Individual Differences in Electric Fishes: An Animal Model of Personality

by

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Abstract

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Advisor: Christopher B. Braun

Individual differences in animals have recently been described in behavioral ecology as behavioral syndromes: suites of correlated behaviors within individuals that are consistent across environmental situations. A central idea behind the behavioral syndromes approach is that behaviors do not occur in isolation; rather, they are integrated with other behaviors within the limited biological machinery of one individual. Electric fish are ideally suited for studies of behavioral syndromes because their behavior can be easily measured and tracked due to their unique electric organ discharges (EODs). It is also a good system to present realistic electric signals that mimic social interactions. Using a cohort of 22 *Microsternarchus* sp. a neotropical knifefish, we carried out a series of behavioral experiments, including a) a free exploration experiment, b) a terrestrial challenge, c) a novelty response experiment, d) a playback experiment with an aggressive sympatric species, and e) a jamming avoidance experiment. With the exception of the playback experiment, all were performed twice on all available individuals over the course of two years. Behavioral responses including EOD rate, locomotor activity, responses to novel as well as threatening stimuli, and reaction times were measured. Through principal components analysis and correlational analysis we determined that *Microsternarchus* sp. exhibit behavioral syndromes in activity, reactivity, aggression/dominance and possibly behavioral flexibility, integrating electric signaling behaviors with components of exploratory behavior and responses to stimuli. For example, individuals with the highest EOD rates spent

more time swimming around a novel environment, than individuals with lower EOD rates, thus these behaviors form part of an activity syndrome.

Preface

Over the course of two years, five different experiments were carried out (and four out of the five were replicated) to determine if *Microsternarchus* sp. display individual differences in behavior that can be organized into behavioral syndromes. The description of these experiments is here organized into three chapters by stimulus type, with a fourth chapter devoted to the analysis of all experimental data combined. In the first experimental chapter (Chapter 2) we will analyze the free exploration experiment. This experiment was performed to determine if individuals differ in their exploratory behavior in a novel environment. In Chapter 3 we will analyze two experiments involving non-social, potentially stressful, environmental stimuli, namely the novelty response experiment and the terrestrial challenge. The stimulus used in the novelty response experiments was a mechanical tap on the tank wall, In the terrestrial challenge animals were subjected to an 'out of water' experience meant to elicit a coping response. Both of these experiments were designed to evaluate how the fish would react following a stressful, non-social event and determine if individuals differ in their coping styles to such events. In Chapter 4 we will analyze experiments involving stimuli that were social in nature, namely the *Gymnotus* playback experiment and the jamming avoidance response experiment. Both these experiments take advantage of the subject's electrosense by playing the recorded EOD of an aggressive and potentially threatening species as well as their own EOD into the water, and monitoring their behavior. These stimuli were meant to elicit social/anti-social behavior, allowing us to determine if individual differences exist in response to social stimuli. Chapter 5 will analyze the combined data from all experiments to determine if behavioral syndromes can be found from correlations between behaviors from different contexts.

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Chapter 1:
Introduction to Animal Personality and Electric Fish

In humans, organized and consistent individual differences in behavior are called personality types (Brody & Ehrlichman, 1998; Derlega, Winsted, & Jones, 1991), but similar patterns of individuality in animals have not been widely appreciated until recently (Sih, Bell, Johnson, and Ziemba, 2004). Historically, behavior in animals was thought to be species-typical and atomizable into separate traits that selection could act upon individually, maximizing fitness in all contexts independently. Individual variation was seen as noise that surrounded a species-typical ideal (Gotceitas & Colgan, 1988). More recently, the organization of individual differences in animals has been described as *behavioral syndromes* (Sih et al., 2004): suites of correlated behaviors within an individual that are consistent across environmental situations. The species or population exhibits a *behavioral syndrome* (e.g., aggression) while the individual exhibits a *behavioral type* (e.g., very aggressive to not aggressive) reflecting their tendency to behave in a similar way across multiple situations. Correlated suites of behaviors represent a significant paradigm shift from the study of species-typical behaviors *across* individuals *within* a situation or context to understanding behavior as an organized whole *within* individuals *across* different situations.

In contrast to optimality theory, which assumes animals behave in to maximize their fitness in all contexts (Maynard Smith, 1978), the behavioral syndromes theory allows for individuals to exhibit adaptive behaviors in one context but potentially non-adaptive behaviors in another (related) context. This may reflect a limit to plasticity, resulting from the challenge of regulating multiple adaptive behaviors with only one nervous system. Differences in behavioral skills, physical characteristics, perceptual mechanisms, and nervous systems set up limitations for behavior overall, such that not every individual will be able to swim as fast, jump as high, or

detect predators as well as another. Given these limitations, compromises must be made -- resulting in individuals that do not always display optimal behaviors in every context. (Sih, Bell, Johnson, & Ziemba, 2004).

This thesis will develop electric fish as a model for studying behavioral syndromes or animal personality. The production and detection of electric signals in these fish provide convenient indicators for the study of individual differences in behavior because discharge rate reflects the animal's active sensing of the environment and varies predictably depending on the animal's behavioral state (e.g., rate increases during periods of activity). Finding relationships between individual differences in electric and locomotor behaviors will allow us to discern the non-communicative significance of electric organ discharge (EOD) rate modulation, an area that has been relatively unexplored in the field of electric fish behavior. The neural circuitry underlying the production and detection of electric signals is well understood in electric fish and this could provide insights into possible brain mechanisms involved in individual differences in environmental exploration, responses to novelty and stressors, as well as sensory processing.

Introduction to Animal Personality/Behavioral Syndromes

Origins of Individual Variation. P.J.B. Slater (1981) proposed three possible explanations for the existence of individual differences in behavior; a) multiple behavioral strategies exist as an evolutionary stable strategy; b) individual variation is a side effect of selection for individual recognition; and c) animals may lack the information they need to make a behavioral strategy decision, consequently creating multiple strategies with variable outcomes, depending on the environmental uncertainties.

An evolutionary stable strategy (ESS) is a behavioral strategy that yields maximum fitness to all the members of a population that use it, such that no other strategy would provide

higher fitness when the majority of the population employs the ESS. Evolutionary stable strategies can be pure in that one strategy is used by all members or they can be mixed in that multiple strategies are used in stable proportions. Mixed ESS occur through two mechanisms, (a) each individual maintains their strategy so that each strategy has a stable proportion of players in a population or (b) each individual varies its strategy, playing different roles at a specific frequency or according to an assessment rule (Goodenough, McGuire & Wallace, 1993, Maynard Smith & Price, 1973). In the first case, having each individual play its own strategy leads to individual differences in the behavior (Mesterton-Gibbons & Adams, 2001).

In order to maximize indirect fitness and avoid inbreeding, it is beneficial for individuals to assess the degree of relatedness between themselves and their potential mates. Female great tits (*Parus major*), for example, have been shown to choose mates based on how similar their song is to her fathers, preferring songs are similar, but not identical to, their fathers song. While in the nest, all great tit hatchlings learn the song of their father, male offspring will later sing this song and female offspring will learn to recognize it. By listening to the song of a male and comparing it to the song she learned from her father, females choose a male whose song sounds somewhat similar to the song of her father, effectively choosing a mate that is a distant relative (McGregor& Krebs, 1982).

Finally, individual differences in behavior may occur due to environmental uncertainty. Female swifts (*Apus apus*), for example, that lay three eggs will fledge more young in years of plenty, while females that lay two eggs will fledge more young in years when food is more scarce. Therefore, without the ability to predict food availability, consistent individual differences within the species are likely to be maintained in a population by a fluctuating environment (Slater, 1981).

The field of animal personality evolved largely independently from the field of human personality. This is likely due, in part, to researchers studying animal personality having to demonstrate that individual differences in animals were real and important and not just the result of anthropomorphization. Terms that describe animal personality (e.g., behavioral syndromes, coping styles, individual differences, and temperament) are reflective of the discomfort researchers have using the term *person*ality to refer to animals. All these terms essentially describe the same phenomenon: individual animals differ consistently in their behavior in a similar, measurable way to humans.

While human personality research is rooted in the assumption that individuals are different from one another, animal personality research began with the need to explain why, under the same conditions, individual animals did not behave in an identically optimal way (as predicted by optimality theory) (Brody & Ehrlichman, 1998; Nettle & Penke, 2010). The disciplines of human and animal personality are quite similar, yet there is relatively little cross-referencing in the literature. This is unfortunate as both sides would benefit from a shared understanding of theory and methodology. For example, in the field of human personality, an individual's personality is considered more stable over time but less stable across different contexts (Nettle & Penke, 2010). Consider the rock climber who appears fearless on the face of a cliff but who is unable to get a date because he fears rejection from women. In the field of behavioral syndromes, finding consistency across contexts is fundamental to defining individual differences in animals, with less focus on consistency over time (Sih, Bell, Johnson, & Ziemba, 2004). This conceptual difference could be due to methodological differences between the disciplines. Much of the human personality research relies on self- or informant-reporting to identify personality traits (Furr, 2009). It is often easier to see one's own behavior (or that of a

well-known other) as situationally dependent due to the vast amount of experience the rater has with behaviors across a variety of contexts. Alternatively, much of the animal personality research relies on direct measure of discrete behaviors, chosen to demonstrate variation among similar behaviors in multiple contexts.

Methodology in Animal Personality/Behavioral Syndromes Research

Most human personality researchers have agreed on five broad dimensions of personality, established through various methods of factor extraction or factor analysis (Goldberg, 1990). This model is referred to as the "Big Five Structure" of trait dimensions and includes axes for: (a) neuroticism versus emotional stability, (b) agreeableness versus antagonism, (c) extraversion versus introversion, (d) openness to experience versus closed-mindedness, and (d) conscientiousness versus impulsiveness (Derlega et al., 1991). Animal personality research, still in its infancy when compared to the field of human personality, has not yet come to a general consensus about the description of axes of personality in animals. Like studies of human personality, factor analysis or more commonly, principal components analysis, is used in animal personality research. While these methods can and have been used successfully, they are not without potential pitfalls. Unfortunately these techniques are at times applied incorrectly: using small sample sizes or over-interpreting the results (Budaev, 2010). In other animal studies of personality, correlation coefficients are used to asses which behaviors trend together within individuals. Labels, such as *aggressive* or *bold*, are then applied to these correlations. This method, however, has a high cumulative probability of type I errors when many behaviors or variables are being correlated. Combining this technique with principal components analysis to first reduce the number of variables may partially compensate for this issue when the number of variables is very high (Bell, 2007).

Known Behavioral Syndromes

Due to a recent explosion in the popularity of this topic, many examples of personality or behavioral syndromes in animals have been described for a variety of traits in a wide assortment of taxa (*Horses:* Anderson, Friend, Evans, & Bushong, 1998; *Yellow-bellied marmots:* Armitage, 1986; *Mice:* Benus, Den Daas, Koolhaas, & Van Oortmerssen, 1990; *Rainbow trout:* Biro & Stamps, 2010; *Guppy:* Budaev, 1997; *Great tits:* Carere, Drent, Privitera, Koolhaas, & Croothuis, 2005; *Sheep:* Cook, Massland, & Devine, 1996; *Merriam's kangaroo rats:* Dochtermann & Jenkins, 2007; *Octopuses:* Mather & Anderson, 1993, *Dumpling squid:* Sinn & Moltschaniwskyj, 2005). These include, but are not limited to, activity, aggression, and responsiveness.

Activity. Activity is one of the more obvious attributes that differ across individuals. Animals that are highly active show more movement and ambulation than those that are less active. Activity is associated with the biological necessities of foraging and mating, but increases in exploratory activity also lead to an increased probability of falling prey to a predator (McNamara & Houston, 1994; Sih, 1997) and potentially higher metabolic costs.

For example, Golden Spiny Mice (*Acomys russatus)* display consistent individual differences in their activity patters when kept on a 12:12 LD cycle (Cohen & Kronfeld-Schor, 2006). Some individuals were diurnally active compared to the others that were nocturnal (defined as having more than 50% of their activity during the night). Even among those that were nocturnal, some individuals showed continuous activity at night while others had various periods of activity punctuated by rest.

Aggression. Aggression is another example of a trait that varies by individual (Gosling & John, 1999). While aggression levels certainly vary in different contexts (e.g., territoriality vs.

foraging) often, certain individuals are consistently more aggressive in all contexts when compared to conspecifics (Huntingford, 1976; Sih, Bell, Johnson, & Ziemba, 2004).

In Three Spined Sticklebacks (*Gasterosteus aculeatus)* boldness towards predators was positively correlated with territorial aggression toward conspecifics (Huntingford, 1976). Bold fish showed little change in ongoing behaviors and/or approached the predator, rendering them more at risk of predation. Animals with high territorial aggression showed more bites and lunges at an intruder. Individuals that successfully maintained a territory (and thus benefit from increased fitness in terms of potential breeding success) were also more likely to be vulnerable to predation (and thus is subject to decreased fitness).

Responsiveness. Behavioral syndromes that occur in response to stressful conditions have historically been called *coping styles*. However, they are, in essence, a specific type of behavioral syndrome, with consistent responses (both behavioral and physiological) that differ among individuals while they are under stress (Coppens, de Boer, & Koolhaas, 2010; Koolhaas et al., 1999). Responsiveness can include aspects of activity, boldness, fearfulness, and exploration at the same time (Sih, Bell, & Johnson, 2004).

Two distinct styles of responsiveness represent the two extremes of one axis. Active response styles are referred to as *proactive* and more withdrawn responses are referred to as *reactive* (Koolhaas et al., 1999). Proactive individuals respond to challenges and or stressful events by attempting to obtain control their environment, while reactive individuals take a less active approach, avoiding or sitting and waiting until the event passes (Carere et al., 2005; Koolhaas et al., 1999; Verbeek, Drent, & Wiepkema, 1994). Reactive individuals are also much more aware of the environment and environmental cues than their proactive counterparts

For example, wild-type rats *(Rattus norvegicus*) subjected to two different behavior tests, *proactive* males quickly attacked an intruder in their home cage and were more likely to bury a shock prod inserted into a test cage (Koolhaas et al., 1999). Reactive mice took longer to attack and intruder and simply avoided the shock prod rather than burying it. Proactive individuals tend to form routines readily indicating limited behavioral flexibility. Mice (*Mus musculus omesticums*) selectively bred to display proactive behavior (quickness to attack) took longer to find a food reward after it had been moved from its original location than those bred to display reactive behavior (Benus et al., 1990). Moreover, reactive individuals tend to be guided more by environmental cues than routines. When researchers changed a small aspect of a familiar maze (adding a small piece of tape on the floor) reactive individuals took longer to reach the goal box and made more errors compared to proactive individuals (Benus et al., 1990).

Studies of any personality dimension typically require many hours of observation and a trained behavioral observer in order to accurately record the behaviors of interest. In the field these studies can be intrusive, just the presence of the observer may limit the visibility of the some members of the population. Weakly electric fish provide an opportunity observe behavior in the laboratory without a human observer present or visible, making them particularly useful for the study of individual differences. In humans, individual differences are a basis of personality and have clear implications for mental health. Reactions to novel stimuli or new situations are an indication of individual susceptibility to depression and anxiety disorders, and startle responses in particular have been shown to be exaggerated in patients with anxiety disorders (Grillion, 2002). Animal models of individual differences provide the means to determine the neural basis of individual differences and their ecological relevance. The neural circuitries involved in both generating and sensing electric fields have been worked out in a few

species of electric fish (Carlson, 2002; Kawasaki & Heiligenberg, 1989; Metzner, 1999) which could provide an important avenue for research into the brain mechanisms involved in individual differences in environmental exploration, responses to novelty and stressors, as well as sensory processing.

Introduction to Electric Fish

Weakly electric fish have evolved electrogenic and electrosensory systems that allow them to generate and detect weak electric fields (*Reviews:* Bullock, Hopkins, Popper, & Fay, 2005; Kramer, 1990, Moller, 1995). Since electric fish are mostly nocturnal, their ability allows them to navigate in the dark when visibility is low and to communicate effectively over short distances (*Review*: Ladich, Collin, Moller, & Kapoor, 2006).

Electric fields are generated in these fishes by specialized electric organ (Figure 1.1a) that consist of a set of modified muscle or nerve cells containing many individual electrocytes (Figure 1.1b) stacked together (Figure 1.1c) (Bass 1986; Reviews in Bullock & Heiligenberg, 1986; Bullock et al., 2005; Kramer 1990). The electroreceptor cells that detect electric fields are found embedded in cavities covering the surface of the skin (von der Emde, 1998).

Electric communication and electrolocation have evolved in at least two unrelated groups of teleost fishes: the African Mormyriformes and the South American (Neotropical) Gymnotiformes (Bullock et al., 2005; Hopkins, 1999; Hopkins & Westby, 1986; Kramer, 1990; Ladich et al., 2006; Moller, 1995, 2006; von der Emde, 1998). Weakly electric fish are further distinguished by their discharge patterns into wave-type and pulse-type fish (Figure 1.2). Wave-type fish produce a continuously oscillating voltage potential, in contrast, pulse-type fish produce brief (<5 ms) EODs with inter-pulse intervals ranging from approximately two to 10 times the duration of the EOD itself, depending on the species (see Moller, 1995 for review).

The EOD is characterized by both its waveform and the rate at which EODs are produced. The waveform, defined as the repeatable temporal pattern of voltage change, communicates information about species, sex, and possibly individual (Bass & Hopkins, 1983; Crawford, 1992; Hagedorn & Carr, 1985; Hopkins, 1972; Hopkins & Bass, 1981; Hopkins, Comfort, Bastian, & Bass, 1990; McGregor & Westby, 1992). Individual waveforms are described in terms of their amplitude (mV/cm), duration (ms), and the number and shape of individual phases (Figure 1.3).

Electrolocation. Fish use their sense of electrolocation to explore the local environment. The electric signals are a non-propagating electric dipole field (Hopkins, 1999). Thus effective signal amplitudes are only found fairly close to the body of the fish, forming an area of current flow around the fish. Objects that conduct electricity more than the surrounding water cause the current around the fish's body to *concentrate,* while resistive objects *diffuse* the current. These changes in the electric field cause the pattern of transdermal current flow at the electroreceptors to change as well (von der Emde, 1999). In other words, the near-by presence of objects that differ in conductivity from the aquatic medium affects an electric image on the fish's body surface.

Electric fish change their discharge rate in a variety of situations that affect both electrolocation and communication. In pulse fishes, for example, the discharge rate is correlated with the locomotor activity of the animal, such that at night while they are actively moving about and exploring, they discharge at a higher EOD rate than they do during the day while at rest (Black-Cleworth, 1970; Capurro et al., 2001; Hopkins, 1999; Lissmann & Schwassmann, 1965; Moller, 1995; von der Emde & Schwarz, 2002). Higher sampling frequencies provide the fish with a more accurate "electric image" particularly if they or other objects are actively moving.

Many species of electric fish respond to novel objects or stimuli (e.g., mechanical and electrical stimulation, vibration in the water, and sudden change in light levels or noise) with a sudden short-lived increase in EOD rate, called the novelty, startle, or orienting response (Ciali, Gordon & Moller, 1997; Lissmann, 1958; Lopes Correa, Hoffmann, & Grant, 1998; Post & von der Emde, 1999). Increases in EOD rate presumably allow the fish to identify the source of the novel stimuli faster (Barrio, Caputi, Crispino, & Buno, 1991).

Electric fish also modify their discharge rate while performing a "jamming avoidance response (JAR)". The overlapping discharge of two fish with similar EOD rates creates a beat pattern. When the EOD rates are similar the beat pattern affects a change in the fish's sensory receptors that is similar to the change affected by an electrolocative object. In order to disrupt this interference and avoid being jammed, the fish change their EOD rates to increase the frequency difference. (Capurro, 2004; Heiligenberg, 1986; Kramer, 1985; Westby, 1981). The JAR is an effective way of maintaining the ability to electrolocate while sharing space with other discharging fish.

Study Species

Microsternarchus is currently a monotypic genus with only one named species, *M. bilineatus* (Fernández-Yépez, 1968; Mago-Leccia, 1994). However, it has recently become apparent that multiple lineages of *Microsternarchus* exist in the Rio Negro of Brazil, and the validity of *bilineatus* is in question (Nogueira, 2010). Nogueira identified at least five distinct lineages within the genus and proposed that at least two be considered new species by analyzing aspects of EOD behavior, morphology, geographic distribution and mitochondrial DNA, Due to this current taxonomic uncertainty, we refer to the individuals studied herein as *Microsternarchus* sp. We suspect, based on morphological characteristics (size and color), that

our animals are not part of either of the newly proposed species. However, the species assignment of the remainder of the sampled individuals is unclear, thus tissue samples from many of our subject animals have been deposited at INPA, Brazil (Instituto Nacional de Pesquisas da Amazônia) for future molecular and systematic analysis.

To date, *Microsternarchus* has not been extensively studied in the laboratory but display interesting characteristics that make them stand out as a unique pulse-type fish (faster EOD rates, lower ongoing variability). This project will not only identify individual differences but will also provide more details about the behavior of this somewhat unique species.

General Methods

Subjects

Our subjects were 22 *Microsternarchus* sp. Animals were collected in March 2005 from the Rio Negro using electric-fish detectors and hand nets along the shoreline of shallow streams near Manaus, Brazil (Amazonas State). Our fish came from three different neighboring collection sites along the Rio Negro, nine individuals from Igarapé Adalto (geographic coordinates S 03° 51’ 19.7” , W 60° 58’ 7.6”), five individuals from Igarapé Lauara (S 03° 12’ 39.3”, W 60° 34’ 30.1”), and eight individuals from Igarapé Mato Grosso (S 02° 48’ 46.1”, W 60° 55’ 29.6”). Animals were collected and exported in accordance with both Brazilian and U.S. Laws. Upon arriving in New York, they were housed in communal 37.85 L tanks (five to eight animals per tank) and fed small live oligochaete “blackworms” (*Lumbriculus* sp.*) ad libitum*. In all home tanks and throughout all experiments animals were maintained in low conductivity water characteristic of non breeding conditions (between 115 and 125 μsiemens/cm^2) with a pH between 4.5 and 6.0 at 24°C± 1°C and on a 12hour:12hour light:dark cycle. A water conditioner, Blackwater expert (Kent) was also added to the tanks to maintain conditions similar to the Rio

Negro. The sex of these animals was not readily apparent. Sexing these animals can only be done if a female spontaneously becomes gravid or upon necropsy, although animals kept in nonbreeding conditions may have de-differentiated gonads.

Shelters were provided in each home tank in the form of multiple plastic 'plants' (sections of dark plastic garbage bags that are weighted and cut into strips such that they form conical, multi-layered hide-a-ways) that allow the fish to bury themselves during the day.

Individual Identification. To identify individual animals, visible implantable elastomers (Northwest Marine Technology, Inc.) were injected into the hypaxial muscle in the tail of the fishes. Photos of each fish were taken for future identification (Figure 1.4). All fish were measured (total length in cm) at the time of marking (see Table 1.1 for measurements).

Experimental Outline

This project included five separate experiments conducted between April of 2006 and April of 2008: (a) a free exploration experiment, (b) a terrestrial challenge experiment, (c) a novelty response experiment (d) a *Gymnotus* playback experiment, and (e) a JAR experiment. Experiments were conducted on all individuals and subsequently repeated once at least 3 weeks later (with the exception of the *Gymnotus* playback experiment which was not repeated). The order of experiments was the same for each individual; however the order in which individuals were used in each experiment was random. Throughout the course of the experiments individual animals died or became otherwise unavailable; therefore, not all experiments include all individuals. Figure 1.5 shows the timeline of experiments for each individual.

This research is exploratory in nature and not necessarily hypothesis driven. Prior to forming hypotheses about *why* animals show individual differences in behavior our task herein was to first determine *if* they display individual differences in behavior. Therefore, our aims and

subsequent predictions focus on determining if individual *Microsternarchus* sp. differ consistently in their behavior.

The experiments are organized into separate chapters, as follows.

Beginning with Chapter 2 (Free Exploration) we will discuss the free exploration experiment, along with a supplemental experiment only performed on a small subset of individuals to measure the fine temporal structure of EOD rate patterns. During the free exploration experiment animals were removed from their home tanks and placed individually in a novel environment. Locomotor activity and EOD rates were monitored for three consecutive days and nights. For this chapter we have one specific aim (aim numbers reference the chapter in which they are being addressed, our predictions regarding these aims can be found within the individual chapters):

- Specific Aim 2.1: to describe relationship between changes in EOD rate and locomotor activity (measured by percentage of time spent out of a shelter) in *Microsternarchus* sp. as well as document if consistent individual differences in EOD rate and locomotor activity form the basis for an activity syndrome.

In Chapter 3 (Terrestrial Challenge/Novelty-Response) we will describe the terrestrial challenge experiment and the novelty response experiment, both of which are environmental challenges. For the terrestrial challenge experiment, animals were subjected to an out-of-water experience that included being removed from the water and placed on a moist platform. The time it took the animal to return to the water was measured. Novelty response experiments were used to evaluate each individual's EOD rate in response to a novel stimulus which, in this case, consisted of percussive tapping on the tank wall. For this chapter, we have the following specific aims:

- Specific Aim 3.1: determine if individuals differ consistently in response to a terrestrial challenge indicative of differences in coping responses;
- Specific Aim 3.2: identify if individual differences in the amplitude and duration of novelty responses are indicative of differences in coping responses; and
- Specific Aim 3.3: if differences are seen among individuals in their response to a terrestrial challenge and their novelty responses, determine if patterns of individual differences described within each experiment correlate and conform to established ideas about proactive and reactive coping styles.

In Chapter 4 (*Gymnotus* Playback and JAR) we will describe the *Gymnotus* playback experiment and the JAR experiment. Both these experiments use electrical stimuli to simulate social or intraspecific challenges. The *Gymnotus* playback experiment was conducted in the same experimental tank as the free exploration experiment with the addition of two pairs of T-electrodes through which the EOD signal of an aggressive sympatrically occurring species was played. This playback signal was meant to represent an aggressive intruder. Each individual's locomotor activity and EOD rate was monitored, along with approaches to the playback electrodes, on two consecutive nights and days. Finally, in the JAR experiment, we exposed each fish to 10-s bouts of playback of their own EOD at eight different fixed rates relative to the fish's own starting EOD rate (thus the synthetic signal was constructed to be between 1 and 5 Hz above or below the subject fishes EOD rate). This playback signal was meant to represent a non-aggressive, conspecific. The subject fish's EOD in response to the playback was monitored. For this chapter, we have the following specific aims:

- Specific Aim 4.1: determine if individuals differ in their response to *Gymnotus* playback indicative of differences in social or anti-social behavior.

- Specific Aim 4.2: to characterize the JAR in *Microsternarchus*.
- Specific Aim 4.3: determine if consistent individual differences are seen in the JAR of *Microsternarchus* that would be indicative of differences in social or anti-social behavior.
- Specific Aim 4.4: if individual differences exist in response to *Gymnotus* playback and JARs, determine if patterns of individual differences described within each experiment correlate and conform to established ideas about social or anti-social behavior.

In Chapter 5, we will report on the principal components analysis of the data from each experiment and subsequently identify correlations between principal components extracted from each experiment. In this chapter, we have the following specific aim:

- Specific Aim 5.1: to combine the data from individual experiments to determine if individual variability seen within behaviors from each experiment correlate to form behavioral syndromes.

Finally, in Chapter 6, we will draw conclusions, suggest improvements to our experimental design, and offer suggestions for the potential future directions.

Table 1.1
Length measurements for each individual

Fish ID:	Length:
ADA101	13cm
ADA102	14cm
ADA103	12cm
ADA104	12cm
ADA105	13cm
ADA106	12cm
ADA107	13cm
ADA109	12cm
ADA110	10cm
LAU101	11cm
LAU102	14cm
LAU103	12cm
LAU104	12cm
LAU105	13cm
MAT101	15cm
MAT103	13cm
MAT104	14cm
MAT106	13cm
MAT107	13cm
MAT108	13cm
MAT109	14cm
MAT110	12cm

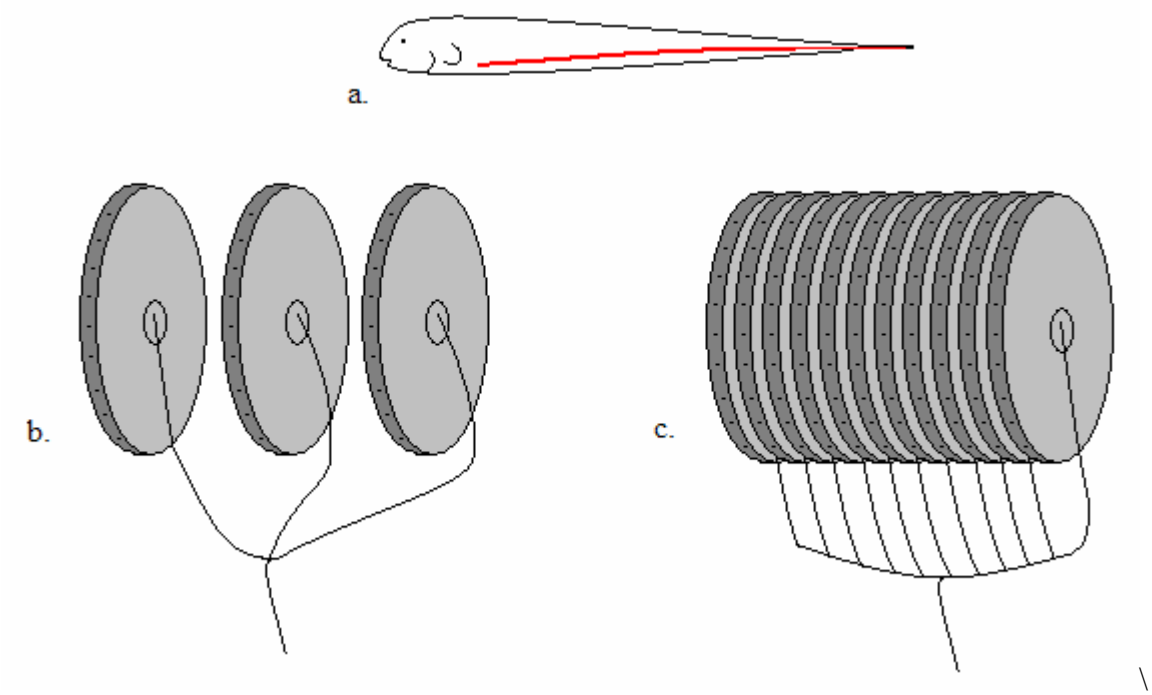


*Figure 1.1.*The location and composition of the electric organ. (a) The location of the electric organ in a typical gymnotiform. (b) The electric organ is made up of many individually innervated electrocytes tightly stacked together (c) throughout the entire organ.

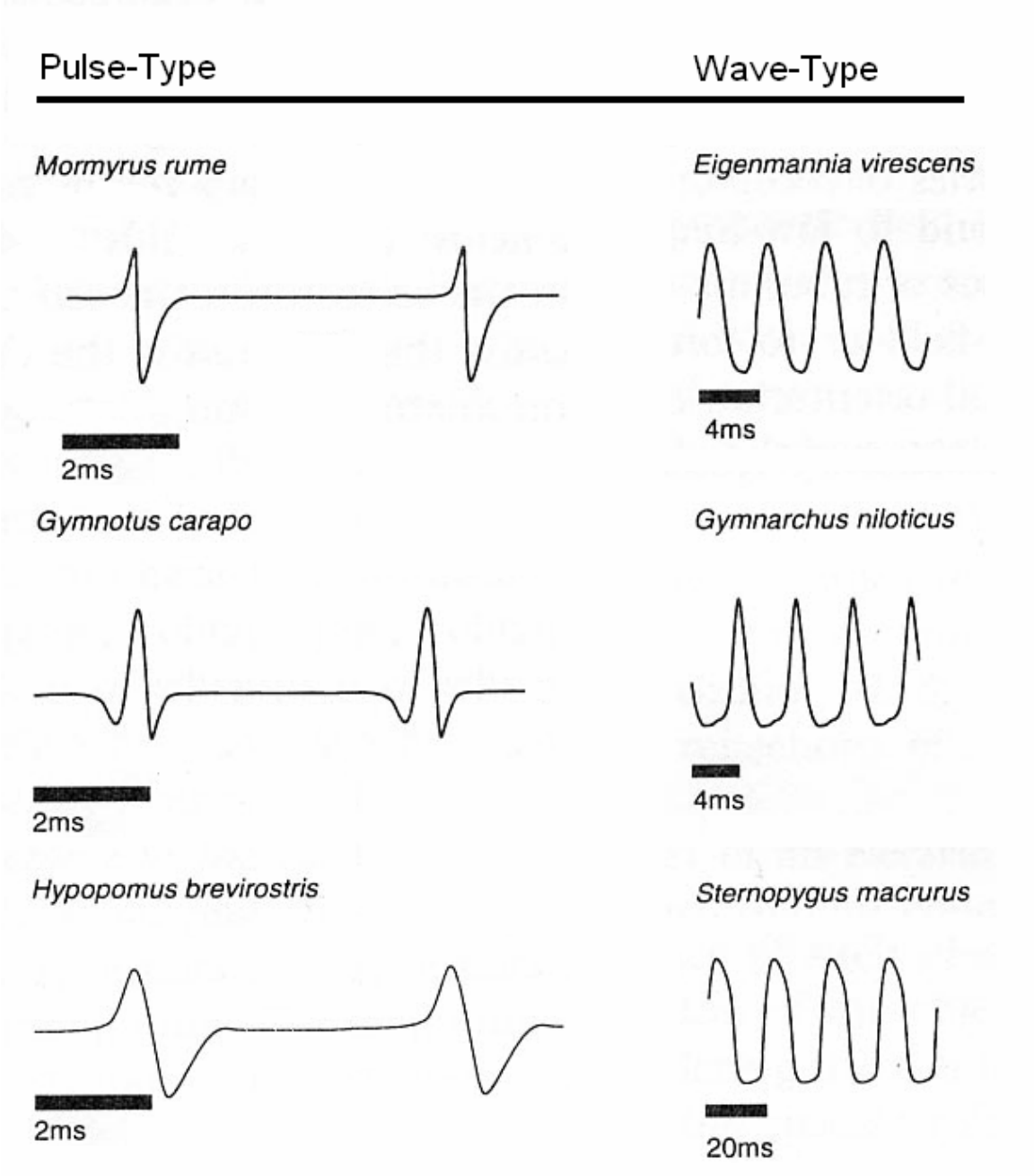


Figure 1.2. The EOD waveforms of pulse-type and wave-type fishes. Wave-type fishes show ongoing oscillation of the voltage potential while pulse-type fishes have brief periods of inactivity (the inter-pulse interval) between EODs. Figure adapted from Figure 5.2 of (Moller, 1995).

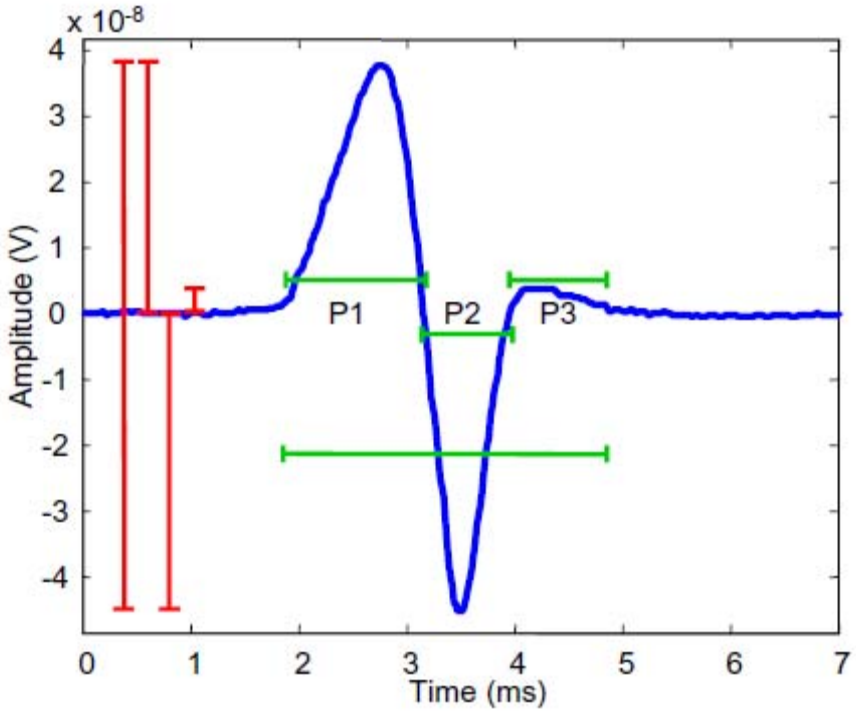


Figure 1.3. Diagram of a single EOD showing phase distinctions. Red bars represent the amplitudes of each phase and the total amplitude of the EOD. Green bars represent the duration of each phase and the total duration of the EOD.

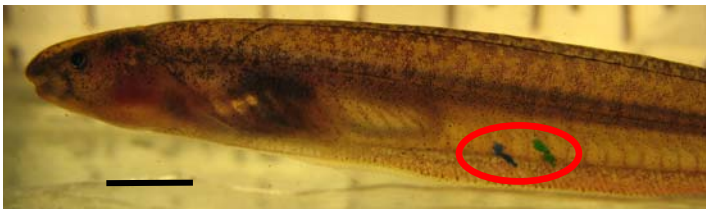
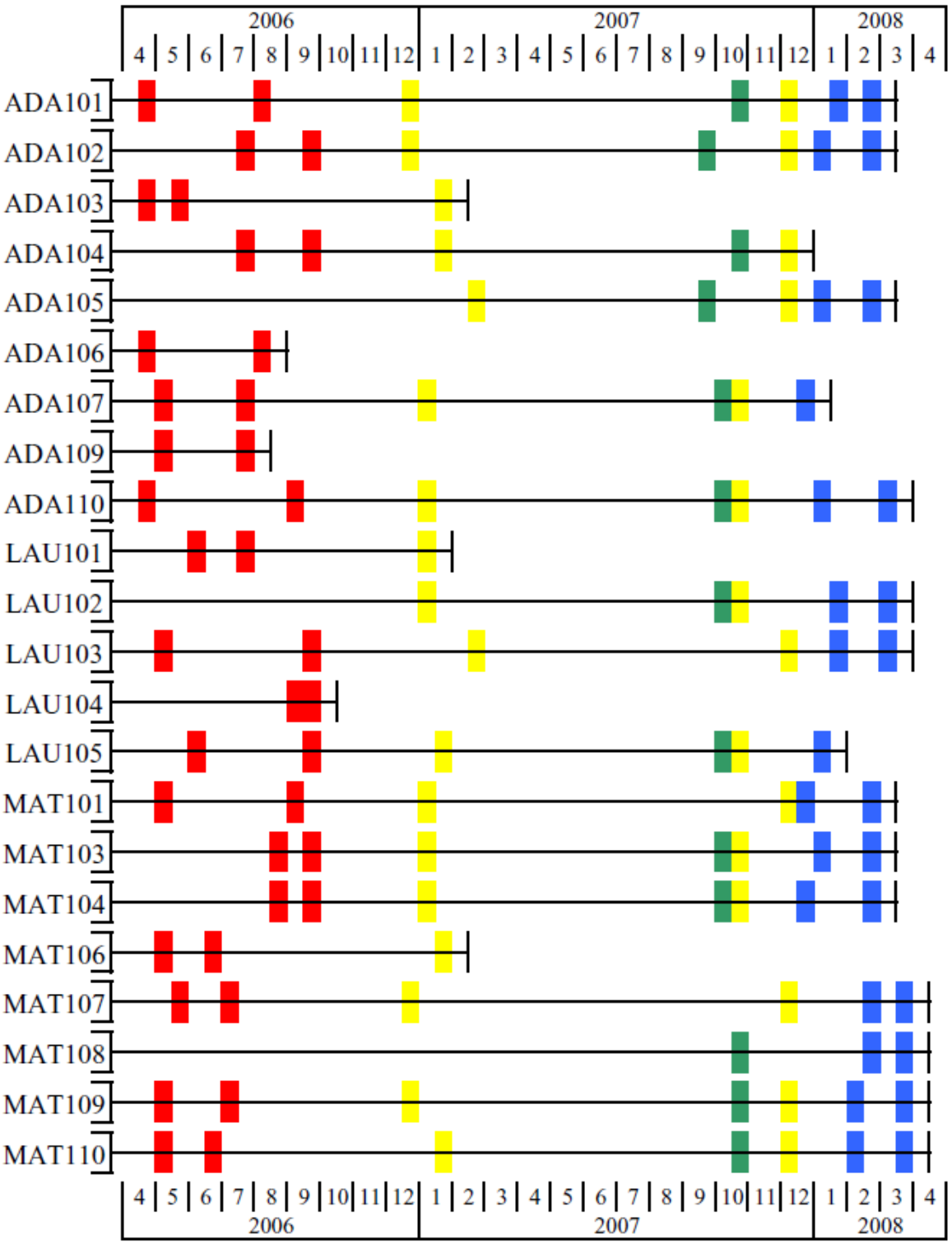


Figure 1.4: Partial view of a *Microsternarchus* sp. showing elastomer injection (inside the red circle). Black bar indicates 1cm.


2006
2007
2008
4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4
ADA101
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MAT110
4 5 6 7 8 9 10 11 12 1 2 3 4 5 6 7 8 9 10 11 12 1 2 3 4
2006
2007
2008

Figure 1.5. Timelines for each individual's experimental series. Blocks of time are accurate to within a period of about 15 days (each month being divided into two halves). Red bars indicate the two replicates of the free exploration experiment, yellow bars represent the terrestrial challenge, green bars indicates the *Gymnotus* playback experiment, and blue bars represent both the novelty response experiment and the JAR experiment which were run sequentially on the same day (JAR experiments always following the novelty response experiment after a 30 minute rest period).Deceased animals have truncated time lines.

Chapter 2:

Rate Equals State: Modulations in electric organ discharge rate consistently differ between individuals, reflecting attention and exploratory motivation in the pulse gymnotiform, *Microsternarchus* sp.

Introduction

When entering a novel environment, animals have the choice to find secure surroundings (e.g., a shelter) or to explore the new and possibly dangerous setting. Exploration and activity can be measured in a variety of ways. Open-field arenas test animals in an environment with no refuges and measure how much they move (Montiglio, Garant, Thomas, & Reale, 2010). Other experiments measure an individuals' latency to emerge from a sheltered area to explore an open arena (Harris, Ramnarine, Smith, & Pettersson, 2010; Popov & Klinov, 2009). Individual animals explore and move about novel environments differently, and these differences have proven to be variable between individuals and consistent within individuals over time (*review:* Bell, Hankison, & Laskowski, 2009; *great tits:* Dingemanse, Both, Drent, Van Oers, & Van Noordwijk, 2002;, *rats:* Mällo, Alttoa, Kõiv, Tõnissaar, Eller, & Harro, 2007;, *great tits:* Verbeek, Drent, & Wiepkema, 1994). Electric fish offer a unique opportunity to measure their activity because we can not only monitor their behavior in the traditional sense (movement of the animal in space) but also measure their ongoing EOD discharge rate, which is an intrinsic measure of active sensing of the environment and possibly attention. Swimming fish consistently discharge at higher EOD rates than resting fish (Black-Cleworth, 1970), implying that exploration and active sensing are related.

Electric fish exhibit light-dark cycle related activity patterns and there appear to be consistent individual differences within these rhythms (Lissmann and Schwassmann, 1965).

Electric fish are more active at night, and their EOD rates increase during this period as well, compared to daytime activity and EOD rates (Dewsbury, 1966; Franchina, 1993; Lissmann & Schwassmann, 1965; Schwassmann & Assuncao, 1989; Stoddard, Markham, Salazar, & Allee, 2007). *Gymnorhamphichthys hypostomus* displays individual differences in the onset of their activity with some individuals shortening their active period and others lengthening it (Lissmann and Schwassmann, 1965).

Among weakly electric fish, EOD rate can vary from highly stable to highly variable depending on the species. Wave-type gymnotiforms are extremely regular in their discharge rate (or its inverse, the inter-pulse interval [IPI]). This extreme regularity, represented by the coefficient of variation (CV: standard deviation divided by the mean; lower values indicate regularity, while higher numbers indicate variability) can be as low as a 2×10^{-4} over a period of 200 cycles, surpassing the regularity of other biological pacemaker systems (e.g. cardiac, respiratory) by an order of magnitude or more (Moortgat, Keller, Bullock, & Seinowski, 1998).

Pulse-type gymnotiforms typically have more variable EOD rates, represented by CVs that are larger than wave-type gymnotiforms. The CV of a pulse-type fish is on the order of 10^{-2}, but some species may have periods of relatively stable EOD rate with CV = 10^{-3} (Capurro et al., 2001; Jones, Alves-Gomes, & Braun, 2004). Still, this is an order of magnitude greater than those seen in wave-type fishes. There are two general types of variation in EOD rate among pulse-type fishes: ongoing variability during an otherwise stable baseline and active modulations in EOD found in a variety of situations, including (a) sensory stimulation in the form of lights or sounds (Correa & Hoffmann, 1998; Kramer, Kirschbaum, & Markl, 1981; Lissmann, 1958), (b) social interaction (Black-Cleworth, 1970; Valone, 1970; Westby, 1981)**,** (c) changes in activity level brought on by nightfall (Black-Cleworth, 1970; Lissmann, 1958; Silva, Perrone, &

Macadar, 2007; Stoddard et al., 2007), and (d) during Mauthner cell activation (Curti, Falconi, Morales, & Borde, 1999; Falconi, Borde, Henandez-Cruz, & Morales, 1995).

The EOD behavior (rate, variation, etc) of our focal species, *Microsternarchus* sp., has not been well documented. Therefore, this chapter will focus on characterizing the species as well as possible individual variations.

Specific Aim 2.1: To describe the relationship between changes in EOD rate and locomotor activity (measured by percentage of time spent out of a shelter) in *Microsternarchus* sp. as well as document if consistent individual differences in EOD rate and locomotor activity form the basis for an activity syndrome.

Prediction 2.1: Animals will display consistent differences in locomotor activity and EOD patterns. These differences will be correlated and thus suggest a behavioral syndrome for activity with high EOD rate individuals also spending the most time out of the shelter.

General Methods

Subjects

Subjects were 19 *Microsternarchus* sp. Individuals measured between 10 and 15 cm in length and were all presumably mature at the time of the experiments. All fish were tagged so that results could be tracked in individual fish over the course of the experiments (see Chapter 1).

These experiments were performed between March 2006 and January 2007. None of the animals appeared to be in breeding conditions at the time of data collection (they were not visibly gravid). Two identical experimental tanks were used so that two individuals could be monitored at once. Individuals were chosen at random for use in the experiments. All experimental procedures and housing conditions adhered to the standards of and were approved

by the Institutional Animal Care and Use Committee of Hunter College (Protocol CB signaling 11/08-02).

Experimental Arena:

Like other electric fish, *Microsternarchus* are nocturnally active and seek out sheltered areas for rest during the day (Dunlap & Oliveri, 2002; Moller, 1995). The recording aquaria were modified from Franchina and Stoddard's (1998) design in which the tank (37.8L) was partitioned into three sections with black plastic mesh. The outer two sections were connected by a central tube through the middle section that was otherwise inaccessible to the fish (Figure 2.1). This central tube served a joint purpose as a passage between the outer sections and as the only shelter in the tank. Animals spent the vast majority of their resting time in this central tube, which was additionally sheltered by the "leaves" of a plastic plant in the central section of the tank. Fish that were out of the tube were defined as *active* and those that were in the tube were deemed *resting*.

A pair of carbon rod recording electrodes was installed at opposite ends of the tank and in-line with the tube, thus placing a resting fish directly between these electrodes. This arrangement guaranteed high-fidelity electrical recordings with highest amplitudes whenever the fish was in the tube. Animals were maintained in low conductivity water (between 115 and 125 $\mu S/cm^2$) with a pH between 4.5 and 6.0 at 24°C ± 1°C and on a 12hour:12hour light:dark cycle.

Experiment 1: Free Exploration

Materials and Methods

Procedure. At the beginning of the experiment, individuals were removed from communal housing and placed singly in a recording aquarium for 72 hours beginning around 12 pm. Individuals were chosen at random. All animals (N = 19) were monitored for three consecutive days and nights using electrical techniques (see detection measures below). To

evaluate behavioral consistency within individuals, the experiment was repeated for all individuals, with at least three weeks between replicates (fish were not tested in same order both times).

Detection Measures. Due to the orientation of the tube in reference to the electrodes the amplitude of the fishes EOD was highest when the fish was within the tube. The voltage across the recording electrodes was amplified 500–1,000 times and band-pass filtered between 0.1 Hz and 10000 Hz (AM Systems model 3000). The signal was then passed to a portable data acquisition device (Tucker-Davis Technologies RM 2.1) controlled by a laptop computer running custom Matlab® routines. The unamplified signal was simultaneously passed to a second amplifier and a custom-made multi-channel window discriminator. Two thresholds were set on the window discriminator. One of these thresholds was set low so that a trigger pulse (TTL voltage) would occur at the start of every EOD, regardless of the subject's position and orientation. The number of EOD-triggered events was counted every second and saved to disk. The other threshold was set high so that a TTL pulse would only occur when the recorded EOD voltage was highest (i.e., when the fish was directly inside the tube).

The high-threshold channel's TTL pulse signaled the presence of the fish in the tube and was sampled once per second. Once every 10 min, this TTL pulse also triggered a 1-s recording from the analog signal, digitized at 50 kHz. To differentiate active fish (those swimming through the shelter or hovering partially within it) from inactive fish (those resting in the shelter), we considered animals at rest when they had been in the shelter (as indicated by the triggered TTL pulse) for at least 30 consecutive seconds. All other behaviors were classified as active, including brief shelter visits and pass-throughs. While this conservative approach may include some short rest periods in *active* estimates, it ensures that the majority of data come from clearly

distinguished active or inactive periods. Unfortunately, this triggering method cannot distinguish among individuals that are actively swimming out of the shelter and those that are resting out of the shelter. Therefore we relied on the animal's strong preference to rest in sheltered locations to maintain our active/resting distinction (this was confirmed with video recordings in a later experiment, as there was no video analysis in this experiment).

Data Analysis. For statistical analysis, the 72 hours of EOD and locomotor activity data were subdivided into six periods representing successive nights (lights out) and days (lights on). The EOD rate following handling and transport was much higher than the typical daytime rate when compared to the subsequent days. This is presumably due to the stress of transfer to a novel environment. EOD rates remained high into the first night. Consequently, we limited our analysis of EOD rate and locomotor activity to 48 hours beginning with lights-on the morning following transport. The dependent variable, EOD rate was analyzed using the following independent variables; day, night, active, and resting, including day-active, day-resting, night-active and night-resting. Statistical analysis was done in SPSS (a repeated measures general linear model and correlation analysis) using an alpha of .05.

Results

During the first replicate, individual animals made up to 138 separate excursions out of the shelter per night, with an average of 49 excursions (Figure 2.2a). On average, animals were active for 7% of the day and 82% of the night. The total duration of individual excursions out of the shelter at night varied from a few seconds to 11.3 hours with excursions of 1–5 min being the most common (Figure 2.2b). During the day, animals were less likely to venture out of the shelter. Individual fish made between 7 and 53 (mean = 24) separate excursions out of the shelter and rarely spent more than a minute out before returning (Figure 2.2a). The pattern of activity

during the second replicate was similar to that listed above (Figure 2.2a–c). It should be noted that only one individual was observed resting outside the shelter during the day, and did so during both replicates but was not excluded from any data analysis.

Microsternarchus displayed a typical gymnotiform pattern of daily EOD rate modulations, discharging at higher rates at night (Figure 2.3). Individual fish varied in the degree of night-time rate increase, ranging from 5% to 36% (or 4.4–35.5 Hz). EOD rate increased gradually in the late afternoon around 5:30 p.m. and then abruptly spiked in response to lights-off at 7:30 p.m. (Figure 2.4). EOD rate remained high and stable for the first few hours of the night, and decreased slightly (1–4%), becoming more variable across individuals as the night progressed into the early morning (Figure 2.3).

Animals discharged at higher EOD rates at night (97.9 Hz ± 5.56) than they did during the day (84.6 Hz ± 9.30) as indicated by a significant effect of time of day on EOD rate $F(1,18) = 9.8$, $p < .001$ and significant post hoc tests $t(18) = -7.37$, $p < .001$. Animals also had higher discharge rates while active (98.1 Hz ± 5.37) than while resting (85.8 Hz ± 7.13) as evidenced by a significant effect of activity $F(1,18) = 52.0$, $p = .006$, as well as significant post hoc tests $t(18) = 9.3$, $p < .001$ (Figure 2.5a). Fish showed the same pattern in the second replicate as well (Figure 2.6a) having higher EOD rates at night (97.2 Hz ± 5.45) than during the day (84.2 Hz ± 9.68) $F(1,18) = 10.3$, $p = .005$ (post hoc $t(18) = -6.87$, $p < .001$), as well as higher EOD rates while active (98.4 Hz ± 5.82) than while resting (85.4 Hz ± 8.44) $F(1,18) = 61.3$, $p < .001$ (post hoc $t(18) = -5.64$, $p < .001$).

Subjects also used a larger *range* of EOD rates during the day than they did at night. The change in EOD rate between periods of rest and periods of activity was different during the day when compared to the night ($F(1,18) = 9.9$, $p = .006$). On average, active EOD rates were 13.8%

higher than resting discharge rates during the day (95.9 Hz ± 10.44 active vs. 84.4 Hz ± 8.5 resting). At night, however animals discharged at rates that were only 6% higher while active than while resting (99.2 Hz ± 4.10 active vs. 93.0 Hz ± 6.72 resting; Figure 2.5b.). Both differences, however, were statistically significant (active vs. resting during the day, $t(18) = 6.0$, $p < .001$ and active vs. resting at night, $t(18) = 7.1$, $p < .001$). Once again, a similar pattern was seen during the second replicate (Figure 2.6b), with animals having a larger difference in active (97.7 Hz ± 10.71) vs. resting (83.9 Hz ± 9.24) EOD rates during the day $t(18) = 5.6$, $p < .001$, compared to active (99.1 Hz ± 4.5) vs. resting (91.3 Hz ± 6.48) EOD rates at night, $t(18) = 8.3$, $p < .001$.

Correlational Analysis. Animals that spent the greatest percentage of nighttime hours active were likely to have higher of EOD rates at night, as evidenced by significant Pearson correlations (Replicate 1: $r = .73$, $p < .001$, Replicate 2: $r = .58$, $p = .009$). Furthermore, animals with the greatest percentage of nighttime hours active were likely to have higher EOD rates while active at night (Replicate 1: $r = .63$, $p = .004$, Replicate 2: $r = .47$, $p = .04$).

Animals with the lowest EOD rates during the day had the greatest changes in EOD rate from day to night (Figure 2.5a and 2.6a, different colors) as evidenced by significant correlations between EOD rate during the day and the percentage change in EOD rate from day to night (Replicate 1: $r = -.85$, $p < .001$, Replicate 2: $r = -.86$, $p < .001$). Similarly, animals with the lowest EOD rates while resting had the largest difference in rate when comparing resting discharge rates to active discharge rates (Replicate 1: $r = -.76$, $p < .001$, Replicate 2: $r = -.78$, $p < .001$). Percentage change in EOD rate for each individual is given in Table 2.1.

We found similar results when analyzing the percentage change in active vs. resting EOD rate at night and day separately (i.e., active vs. resting during the day and active vs. resting at

night) (Figure 2.5b and 2.6b). Individuals with the lowest resting rates during the day and at night showed the greatest amount of change between active and resting rates i.e. percentage change in resting vs. active EOD rates was negatively correlated with resting EOD rates both during the day (Replicate 1: $r = -.72$, $p = .001$, Replicate 2: $r = -.91$, $p < .001$) and at night (Replicate 1: $r = -.89$ $p < .001$, Replicate 2: $r = -.79$, $p < .001$). Finally, individuals with the lowest active rates at night showed the greatest amount of change between active and resting rates at night, i.e. active EOD rates at night were correlated with the percentage change in resting vs. active EOD at night but only during the first replicate ($r = -.52$, $p = .02$).

Individuals showed consistency in how they behaved both in locomotor activity and EOD rate across replicates. Every measure evaluated (e.g., EOD rate, percentage of time exploring, changes in EOD rate, *et cetera*) except change in EOD rate from resting to active during the day (which fails significance based on one appreciably inconsistent individual on this measure: ADA107, removing this individual yields a significant correlation), showed statistically significant correlations between the first and second replicates (see Table 2.2).

Experiment 2: Fine Temporal Structure of EOD

Methods

To examine the fine temporal structure of the changes in EOD rate, a subset of the animals ($n = 4$) were returned to the recording aquaria for periods ranging from 12 to 72 hours depending on the quality of data obtained. Data collection was as described above with the addition of a continuous interval analysis of the EOD. The amplified signal was continuously digitized at 50 kHz using the TDT RM2.1. A spike discrimination routine and digital timers (using TDT's Visual Design Studio programming environment) were used to extract the interval (in seconds) between successive EODs. These intervals were continuously stored to a buffer,

which was uploaded to a personal computer every 15,000 intervals (approximately every 2.5 min).

To guarantee all individuals had high-fidelity measurements, we limited data analysis to selected periods where the animal was at rest within the tube. Based on this criterion we measured CV (SD/mean) during 48 periods of approximately 5 min from the four individuals. The day was divided into quadrants: morning, beginning with lights on (7:30 to 13:30), afternoon (13:30 to 19:30) ending with lights out, night (19:30 to 0:30), and early morning (0:30 to 07:30). These 5-min samples were grouped according to time quadrant resulting in 12 samples from each quadrant. We measure CV over a period of 200 intervals with a 50% overlap between successive periods (Moortgat et al, 1998).

Results

Animals were fairly consistent in EOD rate over the time intervals analyzed (morning, afternoon, night, and early morning). We analyzed a total of 14265 observations and, in 95% of these observations the CV was in the range of 1–5 x 10^{-3}. The CV of the interval duration was quite low and did not appear to vary systematically with time of day. The overall mean CV was 2.4 x 10^{-3} and did not vary significantly across quadrants of the day (morning = 2.4 x 10^{-3}, afternoon = 2.5 x 10^{-3}, night = 2.5 x 10^{-3} and early morning = 2.3 x 10^{-3}).

Occasionally, the CV increased abruptly by as much as an order of magnitude due to active rate changes by the animal (while still in the tube). An example of this behavior is illustrated by Figure 2.7. In the first time period (Figure 2.7a), the animal's EOD rate remained stable with a slight drift downward. Similarly, the CV also remained very stable, mostly between 1–4 x 10^{-3}. Small fluctuations in rate gave rise to similarly small changes in CV. In the second period (Figure 2.7b) the animal (while still resting immobile), performed four abrupt increases in

EOD rate, which caused large changes in the corresponding CV, which rose to a maximum 4.47 x 10^{-2}.

General Discussion

Compared to most pulse-type gymnotiforms, *Microsternarchus* has a very high and stable EOD rate, with an ongoing CV of less than 4 x 10^{-3} measured over 200 cycles. There are no comparable measures of CV for pulse fishes documented, but our own observations suggest that most pulse fish have less regular discharge patterns. Crampton and Albert (2006) report CVs for several genera, but over numerous behavioral contexts (that is, including both day and night samples) rather than measuring baseline variability during a single behavioral state. Accordingly, variability is greater -- their CVs are much higher than those measured in our experiments. Measurements of CV like those presented here and in Moortgat et al. (1998) will be needed to make comparisons within pulse species and across all electric fish species. The overall range of EOD rates appears to have interesting variation across the gymnotiforms.

Compared to other pulse-type gymnotiforms, the nocturnal increase in EOD rate in this genus is a modest change from the daytime rate, typically increasing only by around 13%. Some of the slower discharging pulse-type species increase their nighttime EOD rate 50–100% or more over their average daytime EOD rate. Examples include: *Gymnotus carapo:* 40 Hz during the day, 70 Hz at night (Black-Cleworth, 1970), *Gymnorhamphichthys rondoni*: 15–20 Hz during the day, 70–100 Hz at night; (Crampton & Albert, 2006; Lissmann & Schwassmann, 1965), and *Brachyhypopomus pinnicaudatus*: 1–2-fold increase at night (Stoddard et al., 2007). Other faster discharging species that have rates more similar to *Microsternarchus* have nighttime rates that are 35% higher than their daytime rates e.g., *Gymnorhamphichthys rosamariae*: 80–90 Hz during the day 110–117 Hz at night (Crampton & Albert, 2006).

The behavior of *Microsternarchus* in terms of overall EOD rate, EOD rate regularity and change in EOD rate between day and night appears to place them in-between pulse type (slow, irregular and variable) and wave type species (fast, regular and constant). Whether this also has evolutionary implications has yet to be seen.

The lowest resting rates used during the day are never achieved at night, indicating that the time spent in the shelter at night may not necessarily be "inactive" time. Nocturnal discharge rates in *Microsternarchus* are higher and more consistent (smaller range of EOD rates) than they are during the day. This pattern is well known in pulse gymnotiforms (Black-Cleworth, 1970; Lissmann & Schwassmann, 1965; Silva et al., 2007; Stoddard et al., 2007). The high level of consistency in EOD rates used at night is likely due to animals having a more consistent behavioral state at night (i.e., they are active nearly all of the time). Daytime behavioral states range from full rest to brief periods of high activity, and these differences are mirrored in the EOD rate.

Furthermore, the measured range of EOD rates was smaller while animals were out of the shelter compared to when they were in it. Animals that are outside the shelter are exploring and navigating the arena nearly constantly. Conversely, animals that are inside the shelter can be truly resting and thus discharging at their lowest EOD rate or simply taking shelter and simultaneously maintaining high vigilance. Additionally, the larger range of rates seen while at rest could also be influenced by the time it takes for an animal to lower its EOD rate after being active.

The EOD rate of *Microsternarchus* is reflective of their underlying behavioral state (whether they are resting or active). Higher EOD rates accompanied locomotor activity both during the day and at night. We have clearly demonstrated that EOD rate is closely tied to

behavioral state and is not just a reflection of time of day. When fish become active, they discharge at a higher EOD rate. However, fish do not have to be swimming to show increased EOD rates. Often, while they are immobile, EOD rate will show rapid increases for brief periods of time. We believe this is an external indicator demonstrating increased attention to their surroundings, likely triggered by some unidentified stimulus.

We demonstrated that differences in both EOD rate and locomotor activity are consistent across time within individuals. Our data supported Prediction 2.1 that animals would display consistent differences in locomotor activity and EOD patterns and that differences would be correlated suggesting a behavioral syndrome for activity with high EOD rate individuals also spending the most time out of the shelter. Active individuals did, in fact, have higher EOD rates. This correlation implies an underlying organization of behavior within the individual and thus a behavioral syndrome for heightened activity of all kinds. It is possible that the higher EOD rates are simply a side effect of increased activity or that both traits are an indicator of an underlying tendency for activity within the individual. It would be interesting to see if immobilized the animals (either with drugs or restraints) continued to display the same characteristically high or low EOD rates in the absence of locomotor activity.

There was also an interesting relationship between overall EOD rate and the amount of change in EOD rate that an individual underwent during day/night or active/resting. Animals with lower discharge rates had greater changes in EOD rate as they changed states. This was mostly due to differences in daytime or resting rates since EOD rates at night were more similar between resting and active periods. At this point it is unclear why some animals have characteristically lower EOD rates than others, but we can speculate that these individuals are either showing less discomfort in the novel environment since they are able to decrease their

attentiveness more during periods of rest, or there is something that makes them less active overall. It is possible that underlying differences in individual metabolic rate could account for individual differences in activity (Biro & Stamps, 2010). If individuals differ in their resting metabolic rate, this would cause some individuals to grow faster and have higher food requirements than others, setting up differences in overall activity. This relationship has been shown in a variety of species including birds and mammals (review: Biro and Stamps 2010).

Table 2.1

Percetange change between average active and resting EOD rates for all individuals in both replicates.

Rep. 1	Day/Night	In/Out	Day- In/Out	Night-In/Out
ADA101	17.7%	19.9%	21.9%	11.8%
ADA102	10.1%	11.3%	15.4%	5.2%
ADA103	21.4%	21.7%	17.3%	7.8%
ADA104	9.9%	10.9%	13.6%	6.1%
ADA106	5.0%	6.2%	8.1%	4.0%
ADA107	25.6%	22.2%	1.3%	1.0%
ADA109	7.2%	7.7%	10.1%	3.4%
ADA110	11.0%	11.3%	12.4%	3.2%
LAU101	10.9%	10.3%	14.4%	3.4%
LAU103	56.6%	4.9%	-15.4%	9.2%
LAU104	26.3%	27.5%	36.1%	11.8%
LAU105	21.9%	32.1%	41.2%	22.6%
MAT101	10.3%	11.9%	11.6%	7.4%
MAT103	16.2%	16.8%	18.9%	6.7%
MAT104	17.4%	15.6%	22.5%	4.2%
MAT106	5.0%	5.6%	7.4%	3.1%
MAT107	31.3%	23.4%	14.5%	4.3%
MAT109	4.8%	6.3%	9.1%	12.6%
MAT110	10.2%	10.2%	6.5%	3.4%
Median	11.0%	11.3%	13.6%	5.2%
Rep. 2	Day/Night	In/Out	Day- In/Out	Night-In/Out
ADA101	12.6%	15.5%	19.1%	11.9%
ADA102	8.6%	9.8%	12.0%	5.7%
ADA103	28.1%	29.8%	27.9%	14.4%
ADA104	10.4%	8.5%	3.0%	5.5%
ADA106	-2.7%	-0.8%	1.4%	2.8%
ADA107	45.0%	43.1%	40.8%	13.9%
ADA109	8.5%	9.7%	13.3%	5.7%
ADA110	9.9%	10.7%	16.4%	5.3%
LAU101	12.4%	12.3%	17.4%	6.0%
LAU103	32.7%	6.6%	-23.1%	17.0%
LAU104	13.8%	18.8%	24.7%	15.8%
LAU105	19.0%	25.7%	32.2%	18.0%
MAT101	20.1%	22.7%	25.5%	13.7%
MAT103	24.9%	23.4%	29.4%	5.8%
MAT104	18.5%	16.7%	23.1%	4.6%
MAT106	2.7%	4.0%	5.8%	3.1%
MAT107	32.7%	30.1%	36.1%	5.0%
MAT109	5.7%	6.9%	8.4%	7.7%
MAT110	11.0%	11.2%	14.5%	4.3%
Median	12.6%	12.3%	17.4%	5.8%

Note. Colors match the colors used in Figure 2.5 and 2.6 such that red is the median, blue highlights individuals with a percentage change less than or equal to the median and black are individuals that are greater than the median.

Table 2.2

The correlation coefficient for each measure between the first and second replicates of the free exploration experiment

Measure:	*r:*
% Day Active	.687***
% Night Active	.758**
EOD rate:	
Day	.807***
Night	.666**
Resting	.808***
Active	.776***
Day-Resting	.780***
Day-Active	.816***
Night-Resting	.634**
Night-Active	.616**
Change in EOD rate:	
Day/Night	.729***
Resting/Active	.772***
Resting/Active Day	n.s.
Resting/Active Night	.647**

Note. ** $p < .01$ ***$p < .001$

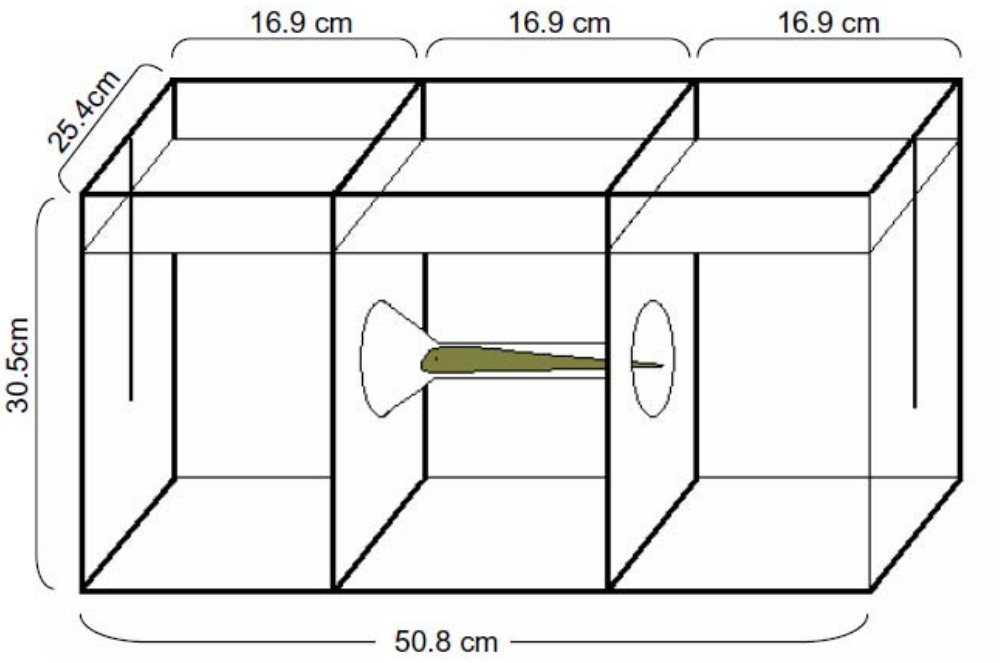
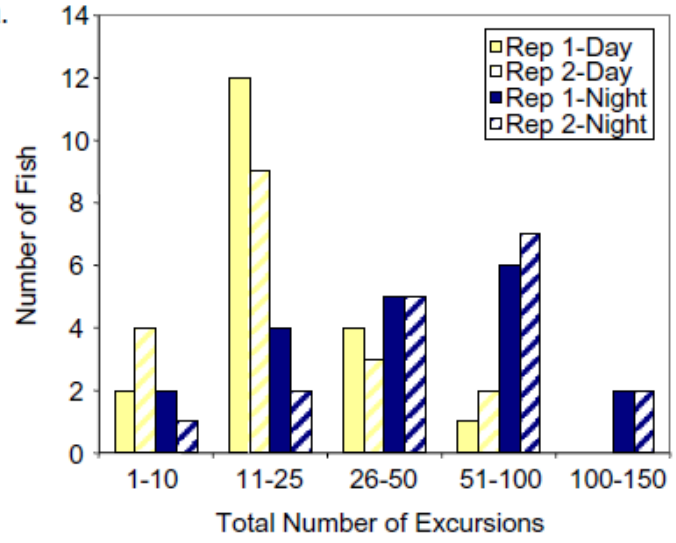
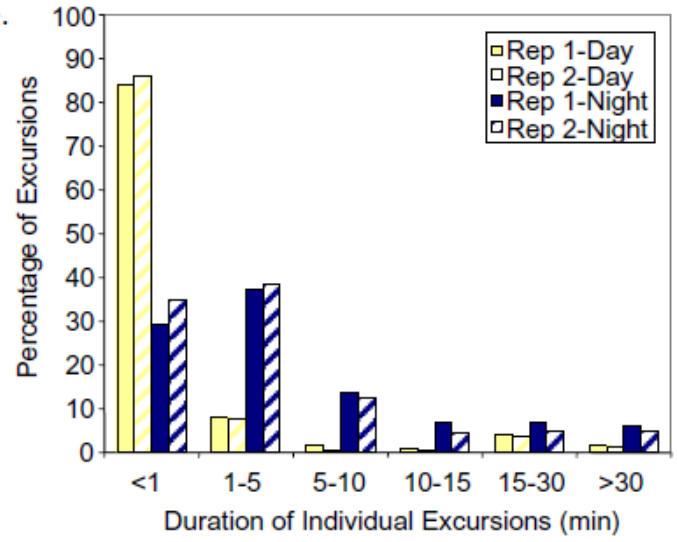


Figure 2.1. Diagram of the free exploration experimental tank with dimensions. This was a modified design from Franchina and Stoddard (1998), where the inner section connects the outer two sections with a central tube but was otherwise inaccessible to the fish. A black plastic plant (not shown) was added to the central potion of the tank surrounding the tube to darken the area and make it an even more attractive shelter.

a.


Rep 1-Day
Rep 2-Day
Rep 1-Night
Rep 2-Night
Number of Fish
0
2
4
6
8
10
12
14
1-10
11-25
26-50
51-100
100-150
Total Number of Excursions

b.


Rep 1-Day
Rep 2-Day
Rep 1-Night
Rep 2-Night
Percentage of Excursions
0
10
20
30
40
50
60
70
80
90
100
<1
1-5
5-10
10-15
15-30
>30
Duration of Individual Excursions (min)

c.

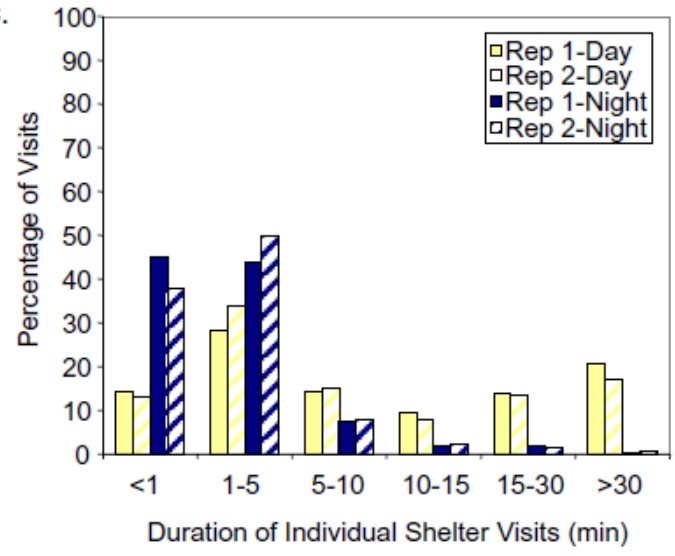

Rep 1-Day
Rep 2-Day
Rep 1-Night
Rep 2-Night
Percentage of Visits
0
10
20
30
40
50
60
70
80
90
100
<1
1-5
5-10
10-15
15-30
>30
Duration of Individual Shelter Visits (min)

Figure 2.2. Activity patterns in *Microsternarchus* during the first and second replicates of the free exploration experiment. Values shown are averages over two consecutive days and nights, respectively. (a) The total number of excursions from the shelter during the day (light bars) and night (dark bars) in Replicate 1(solid bars) and Replicate 2 (hashed bars). (b) The frequency distribution (% of total) of the duration of individual excursions (in minutes) during the day and night. (c) The frequency distribution (% of total) of the duration of individual shelter visits.

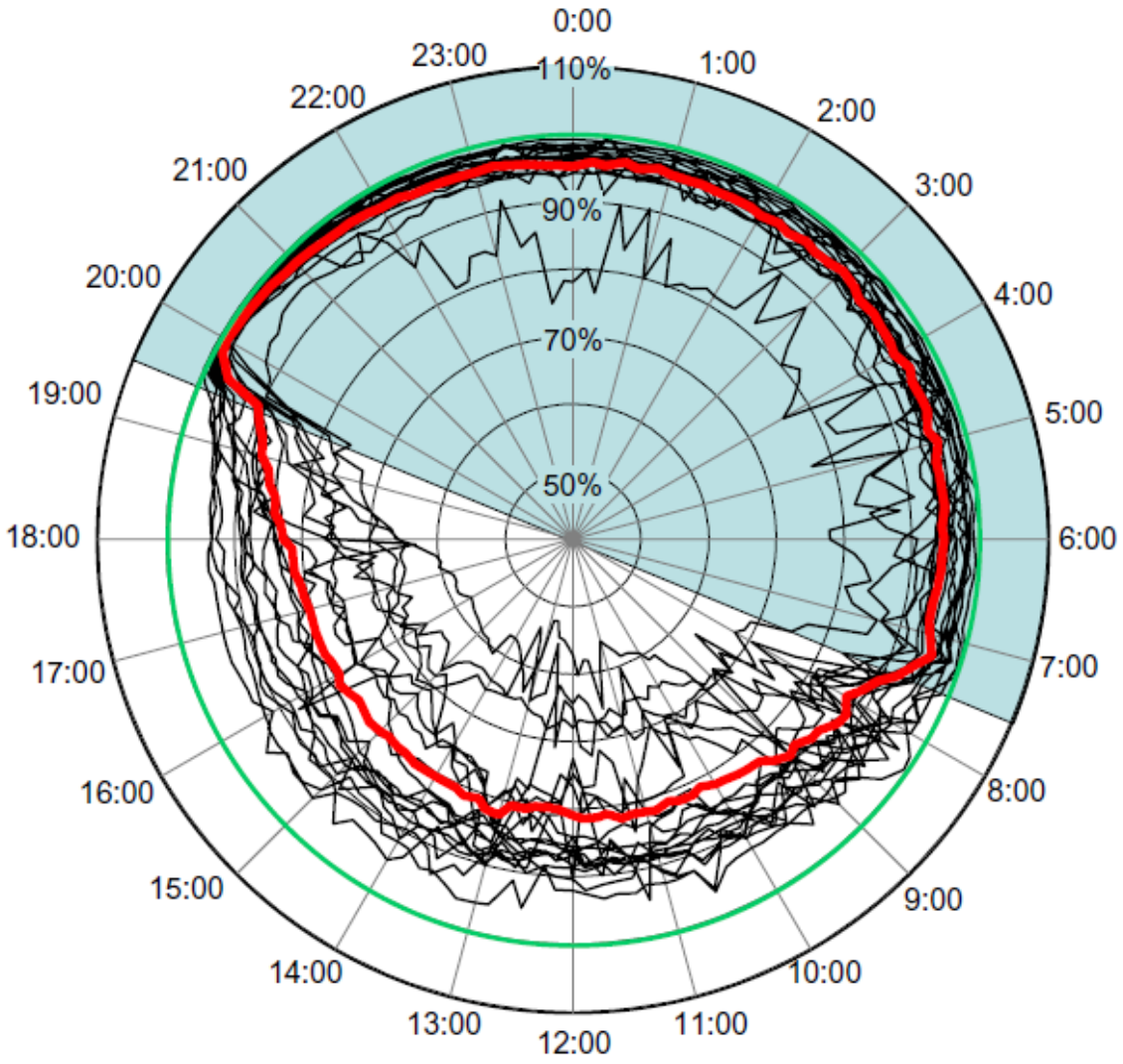


Figure 2.3. Diel Changes in EOD rate. Normalized EOD rate is plotted against time of day on a circular plot. Values plotted are 10 min averages, normalized as a percentage of each individual's maximum EOD rate. Each animal is shown as an individual trace. The heavy red trace plots the mean of all individuals, and the green circle highlights the 100% level. The shaded portion indicates the animals' subjective night.

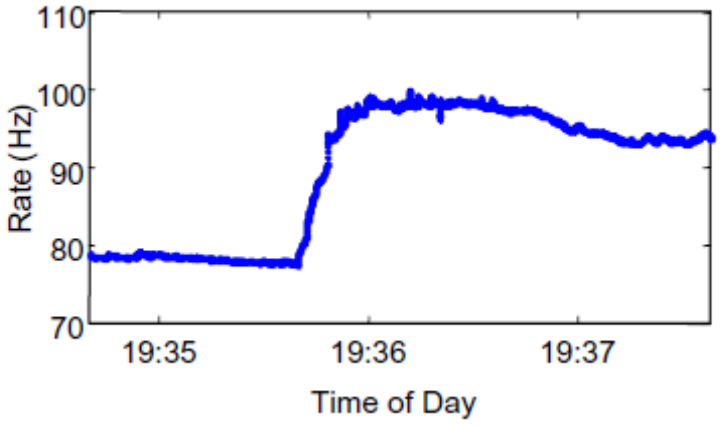


Figure 2.4. Instantaneous changes in EOD rate with transition to darkness. A representative period showing the EOD rate change that occurred when the lights were switched off. The individual's instantaneous rate is plotted, measured at every interval (1/interval duration). Lights out occurred just prior to 19:36.

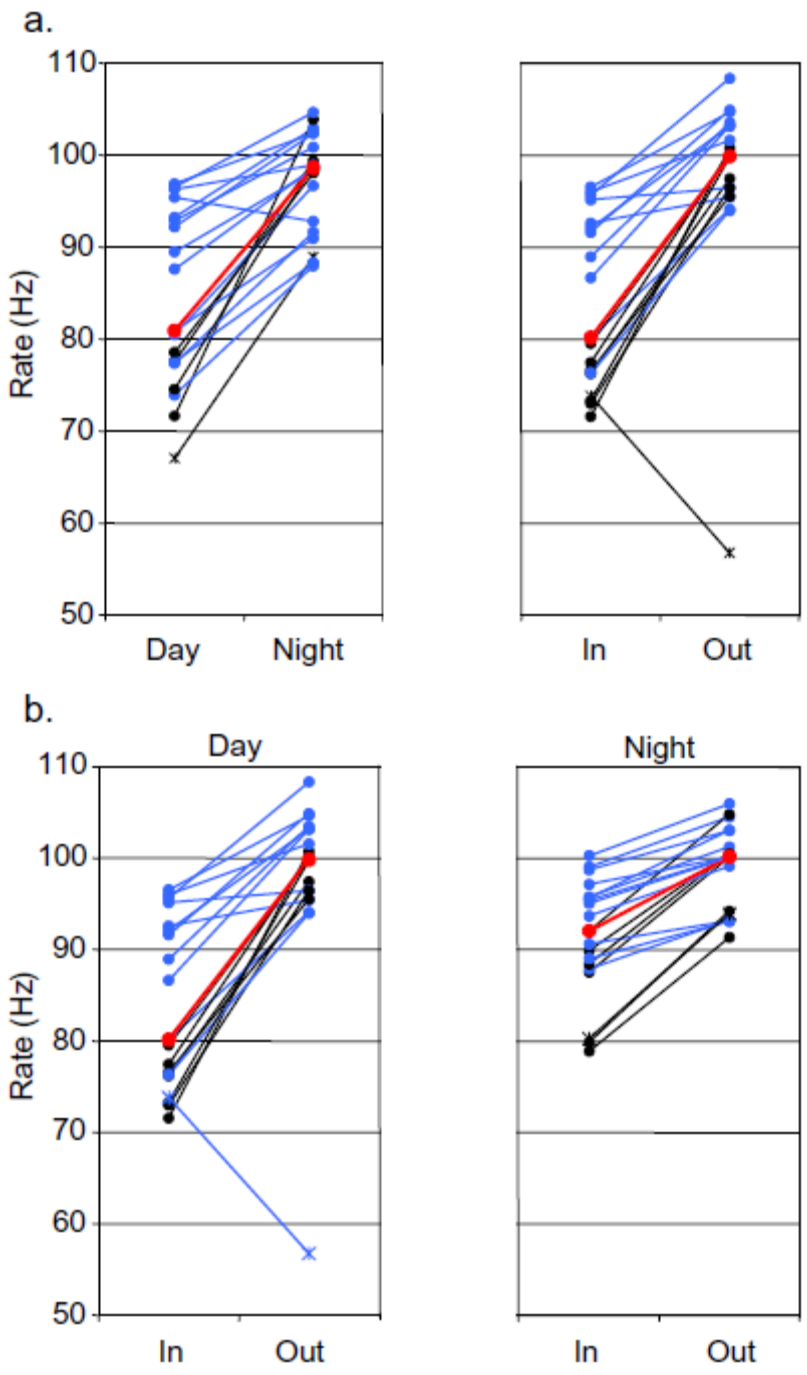

a.
110
100
90
80
70
60
50
Rate (Hz)
Day
Night
In
Out
b.
Day
Night
110
100
90
80
70
60
50
Rate (Hz)
In
Out
In
Out

Figure 2.5. EOD rate modulation in Replicate 1 of the free exploration experiment. (a) Mean EOD rates for all 19 individuals during the first replicate as a function of circadian period (left) and location (right) (b) EOD rate separated by location within each time period, day (left) and night (right). The red lines indicate the median of all individuals, blue lines highlight individuals with a percentage change (between the two states) less than or equal to the median while, and black lines are individuals with a percentage change greater than the median (see Table 2.2 for exact values for each individual). Data point labeled with * indicates the individual that did not conform to the active and resting distinction.

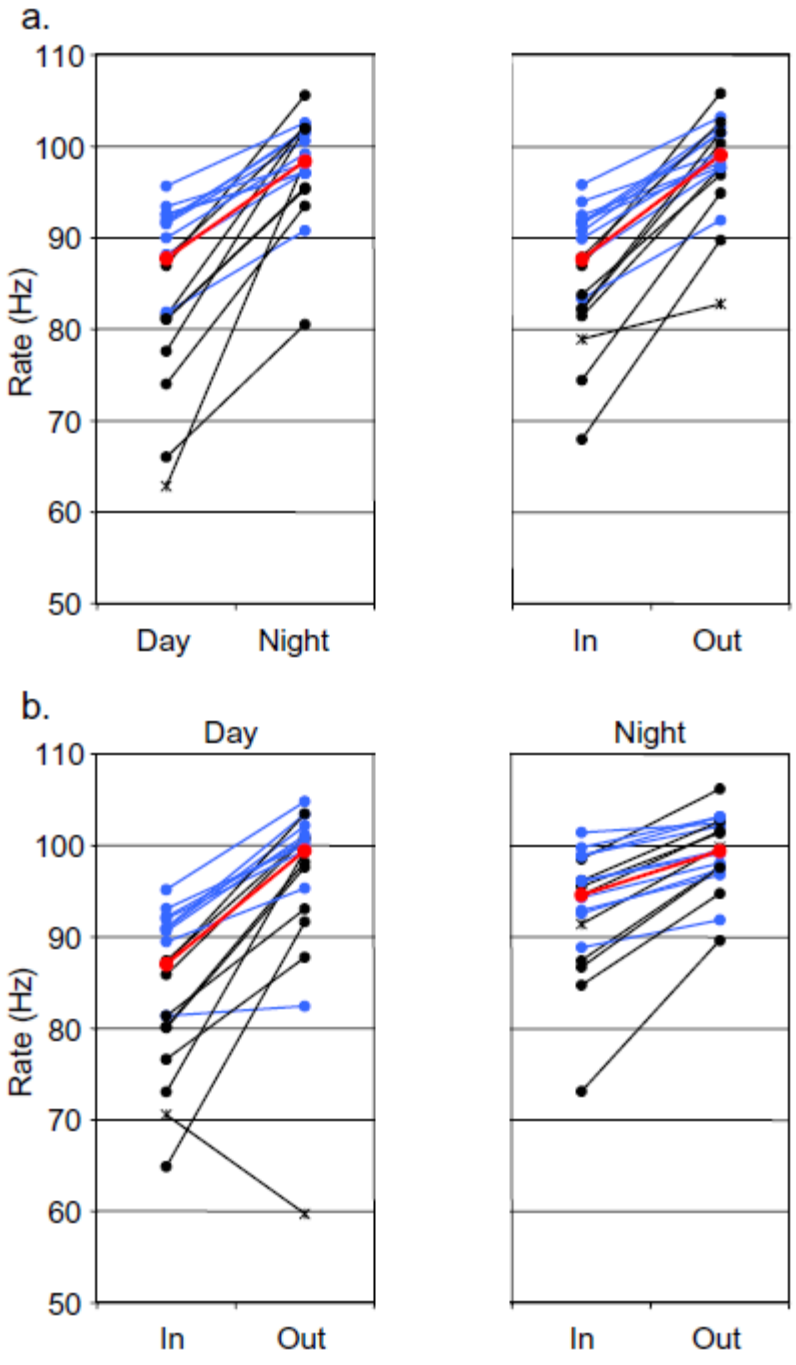

a.
110
100
90
80
70
60
50
Rate (Hz)
Day
Night
In
Out
b.
Day
Night
110
100
90
80
70
60
50
Rate (Hz)
In
Out
In
Out

Figure 2.6. EOD rate modulation in Replicate 2 of the free exploration experiment. (a) EOD rates for all individuals during the second replicate as a function of circadian period (left) and location (right) (b) EOD rate separated by location within each time period, day (left) and night (right). Red lines indicate the median of all individuals, blue lines highlight individuals with a percentage change between the two states less than or equal to the median while, and black lines are individuals with a percentage change greater than the median (see Table 2.2 for exact values for each individual). Data point labeled with * indicates the individual that did not conform to active and resting distinction.

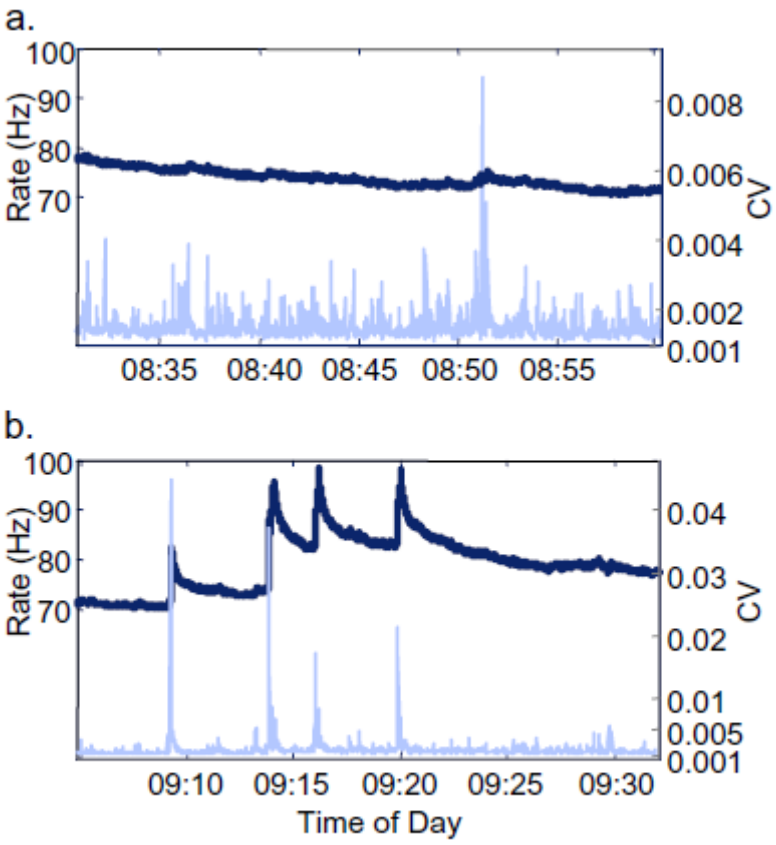


Figure 2.7. Instantaneous EOD rate plotted (dark blue) with the corresponding CV (light blue) on the same x axis. (a) shows a period of relative stability of EOD rate, while (b) shows a period where four rapid changes in EOD rate occur while the animal was resting immobile in the tube. Note that the CV y-axis differs in scale from a to b.

Chapter 3:

Behavioral responses to non-social, environmental challenges imply the existence of coping styles in *Microsternarchus* sp.

Introduction

Researchers define coping styles (similar to personality and behavioral syndromes) as the combination of correlated behavioral traits or physiological responses that are consistent over time within an individual and across contexts (Koolhaas et al., 1999). Within a particular coping style, animals are said to display a coping response to deal with a challenge or stressor in their environment. Coping responses can be either behavioral or physiological and generally function to control the stressful event or situation (Koolhaas et al., 1999; Wechsler, 1995). Of the terms used to describe personality in animals, researchers who use the term coping style provide the most details regarding what specific behavioral traits and physiological responses an individual will display depending on the specific coping style it possesses.

There are two commonly recognized types of coping styles: proactive coping and reactive coping (Carere, et al., 2005; Koolhaas et al., 1999). Proactive coping is an active coping style characterized by high levels of territoriality or aggression, active avoidance, and other active attempts to counteract the stressor. Reactive coping, on the other hand, is a conservative or withdrawing coping style characterized by freezing and nonaggressive responses to stressors (Koolhaas et al., 1999; Overli et al., 2007). These two coping styles have been particularly well studied in mammals, but similar work has also been done in birds, fish, and invertebrates (although the vocabulary used is sometimes different; for example the terms *bold* and *shy* are often used instead of *proactive* and *reactive*). In rats and mice in particular, a suite of behaviors has been described that differ between proactive and reactive individuals, including attack

latency, active avoidance, defensive burying, routine formation, and behavioral flexibility (Koolhaas et al., 1999). Proactive individuals attack more quickly, actively avoid stressors, show more defensive burying, and are quick to form routines, showing lower behavioral flexibility once routines are established. Reactive individuals display the opposite tendencies.

In rainbow trout, two genetic lines have been established that differ in their cortisol levels following a stress test and also differ in their reactivity to environmental changes. These two lines, based on coritsol levels, show marked similarity to proactive and reactive coping styles. High-level (HR) individuals display characteristics that are similar to the reactive type of coping style (low social dominance, high locomotor response to stress, and stress-induced anorexia) while low-level (LR) individuals are more similar to proactive styles (Overli et al., 2007). More recent studies have shown that HR individuals appear more aware of changes in their environment, reacting more fearfully to novelty than their LR counterparts (Ruiz-Gomes, Huntingford, Overli, Thorqvist, & Hoglund, 2011).

In an effort to better understand the coping responses of *Microsternarchus* sp. to mildly stressful events, we subjected individuals to two mildly stressful environmental challenges: a terrestrial challenge and a novelty response experiment.

Terrestrial Challenge

Microsternarchus live in second- and third-order tributaries that are subject to great seasonal variation in water depth. During the dry season, animals may get caught in stream beds that will completely dry up. As such, fish that can maneuver from streams that are drying up to those that are deeper are more likely to survive. Thus, we hypothesize that coping successfully with being out of the water is an ecologically relevant behavior in this species. This experiment

involves a brief displacement from the water to a platform just above the surface ("terrestrial challenge") and the time it takes for an individual to return to the water is measured.

Specific Aim 3.1: Determine if individuals differ consistently in response to a terrestrial challenge indicative of differences in coping responses.

Prediction 3.1: *Microsternarchus* will display consistent individual differences in response to a terrestrial challenge in that some individuals will react quickly to return to the water after displacement, thus displaying a proactive coping response, while other individuals will respond more slowly or with a *sit and wait* approach, thus displaying a reactive coping response.

Novelty Response Experiments

Many species of electric fish respond to novel stimuli with an increase in the repetition rate of their electric organ discharge (EOD). This response has been variously termed the novelty, startle, or orienting response (Ciali, Gordon, & Moller, 1997; Lissmann, 1958; Lopes Correa, Hoffmann, & Grant, 1998; Post & von der Emde, 1999). Successive EODs create an image of the fish's immediate environment; therefore, increasing the number of EODs per unit time increases the temporal resolution of the electric image. Presumably then, fish transiently increase their EOD rate in order to better identify the source of the novel stimulus (Barrio, Caputi, Crispino, & Buno, 1991).

Gnathoneus petersii (Post and von der Emde, 1999; Ciali et al, 1997) and *Gymnotus carapo* (Barrio et al., 1991; Correa & Hoffmann, 1998; Caputi, Aguilera, & Castello, 2003) have been frequently used in novelty response experiments because they have low resting EOD rates (5-10 Hz and 30-60 Hz respectively) and show large obvious changes in response to novelty. In

contrast, our focal species, *Microsternarchus* sp., have resting EOD rates between 70 and 90 Hz increasing as high as 120 Hz when active.

While individual differences have been largely ignored in novelty studies on electric fish, Barrio et al. (1991) suggested that the five individuals they tested had characteristic response profiles to novel mechanical stimuli. In another species of fish, rainbow trout (*Oncorhynchus mykiss),* individuals that display a more reactive coping response have been shown to be more aware of changes in their environment and more sensitive to novelty (Ruiz-Gomes, Huntingford, Overli, Thorqvist, & Hoglund, 2011) we predict that some of our individuals will be more sensitive to changes in their environment and therefore have larger EOD responses (larger change from baseline EOD rate) to a novel mechanical stimulus than other individuals, thus adopting a more reactive coping response.

Specific Aim 3.2: Identify if individual differences in the amplitude and duration of novelty responses are indicative of differences in coping responses.

Prediction 3.2: Individuals will behave differently in response to a novel stimulus but novelty response amplitude and duration will be correlated. Individuals with larger and longer novelty responses will be displaying a reactive coping response (more sensitive to their environment), while individuals with smaller and shorter novelty responses will be displaying a proactive coping response (less sensitive to their environment).

Specific Aim 3.3: If differences are seen among individuals in their response to a terrestrial challenge and their novelty responses, determine if patterns of individual differences described within each experiment correlate and conform to established ideas about proactive and reactive coping styles.

Prediction 3.3: Individuals that display a proactive coping response in the terrestrial challenge (quick escape from the platform) will also show a proactive coping response in response to novelty (smaller novelty responses). Conversely, those individuals that display a reactive coping response in response to a terrestrial challenge (slow escape from the platform) will also display a reactive coping response to novel stimuli (larger novelty responses).

General Methods

Subjects

Subjects included between 13 and 19 *Microsternarchus* sp. The sample size for each experiment and replication varied slightly, as some animals died during the course of the experiments. Individuals were between 10 and 15 cm in length and were all presumably mature at the time of the experiments. All fish were tagged so that data could be tracked in individual fish over the course of the experiments. (For further details on our subjects, see Chapter 1.)

Overview

In the two experiments reported here, individual animals were presented with two types of environmental challenges and their responses were recorded. The first challenge removed the fish from water and measured the time it took them to return to it (terrestrial challenge), and the second introduced a series of novel mechanical stimuli. In the terrestrial challenge we recorded overt behaviors (with no EOD recordings), while in the novelty response experiment we measured changes in the EOD (e.g. changes in the animals' inter-pulse interval [IPI]). Each type of challenge was presented twice (at least three weeks after the first, both replicates of the terrestrial challenge were presented before beginning novelty response experiments) to determine if there were any consistent individual differences in both responses. Statistical analysis was done in SPSS using an alpha of .05. We computed Pearson correlation coefficients between all

behavioral measures (further details below) including those that were dichotomous since Phi coefficients (special case Pearson correlation between two dichotomous variables) and point biserial correlation coefficients (special case Pearson correlation between a dichotomous variable and an interval variable) are numerically identical to Pearson correlation coefficients (Weinberg & Abramowitz, 2008). Hunter College Institutional Animal Care and Use Committee (IACUC) approved all experiments (Protocol CB signaling 11/08-02). Animals were maintained in low conductivity water (between 115 and 125 μsiemens/cm^2) with a pH between 4.5 and 6.0 at 24°C ± 1°C and on a 12hour:12hour light:dark cycle in their home tanks.

Experiment 1: Terrestrial Challenge

Methods

Subjects. The first replicate of the terrestrial challenge occurred in December 2006 and January 2007 and included 19 subjects. The second replicate occurred between October and December 2007 and included 15 of the original 19 animals.

Materials. The test arena was a clear acrylic tank, 39.5 cm x 44.5 cm x 15 cm (1.5 cm thick) with an 11.5 cm diameter platform (an inverted plastic flower pot) in the center of the arena. The platform was covered by a moist paper towel that was cut to fit, and the arena was filled with water of similar conductivity, pH, and temperature to the animal's home tank. The top of the platform was approximately 8 cm above the surface of the water (Figure 3.1).

Procedure Animals were netted in the home tank and placed into a holding bucket (containing water matched to the home tank and test arena) where they were allowed to rest for 5 min. Experiments were conducted during the animals subjective day. Animals were chosen randomly and two animals from the same home tank were used sequentially (each had its own holding bucket) Video recording was started and the experimenter captured animals from the bucket

in a net specifically designed to fit the platform. The netted animal was immediately moved to the platform and inverted over it so that the animal was resting on the platform. The empty net remained beneath the fish, covering the base of platform. We measured the latency for an animal to escape from the platform and find the tank of water. Generally this behavior requires the fish to perform rapid flipping motions from side to side until it falls off the side of the platform. Animals that did not move after 20 seconds were gently nudged into the water and the trial terminated. This 20-s limit was arrived at through consultation with the IACUC. In addition we measured three other behaviors: (a) whether or not the fish was swimming in the holding bucket after the 5-min rest period (yes or no), (b) how much the fish struggled in the net during transfer (visually ranked high, low or none), (d) and whether fish moved on the platform at all (yes or no). One human observer rated all individuals from both replicates using the recorded videos. These variables were non-parametric and were analyzed using Pearson correlation coefficients as mentioned above.

Results

Individual length did not correlate with any of the dependent variables in this experiment. During the first replicate of the terrestrial challenge, only three of 19 individuals swam in the bucket after the 5-min rest period (Figure 3.2a). All of the animals struggled in the net during transfer—10 with high intensity, nine with low intensity (Figure 3.2b). Fifteen animals moved on the platform after placement while the other four remained motionless (Figure 3.2c). Of the 15 animals that moved on the platform, 11 escaped from it (Figure 3.2d). Average time to escape (not including terminated trials) was 0.88 s ± 0.30. During the second replicate only one animal was swimming in the bucket after the 5-min rest period and only one animal of 15 failed to struggle in the net during transfer (not the same individual in each case). Seven individuals had high intensity struggles in the net and seven had low intensity struggles. Ten animals did not escape the platform, five of which

were completely motionless. The other five individuals escaped from the platform in less than 2 s, many in less than 1 s (mean time to escape = 0.94 s ± 0.38).

Individual fish did not behave similarly across replicates (Figure 3.2). For instance, the likelihood of swimming in the isolation bucket was the only behavior that was consistent across replicates ($r = .535$, $p < .05$) with 13 of 15 animals remaining consistent in their behavior between replicates (Figure 3.2a). Only 8 fish consistently struggled in the net in both replicates (Figure 3.2b). Moving on the platform proved to be the least consistent of all behaviors, with only six animals remaining consistent (Figure 3.2c). Lastly, eight (of 15) individuals escaped from the platform in both replicates (Figure 3.2d).

Seven animals were consistent in at least three out of the four behaviors (of those, three were consistent in all four behaviors), while the other eight were consistent in two or fewer. During the first replicate, the more an animal struggled in the net, the more likely they were to get off the platform quickly ($r = -.69$, $p = .001$). This was the only significant correlation from this experiment.

Discussion

Behavior differed between individuals. Animals either escaped from the platform in less than 2 s or they had to be removed from it after 20 seconds. Animals that quickly escaped from the platform in the terrestrial challenge thus displayed attributes of a proactive (fight or flight) coping style while animals that remained on the platform displayed a component of the reactive (sit and wait) coping style. Since coping styles are meant to effectively deal with stressors, sitting motionless out of the water may result only in desiccation and thus may not actually be considered a coping response. However, we cannot speculate as to whether individuals would have remained motionless to the point of death or if they had yet to reach some internal threshold (of low oxygen levels for example) before attempting to escape.

Animals were not consistent in their behavior from one replicate to another (all correlations not significant). This does not support our prediction that individuals would differ consistently across replicates. There are a few possible explanations for this. First, it is possible that behavioral consistency itself shows enough individual variation (i.e., some animals are consistent and some are not) that a correlation would not effectively measure it. Another possibility is that we did not account for extraneous variables within our experimental design. Where the animal landed on the platform, which side of its body it landed on, and/or how hard or fast the animal landed, for example, may have affected the behavior of each animal (handling effects).These variables would be difficult to control for with the current experimental design, and consistent behavior might not be a realistic outcome because of that. It was often difficult to determine from the video which side of its body an animal landed on (some move so quickly in real time that this would also be impossible to determine during the course of the experiment) but it was clear that animals were not all landing on the same side or in the same location. It is possible that animals have a dominant side and those that landed on this side were more likely to move around. As for the effect of location on the platform, anecdotally, the one animal that landed the most off center, with a quarter of its body, including its head, off the platform, did not make any attempt to escape the platform. Finally, it is possible that a one-year gap between replicates is simply too long a time frame to show good behavioral consistency in these behaviors in this species.

During the first replicate, animals that struggled the most in the net were the most likely to get off the platform. This correlation makes sense in light of the fact that if the animal was already moving in the net it was likely still moving when placed on the platform and therefore more likely to get off the platform. This was not true during the second replicate. It is unclear

what role experience would play in this experiment, however a one year delay makes learning effects rather unlikely. We suspect that behavior from the first replicate would be more representative of wild fish as this replicate occurred closer in time to the fishes' capture (21 months in captivity vs. 32 months). Since animals tended to change their behavior to become more reactive (less proactive) it is possible that captivity induced some learned helplessness behavior. Another possible explanation would simply be the age of the individual. Older individuals may be less likely to expend the energy needed to get off the platform. We do not have age information on these individuals since they were wild caught.

Experiment 2: Novelty Response

Materials and Methods:

Subjects. Fifteen *Microsternarchus* sp. were involved in the first replicate of the experiment and 13 in the second. These experiments were performed between December 2007 and February 2008. At least three weeks separated replicates for each individual. Individuals were chosen for experimentation at random until all individuals were completed.

Materials. All water was matched for conductivity, temperature and pH to the fish's home tank. Experimental tanks, which were identical to those used in the free exploration experiment (Chapter 1 Experiment 1), contained a central tube connecting the two outer sections of the tank. This tube served as the only suitable shelter in the tank (Figure 3.3). To eliminate effects of external stimuli, tanks were placed on a vibration isolation table (Nano-k, Minus k Technology) in a sound dampening booth (single-wall Industrial Acoustics Corporation).

Procedure. Animals were removed from their home tanks and transferred to the experimental tanks in a holding bucket. Individuals were placed in the experimental tank around 10:00 a.m. and allowed to acclimate for 30 min. After initial placement in the tank, fish were

provoked to enter the tube to standardize the position of all individuals with reference to the stimulus. They then voluntarily remained inside for the duration of the experiment (approximately 2.5 hrs). After the acclimation period, precisely timed stimuli (see Stimulus Generation) were delivered to the outside of the tank in line with the central tube. Ten stimuli were presented consecutively on three inter-stimulus interval (ISI) schedules (30, 10, and 5 s). After each presentation of 10 stimuli, 10 min elapsed before the start of the next set. Subsequently, these sets of 10 stimuli will be referred to as a trial. The order of ISI schedules was the same for all animals, such that a 30-s ISI trial was presented first followed by a 10-s ISI trial, followed by a 5-s ISI trial. This sequence was repeated at least five times. The order was kept consistent for all animals so that differences in behavior could not be attributed to differing order effects. If an individual moved from the shelter during stimulus presentation, that trial was discarded and repeated at the end of the experiment until five clean trials were obtained at each ISI.

Stimulus Generation. Each stimulus consisted of a series of three percussive taps to the tank wall (30 ms at 100 Hz) delivered by a mini-shaker (Model 4810 Brüel & Kjær) attached to an 8 mm diameter polyvinyl sphere inserted onto a 15-cm shaft (a 16-gauge Hamilton syringe needle). The sphere was placed so that at rest it was approximately 0.2 mm away from the glass. Prior to each experiment, a miniature hydrophone (Model 8103 Brüel & Kjær) placed just above the central tube was used to calibrate the stimuli to a sound pressure level of 150±3 dB re: 1µPa. Preliminary experiments showed that this stimulus was effective in eliciting a novelty response from this species.

Detection measures. The animals' inter-pulse interval (IPI) was continuously monitored using custom-made carbon rod electrodes. The voltage across the recording electrodes was

amplified 500-1000x and band-pass filtered between 0.1Hz-10,000Hz (A-M Systems model 3000). The amplified signal was continuously digitized at 50 kHz using the Tucker Davis Technologies RP2.1 (TDT). Spike discrimination routines and digital timers (using TDT's Visual Design Studio programming environment = RPVDS) were used to extract the interval (in seconds) between successive EODs. A digital trigger activated by the experimenter initiated IPI collection. After 20 IPIs were collected, a single stimulus was presented and the following 180 IPIs were collected for a total of 200 IPIs. After waiting the appropriate inter-trial interval (30, 10 or 5 s), the computer automatically delivered the next stimulus until all 10 stimuli were delivered for that trial. The median of each inter-pulse interval over the five trials of the same ISI was taken for each stimulus and was used for analysis. This method created one set of responses at each ISI for every animal.

Data Analysis. To analyze the specific EOD response properties (amplitude and duration) a baseline EOD rate was established for each response using the mean duration of 10 IPIs prior to the stimulus presentation. For measurements involving the amplitude of the response, intervals were normalized (in percentage) to the baseline. For measurements of duration, the raw intervals were summed. The lower confidence interval of the baseline was defined by subtracting 1.5 times the standard deviation of the duration of the first 10 IPIs from the baseline. The response of the fish was considered to begin when the IPI fell below this criterion for two consecutive inter-pulse intervals. The amplitude of the response was defined as the percentage change between baseline and the shortest inter-pulse interval among the consecutive intervals below criterion. The duration of the response was calculated by summing the intervals between the start of the response and the interval that reached a value halfway between the start value and the smallest value as the response returned to baseline. To measure

habituation of the EOD response over the course of the experiment, we calculated a habituation quotient by averaging the response amplitudes to the first stimulus at each of the ISIs as well as the response amplitude to the last stimulus at each ISI, and subsequently calculating a percentage change (average response to Stim.1 from Trial 1 – average response to Stim.10 from Trial 5)/average response to Stim.1 from Trial 1). Therefore, larger habituation quotients indicate greater habituation.

Results

All fish responded to controlled taps on the tank wall in a repeatable way (e.g., see Figure 3.4). Prior to any stimulus presentation, animals had an average EOD rate of 77 Hz ± 14.3.

Response Amplitude. The average normalized response amplitude (percentage change from baseline) was 0.55% ± 0.31 and showed variation across individual, ISI, and trial number. To illustrate changes in response amplitude over successive stimuli, all individuals' median responses were averaged by stimulus number for each ISI (Figure 3.5). During the first replicate animals had larger amplitude novelty responses to stimuli presented on a longer ISI (mean amplitude 30 s ISI = 0.75% ± 0.32, 10 s ISI= 0.68% ± 0.28, 5 s ISI = 0.64% ± 0.27) as evidenced by a significant effect of ISI ($F(2,28) = 12.0$, $p < .001$) on response amplitude as well as significant post hoc pair-wise comparisons, using Bonferroni adjustments for multiple comparisons (5 s vs. 10 s $p < .05$, 10 s vs. 30 s, $p < .05$, 5 s vs. 30 s, $p = .001$). Animals were also more likely to have a larger response amplitude to the first stimulus compared to other stimuli (mean amplitude of Stimulus 1= 0.84% ± 0.37, all other stimuli = 0.67% ± 0.28) as evidenced by a significant effect of stimulus number ($F(9,126) = 12.2$, $p < .001$) and significant pair wise comparisons of Stimulus 1 with all the others ($p < .05$).

During Replicate 2 animals had larger responses to stimuli presented on a 30 s ISI but the same amplitude to both 10 and 5 s ISIs ($F(2,24) = 9.8$, $p = .001$; pair wise comparisons: 30 s vs. 10 s $p < .05$, 30 s vs. 5 s $p < .05$). The mean amplitude of 30 s ISI = 0.78% ± 0.52, 10 s ISI= 0.68% ± 0.40, 5 s ISI = 0.65% ± 0.38. While stimulus order had an effect the amplitude of the response ($F(9,108) = 5.0$, $p < .001$); the response to Stimulus 1 was only significantly larger than Stimulus 2 ($p < .05$) while all others were not significantly different (mean response amplitude to Stimulus 1 = 0.86% ± 0.59, Stimulus 2 = 0.75% ± 0.53).

In addition to significant contributions of ISI and stimulus number to EOD, the individual performing the behavior also appeared to be a strong determinant in the overall amplitude variation. Based on the histogram of median responses from all individuals across all ISIs and stimuli (Figure 3.6a), we defined *large responses* as those larger than 0.75% and subsequently determined what percentage of each individual's median responses were large responses (Figure 3.6b). Individuals were classified as either large responders (six individuals have more than 75% large responses) or small responders (the other nine individuals have less than 37% large responses; of those, eight individuals have less than 1% large responses).

Animals habituated to the stimulus, having smaller amplitude responses to stimuli that occur later in the experiment compared to those that occur early. Habituation of response amplitude across the entire experiment is shown in Figure 3.7. Order of presentations can be read left to right, 30 s ISIs occurred before 10 s ISIs, which occurred before 5 s ISIs (10-min breaks were given between each). Average habituation quotient for all individuals was 56.1% ± 18.2 during Replicate 1 and 35.1% ± 29.8 during Replicate 2.

Response Duration. Response durations averaged 0.24 s ± 0.13, and this measure also displayed variation across individuals, ISI, and stimulus number. Figure 3.8 shows changes in

duration that occur with successive stimuli. ISI was averaged across animals for each ISI across stimuli. During the first replicate, animals had significantly longer responses to stimuli presented on a 30 s ISI than either a 10 s or 5 s ISI (mean duration 30 s ISI = 0.26 s ± 0.13, 10 s ISI= 0.24 s ± 0.12, 5 s ISI = 0.22 s ± 0.13) as evidenced by a significant effect of ISI and significant pair-wise comparisons of ISI using Bonferroni adjustments for multiple comparisons (p = .002 for both comparisons). Stimulus order also affected the response duration, F (9,126) = 5.9, p < .001 in that animals had significantly longer responses to Stimulus 1 compared to Stimulus 6 (p = .04) and to Stimulus 10 (p = .01). Mean response duration to Stimulus 1= 0.32 s ± 0.16, Stimulus 6 = 0.25 s ± 0.12 and Stimulus 10 = 0.23 s ± 0.13.

During the second replicate animals response durations were not affected by ISI but were affected by stimulus number F(9,108) = 6.0, p < .001. Animals had longer responses to Stimulus 1 compared to Stimuli 7, 9 and 10 as evidenced by significant pair-wise comparisons for these stimuli (for each comparison p < .05). Mean response duration to Stimulus 1= 0.29 s ± 0.19, Stimulus 7 = 0.20 s ± 0.14, Stimulus 9 = 0.19 s ± 0.13 and Stimulus 10 = 0.19 s ± 0.12.

As with response amplitude, we used the frequency histogram of response durations to define *long responses* as those longer than 0.2 s (Figure 3.9a). This organization did not show a clear dichotomy of individuals as long or short responders (Figure 3.9b). Rather, there appeared to be a more graded scale of individuality in terms of response duration.

Correlational Analysis. There were many significant correlations among variables within this experiment (see Table 3.1 for correlation coefficients). Animals that had larger amplitude responses also had longer duration responses. Animals with the lowest baseline EOD rates had the largest amplitude and longest duration novelty responses. Finally, baseline rate was also significantly correlated with habituation such that animals with the highest baseline EOD

rate showed less habituation of the response. All correlations mentioned above were significant in both replications. All measures except percentage of long responses were significantly correlated between replicates.

There were also a number of significant correlations between behaviors measured in the Terrestrial Challenge and Novelty Response Experiment (Table 3.2). Individuals that failed to escape from the platform in the first replicate of the terrestrial challenge had lower baseline EOD rates as well as larger amplitude and longer duration responses to novel stimuli during the second replicate of the novelty response experiments. Additionally, the less an animal moved on the platform during the first replicate of the experiment, the larger and longer their EOD responses were to novel stimuli. Finally, animals that struggled more in the net during the second replicate had lower baseline rates and higher amplitude and duration novelty responses

Discussion

All individuals reliably responded to novel mechanical stimuli by increasing their EOD rate. There were also consistent differences between individuals in both EOD amplitude and EOD duration and these variables were correlated, with some individuals having larger amplitude and longer duration novelty responses than others. This supported Prediction 3.2 that some individuals would show a more proactive coping response having smaller and shorter novelty responses while others would show a reactive coping response with larger and longer novelty responses.

Response Amplitude. Differences in the effect of inter-stimulus interval on novelty response amplitude across replicates are likely an exposure/habituation effect. In the first replicate, all three ISIs proved to be significantly different from each other, but in the second replicate only the 30-s ISI was significantly different from the other two. Since the order of ISIs

was the same for every animal (i.e., 30, 10, 5, 30, 10, 5, etc.), it is possible the order of presentation influenced the significant effect in the first replicate but that the novelty had worn off by the second replicate, showing that they perceive 10- and 5-s ISIs as essentially the same. The novelty response was also affected by stimulus number, such that it decreased or habituated after the first stimulus. During the first replicate the novelty response amplitude to Stimulus 1 was larger than all other stimuli. In the second replicate Stimulus 1 was only significantly different from Stimulus 2.

Judging from the high degree of correlation for all measures of response amplitude between the first and second replicates, individuals appear to have characteristic response amplitudes to novel stimuli. This characteristic is likely caused by an underlying predisposition for responsiveness possibly set up by individual differences in cortisol levels (Ruiz-Gomes, Huntingford, Overli, Thorqvist, & Hoglund, 2011).

Individuals that started the experiment with lower baseline EOD rates had larger novelty responses than those that began with higher baseline EOD rates. Perhaps, then, individual differences in baseline EOD rate (i.e., resting rate) are an indicator of how sensitive individuals are to changes in their environment.

Response Duration. The duration of the EOD response was significantly affected by ISI only during the first replication and, even then, only the 30-s ISI was different from the other two. Once again, this is could be an exposure effect. Since the 30-s ISI always occurred first, the earlier stimuli would have been more novel than later ones, thus eliciting larger responses. Intermixing the trails, but still keeping the same order across individual would have allowed us to make stronger conclusions about the effect of ISI.

The duration of the novelty response was also affected by the number of stimuli delivered to the fish, however, the exact stimulus numbers that were different were inconsistent between the first and second replicates. In the first replicate, the response to Stimulus 1 was different from the responses to Stimulus 6 and 10 and, in the second replicate, the response to Stimulus 1 was different from the responses to Stimuli 7, 9, and 10. For both replicates, Stimulus 1 was significantly longer than Stimulus 10, indicating that response duration was decreasing or habituating over the course of 10 stimuli.

Once again, animals appear to have characteristic response durations based on the correlations between measures of response duration between the first and second replicates. The percentage of long responses was not significantly correlated between replicates, however, it is likely that response duration is simply less dichotomous than response amplitude, and splitting the responses this way is not effective for individual classification. Since response amplitude and response duration were correlated, it seems likely that these are both indicators of underlying qualitative differences between individuals—namely that some individuals are more sensitive to changes in their environment and, therefore, produce more robust novelty responses than others. This difference could be caused by underlying differences in neurosympatheic activity or cortisol release in response to challenging or stressful situations (Korte, Beuving, Ruesink, & Blokhuis, 1997).

Defining the Response. It is indisputable that this response is a startle or novelty response in that it occurs reliably following a novel startling stimulus. Orienting responses are a specific type of novelty response that function to increase the perceptibility of a stimulus by bringing the appropriate receptors into proper alignment with the stimulus (Sokolov & Cacioppo, 1997). A true orienting response includes a rapid response in the presence of novelty, habituation

after a series of repeated stimuli, and response renewal after a change in the stimulus (Sokolov, 1990). Post and von der Emde (1999) described a similar response in *Gnathonemus petersii,* as it occurred to low and medium intensity stimuli, as fitting the key features of an *orienting response*.

It remains to be seen if we can technically call the response in *Microsternarchus* sp. an orienting response. Theoretically, increasing the EOD rate following a novel stimulus would increase the temporal resolution of the image the fish forms of its environment. The quicker the animal receives information about a stimulus, the faster they can respond to it. In a species like *G. carapo,* novelty responses affect an increase in EOD rate from 32 Hz to 84 Hz (Correa & Hoffmann, 1998). This change in rate would mean a decrease in IPI from 31 ms to 12 ms. Assuming it takes three pulses to identify a novel stimulus (Heiligenberg, 1976, 1980), this increase in rate would yield a difference of 68 ms in detection time. In *Microsternarchus,* we measured the average change in EOD rate during a novelty response to be from 79.9 Hz to 80.5 Hz, which translates to a difference of 0.3 ms over the course of three pulses. Compared to the 68 ms difference in *G. carapo*, it seems unlikely that an increase of 0.3 ms would make a perceptual difference to the fish.

In *G. carapo,* an EOD rate increase provides an improvement in electrolocation up to about 80 Hz (Schegel, 1973); however, it is unlikely that changes of 0.5 Hz would be a large enough increase to affect an improvement. Despite the fact that *Microsternarchus* reliably perform novelty responses, it is possible that the change in EOD in this species is not functionally important for stimulus detection but simply an artifact of overall arousal of the nervous system. If this were the case, the response would *not* be called an orienting response in this species. One could speculate that this response evolved as an orienting response in slower

pulse species and is simply a vestigial characteristic that remains despite the lack of functionality in this higher rate pulse species.

Furthermore, compared to the stimuli used in the experiments by Post and von der Emde (1999), stimuli used in this experiment fall into the high intensity range. According to their conclusions, this intensity should elicit a defensive electric response instead of an orienting response. The set of experiments performed herein does not include the data necessary to distinguish between these types of responses (namely the use of long duration stimuli to test responses to stimulus offset. A disappearing stimulus should not elicit a defensive response since it does not cause harm or pain). However, given the small amplitude of our response compared to that of other species, it may simply be the case that stimuli must have high intensity in order to get any measurable response.

General Discussion

There was a lack of consistency in the individuals' behavior between replicates in the terrestrial challenge. This makes the correlations between the two experiments more difficult to interpret. Behaviors from the first replicate of the terrestrial challenge seem to correlate only with behaviors from the second replicate of the novelty response experiment, if they correlate at all. The only behavior to correlate across experiments from the second replicate of the terrestrial challenge (struggling on the platform) correlated with some behaviors from each replicate of the novelty-response experiments. While more overlap would be preferred, interpreting the correlated behaviors themselves while ignoring the complication of which replicate they came from does yield some good parallels with established definitions and examples of proactive and reactive coping styles.

Animals that displayed larger amplitude and longer duration novelty responses were less likely to move on- and get off of- the platform. Moving on the platform is likely an extension of escaping the platform rather than a separate behavior in its own right. Therefore, it makes sense that these two measures would both be correlated with the same behaviors from the novelty response experiments. This correlation supports our prediction about the existence of coping styles in *Microsternarchus* sp.*,* since the reactive coping style is characterized by conservative responses to stress (not moving on the platform) and by high attention for changes in the environment (Bolhuis, Schouten, Leeuw, Schrama, & Weigant, 2004; Koolhaas et al., 1999; Ruiz-Gomes et al., 2011), thus, more robust responses to novel stimuli.

In the chapters that follow, additional experiments may shed light on further aspects of personality and coping styles in *Microsternarchus* sp.

Table 3.1

Correlation coefficients for comparisons within the novelty response experiment .

	r:	
Measure:	Replicate 1	Replicate 2
Starting EOD rate		
% large responses	-.60*	-.75**
% long responses	-.80***	-.90***
Average amplitude	-.76**	-.79**
Average duration	-.89***	-.85***
Habituation	-.55*	-.58*
% large responses		
% long responses	.87***	.84***
Average duration		
Average amplitude	.85***	.95***
Amplitude consistency	.57*	n.s.
Rate consistency		
Starting EOD rate	.71**	n.s
Average duration	n.s.	.67*

The correlation coefficient for each measure between the first and second replicates of the novelty response experiment

Measure:	*r:*
Starting EOD rate	.75**
% large responses	.63*
% long responses	n.s.
Average amplitude	.76**
Average duration	.82**
Habituation	.60*

Note. * $p < .05$ ** $p < .01$ *** $p < .001$

Table 3.2

Correlation coeficents for comparisons between the terrestrial challenge and the novlety response experiments

	r:	
Measure:	Replicate 1	Replicate 2
Time to flip off platform (Replicate 1)		
EOD Start Rate	n.s.	-.79**
% large responses	n.s.	.65*
% long responses	n.s.	.76**
Average amplitude	n.s.	.59*
Average duration	n.s.	.68**
Moving on the platform (Replicate 1)		
% large responses	n.s.	-.62*
% long responses	n.s.	-.62*
Average amplitude	n.s.	-.67*
Average duration	n.s.	-.72**
Struggle in net (Replicate 2)		
EOD Start Rate	-.65**	n.s.
% large responses	.95***	.63*
Average amplitude	.64*	.78**
Average duration	n.s.	.90***

Note. * $p < .05$ ** $p < .01$ *** $p < .001$

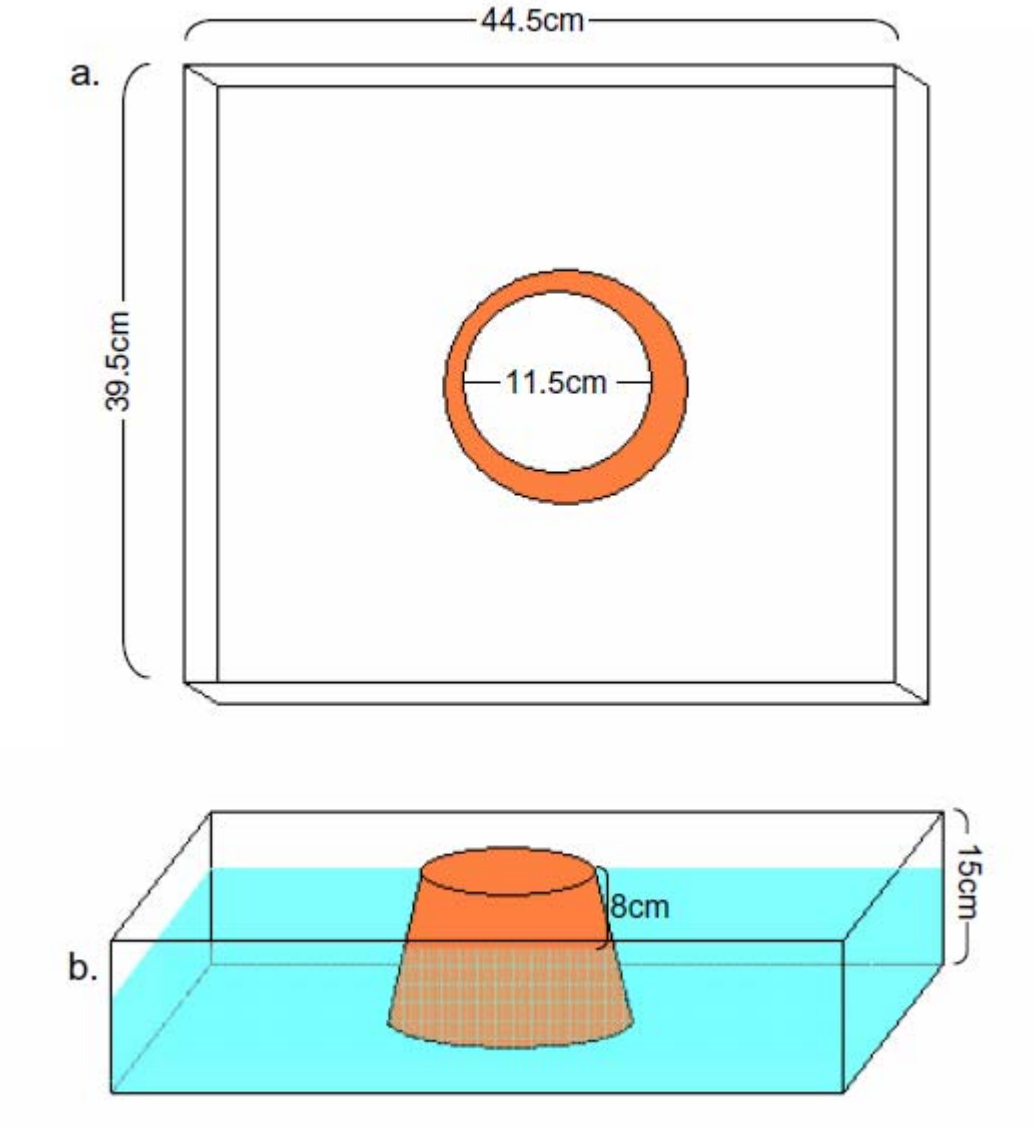


Figure 3.1. Diagram of the terrestrial challenge arena with dimensions. (a) shows the view from the top (b) shows the view from the side.

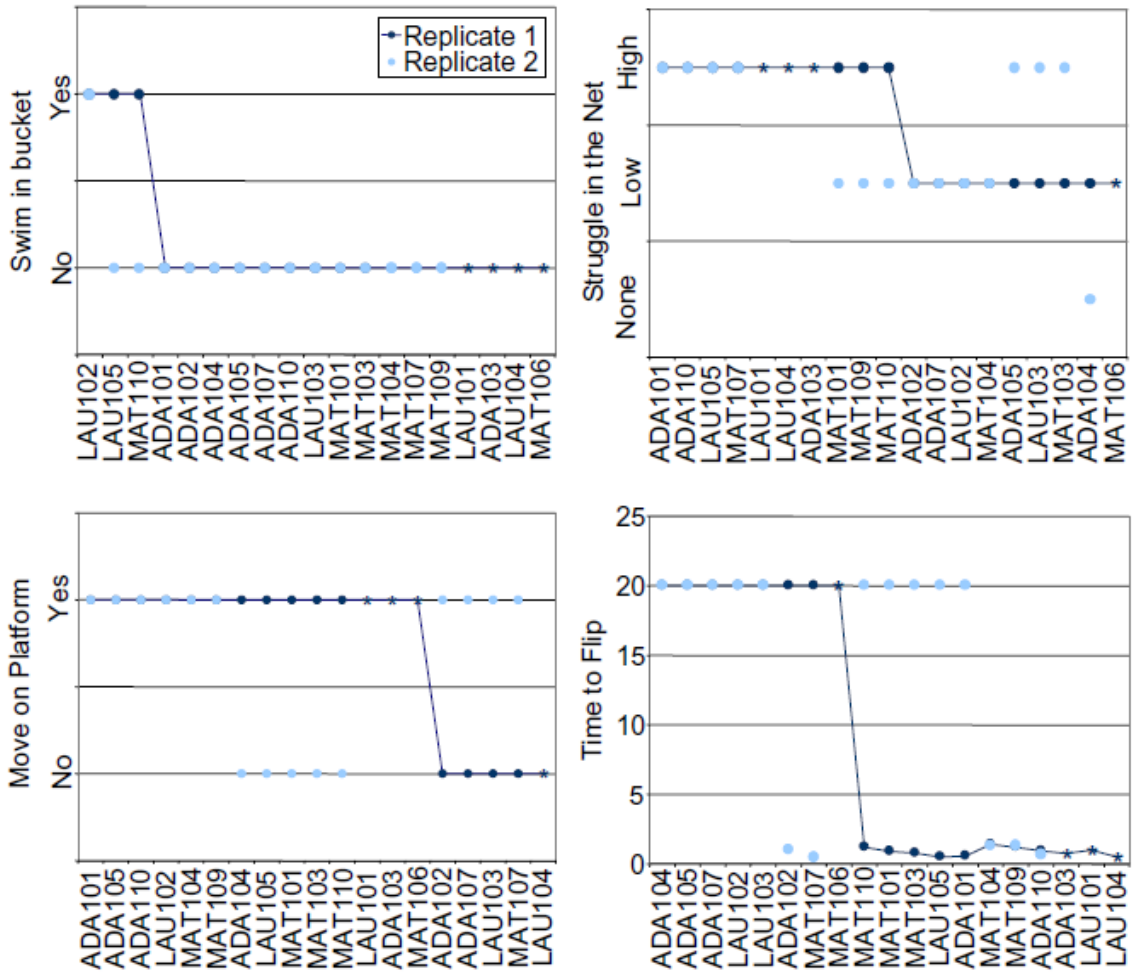


Figure 3.2. Comparison of the four behaviors measured during the terrestrial challenge between Replicate 1 (dark blue circles, and stars when there was no Replicate 2) and Replicate 2 (light blue circles). Whether or not animals were swimming in the bucket after the 5-min rest period (a), the intensity of struggling in the net (b), whether or not animals moved on the platform (c), and the time it took each animal to escape from the platform (d). The maximum allowable time on the platform was 20 s.

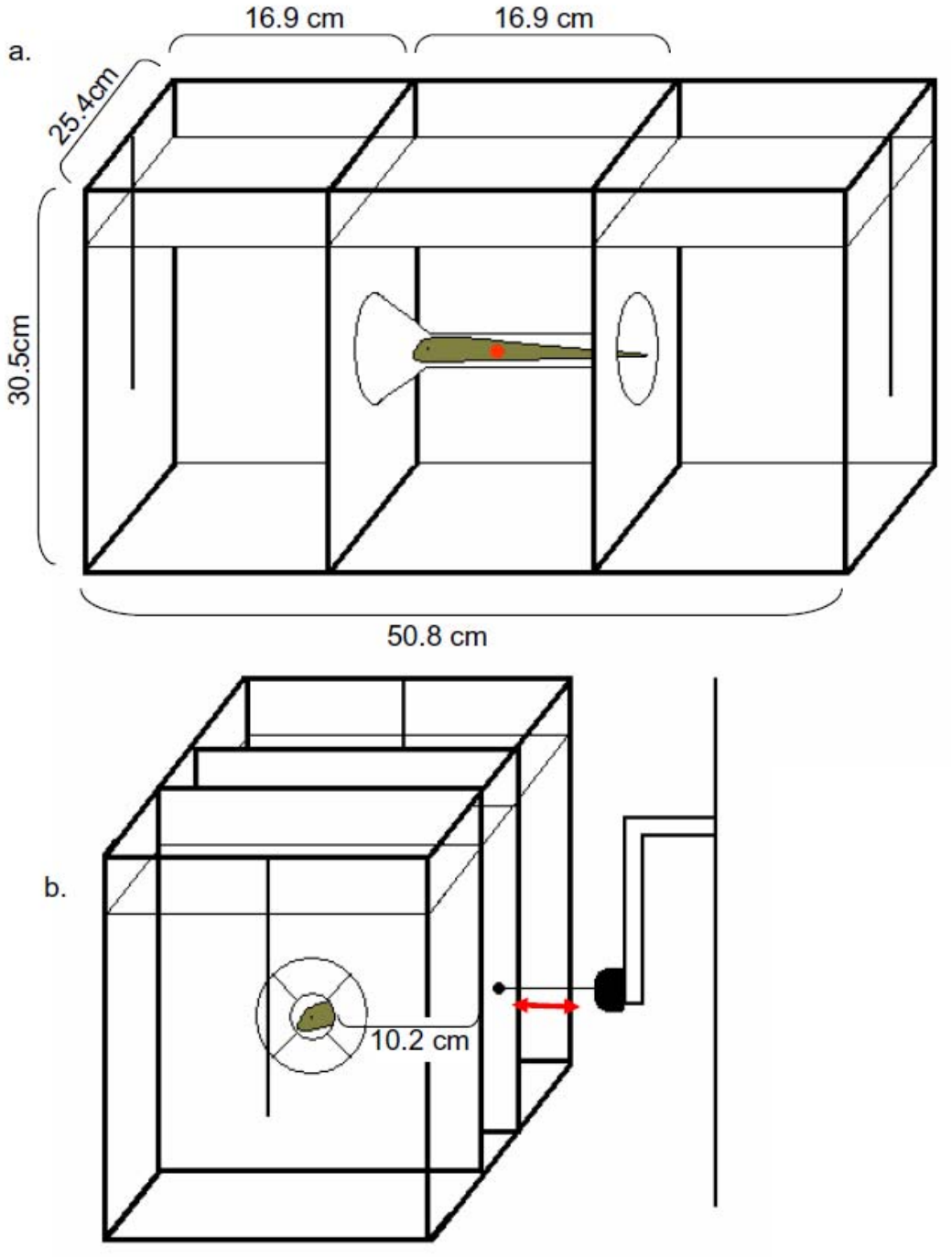


Figure 3.3. Diagram of the novelty response experimental tank with dimensions. Side view (a) showing position of the tap on tank wall (red dot) relative to the fish in the tube. Front view (b) showing the distance between the tank wall (where the tap occurs) and the shelter.

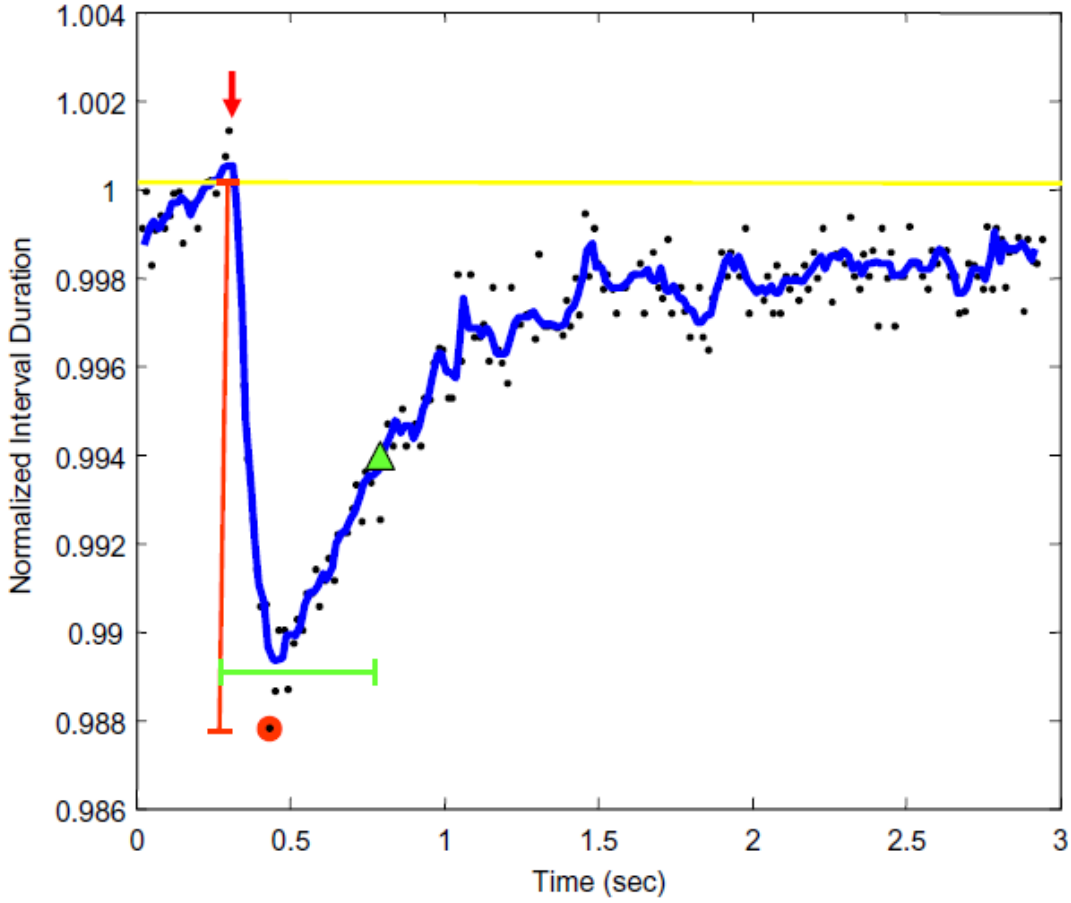
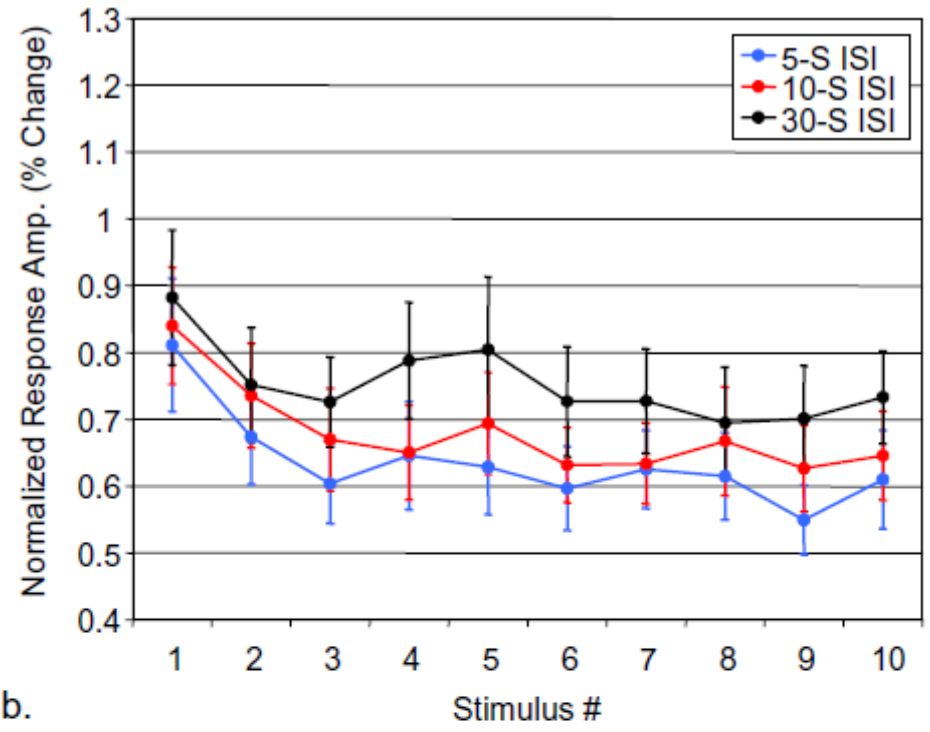


Figure 3.4. A characteristic sample of the novelty response in *Microsternarchus* sp. with labels to show measured variables. An average of five novelty responses (normalized to baseline) from one individual at the same stimulus number across five trials with the same ITI is plotted. The red arrow represents the time of stimulus delivery, black dots show the actual averaged intervals, and the blue line is a smoothed fit line. For analysis, a baseline is calculated (yellow line) from the 10 intervals before stimulus delivery. A criterion for response is set by subtracting 1.5 times the standard deviation of the baseline. The amplitude of the response (shown by the red line) is defined as the percentage change between baseline and the shortest inter-pulse interval (highlighted by the red dot) among the consecutive intervals below criterion. The duration of the

response (shown by the green line) is calculated by summing the intervals between the start of the response and the interval that reached a value halfway between the start value and the smallest value as the response returns to baseline (indicated by the green triangle).

a.


Normalized Response Amp. (% Change)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
1
2
3
4
5
6
7
8
9
10
Stimulus #
5-S ISI
10-S ISI
30-S ISI

b.

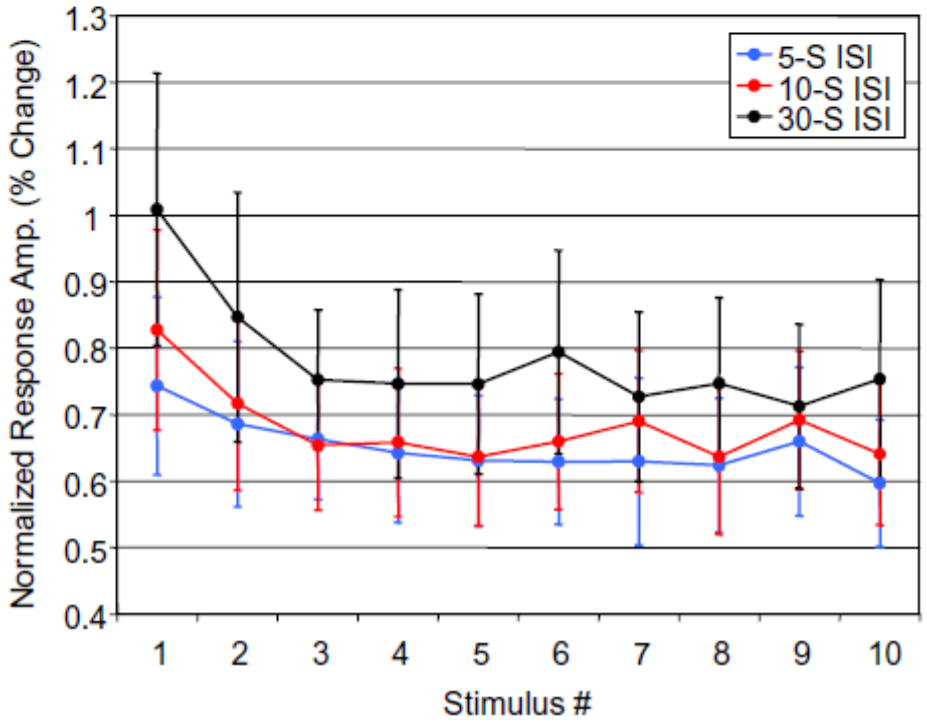

Normalized Response Amp. (% Change)
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
1
2
3
4
5
6
7
8
9
10
Stimulus #
5-S ISI
10-S ISI
30-S ISI

Figure 3.5. The effect of ISI and stimulus number on novelty response amplitude. Longer ISIs and earlier stimuli elicit larger novelty response amplitude. Averages of all individuals' median response amplitude (percentage change from baseline) for each stimulus at each ISI are plotted. Replicate 1is shown in (a) and Replicate 2 is shown in (b). Error bars represent ± one standard error of the mean. Colored lines illustrate the different ISIs: black for 30 s, red for 10 s, and blue for 5 s.

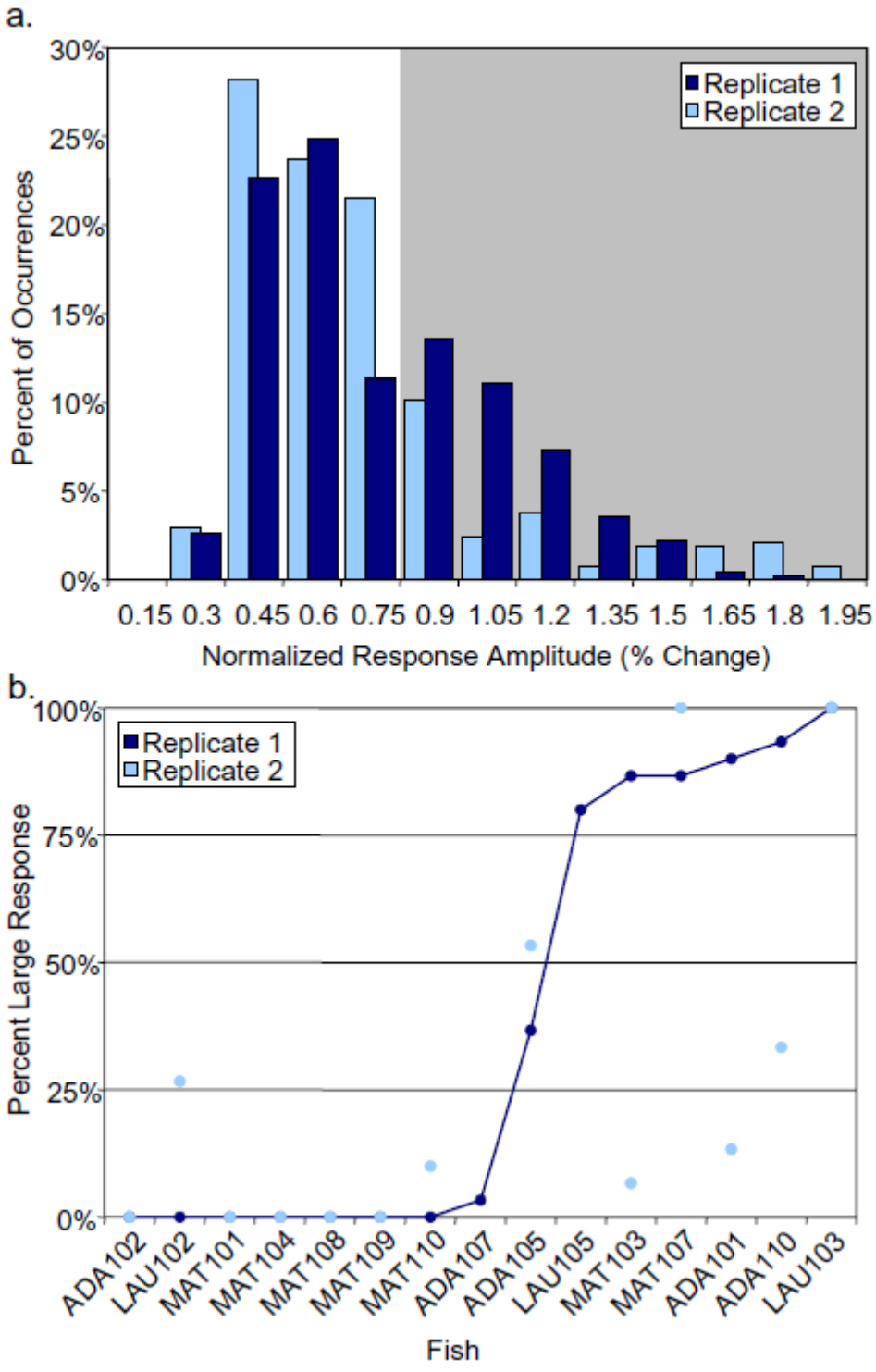
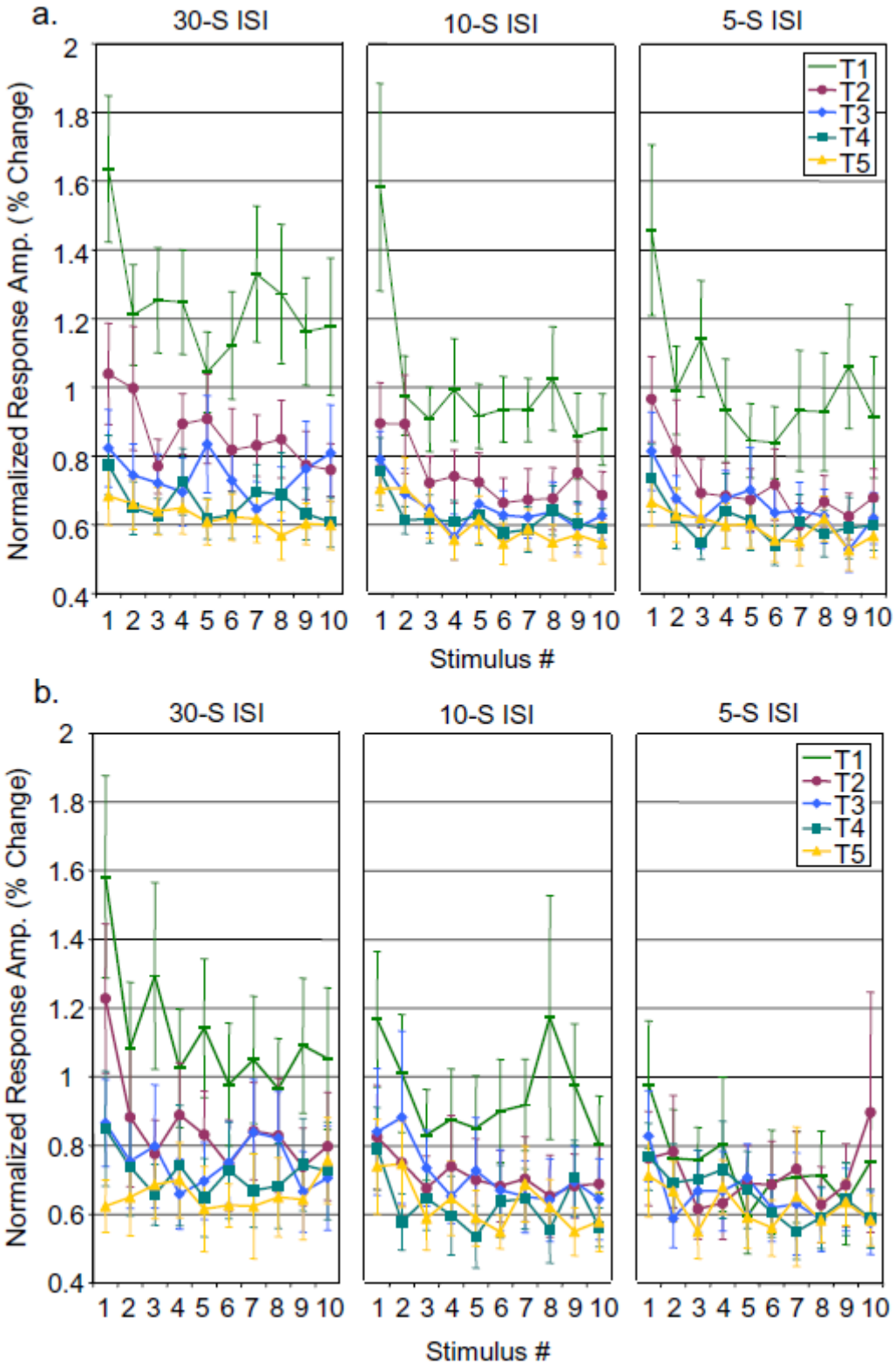


Figure 3.6. Relative frequency histograms of novelty response amplitude in and a classification of individual based on the percentage of large responses. Replicate 1 is show in dark blue and

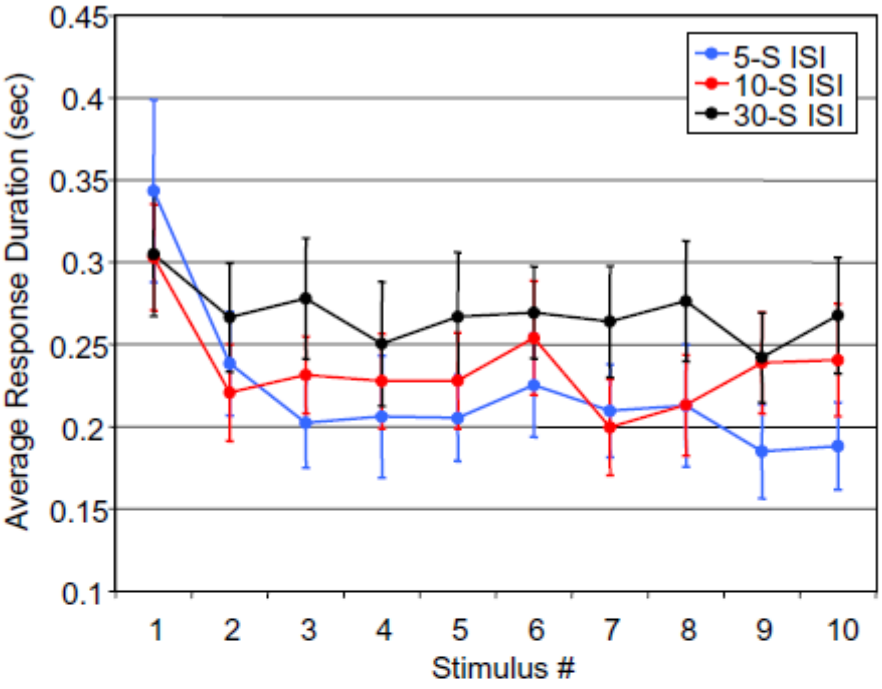
Replicate 2 is shown in light blue. The shaded portion represents those amplitudes which are further classified as large responses. X-axis labels represent the upper limit of each bin, the lower limit being just larger than the previous bin, or zero if there is no previous bin (a). Classification of individuals based on the percentage of their responses that are large responses is plotted in (b).


a.
30-S ISI
10-S ISI
5-S ISI
T1
T2
T3
T4
T5
Normalized Response Amp. (% Change)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
1 2 3 4 5 6 7 8 9 10
Stimulus #
b.
30-S ISI
10-S ISI
5-S ISI
T1
T2
T3
T4
T5
Normalized Response Amp. (% Change)
Stimulus #

(b)

Figure 3.7. Habituation of the novelty response amplitude over the course of the novelty response experiment. Each line represents a trial series averaged across all individuals for Replicate 1 (a) and Replicate 2 (b). Each trial can also be read left to right as it occurred in time. Ten minutes of silence occurred between trials.

a.


0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
Average Response Duration (sec)
5-S ISI
10-S ISI
30-S ISI
1
2
3
4
5
6
7
8
9
10
Stimulus #

b.

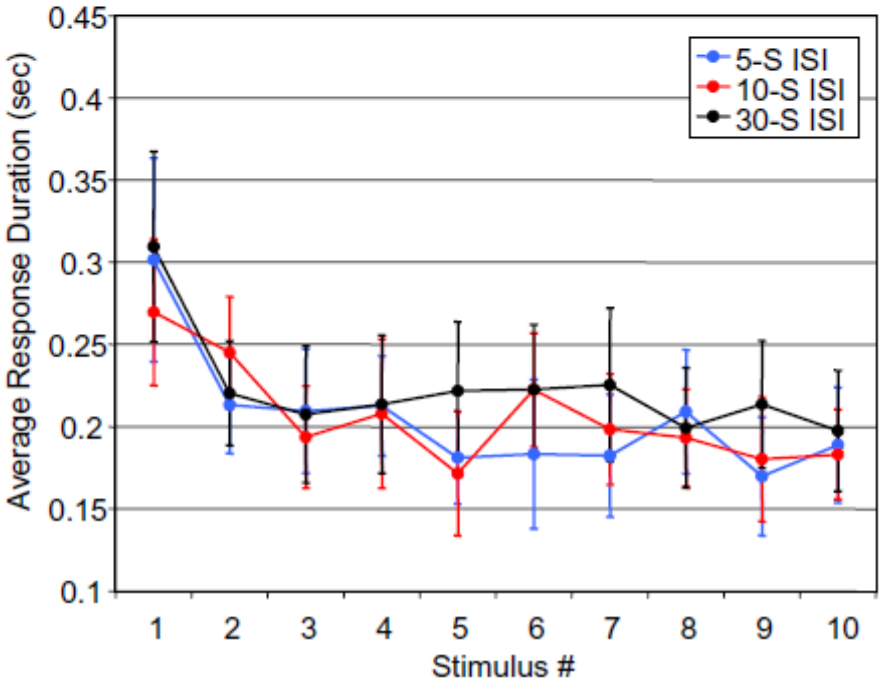
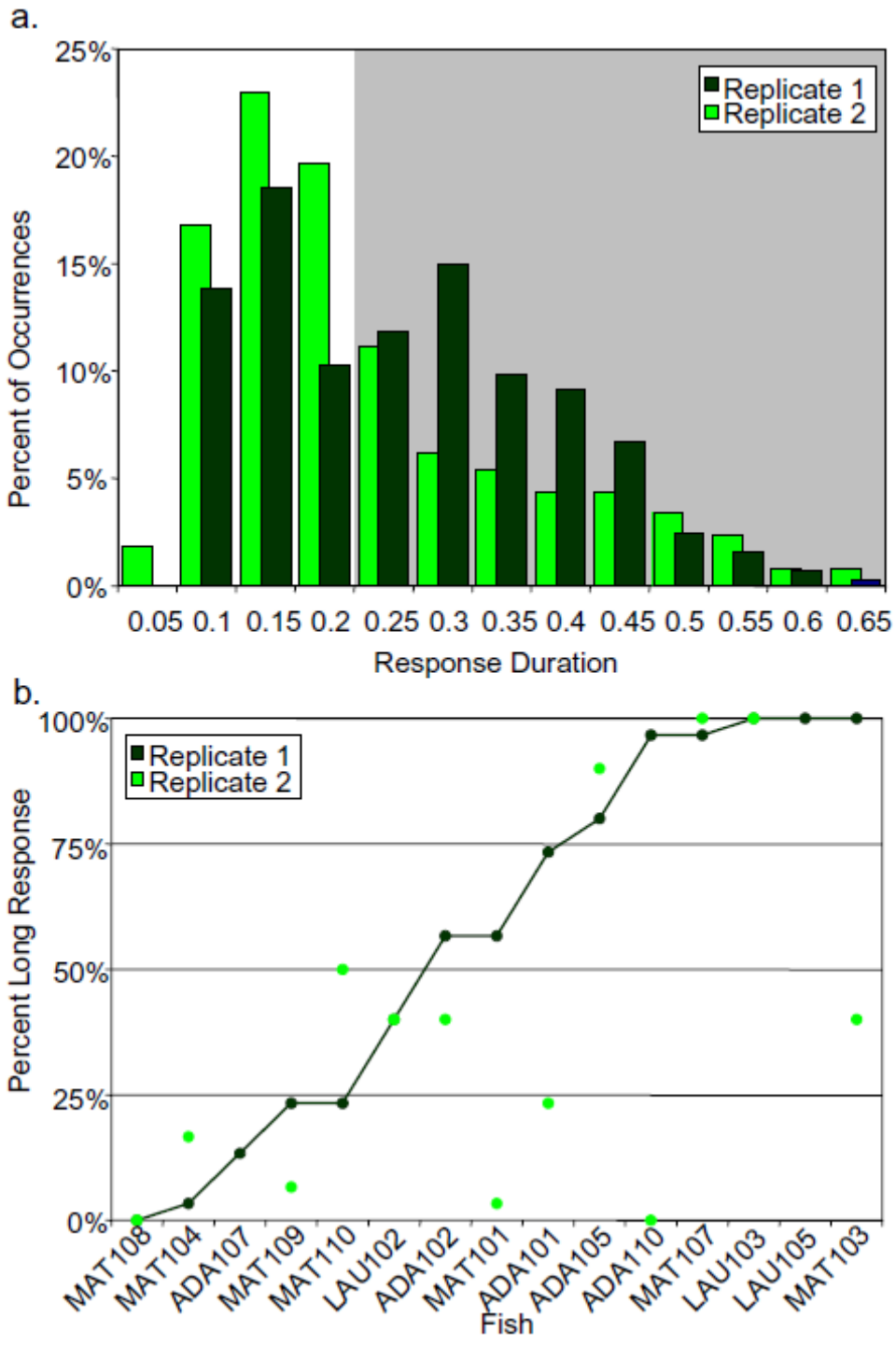

0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
Average Response Duration (sec)
5-S ISI
10-S ISI
30-S ISI
1
2
3
4
5
6
7
8
9
10
Stimulus #

Figure 3.8. The effect of ISI and stimulus number on novelty response duration. Thirty second ISIs (only in Replicate 1) and earlier stimuli (in both replicates) elicit longer novelty response duration. Averages of all individuals' median response durations for each stimulus at each ISI are plotted. Replicate 1 is shown in (a) and Replicate 2 is shown in (b). Error bars represent ± one standard error of the mean. Colored lines illustrate the different ISIs: black for 30 s, red for 10 s, and blue for 5 s.


a.
25%
20%
15%
10%
5%
0%
Percent of Occurrences
Replicate 1
Replicate 2
0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65
Response Duration
b.
100%
75%
50%
25%
0%
Percent Long Response
Replicate 1
Replicate 2
MAT108
MAT104
ADA107
MAT109
MAT110
LAU102
ADA102
MAT101
ADA101
ADA105
ADA110
MAT107
LAU103
LAU105
MAT103
Fish

3.9. Relative frequency histograms of novelty response duration in and a classification of individuals based on the percentage of long responses. Replicate 1 is shown in dark green and Replicate 2 is shown in light green. The shaded portion represents those durations which are further classified as long responses. X-axis labels represent the upper limit of each bin, the lower limit being just larger than the previous bin, or zero if there is no previous bin (a). Classification of individuals based on the percentage of their responses that are long responses is plotted in (b).

Chapter 4:

Behavioral responses to electrical stimuli imply the existence of a behavioral syndrome for aggression *Microsternarchus* sp.

Introduction

Aggression has wide-reaching effects on an individual's fitness, because it comprises aspects of territory acquisition and defense, access to and defense of mates, foraging success, and responses to predators (Logue, Takahashi, & Cade, 2011; Reichmuth, MacDonald, Ramirez, & Weis, 2011; While, Sinn, & Wapstra, 2009). Typical aggressive behaviors include attacking conspecifics, predators or intraspecific intruders, infanticide, siblicide, or any other behavior that serves to increase the aggressor's social dominance over other individuals (Ferguson & Beaver, 2009). Behavioral syndromes for aggression are defined as correlations among a suite of aggressive behaviors across different contexts. For example, in Three-Spined Sticklebacks, individuals that are the most aggressive toward a conspecific intruder are also bold in the face of a predator, resulting in a correlation between how territorial they are and how they respond to predators (Huntingford, 1976).

Our focal species, *Microsternarchus* sp., is a widely distributed small knifefish found in second and third order tributaries of the lower Rio Negro in Brazil. Very little is known about the social behavior of *Microsternarchus* sp. Our own observations suggest that it is a docile species, showing little to no aggression when housed communally under non-breeding conditions (i.e. low conductivity water). When two animals are placed in a tank with only one shelter, they typically share it, and overt aggression is never observed. Even when multiple shelters are available it is not uncommon for more than one fish to share the same shelter. It is not known if aggression escalates during the breeding season.

Response to Potential Threat. Chapter 2 established patterns of locomotor activity and electric organ discharge (EOD) rate in a novel environment, individuals with the highest EOD rates also were also the most active. Here we increased the level of perceived risk in the environment (playing the EOD of an aggressive sympatric species) and measured whether fish altered their electric and exploratory behaviors in the presence of a threat. Presumably the most aggressive individuals will respond to the threat by attacking it or becoming more active, while the least aggressive individuals will respond with decreased activity during playback.

We introduced the electrical playback of an aggressive sympatrically occurring species of electric fish, *Gymnotus* sp.cf. *carapo,* into the experimental tank. The aggressive behavior of *Gymnotus* is well documented (Black-Cleworth, 1970; Capurro, Reyes-Parada, Olazabal, Perrone, Silveira, & Macadar, 1997; Westby, 1975a) and they are likely the most aggressive of all weakly electric fish. The simple presence of two or more conspecifics in one tank is enough to induce aggression and create clear dominance hierarchies. While *Gymnotus* is not likely a predator to full-grown *Microsternarchus* sp., members of both genera are found living sympatrically in the locations where our fish were collected. There are no data on interspecific aggression between *Gymnotus* and *Microsternarchus;* however, *Gymnotus* will readily approach and bite electrodes that are playing simple sine waves (Capurro et al., 1997). Thus, it is likely that other electric fish are also subjected to aggressive attacks on occasion.

Specific Aim 4.1: Determine if individuals differ in their response to *Gymnotus* playback indicative of differences in social or anti-social behavior.

Prediction 4.1: *Microsternarchus* will show individual variation in their responsiveness, to playback of *Gymnotus* EODs. Individual's behavior will be organized around a basic aggressive response with some individuals showing greater changes in locomotor activity or

EOD rate during playback. Individuals that increase their activity the most in the presence of a threatening stimulus will also will approach the playback electrodes more.

Jamming Avoidance Response Experiments. When two or more electric fish are in close proximity to each other, they may experience some degree of electrosensory interference due to coinciding EODs. The more similar the EOD rates, the more interference each fish will experience (Bullock, Hamstra, & Scheich, 1972, Heiligenberg, 1974, 1980; Westby, 1975c). To reduce this electrosensory interference, electric fish perform a behavior called the jamming avoidance response or JAR (Heiligenberg, 1986; Westby, 1981). In some species, only the fish with the higher baseline rate will increase its EOD rate, while other species show a change in both directions, with the higher EOD rate fish further increasing its EOD rate at the same time the fish with the lower starting EOD rate further decreases its EOD rate (Bullock et al., 1972; Capurro, Macadar, Perrone, & Pakdaman, 1998; Caputi, 2004; Heiligenberg, 1975, 1976, 1986; Heiligenberg, Baker, & Bastian, 1978; Kramer, 1990, Westby, 1979). Jamming avoidance allows each fish to maintain private frequencies to perform electrolocation (in wave species specifically) and also increases the beat frequency of the interference. The beat frequency created by overlapping EODs can often distort the perception of the EOD in ways that are similar to the distortion created by objects in the fish's environment.

Westby's model (1979) of directional sensitivity indicates that fish have a detection window which gradually starts a few ms prior to emitting an EOD (how long before is likely variable depending on the species), and rapidly closing following the emission of the EOD. Due to this differential sensitivity based on the position of the EODs in relation to one another, the fish with the higher EOD rate has a different sensory experience than the fish with the lower EOD rate. When the foreign EOD has a lower rate, each successive foreign EOD will advance in

phase compared to the subjects EOD (visually move from left to right on an oscilloscope display) and, therefore, will approach the subject fish's pulse in a *detectable* time-direction before it interferes with electrolocation abilities. On the other hand, when the foreign EOD has a higher discharge rate, the foreign EOD will decrease successively in phase compared to the subjects EOD (visually move from right to left) and, therefore, will become most detrimental to the subject fish at the first moment the EOD is detectable (Westby, 1979).

In *G. carapo,* jamming avoidance can also become relevant for dominance relationships and aggressive behavior, in that a dominant or aggressive fish will actively place its own discharges in the most sensitive period of its opponent while keeping the opponent's discharges in the least sensitive period of its own discharge (Capurro, Pakdaman, Perrone, & Macadar, 1999; Westby, 1979). This situation will effectively jam the opponent but keep its own electrolocation abilities intact.

Specific Aim 4.2: To characterize the JAR in *Microsternarchus*.

Prediction 4.2: *Microsternarchus* will display JARs similar to other electric fish, increasing their EOD rate in response to jamming stimuli with lower EOD rates and decreasing their EOD rate in response to jamming stimuli with higher EOD rates. Animals will alter their EOD rates further and faster in response to stimuli with EOD rates closer to their own.

Specific Aim 4.3: Determine if consistent individual differences are seen in the JAR of *Microsternarchus* that would be indicative of differences in social or anti-social behavior.

Prediction 4.3: Individuals will vary consistently in the degree of responsiveness to jamming stimuli. Individual's behavior will be organized around an aggressive response or dominance assertion. Individuals with larger magnitude JARs (particularly to lower rate stimuli) will also intentionally jam the stimulus more frequently.

Specific Aim 4.4: If individual differences exist in response to *Gymnotus* playback and JARs, determine if they correlate and conform to established ideas about social or anti-social behavior.

Prediction 4.4: Individuals that have the most electrode approaches during *Gymnotus* playback will also have more instances of jamming the stimulus implying the existence of an aggression or dominance syndrome.

General Methods

Subjects

Subjects included up to 15 *Microsternarchus* sp. The sample size for each experiment and replication varies slightly as some animals died during the course of the experiments. Individuals measured between 10 and 15 cm in length and were all presumably mature at the time of the experiments. All fish were tagged so that data could be tracked by individual fish over the course of the experiments. (For further details on our subjects see Chapter 1.)

Overview

In the two experiments reported here, individual animals were presented with electrical stimuli in two different contexts and their responses were recorded. The first experiment was the intermittent long-term playback of the EOD of a sympatrically occurring aggressive species of electric fish, and the second was acute playback of the fish's own EOD waveform in a jamming avoidance paradigm. Responses recorded for the *Gymnotus* playback experiment included locomotor activity, changes in EOD rate, and approaches to the playback electrodes. During the JAR experiment we measured changes in the animal's inter-pulse interval (IPI) while the animals were motionless within a shelter. The JAR experiment was performed twice to determine the individual consistency of behavioral responses. After analyzing the results of each experiment

individually, we also describe how behaviors relate across experiments within individuals. Animals were maintained in low conductivity water (between 115 and 125 μsiemens/cm^2) with a pH between 4.5 and 6.0 at 24°C ± 1°C and on a 12hour:12hour light:dark cycle. Statistical analysis was done in SPSS using an alpha of .05. All experiments were approved by the Institutional Animal Care and Use Committee (Protocol CB signaling 11/08-02).

Experiment 1: *Gymnotus* Playback Experiment

Materials and Methods

Subjects. These experiments were performed in September and October 2007 on 14 *Microsternarchus* sp.

Materials. The experimental aquaria and electrical recordings were identical to those outlined in Chapter 2 with the addition of *two* pairs of custom made T-shaped carbon nub electrodes (Figure 4.1). These electrodes were placed directly beneath either end of the central tube at floor level, perpendicular to the recording electrodes. The electrodes measured 7mm in diameter and 3.8cm end to end.

Procedure. Animals were removed from their home aquaria and placed into an experimental tank (matched for water conditions) for 48 hours. Animals were exposed to the stimulus (see stimulus generation) according to the timeline in Figure 4.2 such that playback alternated between *intermittent* and *silent* every three hours. During intermittent periods, the signal was played for 5-min (referred to subsequently as playback blocks) followed by 4-to-6-min of silence. This could be interpreted as the intruder fish moving out of the detection range, however *Gymnotus* are known to halt its EOD for this length of time (Box & Westby, 1970). Which electrode pair, of the two, that was used for playback was randomized and counterbalanced, alternating between the 5-min playback blocks but not within blocks.

Stimulus Generation. An artificial EOD was created using a recorded *Gymnotus* sp.cf. *carapo* waveform, a known sympatric species and potential threat. The rate of the playback signal was engineered to be 50 Hz with one rate modulation per 5- min block meant to mimic the aggressive frequency bursts that occur in this species (Westby, 1975a; 1975b). This rate modulation always occurred at the start of the third minute. The signal was calibrated to be approximately the same amplitude as a typical *Microsternarchus* signal when played while the T-electrodes were placed next to the tube. Understandably, the level of exposure to the playback signal that each fish received was dependant on their position in the tank during playback. However, exposure level should have averaged out to be approximately equal across all fish, since they were all swimming freely in the tank.

Detection measures. Electrical recordings of the EOD and locomotor activity of the subject fish were identical to those used in the baseline experiment (Chapter 2). In addition to this, we also maintained continuous digital infrared (IR) video surveillance that included indicator IR-LEDs to signal the location and timing of EOD playback.

Video Analysis. Forty-eight video segments of approximately 8 min each were analyzed per fish across the full 48 hours. These time periods were chosen pseudo randomly to include periods of playback and silence during both day and night. During playback blocks this included 1.5 min before, 5 min during and 1.5 min after playback. In addition to our other behavioral measures, the position of the resident fish in the tank and approaches to the playback electrode were documented on a fixed interval observation schedule of 1 s from the video recordings. An electrode approach was defined as the animal's snout coming within 1 cm of the playback electrode. We analyzed the number of electrode approaches in percentage units relative to the amount of video that was watched for each fish during each time period (day with playback,

night with playback, day without playback, night without playback) since the amount of video observed for each time period was not always equal. Due to the video quality in IR conditions and the location of the playback electrodes, it was not always possible to determine if fish were actually biting the electrodes or just investigating them at close range. The approaches were ballistic in nature and generally occurred in rapid succession, with fish making several repetitive snout-to-electrode jabs over successive seconds.

Data Analysis. To compare behavior with and without stimulus presentation, periods of time when the stimulus was actually playing (during the intermittent playback) were compared to the periods of time when the stimulus was silent (not the silent portions of the intermittent playback blocks) as the control. The EOD rate and locomotor activity data presented here only includes the 24-hour period of time following lights on after the first night (i.e., from 7:30 a.m. on Day 2 to 7:30 a.m. on Day 3).

Results

Electrode Approaches. Analysis of the video recordings indicated that animals approached the electrodes more often at night. In fact, most animals were not witnessed making any approaches during the day (Figure 4.3a). Individuals varied in the total amount of time they spent approaching the electrodes (while the electrodes were actively playing stimuli or silent) at night, ranging from 1.85 min to 40.73 min (out of a total of approximately 2.5 hr viewed per fish). Animals spent significantly more time exploring the electrodes while the stimulus was playing than while it was not ($t(13) = -.433$, $p < .01$). Animals approached the electrodes at night, on average, 21.0% of the time during stimulus presentation compared to 3.54% of the time while the electrodes were silent (Figure 4.3a). While individuals varied in how *much* more time they spent approaching electrodes during stimulus presentation compared to when the stimulus

was not playing, the percentage of time spent approaching the active electrodes was correlated with the percentage of time spent approaching the silent one ($r = .812$, $p < .001$). In addition, while the stimulus was playing, animals were more likely to explore the active pair of electrodes rather than the silent pair ($t(13) = 3.49$ $p < .01$). The mean percentage of time spent approaching the active (playing) electrode was 14.7% compared to 6.3% of time spent approaching the inactive (non playing) electrode during playback (Figure 4.3b). As a group, animals failed to discriminate between the two pairs while they were off (Figure 4.3c) (mean percentage of time approaching silent-left = 1.7%, vs. silent-right = 1.9%). While a signal was being played some individuals were more discriminatory than others in that they approached the active electrode more often than they did the inactive one (Figure 4.3d), and animals that were the most discriminatory also approached the playing electrodes the most frequently ($r = .977$, $p < .001$).

Locomotor Activity. Animals spent less time out of the shelter during the day while the stimulus was playing compared to when it was not (Figure 4.4a) as evidenced by a significant interaction effect between time of day and stimulus presence on the percentage of time spent active $F(1, 13) = 14.4$ $p < .01$ as well as significant post hoc analysis comparing periods of playback during the day (mean percentage of time spent active =6.42%) and periods of silence during the day (mean percentage of time spent active=14.16%), $t(13) = -3.63$, $p < .01$. There was no significant difference in the percentage of time spent out of the shelter *at night* with and without playback. Individual animals differed in the amount and direction of the change during the day and at night (Figure 4.4a), in that some individuals increased their activity in the presence of playback, and others decreased activity.

EOD Behavior. Animals discharged at significantly lower EOD rates during the day while the stimulus was playing compared to when it was silent (Figure 4.4b) as evidenced by a

significant interaction between time of day and stimulus presence on EOD rate $F(1, 13) = 5.1$ $p < .05$, as well as significant post hoc analysis comparing periods of playback during the day (mean EOD rate = 91.2 Hz) and periods of silence during the day (mean EOD rate = 93.8 Hz) $t(13) = 2.66$ $p < .05$. Once again, there was no significant difference *at night* when overall mean rate was 112.6 Hz.

There were many significant correlations between variables in this experiment (see Table 4.1). To summarize the relationships, animals that spent more time out of the shelter (with or without playback) have higher EOD rates in general and larger changes in EOD rate in response to playback. Interestingly, individuals that had the most electrode approaches were those that spent the *least* amount of time out of the shelter during playback. The individual's length did not correlate with any measured variables in this experiment.

Discussion

All animals spent more time approaching the playback electrodes at night while the threatening stimulus was on than during any other period of time. Nevertheless, some animals showed a much higher affinity for the electrodes than others. All animals, on occasion, approached the inactive electrode pair during periods while the stimulus was being played from the opposite electrode pair. Some animals were much more likely to approach the active electrode, but others spent nearly equal amounts of time at both. Animals did not discriminate between the electrodes while they were silent.

Playback of *Gymnotus* EODs during the day caused a significant decrease in locomotor activity, although there were a few individuals that increased locomotor activity the presence of playback. While the effect was not significant, animals trended in the opposite direction at night, increasing locomotor activity during playback. There was likely a ceiling effect which limited

our ability to obtain significance in this measure because many animals were already spending over 90% of the night active when the stimulus was silent. In addition, a few animals decreased their activity during playback at night. On the whole, it appeared that most animals were inhibited by the presence of the stimulus during the day and attracted to it at night. Since these animals are nocturnal, it would make sense that they would be more willing to approach a threatening stimulus during their active period and avoid it during their inactive period. An alternative explanation may be that they do not find this stimulus threatening at all, but rather are attracted to it as a social stimulus. However, electrode approaches certainly did not appear *friendly* in nature.

There was a significant decrease in EOD rate during playback during the day. From Chapter 2, we know that animals increase their EOD rate while active and decrease their EOD rate while resting. Therefore, it would be difficult to say whether the decrease in rate was caused directly by the playback or if it was simply caused by the animals' decreased activity (seen in the presence of playback). Unfortunately the number of instances of the subject fish being *active*, during the day while the stimulus was silent and were too infrequent to realistically compare EOD rate during these instances to instances where the fish was *resting* during periods of silence during the day. (In Chapter 2 we compared EOD rate across periods of activity or resting during the day, but further subdividing these time periods by playback presence severely limits the number of observations, particularly when it is understood that animals only spend between 5 and 10% of the day active).

There was an insignificant trend for animals to increase their EOD rate at night during playback, mirroring the trend for increased activity at night during playback. This too could be a

ceiling effect, since animals were already discharging at very high rates at night. It is not clear how much faster they can discharge either physically or realistically.

We predicted that individuals would differ in their responsiveness to *Gymnotus* playback and this was supported by our data. Some individuals increased their locomotor activity and EOD rate during playback while others decreased their locomotor activity and EOD rate during playback (this was also affected by time of day). We further predicted that individuals with the most locomotor activity in the presence of playback would have the most electrode approaches but this was not supported by our data. Animals that approached the electrodes the most spent the *least* amount of time out of the shelter. This correlation could be an indication of how responsive individuals were to the presence of a threatening stimulus, with individuals modifying their behavior in different directions possibly balancing the risk of approaching by retreating to the safety of the shelter.

Experiment 2: Jamming Avoidance Response

Materials and Methods

Subjects. Fifteen *Microsternarchus* sp. were involved in the first replicate of the experiment and 13 were involved in the second replicate. Experiments were performed between December 2007 and February 2008. At least three weeks separated replicates for each individual. Individuals were chosen for experimentation at random until all individuals were completed.

Materials. All water was matched for conductivity, temperature, and pH to each fish's home tank. Experimental tanks were identical to that used in the novelty response experiments (Experiment 2 of Chapter 3) with the addition of a set of custom-made carbon T electrodes (7mm in diameter and 3.8cm end to end) placed next to the central tube (Figure 4.5) just slightly off of

perpendicular to the recording electrodes (which maintained a small stimulus artifact in the recording for timing verification).

Procedure. This experiment occurred after a 30 min rest period following the novelty response experiments (Experiment 2 of Chapter 3). After this length of time resting EOD rates had returned to pre-experimental levels. A sample of each subject's waveform was captured for subsequent playback. In the recorded signal, we refer to the subject as the s1 and the playback, our synthesized fish, as the s2. The s2 signal was played in a stimulus train for 10 s with a fixed EOD rate referenced to the s1 rate at the start of playback. The starting EOD rate difference ranged from 5 Hz below the s1 to 5 Hz higher (this difference is referred to as the Δf). Eight different Δf stimuli were presented in the following order: −1, −2, −3, −5, +1, +2, +3, +5, and each was presented at least five consecutive times. Animals rarely reversed their positions inside the shelter or swam out and needed to be coaxed back in before the start of the next trial. When this occurred, trials were discarded and the assigned Δf was repeated before moving on to the next Δf. There were 2 minutes of rest between all successive presentations of stimulus trains. The order was kept consistent for all animals so that differences in behavior could not be attributed to differing order effects.

EOD Detection Measures. The animals' inter-pulse-interval (IPI) was continuously monitored using custom-made carbon rod electrodes. The voltage across the recording electrodes was amplified 500-1000x and band-pass filtered between 0.1Hz-10,000Hz (A-M Systems model 3000). The amplified signal was continuously digitized at 50 kHz using the Tucker Davis Technologies RP2.1 (TDT). Spike discrimination routines and digital timers (using TDT's Visual Design Studio programming environment = RPVDS) were used to extract the interval (in seconds) between successive EODs.

Data Analysis. We calculated three different dependent variables to characterize the fish's response to the jamming stimuli: (a) mean Δf during each of the 10 s of playback (and a corresponding overall change in EOD rate), (b) jamming/jammed ratio (and a corresponding z-score), and (c) response start time (in number of EODs) The mean Δf is the average difference in EOD rate between the s2 and each fish's EOD rate during each second of playback. This had a corresponding measure of the overall change in EOD rate relating the starting (imposed) Δf to the final mean Δf during the last second of playback. For example, if the s2 was presented at a Δf of −1 and the fish had a mean Δf during the final second of −4, then the overall change in EOD rate in the s1 was −3.

The jamming/jammed ratio was calculated as the difference in the number of pulses in the 3 ms prior to the s2 stimulus to the number of pulses in the 3 ms following the s2 stimulus, both normalized to the total number of pulses in the full 6 ms period (pulses before/ (pulses before + pulses after) − pulses after/ (pulses before + pulses after). We further calculated a z-score of this measure to determine how different (in units of standard deviation) our calculated ratio was from a random distribution of pulses (calculated from a 10,000 iteration simulation of randomly distributed events). We used a z-score of 2 (greater than 95% confidence) as the criterion for determining which jamming/jammed ratio scores were significantly different from random and indicative of a preference for preceding or following positions.

The response start time was scored as the number of EODs the subject produced during s2 playback before shifting its EOD rate. A JAR rate shift was defined as a change in the subject's EOD rate of at least one and a half times the baseline's standard deviation (in either direction) for more than 25 consecutive intervals. Baseline rate was calculated from the 20 IPIs before the s2 started. If the animal never changed its EOD rate from baseline, the start value for

response was set to the maximum number of EODs the animal emitted. These trials are considered nonresponsive. Median values of all responses at each Δf were used for analysis of overall change in EOD rate and response start time, not including non-responses. Median values proved to be more representative of the animal's behavior because of the potential for non-normally distributed responses.

Results

In general, *Microsternarchus* sp. responded to stimuli with lower rates than its own (negative Δfs) by increasing its EOD rate, while stimuli with higher rates (positive Δfs) caused animals to lower their rates (Figure 4.6). Baseline rates averaged 78.7 Hz ± 11.6 during the first replicate and 80.6 Hz ±11.5 during the second replicate. In addition to this simple JAR, other types of responses occurred, which we refer to here as *phase skipping*, *temporary jamming*, and *phase bouncing*. Phase skipping occurred when an individual shortened its IPI (briefly increased its rate) at the moment its own discharges would coincide with the s2 pulse (Figure 4.7a–b). This behavior was only seen in response to stimuli with lower EOD rates (negative Δfs). Temporary jamming occurred when an animal preferentially placed its own pulse just before the s2 EOD (Figure 4.7c–d) for greater than 500 ms. Temporary jamming occurred only in two individuals (ADA110 and MAT110) and only to a Δf of + 1 (stimuli with a EOD rate 1Hz higher than the subject fish). Finally, phase bouncing occurred when an individual's pulse approached or just passed through the s2 pulse and then the animal changed its rate relationship with the stimulus, going from a lower EOD rate to a higher EOD rate than the s2 (Figure 4.7e–f). Phase bouncing always occurred in this direction and, therefore, mostly occurred to positive Δfs (stimuli with higher EOD rates). It did occur once to a negative Δf, as the animal's EOD rate briefly went lower than the s2 before increasing again. Phase bouncing was observed only in four individuals

(ADA110, MAT101, MAT103, and MAT110), and was seen both in sequence (within the same stimulus presentation) with temporary jamming behavior as well as in isolation.

Twelve fish (across both replicates) preferentially discharged more frequently in the 3 ms either just before (had positive ratios, thus prefer jamming the s2) or just after (had negative ratios, thus prefer being jammed by the s2) the s2 pulse (as indicated by having a z-scores during any one second of stimulation with a magnitude greater than 2). In the first replicate, 68% of these ratios were positive, indicating an overall preference for jamming the s2 pulse rather than being jammed by it. In the second replicate, 93% of the ratios were positive, preferring jamming. Smaller magnitude Δfs (s2 stimuli with EOD rates closer to the subject fishes) showed higher instances of animals having a preference (Figure 4.8).

To distinguish between animals that responded to the stimuli quickly from those that performed JARs late in the stimulus presentation we defined *early* responses as those that started before 275 s2 pulses elapsed (about ¼ of the way through). We then used this criterion to determine each individual's *percentage of early responses* to all negative Δfs (lower rate s2s) as well as to all positive Δfs (higher rate s2s). On average, animals responded early to 85% ± 12.7 of negative Δfs and to 70% ± 23.4 of positive Δfs in Replicate 1, and to 82% ± 12.1 of negative Δfs and to 67% ± 18.8 of positive Δfs in Replicate 2 (Figure 4.9). This difference was statistically significant in Replicate 2, $t(12) = 2.5$, $p = .03$, but just missed significance ($p = .06$) for Replicate 1.

Consistency of early responses was measured by finding the slope of the regression line formed between response start time and Δf. Animals had more consistent (early) start times to negative Δfs (lower EOD rate stimuli) and more graded start times in response to positive Δfs (higher EOD rate stimuli). When the stimulus had a lower EOD rate many animals started their

response within 60 jamming pulses (s2) regardless of the magnitude of the Δf or the amount of resulting change in s1 EOD rate. However, there was certainly more variability for Δf stimuli of −3 and −5 (Figure 4.10). For positive Δfs, animals had a more graded relationship (and more variability between individuals) between magnitude of the Δf (difference in EOD rate between the s1 and s2) and response start time, with smaller differences in EOD rate eliciting faster response times. These relationships (comparing the slopes of the regression line for start time compared to the magnitude of Δf for negative vs. positive Δfs) were significantly different from each other for both Replicate 1, $t(14) = 2.8$, $p = .01$ and Replicate 2, $t(12) = 3.2$, $p = .007$. Table 4.2 contains the slope and R^2 values for the regression lines of each individual.

As a group, animals showed larger overall changes in EOD rate in response to smaller differences in EOD rate between the s1 and s2 (smaller magnitude Δfs) for both negative and positive Δfs (Figure 4.11 a, c). This change generally translated to a stable difference of 3.5–5 Hz between the s2 and s1 EOD rates by the end of the 10 s stimulation period regardless of the starting Δf (Figure 4.11 b, d). The overall change in EOD rate by the animal was significantly affected by the difference between the EOD rates of the s1 and s2 for Replicate 1, $F(7, 98) = 177.0$, $p < .001$ and Replicate 2, $F(7, 84) = 68.5$, $p < .001$. In both cases, pair-wise comparisons showed significant differences ($p < .05$) between all cases except the comparison of Δf −3 and Δf −5 in Replicate 1 and Δf −1 and Δf −2 Replicate 2.

Correlational Analysis. Animals showed consistent behavior across replicates in four of the behaviors measured (a) baseline EOD rate ($r = .71$, $p = .007$), (b) overall change in EOD rate to a Δf of −1($r = .62$, $p = .02$), (c) percentage of early responses to negative Δfs ($r = .66$, $p = .014$), and (d) percentage of early responses to positive Δfs ($r = .55$, $p = .05$).

During Replicate 1, animals with higher baseline EOD rates had larger overall changes in EOD rate when the s2 stimulus was 1 Hz below (Δf of −1) the fish's EOD rate ($r = .60$, $p = .03$). Also in Replicate 1, animals that had more consistent early response start times to all negative Δfs (lower slope values) also had larger overall changes in EOD rate when the s2 stimulus was 5 Hz below (Δf of −5) the fish's EOD rate ($r = -.66$, $p = .007$) as well as when the stimulus was 1 Hz above (Δf of +1) the fish's EOD rate ($r = .59$, $p = .02$). During Replicate 2, animals with larger baseline EOD rates had earlier response start times when the s2 stimulus was 3 Hz below ($r = .-72$, $p = .006$) or 2 Hz below ($r = -.60$, $p = .03$), the fish's own EOD rate and later response times when the s2 was 1 Hz below ($r = .62$, $p = .02$) the fishes own EOD rate. Animals with higher baseline EOD rates also had larger overall change in EOD rate when the s2 signal was 3 Hz below ($r = .63$, $p = .02$), 2 Hz below ($r = .71$, $p = .006$) and 1 Hz below ($r = .63$, $p = .02$) the fish's EOD rate. Individuals with higher baseline EOD rates in Replicate 2 also had a higher percentage of early responses to negative Δfs in Replicate 2 ($r = .56$, $p = .046$). Finally, in Replicate 2 the more jamming events an individual showed, the less likely they were to have early response times to stimuli with higher EOD rates ($r = -.79$, $p = .001$).

There were a few correlations between the *Gymnotus* playback experiment and the JAR experiment that were significant and meaningful (not driven by an extreme outlier). Individuals that had a higher number of preferred jamming events during the first replicate of the JAR experiments also had more electrode approaches at night while the electrodes were inactive ($r = .58$, $p = .05$) and while they were active ($r = .59$, $p = .05$). Also, animals with higher baseline EOD rates in the *Gymnotus* playback experiment had higher baseline EOD rates in the JAR experiment for both Replicate 1 ($r = .59$, $p = .04$), and Replicate 2 ($r = .65$, $p = .04$).

Discussion

Microsternarchus sp. reliably exhibits JARs, increasing their EOD rate in response to stimuli with a lower rate and decreasing their EOD rate in response to stimuli with a higher rate. This supports Prediction 4.2 that *Microsternarchus* would exhibit a typical Gymnotiform JAR. This behavior is functionally important to the fish since maintaining the ability to communicate and electrolocate is important for their survival (Kramer, 1990; Heiligenberg, 1975, 1976, 1986; Westby 1979, 1981). This is likely why the amount of individual variation seemed much lower in this experiment than in other experiments. In addition to simply shifting their rate away from the stimulus, other types of EOD strategies were also seen in this species, although they were infrequent. These behaviors (i.e. phase skipping, temporary jamming, and phase bouncing) have been observed in other pulse species as well (Braun, 2009; Heiligenberg, 1974).

Consistent individual differences in JARs can be identified in this species as evidenced by significant correlations across replicates between the percentage of early responses to both higher and lower rate stimuli, as well as in the overall change in EOD rate to a Δf of -1. The other variables measured did not show significant correlations across replicates, but this could be the result of minimal variation in behavior due to the stereotypical nature of the JAR.

We predicted that individuals would vary consistently in their degree of responsiveness to jamming stimuli, organized around an aggressive response or dominance assertion, such that individuals with larger magnitude of JARs (particularly to lower rate stimuli) would also intentionally jam the stimulus more frequently. This exact correlation was not seen, however other correlations did suggest consistent individual variation in JARs, but not necessarily organized around aggressive or dominant behavior. For example animals with more consistently early response start times had larger responses to a few of the jamming stimuli. Consistency of early responses was measured by the slope of the regression lines formed between response start

time and Δf. Individuals with smaller slopes responded consistently early to all Δfs, while individuals with larger slopes show increasing latency to respond to larger Δfs (since all animals displayed early start times in response to a Δf of −1 or +1). This correlation may relate more to an individual's level of responsiveness than to aggression, similar to the correlations found in Chapter 3 between escape time and novelty response amplitude (this will be analyzed further in Chapter 5).

Several studies have shown a direct relationship between discharge rate and dominance status (Dunlap, 2002; Westby, 1975b). Our results indicated that animals with higher baseline EOD rates increased their EOD rates the most (have larger JARs) in response to a stimulus with an EOD rate just below its own. If having a higher EOD rate is a sign of dominance, animals that show the largest increase in their own rates could be signaling their dominance over the foreign fish, with foreigners with the smallest difference in EOD rate being the most threatening. An alternate explanation would be that animals with higher baseline rates have a greater likelihood of discharge at higher rates anyway and are thus more likely to show larger increases. However, if this were the case, one might expect to find that fish with lower baseline rates would show larger *decreases* in EOD rate in response to a stimulus with a higher EOD rate. We did not find this relationship: baseline rate did not correlate with how much animals decreased their rates in response to a stimulus with higher EOD rates. Furthermore, this correlation is in the opposite direction of those we have seen in our other experiments where individuals with the lowest EOD rates had the largest changes in EOD rate in response to stimulation. Therefore, using EOD rate as a signal for dominance seems to be the most parsimonious conclusion for our results.

General Discussion

Jamming interference (intentionally jamming another fish or signal) has been proposed as a way to communicate relative dominance status (Westby, 1979) or as an aggressive display (Tallarovic & Zakon, 2005) in other species of weakly electric fish (*Gymnotus carapo* and *Apteronotus leptorhynchus*). When simulating an intruder in an individual's home tank, the resident will intentionally jam the intruder if the intruder has a higher EOD rate (and, thus, a positive Δf) (Tallarovic & Zakon, 2005).The fish in our sample seemed to show some consistency with this, in that the temporary jamming behaviors that we observed were seen only in response to an s2 signal with a higher EOD rate (Δf of +1)

Westby (1979) theorizes that the fish with the higher discharge rate has *frequency dominance* because all coincidences are predictable for the higher EOD rate partner. That is, the s2 advances in phase with each successive EOD, allowing its future coincidence to be predicted. This may explain why fish have more consistent early response start times when the stimulus has a lower EOD rate compared to when it has a higher EOD rate. Having a lower EOD rate means the fish gets blindsided by each pulse. Therefore, when the number of coincidences is high (as it is smaller differences between the two EOD rates), the fish may be more motivated to change its EOD rate rapidly to avoid being jammed unpredictably compared to when the number of coincidences is lower.

Based on these ideas we predicted that individuals that had the most instances of jamming the stimulus would also have more electrode approaches during *Gymnotus* playback as these are the most agonistic behaviors in each experiment. In fact, our data supported this prediction with a significant correlation between these two behaviors. Animals that approached the electrodes more often also had more instances of jamming the simulated fish (s2) as

measured by jammed/jamming ratios. This indicates that a behavioral syndrome for aggression likely exists in this species, potentially caused by underlying differences in testosterone levels. Individual differences in testosterone have been linked to differences in aggression level in other species (Duckworth, 2006; Kabelik, Weiss & Moore, 2008, While et al., 2009). Further work is required to determine if individual differences in testosterone covary with individual differences in aggressive behavior in this species.

Table 4.1

Correlation coefficients for comparisons within the Gymnotus playback experiment.

Measure:	*r*:
Time Out of Shelter- Night Not Playing:	
Time Out of Shelter- Night Playing	.912***
Change in Rate at Night Stimulus Playing vs. Not	.903***
EOD Rate at Night with Stimulus Not Playing	.575*
EOD Rate at Night with Stimulus Playing	.645*
Time Out of Shelter- Night Playing:	
Change in Rate at Night Stimulus Playing vs. Not	.903***
EOD Rate at Night with Stimulus Playing	.542*
Electrode Approaches at Night with Stimulus Playing	-.744**
Electrode Approaches at Night with Stimulus Playing	
Change in Time out of Shelter at Night Playing vs. Not	-.673**
Change in Rate at Night Stimulus Playing vs Not	
EOD Rate at Night with Stimulus Not Playing	.644**
EOD Rate at Night with Stimulus Playing	.739**
EOD Rate at Night with Stimulus Not Playing	
EOD Rate at Night with Stimulus Playing	.994***
Absolute Value of Change in Rate at Night Playing vs Not	-.681**
EOD Rate at Night with Stimulus Playing	
Absolute Value of Change in Rate at Night Playing vs Not	-.706**

Note. ** $p < .01$ *** $p < .001$

Table 4.2

Slope of the regression line and corresponding R^2 for JAR start time vs. Δf by individual.

	Replicate 1				Replicate 2			
	-Δf		+Δf		-Δf		+Δf	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
ADA101	3.63	0.65	103.10	0.98	-1.77	0.67	107.94	0.71
ADA102	43.00	0.90	60.60	0.89	87.84	0.86	65.70	0.80
ADA105	58.69	0.74	175.54	0.76	39.09	0.48	193.66	0.81
ADA107	22.50	0.65	43.73	0.79	-	-	-	-
ADA110	29.26	0.89	47.93	0.47	8.86	0.43	90.23	0.87
LAU102	7.17	0.81	14.20	0.87	101.77	0.79	11.26	0.84
LAU103	12.37	0.57	39.31	0.55	10.00	0.95	145.34	0.92
LAU105	42.57	0.92	0.74	0.97	-	-	-	-
MAT101	65.66	0.57	19.31	0.71	-7.91	0.22	87.97	0.75
MAT103	2.29	0.00	85.60	0.84	150.54	0.75	123.50	0.79
MAT104	151.66	0.78	97.29	0.54	-0.31	0.00	96.53	0.57
MAT107	25.46	0.02	141.46	0.81	-19.06	0.02	143.14	0.80
MAT108	3.97	0.81	198.00	1.00	39.80	0.66	215.75	0.95
MAT109	3.64	0.30	130.60	0.84	14.71	0.76	125.84	0.75
MAT110	-0.73	0.02	133.10	0.84	15.60	0.91	9.19	0.73

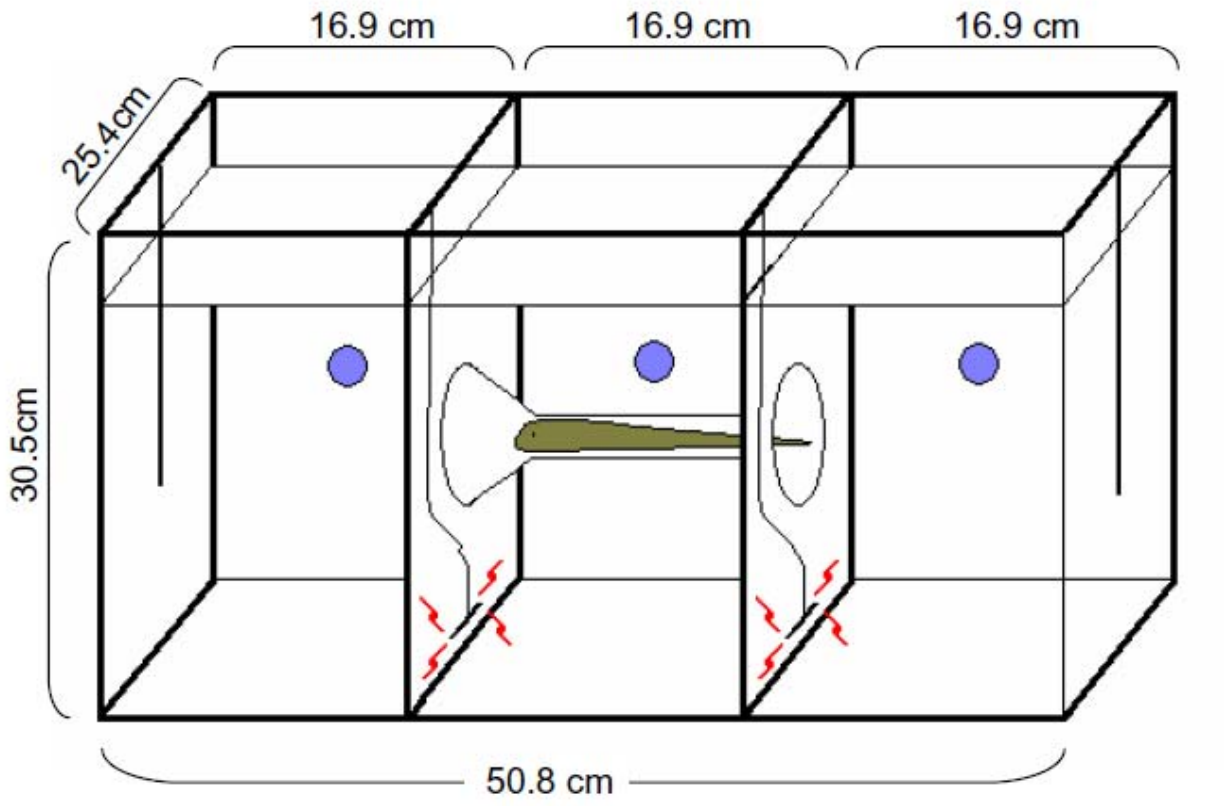


Figure 4.1 Diagram of the *Gymnotus* playback experimental tank with dimensions. Playback electrodes are located beneath the tube on both sides. The purple circles are indicative of the position of the IR-LED indicator lights. The outer two illuminated during (and on the side with) stimulus playback while the center light illuminated at the start of the playback rate modulation. The IR-video was recorded from the illustrated perspective.

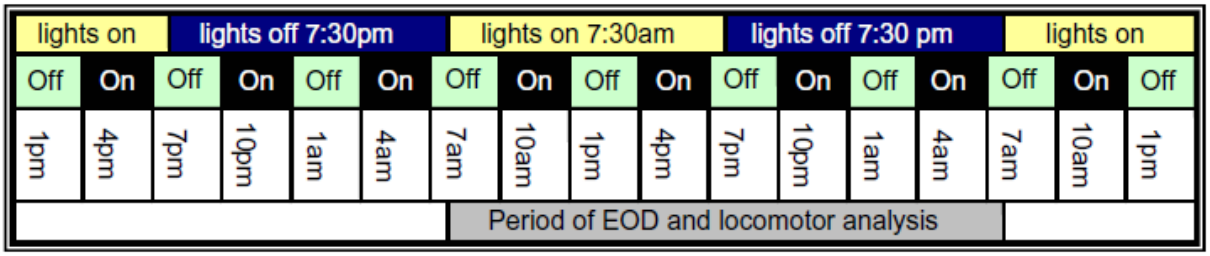


Figure 4.2. Timeline of *Gymnotus* playback presentation and data analysis. Playback alternated between *intermittent* (labeled *On* in the figure for brevity) and *silent* (labeled *Off*) every 3 hours. Intermittent playback included 5 min of playback followed by 4, 5, or 6 min of silence, repeated for the entire 3 hours. The period used for analysis of EOD rate and locomotor activity is indicated by the grey bar.

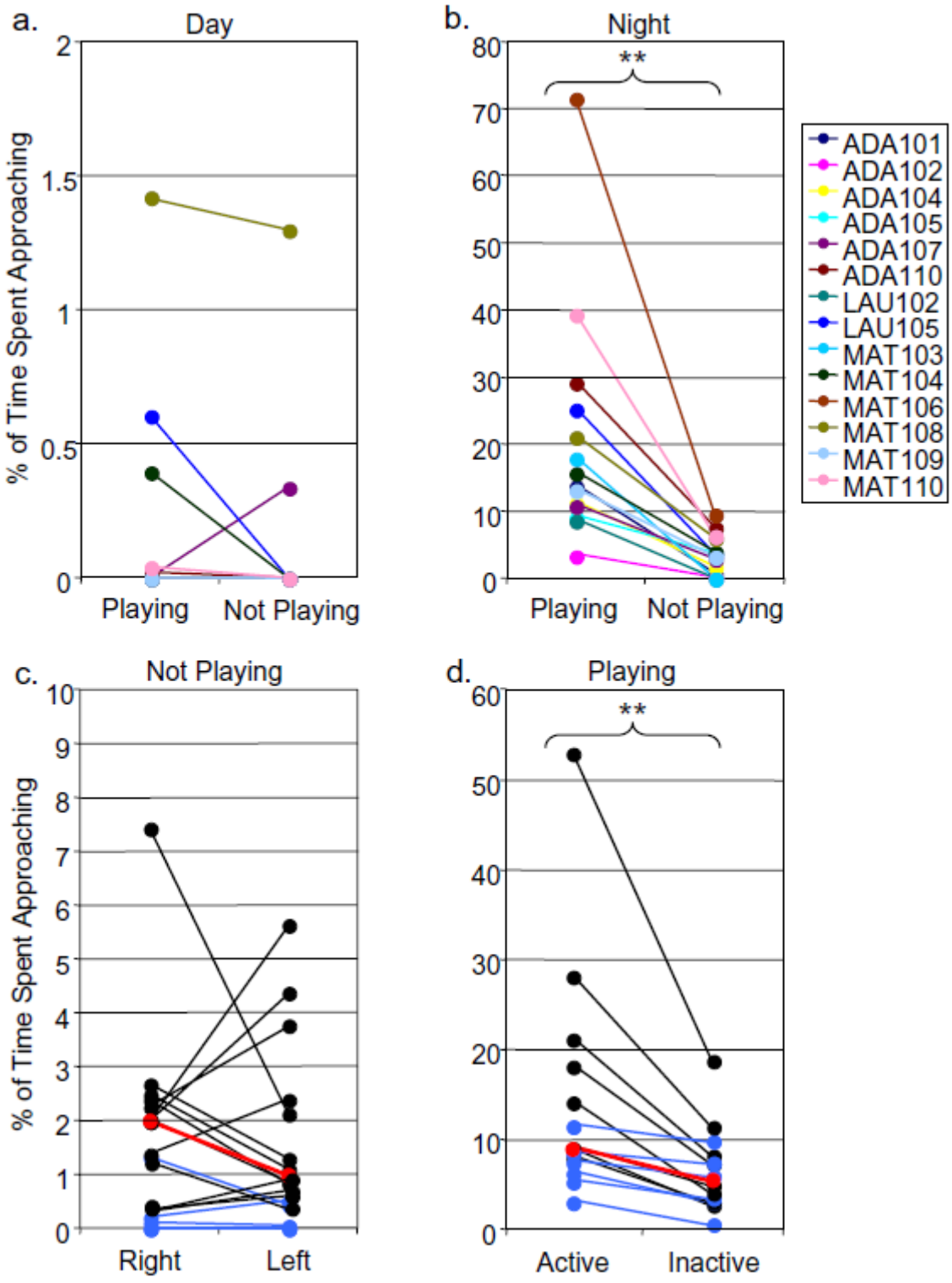

a.
Day
% of Time Spent Approaching
2
1.5
1
0.5
0
Playing
Not Playing
b.
Night
**
80
70
60
50
40
30
20
10
0
Playing
Not Playing
ADA101
ADA102
ADA104
ADA105
ADA107
ADA110
LAU102
LAU105
MAT103
MAT104
MAT106
MAT108
MAT109
MAT110
c.
Not Playing
% of Time Spent Approaching
10
9
8
7
6
5
4
3
2
1
0
Right
Left
d.
Playing
**
60
50
40
30
20
10
0
Active
Inactive

Figure 4.3. Percentage of observed time animals spent approaching the playback electrodes during the *Gymnotus* playback experiment. Upper panel compares when the stimulus was playing and when it was not playing during the day (a) and at night (b). Percentage of observed time spent approaching the left or right electrode during periods without playback (c) or approaching the active or inactive electrode during playback periods (d). The median is shown in red. Individuals that showed less discrimination between electrode conditions than the median are figured in blue, and those that showed greater discrimination are figured in black.

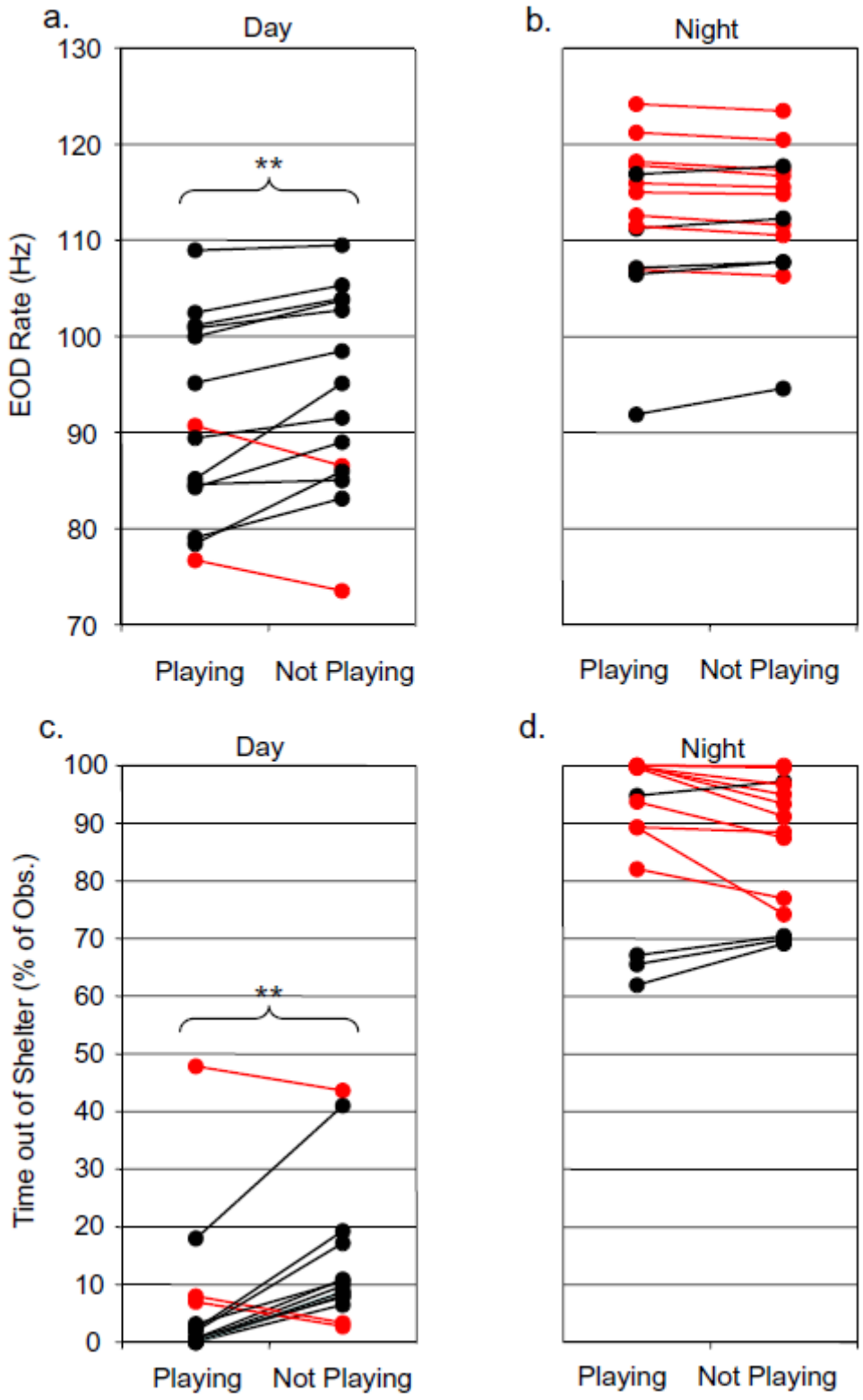

a.
Day
130
120
110
100
90
80
70
EOD Rate (Hz)
**
Playing
Not Playing
b.
Night
Playing
Not Playing
c.
Day
100
90
80
70
60
50
40
30
20
10
0
Time out of Shelter (% of Obs.)
**
Playing
Not Playing
d.
Night
Playing
Not Playing

Figure 4.4. The effect of *Gymnotus* playback on EOD rate and locomotor activity differs between night and day. EOD rate during the day (a) and at night, (b) while the stimulus was playing or silent. Red lines indicate individuals that had higher EOD rates when the stimulus was playing (the majority of animals during the day). Black lines indicate animals that had lower EOD rates while the stimulus was playing (the majority of animals at night). Time out of shelter, in percentage of observations, during the day (c) and night (d) while the stimulus was playing or silent. Red lines indicate individuals that had higher percentage of time out when the stimulus was playing (the majority of animals during the day). Black lines indicate animals that had lower percentage of time out when the stimulus was playing (the majority of animals at night).

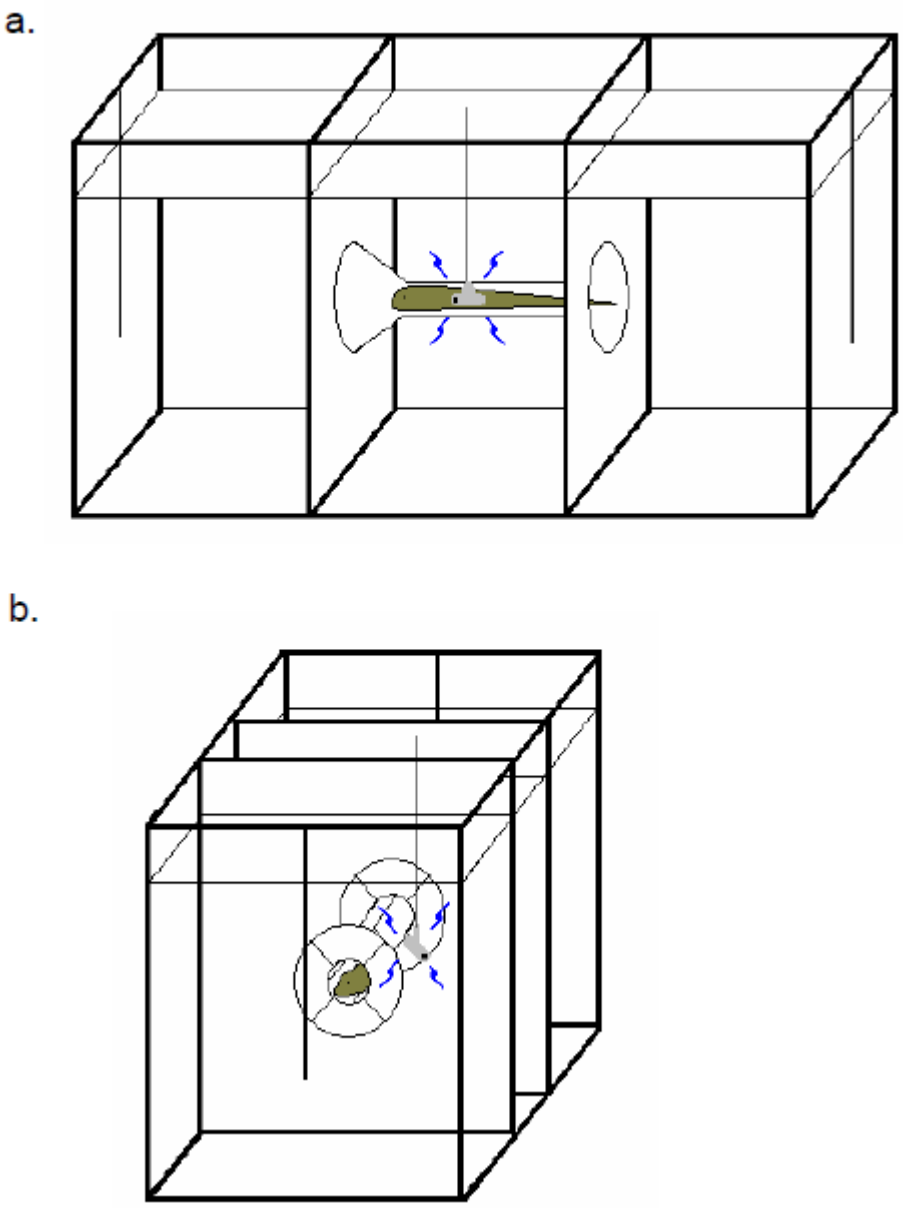


Figure 4.5. Diagram of the jamming avoidance response experimental tank from the side (a) and front (b). The playback electrode was positioned next to the tube at an angle that allowed for a small observable artifact to remain in the recorded signal.

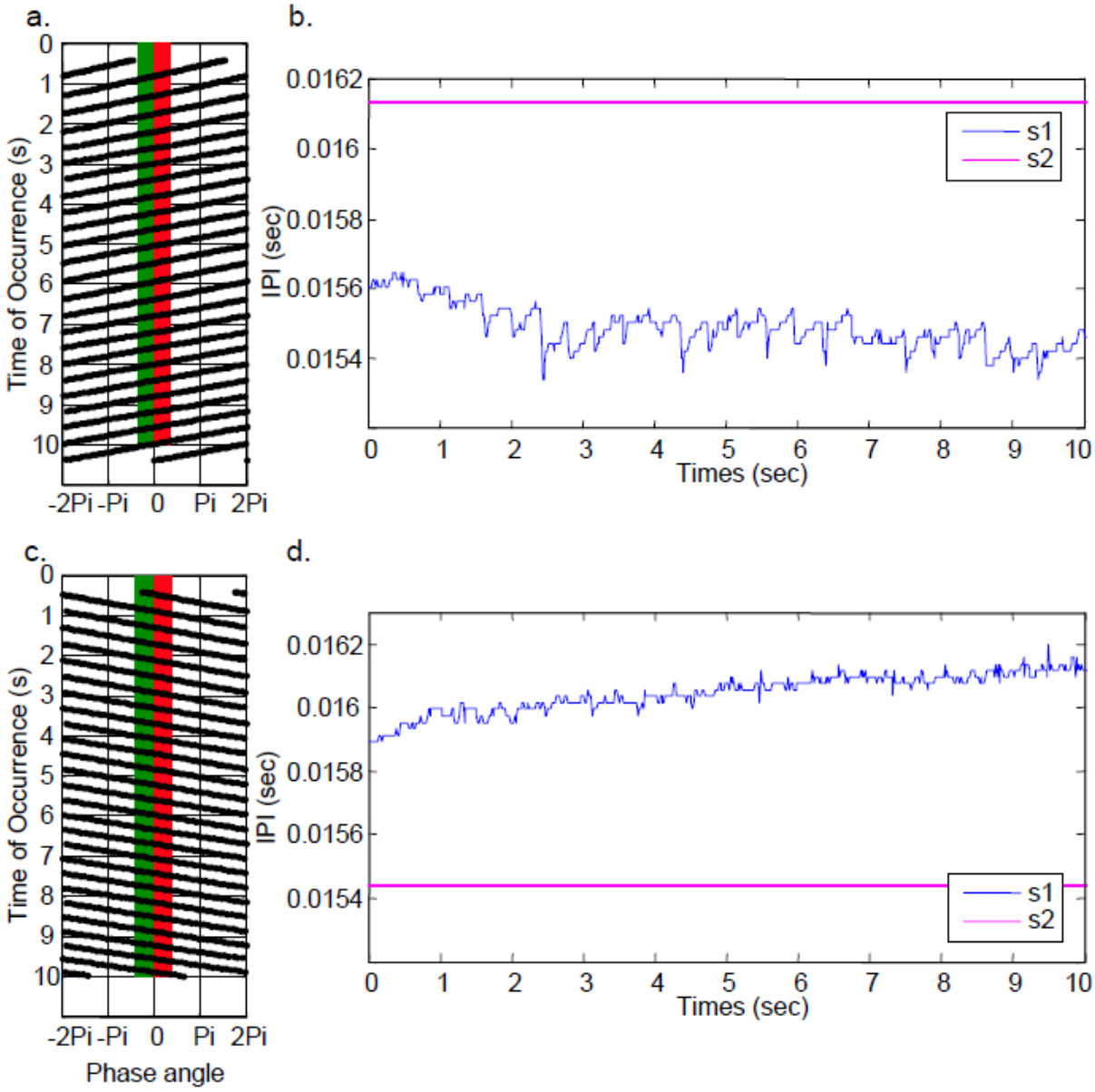


Figure 4.6. Typical jamming avoidance response during stimulus presentations. Graphs on the left (a, c) depict s1 EODs as a function of phase angle relative to s2. Time is plotted on the y-axis and phase angle relative to s2 on the x-axis. Note that two complete cycles are shown. The s2

EOD (zero phase) is presented at the center of the plot. The green shaded area indicates 3 ms just prior to the s2. The red shaded area indicates the 3 ms after s2. Plots on the right (b, d) are the same data plotted in terms of IPI (s) relative to time (s). Note that IPI is the inverse of EOD rate. The s1 is shown in blue. The s2 is shown in magenta. In the phase plots, when the subject discharges at a higher EOD rate (smaller IPI) than the stimulus (negative Δf) consecutive intervals appear successively from right to left displaying a positive slope in time (a). When the subject discharges at a lower rate (larger IPI) than the stimulus (positive Δf) consecutive intervals appear successively from left to right displaying a negative slope (c).

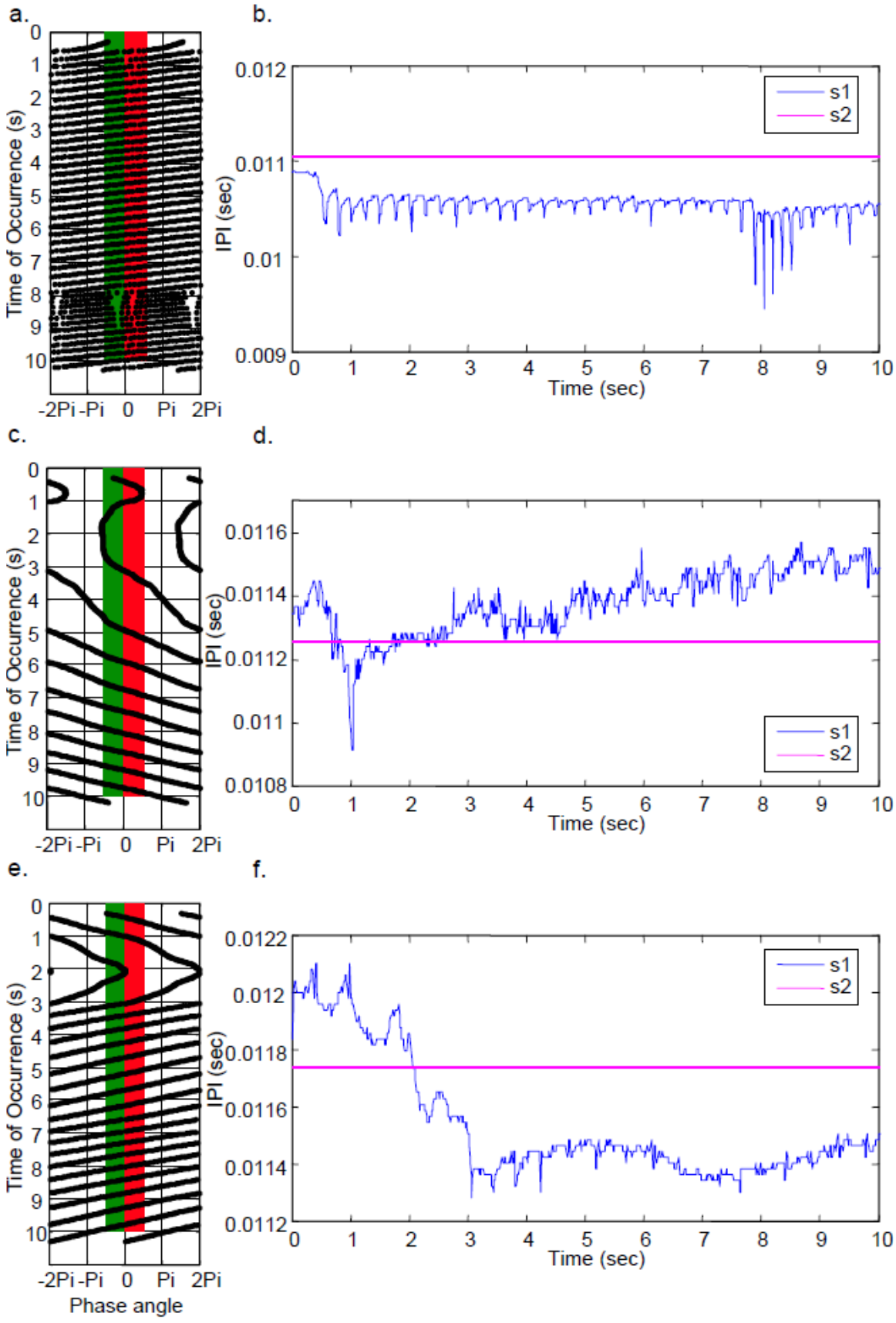
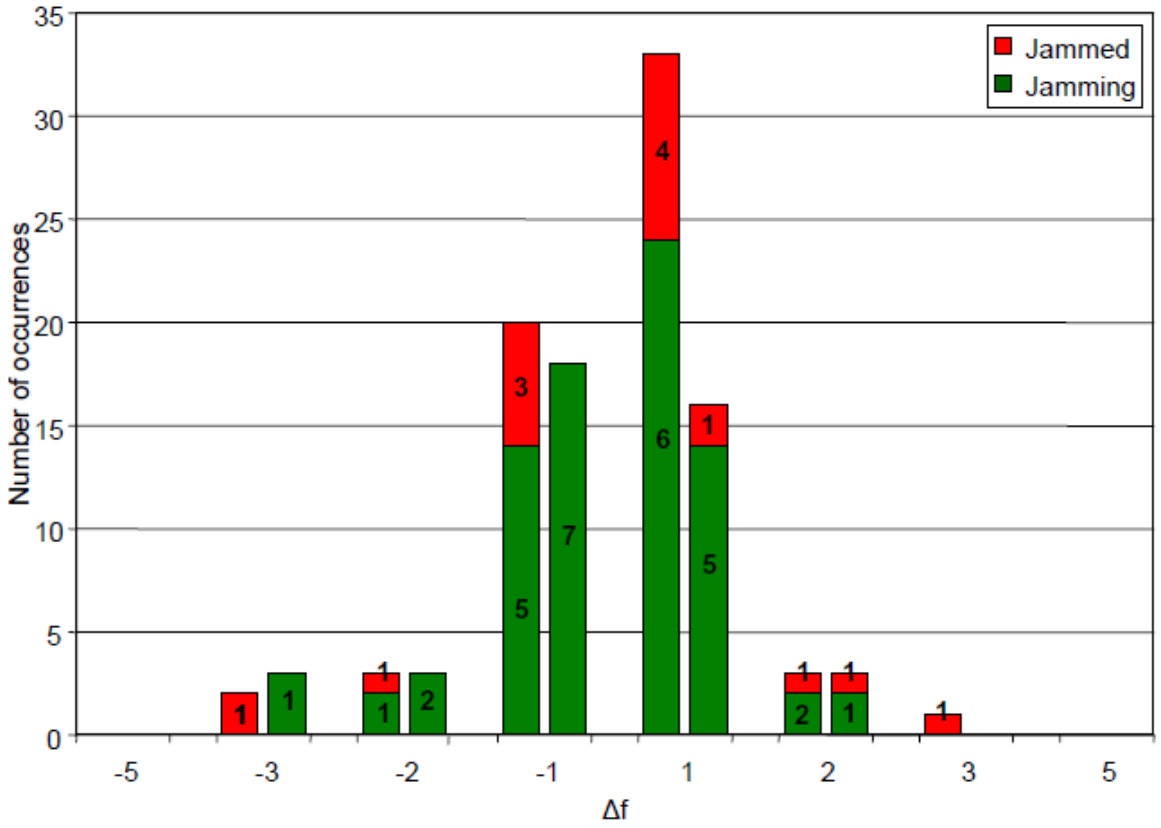

a.
b.
c.
d.
e.
f.
Time of Occurrence (s)
IPI (sec)
Time (sec)
Phase angle
-2Pi -Pi 0 Pi 2Pi
s1
s2

Figure 4.7. Other EOD strategies in response to the stimulus presentation. Plots to the left (a, c, e) show s1 EODs as a function of phase angle relative to s2. Plots to the right (b, d, f) show the same data plotted in terms of IPI relative to time (for more details see Figure 4.4). Phase skipping (a, b). Around 8 s into the presentation, the animal demonstrates phase skipping as successive points appear to jump to the left in the phase plot, also coinciding with an increase in EOD rate (increases in Δf appear as increasing vertical density) (a). In the IPI plot (b), this behavior is visible by large, rapid decreases in the IPI. Temporary Jamming (c, d). Following the first second of the s2 presentation, the animal places its pulse about 3 ms prior to the s2 EOD, as seen in the phase plot (c), potentially jamming the s2. In the IPI plot (d), this behavior appears as the s1 overlaying the s2 as Δf during this time is near-zero. Phase Bouncing (e, f). In the phase plot (e), this is visible as the animal approaching the s2 from the right and briefly passing through it before reversing its relationship to the s2 continually increasing its EOD rate. In the IPI plot (f), this is visible as the s1 is approaching the s2 (increasing s1 EOD rate) and further passing it to obtain an EOD rate that is higher (lower IPI) than the s2. Note that this case is one where the animal changed its EOD rate relationship with s2 (going from a lower to a higher EOD rate).



	-5		-3		-2		-1		1		2		3		5	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
ADA101							0/4			2/0						
ADA102									3/1	3/0						
ADA105					2/0			2/0								
ADA107*																
ADA110									5/2							
LAU102							1/0	0/1								
LAU103						1/0	0/1	2/0			1/0					
LAU105*							0/1									
MAT101							4/0	3/0	2/1	3/0						
MAT103																
MAT104			0/2		0/1		5/0	1/0	3/0	3/0		2/1				
MAT107							1/0	1/1								
MAT108				3/0		2/0		5/0	4/0	3/0						
MAT109										0/2						
MAT110							3/0	4/0	6/5		1/1		0/1			

Figure 4.8. Frequency histogram of instances of preferred jamming (green bars) or being jammed (red bars) during the jamming avoidance response experiment. Graph shows Replicate 1 (left bar of each pair) and Replicate 2 (right bar of each pair) across each Δf (see text for explanation of jamming definitions). Numbers within the bars indicate the number of animals that contributed to each bar. The y-value indicates the total number of occurrences. The table below the graph shows how each individual performed across each Δf and replicate. Green values indicate instances of preferred jamming. Red values indicate instances of being jammed. Individuals marked with an * did not participate in a second replicate.

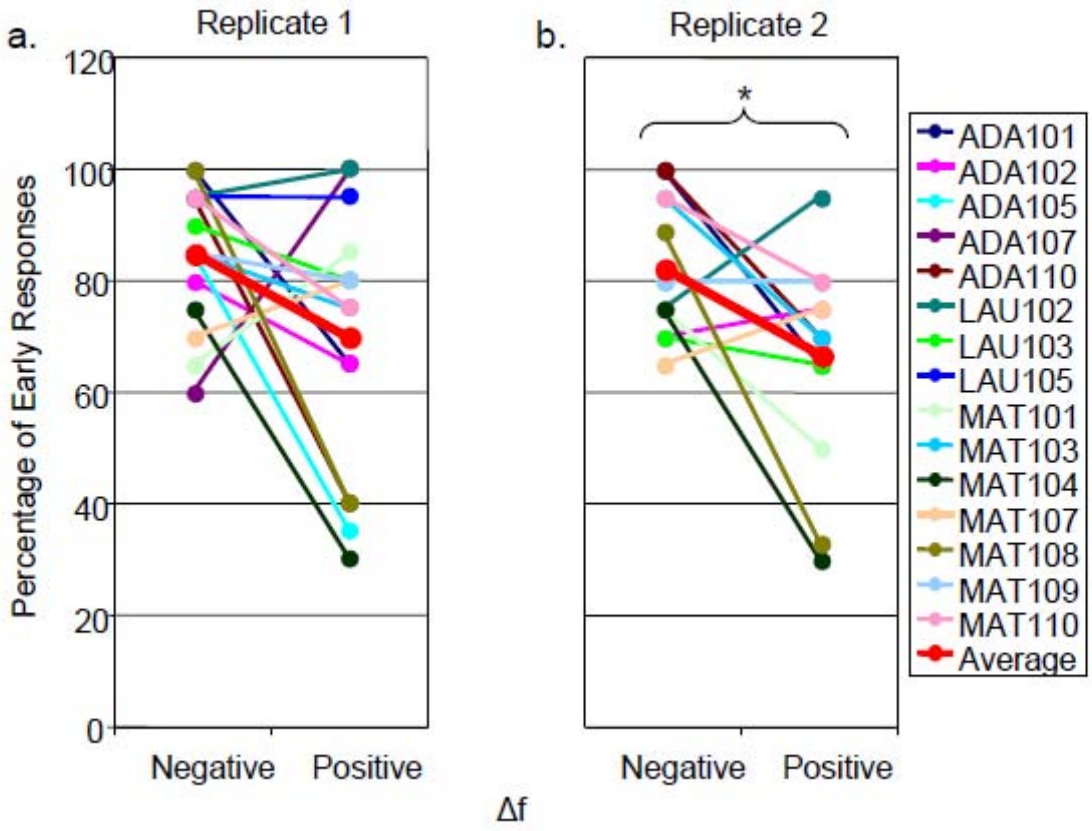
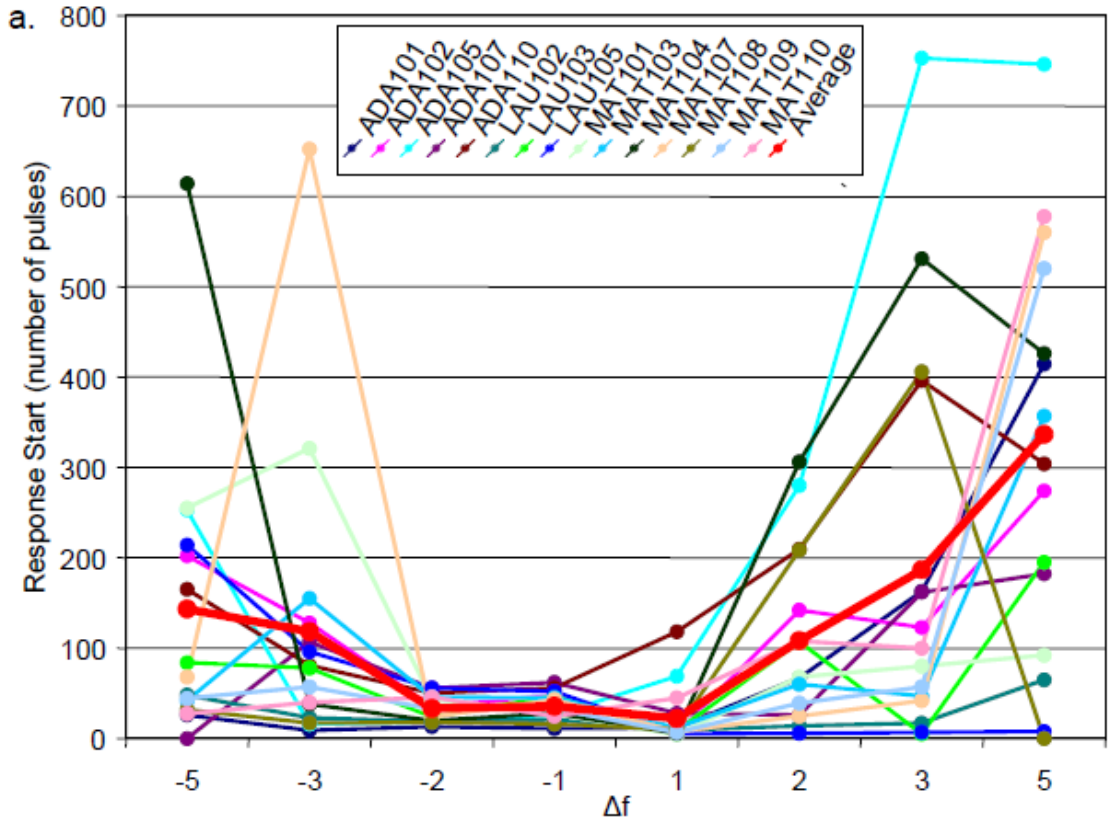


Figure 4.9. The percentage of early responses during the jamming avoidance response experiments. Early response percentage (% of responses that occurred before 275 s2 pulses elapsed) is plotted for both negative and positive Δfs for all individuals in Replicate 1 (a) and Replicate 2 (b). The thicker red line is the average of all individuals.


a.
800
700
600
500
400
300
200
100
0
Response Start (number of pulses)
ADA101
ADA102
ADA105
ADA107
ADA110
LAU102
LAU103
LAU105
MAT101
MAT103
MAT104
MAT107
MAT108
MAT109
MAT110
Average
-5
-3
-2
-1
1
2
3
5
Δf

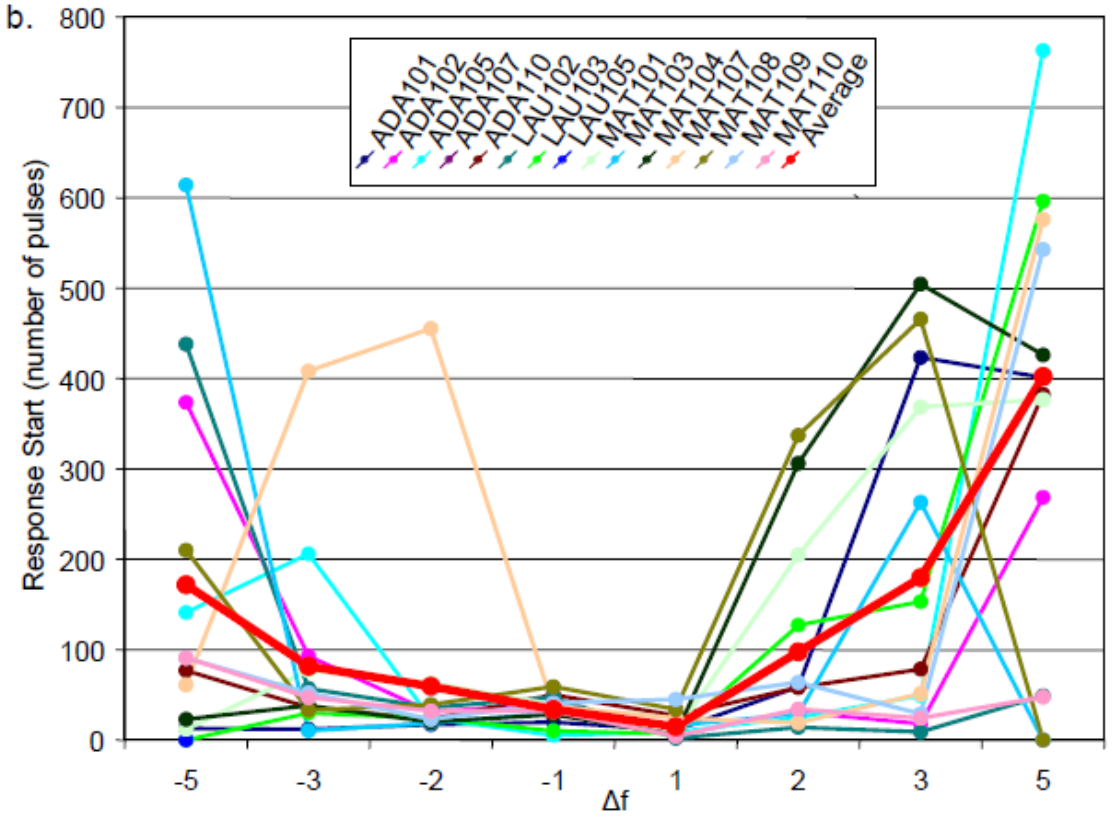

b.
800
700
600
500
400
300
200
100
0
Response Start (number of pulses)
ADA101
ADA102
ADA105
ADA107
ADA110
LAU102
LAU103
LAU105
MAT101
MAT103
MAT104
MAT107
MAT108
MAT109
MAT110
Average
-5
-3
-2
-1
1
2
3
5
Δf

Figure 4.10. Jamming avoidance response start times for all individuals by stimulus type. Response start (in number of EODs) is plotted at each Δf for each individual in Replicate 1 (a) and Replicate 2 (b). The thicker red line is the average for all individuals in each replicate.

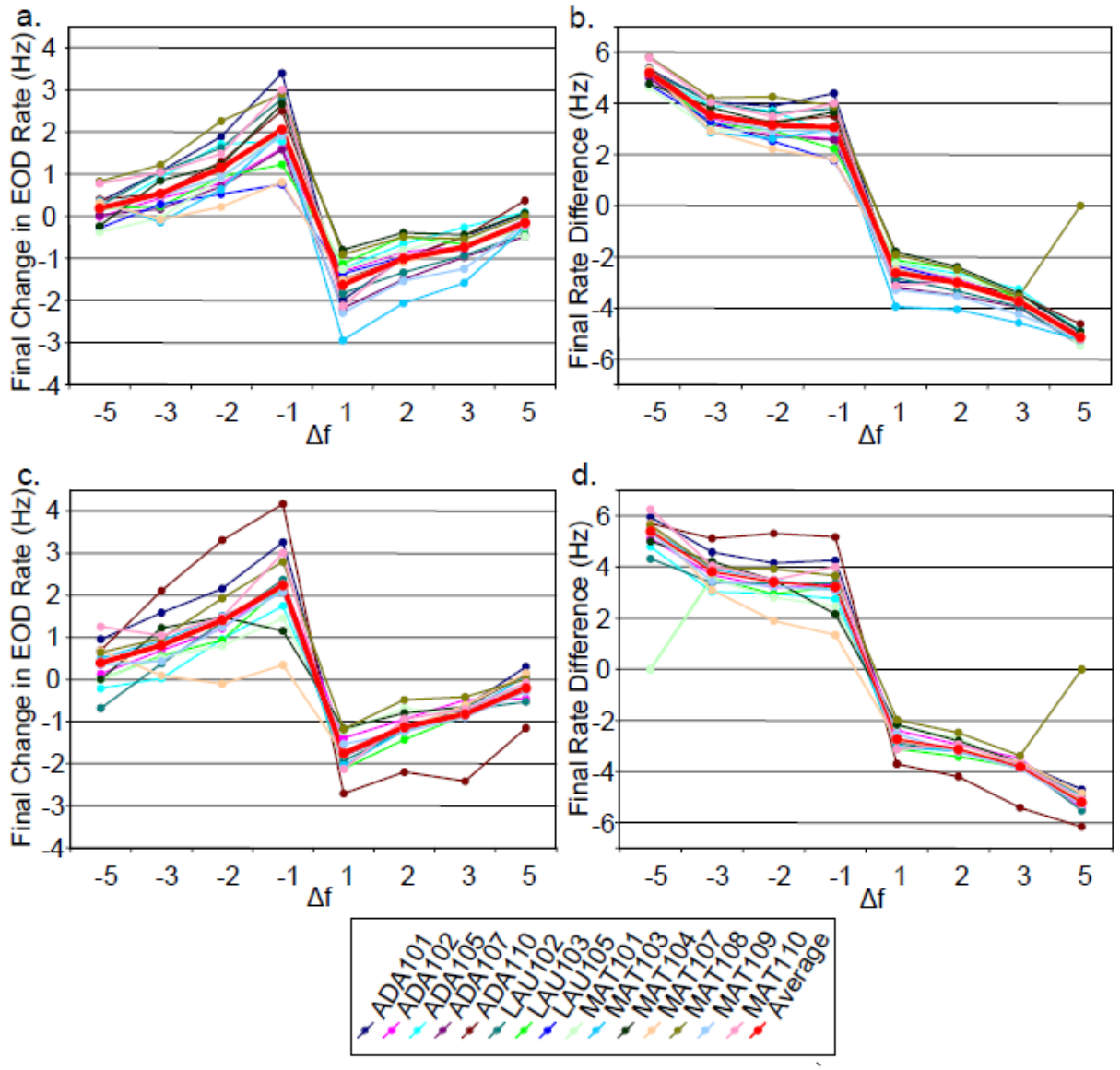


Figure 4.11. Comparison of the final change and the final difference in the subjects EOD rate following 10 s of stimulus presentation. Final change in s1 EOD rate (imposed Δf − mean difference in EOD rate during the final second of presentation between the subject's EOD and s2) for each Δf during Replicate 1 (a) and Replicate 2 (c). The same data, plotted as the resulting difference in EOD rate during the final second between the subject's EOD and s2 for each Δf during Replicate 1 (b) and Replicate 2 (d). Individual animals are indicated by separate lines, with the thicker red line being the average for all individuals.

Chapter 5:

Behavioral Syndromes in *Microsternarchus* sp.

Introduction

Research on behavioral syndromes must meet at least two conditions: (a) individuals must vary in their behavior, and (b) individuals must be measured more than once (Bell, 2007). This is achieved by presenting a cohort of animals with a battery of tests and then determining if individual variation is consistent across the behaviors measured in different tests. While the measurement of a large number of variables is preferred, this requires more sophisticated techniques for appropriate analysis. Correlations are frequently used to evaluate the individual consistency, and having a large number of variables increases the probability that significance will be found erroneously at least once. For example, 15 measured variables will yield a total of 120 comparisons. Using an alpha of .05, six correlations could be significant by chance alone. While adjusting alpha based on the number of tests run is a possibility, more sophisticated techniques are preferred (Bell, 2007; Budaev, 2010).

Principal components analysis (PCA) and factor analysis are two types of exploratory data analysis and are among some of the most widely used statistical procedures in psychological research (Budaev, 2010; Fabrigar, Wegener, MacCallum, & Strahan, 1999). Unlike other statistical tests (e.g., correlations, t-tests, analysis of variance), PCA and factor analysis are generally used to summarize data and generate hypotheses as opposed to testing them. PCA specifically, is used for data reduction—transforming multiple variables into a smaller set of abstract variables (principal components) that account for some majority of the variance within the original data (Gorsuch, 1983). To calculate the principal components, the variables are first correlated and the correlation matrix is subject to specific transformations (Budaev, 2010;

Gorsuch, 1983). These transformations result in a set of linear combinations (the principal components) that each account for a decreasing proportion of the original variance in the data set. A new set of scores can then be created from the correlations between the original variables and each of the principal components. This new set of scores (created from each principal component for each individual in the data set) can then be substituted for the original variables in subsequent statistical tests (Budaev, 2010).

Factor analysis, while similar to PCA, is different type of exploratory analysis that seeks to uncover the latent constructs behind the correlations between variables tested (Budaev, 2010). Factor analysis uses more complex calculations and has an extra factor to account for both random variation between variables and latent factors that may account for variation in only one variable in the set (Fabrigar et al., 1999; Gorsuch, 1983). For this reason, factor analysis is the more appropriate analysis when the researcher is seeking to identify the underlying dimensions of animal personality or behavioral syndromes. Due to the higher degree of complexity of the analysis, very large sample sizes (minimum of 100) and low variable to subject ratios (around 1:5; Gorsuch, 1983) are required in most cases (Budaev, 2010).

To evaluate *Microsternarchus* sp. for behavioral syndromes, we ran a cohort of 22 animals through a series of five experiments (four of which were repeated) over the course of two years. While our data have a total sample size of 22, due to the death or unavailability of certain individuals at different times, we have a *complete* set of data from all experiments and all replicates for only seven individuals. Combining all experiments, we have well over 100 variables, some of which do not pass tests for normality (with little likelihood of effective transformation). Factor analysis under these conditions is strictly forbidden (Gorsuch, 1983). Simply performing correlation analysis on all variables would yield over 5,050 comparisons

which would require a Bonferroni corrected alpha of .0000099. It would thus be rather unlikely to achieve significant findings, given that our sample size is between seven and 19 depending on the comparison. Therefore, given these constraints, the best option is to perform PCA on specific subsets of the variables and then use correlations between the retained principal components to test specific hypothesis about behavioral syndromes. Reducing the number of statistical tests will reduce our cumulative chance of a Type 1 error.

Specific Aim 5.1: to combine the data from individual experiments to determine if individual variability seen within behaviors from each experiment correlate to form behavioral syndromes.

Prediction 5.1: Individual variation will organize into syndromes for activity, reactivity, and aggression/dominance, as evidenced by significant correlations between the principal components from different experiments.

Methods

Following the completion of all experiments, PCAs were conducted. Variables chosen for inclusion in each individual analysis were based on how well they related to each other conceptually and how well the principal components extracted could explain the variation between them. JAR start time (Chapter 4, Experiment 2) was not well suited for PCA because of the extremely non-normal distribution, therefore data were reduced by finding the slope of the regression line formed between response start time and the magnitude of the rate difference (Δf), this calculation reduced 16 variables to 4. Principal components were retained when they explained a large majority of the variation in the data, in most cases, one component was retained but occasionally a second component explained an equal amount of variation and thus it was retained also. Variable loadings (listed with the results), explained how strongly and in what

direction (positive or negative) a particular variable loaded on a principal component. The higher the eigenvector loading the more variation explained by the principal component. Positive loadings indicated a direct relationship between the PC and the original variable (positive correlation), while negative loadings indicated an inverse relationship (negative correlation).

After conducting the PCAs, we analyzed correlations between the extracted principal components, (along with a few other variables in instances in which PCA was not appropriate).

We compared rankings of individuals across principal components or variables (when PCA was not appropriate), by normalizing the principal component scores or variable scores with respect to the highest and lowest scoring individuals. Individuals were thus given a score between zero and one depending on the range of scores within that component. For two principal components (PB_Dynamics_PC1 and PB_Approach_PC1), scores were normalized to the second highest or second lowest score when one individual caused all other scores to be skewed or clustered at one end of the scale. In these instances the extreme animal has a score that was greater than one or less than zero.

Traditionally, principal components have been labeled PC1, PC2 etc. because interpretation of the components is not considered appropriate when performing PCA. This naming strategy only works when a single PCA is performed. In our case we performed multiple PCAs and thus have developed a naming scheme to label each *analysis* in a way that is descriptive of the variables within the analysis and then add PC1, or PC2 to the name of the analysis. The following formula is used to name the *analysis*: *EX_description*, where *EX* is a code to represent the experiment name (FE for free exploration, TC for terrestrial challenge, NR for novelty response, PB for *Gymnotus* playback and JAR for jamming avoidance response experiments) and *description* is a word that attempts to describe the variables within the analysis.

PC1 or PC2 is then added to the analysis name to label the *components* retained from the analysis. For example, *FE_Rate* refers to the analysis of all the EOD rate variables from the free exploration experiment.

Finally, we analyzed baseline EOD rate across experiments and replicates (with the exception of the terrestrial challenge since rate information was not available for this experiment) Correlations and a general linear mixed model were computed to determine if there were any effects of home tank, collection location, experiment or replicate on baseline rates.

Statistics were performed in SPSS (PCA and correlations) and JMP (GLMM) using an alpha of .05. No adjustments were made to the alpha when performing multiple correlations. It is our assertion that performing PCA has sufficiently reduced our cumulative chance of Type 1 error; any further adjustments would excessively limit our ability to draw conclusions.

Results

Principal Components Analysis

The analysis of the free exploration experiment (Chapter 1, Experiment 1) included three different PCAs and reduced 20 variables to four. The first analysis, which we refer to as FE_Rate included all the variables pertaining to EOD rate from both replicates (i.e., average day rate, average night rate, average in rate, average out rate), and the first component (FE_Rate_PC1) from this analysis explained 67.7% of the total variation in the data (eigenvalue of 5.42). All variables loaded positively on this component, meaning they have a positive relationship with the extracted component (see Table 5.1 for eigenvector loadings).

The second analysis included all of the variables pertaining to *changes* in EOD rate across sampling periods from both replicates (i.e., change in rate day vs. night, change in rate in vs. out of the shelter, change in rate in vs. out during the day, and change in rate in vs. out at

night) and was labeled FE_Dynamics. The first component (FE_Dynamics_PC1) accounted for 57.1% of the variation in the data (eigenvalue of 4.57), and all variables loaded positively on this component.

The final analysis from this experiment (FE_Activity) included variables for the percentage of time spent active during both replicates (i.e., percentage of the day spent active and percentage of the night spent active), and two components were retained that explained a total of 87% of the variation in the data. The first component (FE_Activity_PC1) explained 45.2% of the variance in the data (eigenvalue of 1.81). Percentage of the day spent active (in both replicates) loaded positively on this component, while the percentage of the night spent active (in both replicates) loaded negatively. Since the percentage of the night spent active (in both replicates) loaded much more strongly on Component 2, we saved both components from this analysis. Component 2 (FE_Activity_PC2) accounted for 41.9 % of the variance in the data, and all variables loaded positively.

The data from the terrestrial challenge experiment (Chapter 3, Experiment 1) were submitted to two different PCAs reducing a total of six variables to two. Due to the low level of consistency between replicates, we performed one PCA on variables from Replicate 1 (TC_Rep1) and a second PCA on the variables from Replicate 2 (TC_Rep2). The variable for swimming in the bucket had very low variability, since nearly all animals were not swimming in the bucket after the 5-min rest period; therefore, we excluded this variable from the PCAs. For the first replicate, one component explained 63.2 % of the variance (eigenvalue of 1.9). Time to escape the platform loaded negatively, while the other two variables (moving on the platform and struggling in the net) loaded positively (therefore, high values on this component imply short escape times, more struggling in the net, and more moving on the platform) We will

subsequently refer to this component as TC_Rep1_PC1. For the second replicate, one component (TC_Rep2_PC1) explained 54.1% of the variation in the data (eigenvalue of 1.6) and, once again, time to escape the platform loaded negatively while the other two variables loaded positively.

Two PCAs were performed on variables from the novelty response experiments (Chapter 3, Experiment 2) which reduced 12 variables to 2. The first analysis (NR_Responsivness) included all variables from both replicates except those pertaining to habituation measures (i.e., baseline rate, percentage large responses, percentage long responses, average duration, and average amplitude). The first principal component of NR_Responsiveness explained 77.5 % of the variance in these data (eigenvalue of 7.75). All variables loaded positively except baseline rate from both replicates (therefore, high values on this component describe animals with large amplitude and long duration responses to novel stimuli and low baseline rates.). We labeled this NR_Responsiveness_PC1. The second PCA (NR_Habituation) was performed on the habituation quotient from both replicates, and the first component accounted for 80% of the variation in the data (eigenvalue of 1.6). Both variables loaded positively on this component, which labeled NR_Habituation_PC1.

Four PCAs were performed on data from the *Gymnotus* playback experiments (Chapter 4, Experiment 1) which reduced 16 variables to five. The first PCA (PB_Approach) included variables that measured electrode approaches (percentage of time spent approaching the electrodes at night with and without stimulus playback, and percentage of time spent approaching the electrodes during the day with and without stimulus playback). Two components were retained from this PCA accounting for a total of 93% of the variation in the data. Component 1 (PB_Approach_PC1) explained 52% of the variation in the data (eigenvalue

of 2.1), and all variables loaded positively on this component. This second component (PB_Approach_PC2) accounted for 40.4% of the variation in the data (eigenvalue of 1.6). Nighttime approaches loaded positively, while daytime approaches loaded negatively (therefore, high values on this component represent more night time approaches and fewer day time approaches).

The second PCA (PB_Rate) was performed on the EOD rate variables from this experiment (i.e., average night rate with and without playback, and average day rate with and without playback). The first component explained 81.3% of the variation in the data (eigenvalue of 3.2), and all variables loaded positively on this component. This component was labeled PB_Rate_PC1.

The third PCA (PB_Activity) was performed on all variables pertaining to the percentage of time the animal spent active (i.e., percentage of night spent active with and without playback, and percentage of day spent active with and without playback). The first component (PB_Active_PC1) explained 64% of the variation in the data (eigenvalue of 2.55), and all the variables loaded positively on this component.

The final PCA for this experiment (PB_Dynamics) was performed on variables that accounted for the *difference* between behaviors (EOD rate and activity) with and without playback. The first component (PB_Dynamics_PC1) of this analysis accounted for 50.5% of the variation in the data (eigenvalue of 2.0). Differences occurring at night in response to playback loaded negatively, while differences occurring during the day loaded positively.

Finally, four PCAs were performed on data from the JAR experiment (Chapter 4, Experiment 2) which reduced 24 variables to six. The first (JAR_Ratio) was performed on the variables pertaining to the occurrences of animals preferred jamming or being jammed during

both replicates. The first component explained 50.9% of the variance in the data (eigenvalue of 2.0), and all variables loaded positively except preference for being jammed in the second replicate. We labeled this component JAR_Ratio_PC1.

The second PCA (JAR_EarlyRes) was performed on the variables regarding the percentage of early response times to both higher and lower rate jamming stimuli during both replicates (i.e., percentage early responses to positive Δfs and percentage early responses to negative Δfs). The first two components explained a total of 82.5% of the variability in these data. Component 1 (JAR_EarlyRes_PC1) alone accounted for 44.9% of the variability (eigenvalue of 1.8) and loaded positively for lower rate stimuli but negatively for higher rate stimuli. The second component (JAR_EarlyRes_PC2) accounted for 37.6% of the variability (eigenvalue of 1.5) and had positive loadings for all four variables but the strongest loadings for higher rate stimuli (note that high scores on the original variables indicated a higher percentage of early responses).

The third PCA (JAR_Responsiveness − Δfs) was performed on variables from both replicates pertaining to the overall change in EOD rate in response to negative Δfs. One principal component was retained (JAR_Responsiveness − Δfs_PC1), which explained 57.5% of the variation, and all variables loaded positively on this component.

The final PCA (JAR_Responsiveness +Δfs) was performed on the variables from both replicates pertaining to the overall change in EOD rate in response to higher rate stimuli. Two components were retained, accounting for a total of 79.9% of the variance in the data. The first component (JAR_Responsiveness+Δfs_PC1) accounted for 44.1% and loaded heavily with the variables from Replicate 2 (all positive loading), while the second component

(JAR_Responsiveness+Δfs_PC2) explained 35.8% of the variation and loaded heavily with variables from Replicate 1 (again, all positively).

Correlational Analysis

Length (in cm) did not correlate with any of the variables. (Initially, a significant correlation was found between length and JAR_EarlyRes_PC1, but removal of our smallest individual caused this correlation to no longer be significant.) Higher baseline EOD rates correlated with principal components related to greater activity, slower escape times in the terrestrial challenge, and larger changes in rate in response to lower rate stimuli in the JAR experiment, while lower rates correlated with larger responses to various stimuli and increased habitation of responses (see Table 5.2 for correlation coefficients relating to baseline rate).

There were several instances of correlations across principal components within the same experiment (Table 5.3). In the free exploration experiment, higher rates (FE_Rate_PC1) were correlated with smaller changes in EOD Rates between states (FE_Dynamics_PC1). In the novelty response experiment, increased levels of habituation (NR_Habituation_PC1) correlated with larger and longer novelty responses (NR_Dynamics_PC1). Within the JAR experiment early response times to higher rate stimuli (JAR_EarlyRes_PC2) correlated with smaller slopes of start times in response to lower rate stimuli in Replicate 1 (non-PCA variable) and smaller changes in EOD rate in response to higher rate stimuli (JAR_Responsiveness+Δfs_PC1). Larger changes in EOD rate in response to lower rate stimuli (JAR_Responsiveness− Δfs_PC1) correlated with early response times to lower rate stimuli (JAR_EarlyRes_PC1). Finally, exploration time in the *G. carapo* playback experiment (PB_Activity_PC1) correlated with decreases in EOD rate and shelter use during periods of playback (PC_Dynamics_PC1).

We also found a number of significant correlations between principal components from *different* experiments (Table 5.4). Discharge rate in the free exploration experiment (FE_Rate_PC1) correlated positively with discharge rate in the *Gymnotus* playback experiment (PB_Rate_PC1, Figure 5.1a). Also, activity levels at night in the free exploration experiment (FE_Activity_PC2) correlated with EOD rate behaviors in the *Gymnotus* playback experiment, with more active animals having higher EOD rates (Figure 5.1b). Animals that escaped the platform quickly had smaller responses to novelty as evidenced by responses in the first replicate of the terrestrial challenge (TC_Rep1_PC1) being correlated with responses to novel stimuli in the novelty response experiment (NR_Responsiveness_PC1) (note that large scores for TC_Rep1_PC1 indicate shorter escape times; Figure 5.1c). Animals with large responses to novelty also had larger changes in EOD rate between states (Figure 5.1d) as responses to novel stimuli were also correlated with the changes in EOD rate across sampling periods (FE_Dynamics_PC1).

Animals that showed the least amount of habituation showed more approaches to the playback electrodes (Figure 5.1e) seen in the amount of habituation to novel stimuli (NR_Habituation_PC1) being correlated with the number of approaches made to the electrodes (PB_Approach_PC1). Animals with more approaches to the playback electrodes also had more early response start times to lower rate stimuli (Figure 5.1f), as evidenced by PB_Approach_PC1 also being correlated with the percentage of early responses to lower rate stimuli in the JAR experiment (JAR_EarlyRes_PC1). Animals with larger changes in response start time to increasingly higher rate stimuli (that is, their responses started later as magnitude of the rate difference increased) also exhibited larger changes in EOD rates across states (Figure 5.1g), larger increases in behavior in response to playback during the day, and larger decreases in

behavior in response to playback at night (Figure 5.1h). This was indicated by the slope of the response start times to higher rate stimuli in Replicate 2 of the JAR experiment (non-PCA variable) being correlated both with changes in EOD rate across sampling periods in the free exploration experiment (FE_Dynamics_PC1) and changes in behavior in relation to *Gymnotus* playback (PB_Dynamics_PC1).

GLMM

The starting EOD rate (Figure 5.2) in the free exploration experiment and *Gymnotus* playback experiment were comparable but each of these was significantly different from the baseline rates in the novelty response experiment and the JAR experiment (which were also comparable). This was indicated by pair-wise comparisons ($p<.05$) after finding a significant effect of the experiment ($F(3,85) = 20.15$, $p<.001$) on start rate but no effect of replicate, home tank or collection location.

Discussion

Our analysis of starting EOD rate across all experiments indicated that replicate had no effect on individual starting rate, but that experiment type had an effect. Both JAR and novelty response experiments were similar to each other in starting EOD rate but different from the free exploration experiment and the *Gymnotus* playback experiment (which were also similar). These relationships make sense in light of the fact that these were the sets of experiments that were most similar in design and location (in the same room as the fish were housed in vs. a different room in a sound proof booth), in data collection (IPI collection vs. 250 ms averages for rate estimation) and in time (JAR and NR were generally conducted consecutively in each individual). Due to the large number of significant correlations between the starting EOD rates in all these experiments, the differences in the model likely reflect temporary adjustments to their

surroundings or simply reflect differences in measurement, rather than indicating a fundamental difference between discharge behaviors in each set of experiments.

The between-experiment correlations of PC variables organized themselves in such a way that three PC variables (PB_Rate_PC1, NR_Responsiveness_PC1 and PB_Approach_PC1) had two correlates each with other PC variables (Table 5.4). We also have one non-PC variable (Slope + Δfs JAR Rep2) that correlated with two PC variables. We used these four components/variables as *keystone variables* that organize four different behavioral syndromes: (a) activity, (b) reactivity, (c) aggression/dominance, and (d) flexibility (Figure 5.3).

The results from Chapter 2 inferred the existence of an activity syndrome, in that EOD rate correlated with the percentage of time an individual spent active in the free exploration experiment. Correlations between PCs provided solid evidence for the existence of this syndrome. The keystone component was composed of EOD rate variables in the *Gymnotus* playback experiment. This PC correlated with the PCs for both EOD rate and locomotor activity in the free exploration experiment. Figure 5.4a illustrates how normalized scores for individual animals for each of the correlated components compared within this syndrome of activity. It is possible that underlying differences in individual metabolic rate or baseline neuronal activity could account for a behavioral syndrome of activity (*review:* Biro & Stamps, 2010). In deer mice (*Peromyscus maniculatus*), individuals with that ran the most also had high resting metabolic rates (Chappell, Garland, Rezende and Gomes, 2004).

The results from Chapter 3 implied the existence of proactive and reactive coping styles with some individuals displaying quick responses to challenges and limited attention to the environment (i.e. proactive) while other individuals displayed more conservative responses to challenges and greater attention to the environment (i.e. reactive). More specifically, smaller

changes in EOD rate in response to novel stimuli correlated with rapid escape times from the platform during the terrestrial challenge experiment- a proactive style, and vice versa for reactive individuals. Further evidence for the existence of this syndrome was found in the correlations between PC variables across experiments. Individuals that showed the largest changes in response to novel stimuli also displayed longer latencies to escape from a platform as well as larger changes in EOD rate between states (i.e., day to night, resting to active). In this case, the keystone of this syndrome was the PC variable pertaining to changes in EOD behavior in response to a novel stimulus (NR_Responsiveness_PC1). This correlated with the PCs composed of variables from the first replicate of the terrestrial challenge (TC_Rep1_PC1) and with the variables pertaining to changes in EOD rate and activity in the free exploration experiment (FE_Dynamics_PC1).

Figure 5.4b illustrates how individual animal's normalized scores for each of the correlated components compare within this syndrome of reactivity. These correlations may exist because of underlying differences in the reactivity of the sympathetic nervous system. Other model systems have shown that differences in both corticosteroid levels and catecholamine levels in response to stress correlate with behavioral responses (Korte, Beuving, Ruesink, & Blokhuis, 1997; Overli et al., 2007). In Rainbow Trout (*Oncorhynchus mykiss*) individuals that are selected to show high levels of cortisol release during confinement stress display behaviors typically seen in reactive individuals (lower aggression, more submission and increased latency to feed following stress) while individuals with lower levels of cortisol release typically show behaviors more characteristic of proactive individuals which display higher aggression, increased social dominance and shorter latencies to feed following stress (Overli et al., 2007). Additionally, in Senegalese Sole (*Solea senegalensis*) individual differences in oxygen

consumption has been linked to coping styles (Martins, Castanheira, Engrola, Costas, & Conceicao, 2011). Individuals with higher oxygen consumption took longer to escape a confinement (reactive coping style) than individuals with lower oxygen consumption.

The results from Chapter 4 implied a possible aggression/dominance syndrome with some animals displaying more agonistic behaviors than others. Specifically the number of electrode approaches correlated with the number of instances where the animal preferentially placed their EOD in a jamming phase relationship with the stimulus, both of which can be considered agonistic or possibly displays of dominance. Once again, between-experiment PCs correlated in such as way as to also support the existence of this syndrome. Our keystone component for this syndrome (PB_Approach_PC1) was composed of variables pertaining to the percentage of time animals approached the playback electrodes. Animals that approached electrodes most often also habituated to novel stimuli the least and had more early start times to stimuli with lower EOD rates during the JAR experiment. While it is difficult to interpret habituation as a measure of aggression, this correlation implies that aggressive animals may show the least amount of habituation. This relationship is understandable when considering that individuals that do not habituate to a stimulus will subsequently have more aggressive encounters with the stimulus (Bee & Gerhardt, 2001). Furthermore, early response starts to lower frequency jamming stimuli could be indicative of dominance assertion, since the individual is attempting to shift its EOD rate more quickly into a position of increased frequency dominance (Westby, 1975b).

Figure 5.4c illustrates how normalized scores for individual animal's for each of the correlated components compared within this syndrome of aggression/dominance. Potential explanations for differences in aggression or dominance could be found in underlying differences

in testosterone (While et al., 2010) or serotonin signaling activity (Overli, Harris, & Winberg, 1999). Testosterone has been traditionally assumed to activate aggression (Kabelik, Weiss & Moore, 2008) but it is more likely that this relationship is context dependant and related to the breeding season (Duckworth, 2006). For example, in lizards, a negative correlation was found between testosterone and aggression in males at the end of the mating season (While et al., 2010). In Rainbow Trout (*Oncorhynchus mykiss*) an increase in serotonergic activation was seen in all individuals shortly following a social contest before social hierarchy was established but is only seen in subordinate individuals after 24h of social interaction, (Overli, Harris, & Winberg, 1999). This implies that underlying differences in serotonergic activation may contribute to an individual's dominance status.

Finally, one other set of correlations was found between principal components across experiments, although it is somewhat more difficult to interpret. The keystone variable (in this case not a principal component, but a data reduction technique nonetheless) for this syndrome was the slope of the regression line formed between response start time and the magnitude of the difference in EOD rate between the stimulus and the fish (Δf) for higher EOD rate stimuli (+Δf). Individual's with lower slopes have consistently early response start times to all higher rate stimuli, while animals with larger slopes have a more graded relationship, such that stimuli that are further from the fish's EOD rate elicit later response starts compared to stimuli that are closer to the fish's EOD rate. This variable correlated positively with larger changes in EOD rate and activity between states (i.e., day to night, resting to active) during free exploration (FE_Dynamics_PC1) and negatively with changes in EOD rate and activity in response to *Gymnotus* playback (PB_Dynamics_PC1). While it is possible that these behaviors are related to reactivity, since FE_Dynamics_PC1 is also in the reactivity syndrome, there was not enough of

an overlap in the other correlations to justify such a grouping. We tentatively refer to this syndrome as flexibility with the understanding that a larger sample size could cause this syndrome to link more strongly with reactivity.

In the case of PB_Dynamics_PC1 larger values are indicative of *increases* in EOD rate and locomotor activity in response to playback during the day and to *decreases* in EOD rate and locomotor activity in response to playback at night, while smaller values are indicative of larger *decreases* in EOD rate and locomotor activity in response to playback during the day and to larger *increases* in EOD rate and locomotor activity in response to playback at night. This latter relationship (decreasing rate and activity during the day and increasing it at night) implies attraction to the stimulus at night and inhibition by the stimulus during the day, while the former relationship seems to imply inhibition by the stimulus at night but attraction to it during the day. However, during the day, the decreases in behavior (inhibition) are much larger than the increases in behavior (attraction). At night, the magnitude of changes is much smaller overall but show a similar pattern: that increases in behavior (attraction) are larger than decreases in behavior (inhibition). Thus, we should interpret large (more positive) values for PB_Dynamics_PC1 as indicative of more *consistent* behavior (smaller changes) in the presence of the stimulus, and smaller (more negative) values as being indicative of behavioral *flexibility* (higher activity and rate at night and lower activity and rate during the day) in the presence of the stimulus. (Remember that the correlation with the slope values was negative.) FE_Dynamics_PC1 should also be interpreted such that low values indicate increased consistency (smaller changes in EOD rate and activity across states) and high values as increased flexibility (larger changes in EOD rate and activity across states). Figure 5.4d illustrates how individual animals normalized scores for each of the correlated components or variables

compared within this syndrome of flexibility. Behavioral flexibility has been linked to both aggression syndromes (Benus, Den Daas, Koolhaas, & Van Oortmerssen, 1990) and to proactive and reactive coping styles (Bolhuis, Schouten, Leeuw, Schrama, & Weigant, 2004). It is unclear if this syndrome represents an additional axis of behavior or if, upon further experimentation, it will emerge as related to one or more of our other syndromes.

All this evidence together suggests that *Microsternarchus* sp. display behavioral syndromes for activity, reactivity, aggression/dominance, and possibly flexibility. Since these syndromes are based on principal components and not simply correlations between individual variables they are considered more conservative and are likely more valid than simple correlations between primary variables. These principal components were derived from contexts relating to exploration, potentially stressful environmental challenges, social threat and social communication indicating that the personality of individual *Microsternarchus* were consistent across contexts as well as across time.

Table 5.1

Eigenvector loadings for each PCA

Principal Component	Variable:	Eigenvector loading: Replicate 1	Replicate 2
FE_Rate	FE_Rate_PC1 (67.7% of variation explained)		
	Day Rate	.95	.79
	Night Rate	.64	.87
	In Rate	.91	.75
	Out Rate	.80	.83
FE_Dynamics	FE_Dynamics_PC1 (57.1% of variation explained)		
	Change in Rate Day vs. Night	.69	80
	Change in Rate In vs. Out	.85	.82
	Change in Rate In vs. Out - Day	.57	.91
	Change in Rate In vs. Out - Night	.50	.81
FE_Activity	FE_Activity_PC1 (45.2% of variation explained)		
	% Night Active	-.16	-.43
	% Day Active	.89	.89
	FE_Activity_PC2 (41.9% of variation explained)		
	% Night Active	.92	.82
	% Day Active	.29	.27
TC_Rep1	TC_Rep1_PC1 (63.2% of variation explained)		
	Time to Escape the Platform	-.93	
	Struggle in the Net	.82	
	Move on the Platform	.61	
TC_Rep2	TC_Rep2_PC1 (54.1% of variation explained)		
	Time to Escape the Platform		-.80
	Struggle in the Net		.48
	Move on the Platform		.87
NR_Resp.	NR_Responsiveness_PC1 (52% of variation explained)		
	Start Rate	-.86	-.84
	Percent Large Responses	.76	.93
	Percent Long Responses	.83	.86
	Average Amplitude	.90	.92
	Average Duration	.95	.92

Table 5.1 (cont.)

Eigenvector loadings for each PCA

Principal Component	Variable:	Eigenvector loading: Replicate 1	Replicate 2
NR_Hab.	NR_Habituation_PC1 (80% of variation explained)		
	Habituation Quotient	.89	.89
PB_Approach	PB_Approach_PC1 (52% of variation explained)		
	% of Time Approach Silent at Night	.76	na
	% of Time Approach Active at Night	.57	na
	% of Time Approach Silent during the Day	.75	na
	% of Time Approach Active during the Day	.78	na
	PB_Approach_PC2 (40.4% of variation explained)		
	% of Time Approach Silent at Night	.58	na
	% of Time Approach Active at Night	.78	na
	% of Time Approach Silent during the Day	-.60	na
	% of Time Approach Active during the Day	-.56	na
PB_Rate	PB_Rate_PC1 (81.3% of variation explained)		
	Night Rate- Without Playback	.92	na
	Night Rate- During Playback	.91	na
	Day Rate- Without Playback	.93	na
	Day Rate- During Playback	.85	na
PB_Activity	PB_Activity_PC1 (64% of variation explained)		
	Night Activity Without Playback	.84	na
	Night Activity-During Playback	.82	na
	Day Activity-Without Playback	.83	na
	Day Activity- During Playback	.69	na
PB_Dynamics	PB_Dynamics_PC1 (50.5% of the variation explained)		
	Diff. in Rate at Night Active/Silent	-.80	na
	Diff. in Rate during the Day Active/Silent	.84	na
	Diff. in Activity at Night Active/Silent	-.54	na
	Diff. in Acitity during the Day Active/Silent	.62	na

Table 5.1 (cont.)

Eigenvector loadings for each PCA

Principal Component	Variable:	Eigenvector loading: Replicate 1	Replicate 2
JAR_Ratio	JAR_Ratio_PC1 (50.9% of variation explained)		
	Preference for Jamming	.88	.62
	Preference for Being Jammed	.76	-.54
JAR_EarlyRes	JAR_EarlyRes_PC1 (44.9% of variation explained)		
	% Early Response Negative Δf	.74	.85
	% Early Response Positive Δf	-.64	-.32
	JAR_EarlyRes_PC2 (37.6% of variation explained)		
	% Early Response Negative Δf	.55	.32
	% Early Response Positive Δf	.62	.85
JAR_Resp. -Δf	JAR_Responsiveness -Δf_PC1 (57.5% of variation explained)		
	Overall Change in Rate to Δf -5	.60	.46
	Overall Change in Rate to Δf -3	.73	.78
	Overall Change in Rate to Δf -2	.78	.87
	Overall Change in Rate to Δf -1	.87	.87
JAR_Responsiveness +Δf	JAR_Responsiveness +Δf_PC1 (44.1% of variation explained)		
	Overall Change in Rate to Δf +5	-.28	.64
	Overall Change in Rate to Δf +3	.37	.83
	Overall Change in Rate to Δf +2	.57	.91
	Overall Change in Rate to Δf +1	.54	.88
	JAR_Responsivness+Δf_PC2 (35.8% of variation explained)		
	Overall Change in Rate to Δf +5	.66	-.42
	Overall Change in Rate to Δf +3	.86	-.45
	Overall Change in Rate to Δf +2	.78	-.28
	Overall Change in Rate to Δf +1	.78	-.11

Table 5.2

Correlation coefficients (and corresponding N) for baseline EOD rates

	FE_1	FE_2	NR_1	NR_2	PB_1	JAR_1	JAR_2
FE_1		.86***		.81**	.63*	.70*	.90***
	1	19	n.s.	10	11	12	10
FE_2			.64*	.79**	.76**	.82**	.83**
		1	12	10	11	12	10
NR_1				.75**	.67*	.75**	.73**
			1	13	12	15	13
NR_2					.69*	.66*	.97***
				1	10	13	13
PB_1						.59*	.65*
					1	12	10
JAR_1							.71**
						1	13

	FE_1	FE_2	NR_1	NR_2	PB_1	JAR_1	JAR_2
FE_Rate_PC1	.54*	.70**	.71**	.74**	.73*	.73*	.65*
	19	19	12	10	11	12	10
FE_Dynamics_PC1	-.67**	-.71**		-.79**	-.62*		-.795**
	19	19		10	11		10
TC_Rep1_PC1				.73**			.66*
				12			12
NR_Resp._PC1	-.78**	-.79**	-.86***	-.84***		-.70**	-.86***
	10	10	13	13		13	13
NR_Hab._PC1			-.56*	-.61*			
			13	13			
JAR_Ratio_PC1					.73*		
					10		
JAR_EarlyRes_PC1							.56*
							13
PB_Rate_PC1		.63*			.61*	.74**	
		11			14	12	
JAR_Resp.	.66*			.56*	.64*		.67*
-Δf's_PC1	10			13	10		13

Note. * $p < .05$ ** $p < .01$ *** $p < .001$

Table 5.3

Correlation coefficients for comparisons within experiments

Measure:	*r:*	N:
FE_Rate_PC1		
FE_Dynamic_PC1	-.68**	19
NR_Responsiveness_PC1		
NR_Habituation_PC1	.55*	13
JAR_EarlyRes_PC2		
Slope -Δf JAR Rep 1.	-.81**	13
JAR_EarlyRes_PC2		
JAR_Responsiveness+Δf's_PC1	.90**	13
JAR_EarlyRes_PC1		
JAR_Responsiveness-Δf's_PC1	-.57*	13
PB_Activity_PC1		
PB_Dynamics_PC1	-.74**	14

Note. * $p < .05$ ** $p < .01$ *** $p < .001$

Table 5.4

Correlation coefficients for comparisons between experiments

Measure:	*r:*	N:
PB_Rate_PC1		
FE_Rate_PC1	.72*	11
PB_Rate_PC1		
FE_Activity_PC2	.87**	11
NR_Responsiveness_PC1		
TC_Rep1_PC1	-.59*	12
NR_Responsiveness_PC1		
FE_Dynamics_PC1	.72*	10
PB_Approach_PC1		
NR_Habituation_PC1	-.66*	10
PB_Approach_PC1		
JAR_EarlyRes_PC1	.66*	10
Slope +Δf JAR Rep 2.		
FE_Dynamics_PC1	.67*	10
Slope +Δf JAR Rep 2.		
PB_Dynamics_PC1	-.64*	10

Note. * $p < .05$ ** $p < .01$ *** $p < .001$

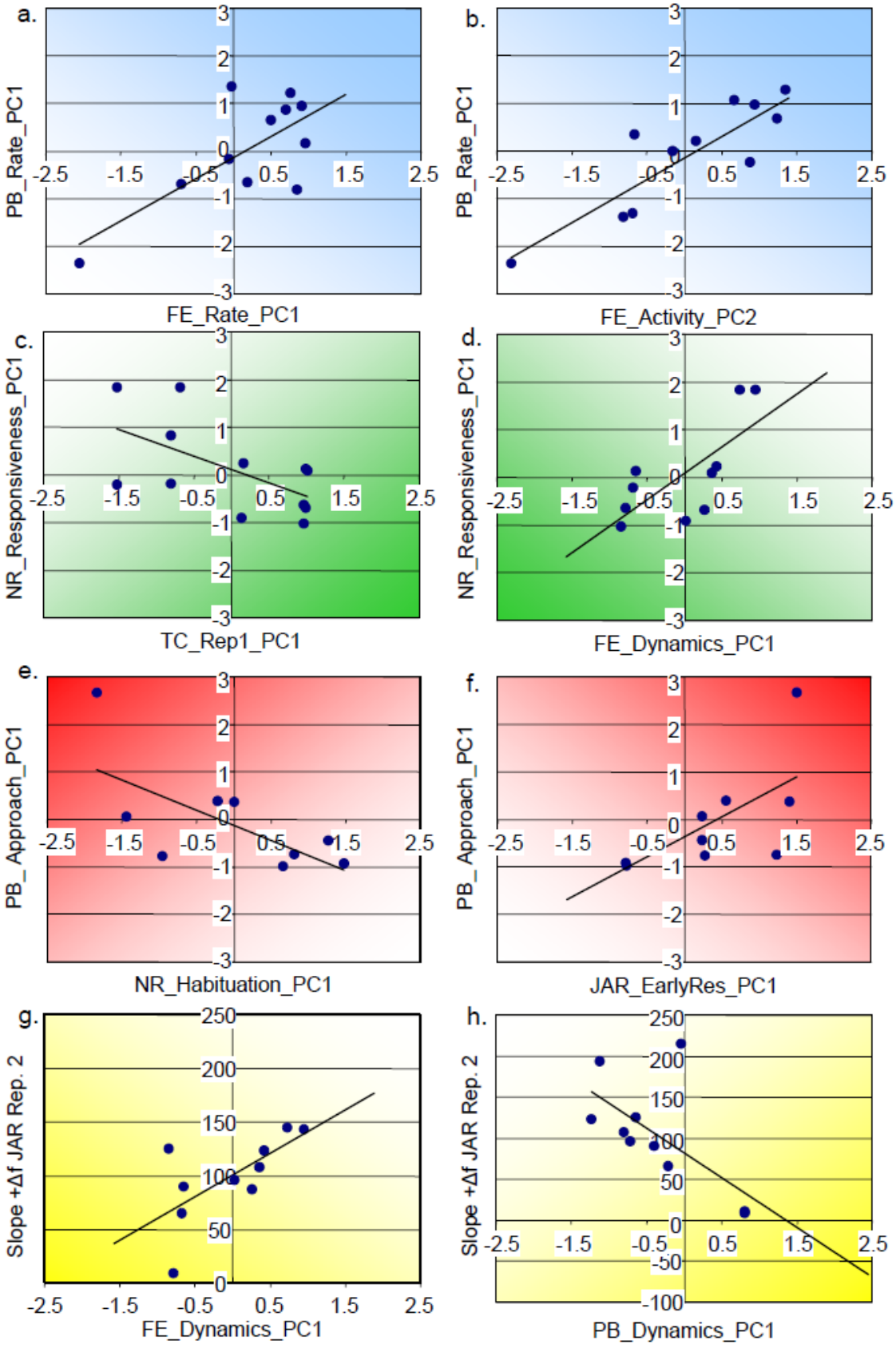

a.
PB_Rate_PC1
FE_Rate_PC1
b.
PB_Rate_PC1
FE_Activity_PC2
c.
NR_Responsiveness_PC1
TC_Rep1_PC1
d.
NR_Responsiveness_PC1
FE_Dynamics_PC1
e.
PB_ Approach_PC1
NR_Habituation_PC1
f.
PB_ Approach_PC1
JAR_EarlyRes_PC1
g.
Slope +Δf JAR Rep. 2
FE_Dynamics_PC1
h.
Slope +Δf JAR Rep. 2
PB_Dynamics_PC1

Figure 5.1. Scatter plots of between-experiment correlations and corresponding regression lines. Graphs are grouped in pairs representing our four behavioral syndromes, with the keystone component given on the y-axis of each graph. Activity Syndrome (a-b), Reactivity Syndrome (c-d), Aggression/Dominance Syndrome (e-f), and Flexibility Syndrome (g-h). Color gradients accentuate the relationships between variables and coordinate with gradients in Figures 5.2 and 5.3. The activity syndrome is always displayed in blue with darker regions indicative of higher activity. Reactivity syndrome is always displayed in green with darker regions being indicative of proactive behavior and lighter regions indicative of reactive behavior. The aggression/dominance syndrome is always displayed in red with darker regions being indicative of more aggressive/dominant behavior. Finally, the flexibility syndrome is always displayed in yellow with regions of higher saturations being indicative of more consistent behavior while lighter regions represent increased flexibility.

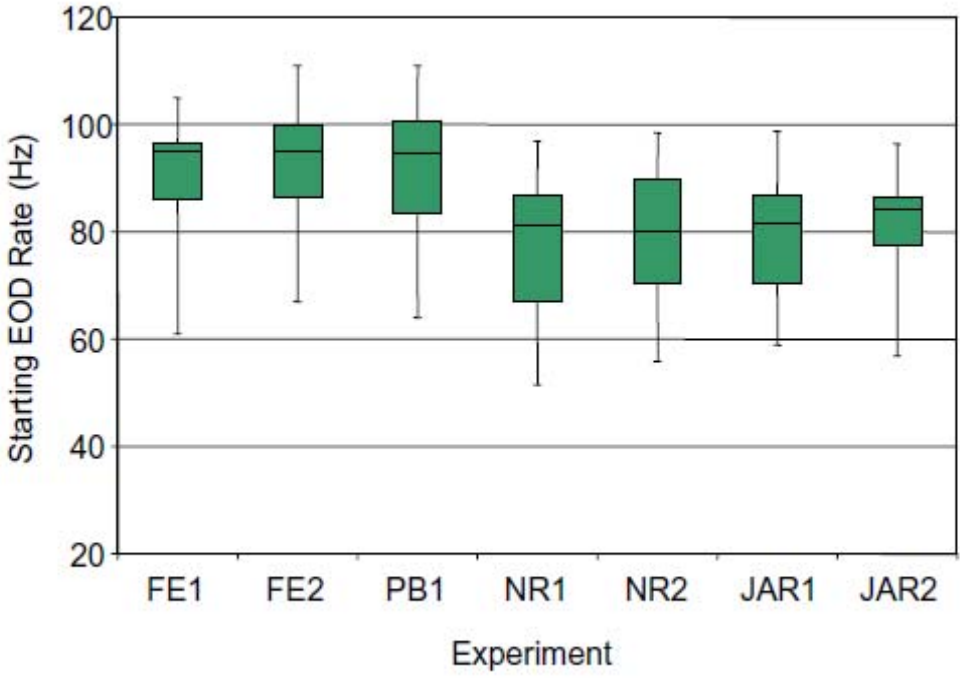


Figure 5.2. Comparison of starting EOD rate for each experiment and replicate. FE= free exploration, PB= *Gymnotus* playback, NR= novelty response, JAR= jamming avoidance response. Boxes show the 25 to 75 percentiles and the median individual is shown by the horizontal line. The whiskers indicate the individual with minimum and maximum observed EOD rate.

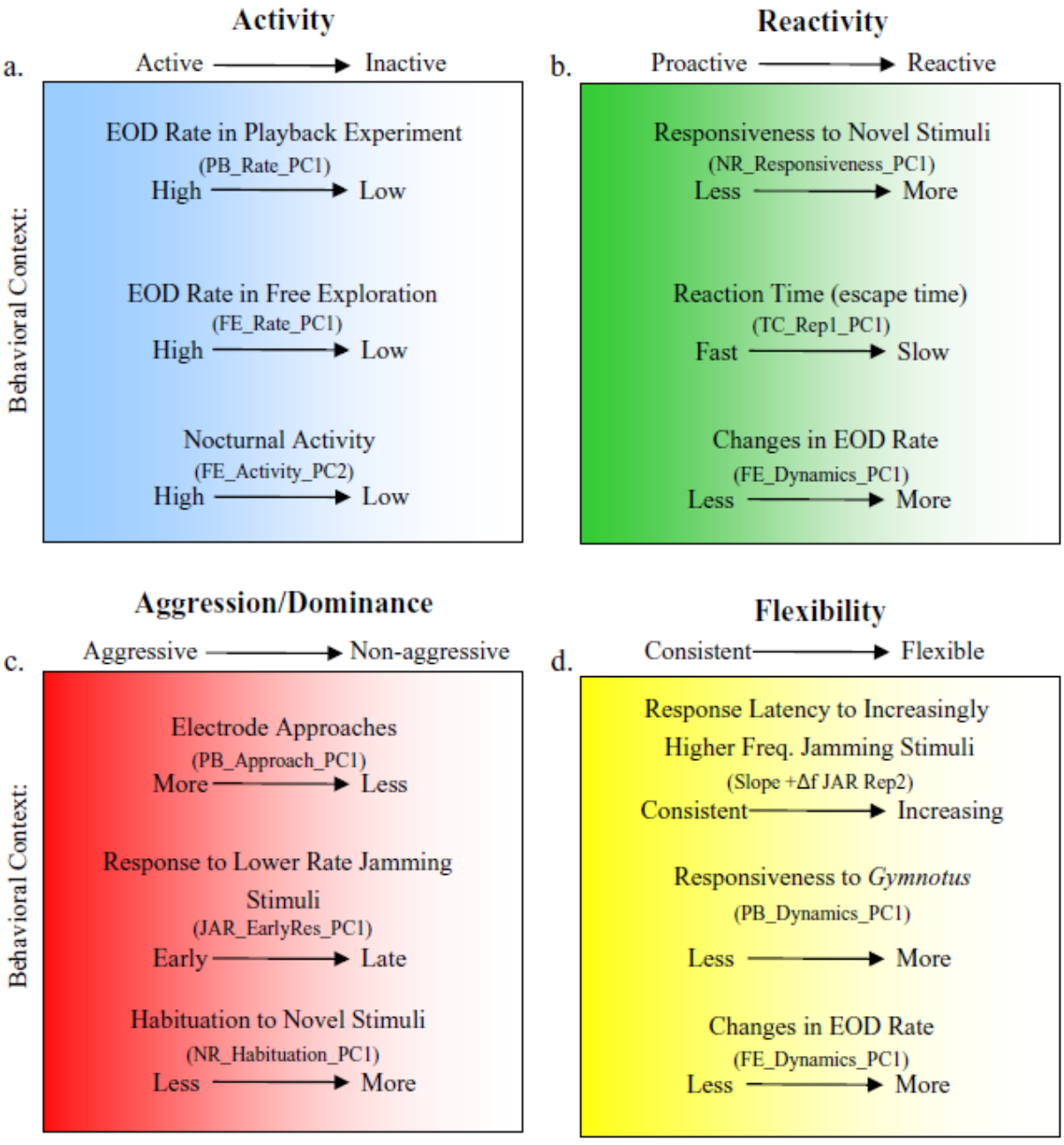

Activity
a.
Active → Inactive
Behavioral Context:
EOD Rate in Playback Experiment
(PB_Rate_PC1)
High → Low
EOD Rate in Free Exploration
(FE_Rate_PC1)
High → Low
Nocturnal Activity
(FE_Activity_PC2)
High → Low
Reactivity
b.
Proactive → Reactive
Responsiveness to Novel Stimuli
(NR_Responsiveness_PC1)
Less → More
Reaction Time (escape time)
(TC_Rep1_PC1)
Fast → Slow
Changes in EOD Rate
(FE_Dynamics_PC1)
Less → More
Aggression/Dominance
c.
Aggressive → Non-aggressive
Behavioral Context:
Electrode Approaches
(PB_Approach_PC1)
More → Less
Response to Lower Rate Jamming Stimuli
(JAR_EarlyRes_PC1)
Early → Late
Habituation to Novel Stimuli
(NR_Habituation_PC1)
Less → More
Flexibility
d.
Consistent → Flexible
Response Latency to Increasingly Higher Freq. Jamming Stimuli
(Slope +Δf JAR Rep2)
Consistent → Increasing
Responsiveness to Gymnotus
(PB_Dynamics_PC1)
Less → More
Changes in EOD Rate
(FE_Dynamics_PC1)
Less → More

Figure 5.3. Summary of the four behavioral syndromes extrapolated from the correlations between principal components. The directionality for each behavior (or behavioral type) is given below the syndrome name (and accentuated with the color saturation within each box) to differentiate between how behaviors relate within each behavioral type (Active vs. Inactive, Proactive vs. Reactive, Aggressive vs. Non-aggressive, Consistent vs. Flexible). The keystone component for each syndrome is listed at the top.

Activity

	PB_Rate_PC1	FE_Activity_PC2	FE_Rate_PC1
Inactive			
LAU105	-0.7	0	0
MAT104	0.05	0.29	0.54
MAT103	0.07	0.74	0.43
LAU103	-	0.88	0.33
MAT107	-	0.5	0.27
ADA101	0.3	0.65	0.44
ADA105	0.37	-	-
MAT108	0.43	-	-
MAT101	-	0.74	0.47
ADA104	0.45	0.71	0.78
ADA102	0.67	0.94	0.74
ADA110	0.77	0.58	0.73
MAT109	0.81	0.91	0.79
MAT110	0.94	0.83	0.77
Active			

Reactivity

	NR_Resp._PC1	TC_Rep1_PC1	FE_Dynamics_PC1
Reactive			
MAT107	0	0.33	0.27
LAU105	-	-	0
LAU103	0.004	0	0.33
ADA105	0.32	0.28	-
ADA104	-	0.28	0.78
MAT103	0.51	0.66	0.42
ADA110	0.54	0.99	0.73
ADA101	0.56	0.99	0.44
ADA102	0.65	0	0.74
MAT110	0.79	0.99	0.77
MAT101	0.8	0.99	0.47
MAT104	0.87	0.65	0.54
MAT109	0.91	0.99	0.79
MAT108	1	-	-
Proactive			

Aggression/Dominance

	PB_Approach_PC1	JAR_EarlyRes_PC1	NR_Habituation_PC1
Non Aggressive			
ADA102	0	0.25	0.25
MAT107	-	0	0.33
MAT101	-	0.12	0.49
MAT103	0.09	0.6	0.74
ADA101	0.11	0.91	0.2
ADA104	0.14	-	-
MAT109	0.24	0.35	0.61
ADA105	0.24	0.59	0.06
MAT104	0.45	0.59	0.88
ADA110	0.6	0.97	0.44
LAU105	0.6	-	-
MAT110	0.61	0.69	0.51
MAT108	1.63	1	1
Aggressive			
LAU103	-	-	-

Flexibility

	Slope +Δf JAR Rep 2.	PB_Dynamics_PC1	FE_Dynamics_PC1
Flexible			
MAT108	0	0.52	-
ADA105	0.11	0.05	-
LAU103	0.34	-	0.33
MAT107	0.35	-	0.27
MAT109	0.44	0.26	0.79
MAT103	0.45	0	0.42
ADA101	0.52	0.19	0.44
MAT104	0.58	0.23	0.54
ADA110	0.61	0.36	0.73
MAT101	0.62	-	0.47
ADA104	-	0.56	0.78
ADA102	0.73	0.44	0.74
MAT110	1	0.89	0.77
LAU105	-	1.6	0
Consistent			

Figure 5.4. The order of individual animals within each behavioral syndrome. The normalized component scores for each individual are listed for each principal component or variable that was correlated in each syndrome. Individuals are ranked by the keystone component (listed at left); those with missing scores are fit based on the available scores. Note that LAU103 did not have any data for the aggression syndrome and is, therefore, omitted from the ranking.

Chapter 6:

Conclusions, Improvements, and Future Directions

Conclusions

This section will summarize our predictions from each chapter and briefly indicate the major findings related to each of our predictions.

In Chapter 2 we discussed animal's exploratory behavior in a novel environment, monitoring their EOD rate and locomotor activity for 72 hours.

Prediction 2.1: Animals will display consistent differences in locomotor activity and EOD patterns. These differences will be correlated and thus suggest a behavioral syndrome for activity with high EOD rate individuals also spending the most time out of the shelter.

Supported. Individual differences seen in EOD rate and locomotor activity were consistent across replicates, as evidenced by significant correlations between these behaviors. Measures of EOD rate and activity were correlated within individuals. This supports the idea that EOD rate and exploratory behavior form a behavioral syndrome for activity. We also found an interesting correlation between EOD rate and the change in EOD rate across states (day vs. night or in vs. out of the shelter) such that individuals with smaller EOD rates had larger changes in EOD rate across states. We also found an interesting correlation between EOD rate and the change in EOD rate across states (day vs. night or resting vs. active) such that individuals with smaller EOD rates had larger changed in EOD rate across states.

In Chapter 3 we discussed animal's responses to two potentially stressful acute environmental (non-social) challenges. The first challenge was an 'out of water' experience where we monitored their latency to return to the water and the second challenge involved the introduction of novel mechanical stimuli and the changes in their EOD rate in response to the

stimuli were monitored. The goal of these experiments was to monitor behavior in response to mildly stressful non-social stimuli and determine if *Microsternarchus* sp. displayed individual differences in how they coped with these stimuli. Subsequently we wanted to know if individuals had similar coping styles to both types of stimuli implying a behavioral syndrome for reactivity.

Prediction 3.1: *Microsternarchus* will display consistent individual differences in response to a terrestrial challenge in that some individuals will react quickly to return to the water after displacement, thus displaying a proactive coping response, while other individuals will respond more slowly or with a *sit and wait* approach, thus displaying a reactive coping response.

Inconclusive. Individuals certainly differed in their response; however, they did not all behave consistently across replicates. This is most likely due to the long latency between replicates, although it could also reflect an underlying difference in individual consistency with some individuals behaving more consistently over time than others. Much larger sample sizes and more replications would be needed to test this hypothesis. Irrespective of the lack of consistency, some individuals did react quickly to return to the water, while others never made any attempt. This opposition in behavior is similar to reported studies of proactive and reactive coping styles.

Prediction 3.2: Individuals will behave differently in response to a novel stimulus but novelty response amplitude and duration will be correlated. Individuals with larger and longer novelty responses will be displaying a reactive coping response (more sensitive to their environment), while individuals with smaller and shorter novelty responses will be displaying a proactive coping response (less sensitive to their environment).

Supported. The novelty response in *Microsternarchus* sp. reflects a modest change in EOD rate from baseline, generally on the order of 0.5 Hz. This is a much smaller response than those seen in other pulse species that will change their EOD rate by several tens of Hz in response to novel stimuli. Response to the stimulus differed consistently in the amplitude and duration and these two variables were correlated, with some individuals consistently having larger and longer responses than others. Once again, this behavioral variation is similar to reported studies of proactive and reactive coping responses with proactive individuals have smaller responses to environmental novelty and reactive individuals having larger responses to environmental novelty. The individual consistency across replicates suggests that individuals can be characterized according to behavioral type.

Prediction 3.3: Individuals that display a proactive coping response in the terrestrial challenge (quick escape from the platform) will also show a proactive coping response in response to novelty (smaller novelty responses). Conversely, those individuals that display a reactive coping response in response to a terrestrial challenge (slow escape from the platform) will also display a reactive coping response to novel stimuli (larger novelty responses).

Partially Supported. Since behavior in the terrestrial challenge was not consistent this prediction is mostly invalid. However, presuming failure to obtain consistent behavior was a flaw of our experimental design (one year delay between replicates) we can test this prediction by limiting our analysis to the first replicate of the terrestrial challenge. There were significant correlations between time to escape the platform in the first replicate of the terrestrial challenge and amplitude and duration of the novelty response, such that animals with larger responses to novelty also showed longer escape times and vice versa. These correlations conform to established ideas of reactive and proactive individuals. Reactive individuals show larger

responses to changes in their environment and a more conservative (or sit and wait) response style to stressful events. Proactive individuals show the opposite trends—that is, minimal responses to changes in the environment and rapid active responses to stressful events.

In Chapter 4 we discussed two experiments in which we presented animals with social stimuli, taking advantage of their electrosense by playing the recorded signal of a potentially threatening sympatric species as well as playing their own EOD back to them in a JAR paradigm. The goal of these experiments was to determine if individuals differed in their social or anti-social behavior in response to these types of stimuli and to see if individuals behaved consistently in response to both types of stimuli indicating a behavioral syndrome around social or anti-social behavior.

Prediction 4.1: *Microsternarchus* will show individual variation in their responsiveness, to playback of *Gymnotus* EODs. Individual's behavior will be organized around a basic aggressive response with some individuals showing greater changes in locomotor activity or EOD rate during playback. Individuals that increase their activity the most in the presence of a threatening stimulus will also will approach the playback electrodes more.

Partially Supported. Individuals did differ in their response to *Gymnotus* playback with some individuals becoming more active in response to playback and other individuals becoming less active in response to playback (and these differences were mirrored in the EOD rate as well). Individuals also differed in how often they approached the playback electrodes. However, individual's that approached the electrodes the most spent the *least* amount of time out of the shelter. This correlation could be an indication of how responsive individuals were to the presence of a threatening stimulus, with individuals modifying their behavior in different directions possibly balancing the risk of approaching by retreating to the safety of the shelter. We

were unable to find solid evidence for a social or anti-social behavioral syndrome within this experiment (we did, however, find correlations *between* experiments that would imply such a syndrome, see below).

Prediction 4.2: *Microsternarchus* will display JARs similar to other electric fish, increasing their EOD rate in response to jamming stimuli with lower EOD rates and decreasing their EOD rate in response to jamming stimuli with higher EOD rates. Animals will alter their EOD rates further and faster in response to stimuli with EOD rates closer to their own.

Supported. *Microsternarchus* sp. displayed the typical JAR, increasing their EOD rate in response to jamming stimuli with lower rates and decreasing their EOD rate in response to jamming stimuli with higher rates. Other phasic (non-rate related) behaviors were also observed. Individuals were also more likely to have larger and faster responses when the EOD rate of the stimulus was more similar to the fish's own EOD rate.

Prediction 4.3: Individuals will vary consistently in the degree of responsiveness to jamming stimuli. Individual's behavior will be organized around an aggressive response or dominance assertion. Individuals with larger magnitude JARs (particularly to lower rate stimuli) will also intentionally jam the stimulus more frequently.

Partially Supported. Individuals were consistent across replicates in their latency to respond to jamming stimuli. Some individuals consistently responded early (as measured by high percentage of early responses) to all stimuli, while other individuals took longer to respond to jamming stimuli that were further from their own EOD rate. While there was no correlation between larger magnitude JARs and intentionally jamming the stimulus there was a correlation between more consistently early response start times and larger responses to a few of the jamming stimuli. This suggests that individuals did vary consistently in their degree of

responsiveness to jamming stimuli, just not in the exact variables we predicted. The correlations within this experiment were not necessarily organized around an aggressive or dominance syndrome but may actually relate more to an individual's level of responsiveness similar to those found in Chapter 3 between escape time and novelty response amplitude.

Prediction 4.4: Individuals that have the most electrode approaches during *Gymnotus* playback will also have more instances of jamming the stimulus implying the existence of an aggression or dominance syndrome.

Supported. These two behaviors, approaching the electrodes and intentionally jamming are likely the most agonistic behaviors from each of these experiments and there was a significant correlation between them. Both these behaviors have been called aggressive in other species of pulse fish (see Chapter 4 for details), although it is also possible that these correlations are indicative of dominant status as well. We also can not discount that there may be a social curiosity component to approaching the playback electrodes, however this behavior certainly did not appear friendly in nature, we labeled the behavior as *approaching* the electrode instead of *attacking* the electrode simply to be conservative and not imply an intention to the behavior.

Prediction 5.1: Individual variation will organize into syndromes for activity, reactivity, and aggression/dominance, as evidenced by significant correlations between the principal components from different experiments.

Supported. The principal components distilled from the raw data were correlated in ways that imply behavioral syndromes for activity, reactivity, aggression/dominance, and possibly behavioral flexibility as well. Animals that are more active show higher EOD rates and higher activity in general. Reactive animals have large responses to novelty, long latencies to escape a platform above the water surface and larger changes in EOD rate across states (in vs. out of the

shelter and day vs. night). Aggressive animals have more playback electrode approaches, more early responses to lower EOD rate jamming stimuli and less habituation to novel stimuli. Finally, flexible animals display increasing latency to respond to higher frequency jamming stimuli, larger changes in behavior in response to *Gymnotus* playback and larger changes in EOD rate across states.

Improvements

While this body of work does demonstrate that *Microsternarchus* sp. makes an excellent model system for behavioral syndromes and should be further explored in the future for the proximate and ultimate causation of behavioral syndromes, our experimental design was not without its caveats. In this section we acknowledge the mistakes made along the way and suggest improvements to our experimental design.

The correlations between principal components are robust and undeniable, however the interpretations of those correlations into behavioral syndromes are subjective. It is possible that other scientists could look at that same set of correlations and decide that they are evidence of entirely different behavioral syndromes. For this reason it would have been ideal to be able to perform factor analysis rather than principal components analysis as this method shows more specific groupings of variables into traits. Unfortunately we did not have a large enough sample size available to us to perform factor analysis.

One way in which our interpretation could be questioned is that EOD rate dynamics were correlated with variables that were labeled reactivity as well as flexibility. Another interpretation of this would be simply to have one axis instead of two, especially since the proactive and reactive axis is known to include aspects of behavioral flexibility (Bolhuis, Schouten, Leeuw, Schrama, & Weigant, 2004; Koolhaas et al., 1999). However, other components did not correlate

between the two syndromes, therefore implying that these behaviors make up their own syndrome, at least for this data set.

While our raw variables implied an aggression syndrome from correlations between electrode approaches and instances of preferred jamming, the principal components containing these variables did not show a correlation. This does not mean the original correlation was invalid but rather that we could not support it with a more conservative and robust analysis of the results. This could simply be a failure of our small sample size. Since the JAR and novelty response experiments were the final in the series, they had the smallest sample sizes (n = 13) and, thus, an even smaller overlap between individuals in this experiment and individuals in the terrestrial challenge experiment (overlap n = 12). The addition of more individuals could render these correlations significant. That said, we did find evidence for an aggression syndrome in the correlations between our principal components as stated above.

A possible confounding variable that we did not control for was the social structure in the animal's home tank. It is possible that EOD rates were affected by the other individuals in the home tank, with each fish establishing a characteristic EOD rate or set of rates that allow for the least jamming interference in the tank. The social nature of these tank environments was largely unknown and, therefore, could have affected individual personality. For example, an individual could be the most dominant individual in the tank, but were it with a different group of animals may not be the most dominant. Since our animals were not kept in breeding conditions and we never observed any overt aggression in the home tanks we have assumed that this confound is small. We also found no effect of home tank on starting EOD rate across experiments so we expect that social environment did not play a large role in our experiments or certainly that the effect was balanced for all individuals.

I would suggest a few improvements to our design that are specific to individual experiments. For example, in the terrestrial challenge experiments, it would have been preferable to have the second replicate occur closer in time to the first replicate and to have recorded the fish's EOD while it was on the platform. This experiment was not part of our original experimental plan; thus, repeating it, while obvious now, was not considered until much later. Due to the high variability in behavior it might also be interesting to replicate this more than just once to understand if this behavior is more related to age, captivity or personality. We also intended to have a second replicate of the *Gymnotus* playback experiment but, due to challenges with the video surveillance equipment, this experiment began much later than anticipated. Due to the length of time it took to conduct the experiment, we made the decision to forfeit the second replicate in order to begin the novelty response and JAR experiments before more animals died. It also would have been preferable to perform the *Gymnotus* playback experiment closer in time to the free exploration experiment so that, in addition to comparing periods when the stimulus was playing to when it was silent within the *Gymnotus* playback experiment, we could have also compared behaviors across the two experiments to determine if there were changes in behavior that lingered even while the stimulus was not playing.

Additionally, in both the JAR experiments and the novelty response experiments, we kept the order of stimuli the same across all individuals so that differences in individual behavior could not be attributed to order effects. However, in doing so, we have limited our ability to draw conclusions about the differences we observed between stimuli. We cannot differentiate between effects caused by the order of presentation and those caused by the stimuli. It would appear that we unintentionally placed stimuli to which we expected would get the largest response at the beginning of the experiments (30 s ISI, and Δf of -1). A better approach would have been to keep

the order the same for all individuals but pseudo randomize the presentation order so that stimuli order is more mixed.

Future Directions

This thesis work has established that behavioral syndromes can be identified in at least this species of electric fish and presumably in others as well. In fact, it is likely that other pulse species may provide even better models for individual variation, since EOD rate dynamics (which are even larger in other species) were so integrated in the syndromes we extrapolated. This work serves to inform the electric fish community that the relative EOD rate of individuals can potentially provide a wealth of information about the individual fish. In species that display a dominance hierarchy, this could be a critical piece of information. For example, in an aggressive species like *Gymnotus,* if group housing is preferred, placing individuals with vastly different EOD rates together in a tank may decrease the mortality rate since the dominance hierarchy can be established quickly, rather than placing two individuals with similarly high EOD rates together as these individuals will likely fight extensively to establish dominance.

This work could also lead to exploration of how the actions of neurotransmitters and hormones alter the personality of an individual by altering the characteristics of the EOD rate in conjunction with other behaviors. Serotonin, for example, has been shown to modulate dominance and subordinate behavior in other species of fish (Overli, Harris, & Winberg, 1999), and has also been shown to increase the amplitude and duration of the waveform of *Brachyhypopomus pinnicaudatus*, another pulse species of electric fish (Stoddard, Markham, & Salazar, 2003). This would be an excellent system to further our understanding of the relationship between personalities and circulating hormones. Furthermore, using a simple battery of tests, it would be possible to isolate only the most reactive (or possibly anxious) fish leading

to new models of psychopathological behavior (Pawlak, Ho, & Schwarting, 2008). These models can subsequently be used to test hypotheses about new drug or hormone treatments that could alleviate various conditions.

Additionally, the neural circuitries of both electroreception and electrogenesis have been worked out in a few species (Carlson, 2002; Kawasaki & Heiligenberg, 1989; Metzner, 1999). Having determined that EOD rate and EOD rate dynamics are an integral part of behavioral syndromes in this (and likely other) species, we have the opportunity to create and test hypotheses regarding the brain mechanisms controlling exploration of the environment, responses to novelty, environmental stressors, and possibly personality itself.

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Positions Held:

2002-2007	Graduate Research Assistant - Hunter College, CUNY
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2000-2002	Research Assistant to Michael Noonan PhD – Canisius College

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Teaching Experience:

Hunter College, CUNY:
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