simultaneously serves in the context of both communication and orientation. For this reason it may be difficult to determine if the discharge patterns associated with specific behaviors serve primarily in communication, to facilitate electrolocation, or even to jam the electrolocation of a neighbor. Parallel display is an excellent example of such a behavior of ambiguous function. Perhaps the most effective way to discern the likely function is to determine whether the conditions associated with such behavior favor electrolocation or if they are better suited for the transmission of information. The EOD patterns, behavior, and physical proximity associated with parallel display suggest the former. If this is the case, body size is the attribute being communicated, not an EOD-based message.

If the EOD serves as a badge of social status, as the results of the free interactions suggest, then parallel display may be a method to test whether such a badge is serving as a reliable indicator of resource holding potential, or if it is instead a bluff. The results of the restricted interactions showed that a dominant-like EOD waveform might be attained in the absence of physical combat. If dominant-like EODs can be bluffed, additional assessment may be necessary. Parallel display may permit rapid assessment with a minimum risk of injury, as the body orientation and high discharge rates associated with it suggest. These results demonstrate that agonistic interactions involve a complex mix of signals and assessment, each of which are likely to involve complex multi-sensory integration.

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