Behavioural syndromes: implications for electrocommunication in a weakly electric fish species

By

Isabelle Shank

Thesis submitted to the
School of Graduate Studies and Research
University of Ottawa
In partial fulfillment of the requirements for the
M. Sc. Degree in the
Ottawa-Carleton Institute of Biology

Thèse soumise à
L'Ecole d'études supérieure et de recherche
Envers la réalisation partielle des exigéances du degré M. Sc.
De l'Institut de Biologie Ottawa-Carleton

Abstract

Behavioural syndromes, defined as suites of correlated behaviours across different contexts, are used to characterize individual variability in behaviours. Males of the weakly electric fish species, *Apteronotus leptorhynchus,* produce electro-communication signals called chirps. Chirps are thought to be involved in agonistic signalling, as their relative incidence increases during agonistic conspecific interactions. However, high levels of individual variability in aggression obscure the role of chirps in mediating aggression. Here, I tested the presence of an aggression-boldness behavioural syndrome, and then considered the implications such a syndrome would have on chirping behaviours. Behavioural tests in anti-predation, object novelty, feeding, conspecific intrusion and novel environment exploration contexts revealed a syndrome involving only object novelty and feeding. We found no correlation between chirping behaviour and the assessed behaviours. Our results demonstrate that chirps represent a more complex communication system than previously suggested.

Résumé

Les syndromes comportementaux, définis comme groupe de comportements corrélés face à différents contextes, sont utilisés afin d'évaluer les variations individuelles de comportements. Les mâles de l'espèce de poisson électrique à faible charge, *Apteronotus leptorhynchus,* produisent des signaux d'électro-communication, les « chirps ». Il est suggèré que les « chirps » jouent un rôle dans la communication agressive, car leur fréquence relative augmente en présence d'un congénère ou d'une simulation d'un congénère. Par contre, les hauts niveaux de variation individuelle dans les taux d'agression obscurent le rôle des « chirps » dans la médiation de conflits. Premièrement, j'ai tenté d'identifier un syndrome comportemental associé à l'audace et à l'agression dans cette espèce. Par la suite, j'ai considéré l'influence d'un tel syndrome sur la production de « chirps ». Les tests comportementaux d'anti-prédation, d'introduction d'un nouvel objet, d'alimentation, d'intrusion territoriale et d'exploration d'un nouvel environnement ont révélé un syndrome seulement pout l'introduction d'un nouvel objet et l'alimentation. Nous n'avons pas trouvé de corrélation entre les comportements examinés et le nombre de « chirps » émis. Nos résultats dèmontrent que les chirps représentent un système de communication beaucoup plus complex que ce précédemment suggéré.

Acknowledgments

I would first like to thank Dr. John Lewis for giving me this amazing opportunity. With his excellent mentorship, support, and patience, I was able to reach my full potential as a researcher and gain intellectual independence. It has been a great journey and I am extremely grateful. I also extend gratitude to the members of my thesis committee, Drs. Katie Gilmour, Jean-Guy Godin, and Vance Trudeau, for their support, input and encouragement. I would also like to thank Bill Fletcher for his assistance.

Through the blood, sweat, and tears, I received endless support from previous and present Lewis Lab members. Whether it was brainstorming sessions, revision of manuscripts or coffee breaks filled with laughter, they were always there. A special thanks to Ginette Hupé for inspiring and helping me throughout the process.

I would also like to thank my family for their love, encouragement, kind words and especially their patience during the most challenging times. Your endless support has made this possible. The cheers offered by friends, has also been essential. Special thanks to Sara Abdallah for her constant encouragement, help and optimism throughout these past years.

To conclude, I acknowledge financial support provided by the University of Ottawa in the form of an admission scholarship. This research was financially supported in part by the Natural Sciences & Engineering Research Council (NSERC) of Canada Discovery Grant to John Lewis.

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List of Abbreviations

AC – Alternating current

AP – Anti-predation test

CI – Conspecific intrusion test

CI adj. – Adjusted Conspecific intrusion test

DC – Direct current

DF – Difference frequency

ELL – Electrosensory lateral lobe

EOD – Electric organ discharge

EODf – Electric organ discharge frequency

EX – Novel environment exploration test

FE – Feeding test

JAR – Jamming avoidance response

N – Sample size

ON – Object novelty test

PCA – Principal component analysis

W1 – Week 1

W2 – Week 2

1. General Introduction

An animal's behaviour serves as the interface among its genotype, phenotype, and the surrounding environment. Behaviours mediate a variety of functions such as maintaining homeostasis, and alternating feeding and reproductive strategies in shifting environmental conditions. Thus behaviours reflect an individual's internal state and condition. However, traditional studies only consider behaviour in a one-dimensional manner, not taking into consideration that the behavioural observations from which conclusions were drawn, only represented snapshots of the animal's behavioural spectrum. Such studies described behaviours as stereotyped reactions to the environment with little room for variation (Dall *et al.*, 2004; Réale *et al.*, 2007; Kappeler and Kraus, 2010; Conrad *et al.*, 2011).

In recent years, the field of animal behaviour has undergone somewhat of a revolution with the acknowledgement of the importance of behavioural variability (Dall *et al.*, 2004; Réale *et al.*, 2007; Kappeler and Kraus, 2010; Conrad *et al.*, 2011). Studies of animal behaviour have now become multi-dimensional, and attempt to understand the whole spectrum of variable behaviours. Rather than attempting to characterize behaviours as distinct stereotyped phenomenon exhibited by all members of a species, the focus has been on characterizing the magnitude of variability and the possible mechanisms that permit them to perpetuate throughout the animal kingdom (Kappeler and Kraus, 2010).

1.1 Behavioural variability

Variability in animal behaviour has been the subject of scientific investigations for more than fifty years and major changes in theories and ideas have occurred within

this topic of behavioural ecology (Kappeler and Kraus, 2010). In general, phenotypic, genetic and behavioural variation provides increased adaptability to alternating selection pressures that arise in dynamic environments. Thus, variation is advantageous for survival, and hence is maintained at multiple organisational levels (Mather, 1966; Bulmer, 1971; Lacy, 1997). Empirical evidence demonstrates that variation in behaviour occurs across species, across populations, across individuals, and even within an individual (Kappeler and Kraus, 2010). The underlying mechanisms believed to be the cause of behavioural variation differ for each level.

1.1.1 Variability across species

It seems somewhat intuitive that animals of differing species will differ in their behaviour. The major factor in shaping these disparities is the difference in life history traits across the animal kingdom (Kappeler & Kraus, 2010). For example, mode of fertilization, egg size, and territoriality are some of the phylogenetic traits that dictate the type of parental care behaviours across fish species (Gross & Sargent, 1985). In North and South American birds, parental care feeding behaviours are influenced by clutch size (Martin *et al.*, 2000). Social behaviours are also affected by the cognitive abilities dictated by the phylogenetic traits of the animal. For example, European starlings (*Sturnus vulgaris*) can visually track objects, a cognitive ability that enables them to discriminate and associate with specific neighbours within a group. Combined with social learning, this cognitive ability gave rise to flocking, a collective animal behaviour (Ballerini *et al.*, 2008). Overall, life history traits that mediate behavioural inconsistencies across species can be generalized into four broad categories: modes of reproduction,

progeny parameters (size, number, etc.), mortality patterns, and body dimensions (Kappeler & Kraus, 2010).

1.1.2 Variability across populations

The majority of studies pertaining to behavioural variability focus on the variance within species. Different populations of a given species often adopt different behavioural solutions when faced with a problem or challenge associated with their local condition (Kappeler & Kraus, 2010). Consequently, a large proportion of variance across populations results from adaptation to local conditions and to phylogenetic traits (Kappeler & Kraus, 2010). A traditional manifestation of this type of variation is seen when comparing populations faced with varying predation risks. Sticklebacks (*Gasterosteus aculeatus*) from high predation areas exhibit stronger anti-predatory responses to a simulated predatory attack compared to individuals from a low predation area (Bell, 2005). High predation conditions selected for shy individuals, resulting in a population that is less gregarious.

1.1.3 Variability across individuals

Within a population, all organisms are faced with the same local condition and have overall the same life history traits. Yet in most populations, we do not find only one optimal behavioural phenotype (Persson *et al.*, 1997; Stamps, 2003; Kappeler and Kraus, 2010). This phenomenon is made obvious with the varying social and hierarchical structures within populations (Persson *et al.*, 1997). For example, in honey bees, colony role is determined by an individual's responsiveness to sucrose. Increased sensitivity to this primary resource leads to the attribution of a foraging role and the development of

foraging behaviours in the respective bee (Scheiner *et al.*, 2004). Animal species that are the subject of animal personality and behavioural correlates studies are also good examples of within population behavioural variability (Bergmüller, 2010). It is suggested that the generation of variance at the population level is a result of the interaction among an individual's genes, hormonal state, environment, and experiences (Persson *et al.*, 1997; Sih *et al.*, 2004A; Kappeler and Kraus, 2010).

1.1.4 Variability within individuals

Intra-individual variation is the primary level from which behavioural variance originates. Seasonal changes, age and associated experiences, social environment, and resource availability are the major modulators of an individual organism's behaviour (Kappeler and Kraus, 2010). In Zimbabwean native impalas (*Aepyceros melampus*), for example, the dimensions of the herd (i.e. the size of the social group), will determine the type of resources an individual will graze upon, thereby modifying an individual's feeding behaviours (Fritz and Garine-wichatitsky, 1996). In Californian mice (*Peromyscus californicus*), social circumstances have direct effects on aggressive encounters with conspecifics. Holding residency in intruder-resident confrontations results in higher winning rates in this species (Fuxjager *et al., 2009)*. Additionally, previous successful experience further enhances the winning probability of an individual via changes in testosterone and progesterone levels, which provides the mouse with increased dominant behaviours (Fuxjager *et al.*, 2009). Hence individual variability in behaviour can originate from one or multiple factors listed above.

1.2 Behavioural syndromes

1.2.1 Defining behavioural syndromes

Behavioural variability implies that animals can successfully adopt different strategies to achieve growth, survival and reproduction (Bergmüller, 2010). Individual variability in behaviour recently has been characterized using the concepts of behavioural syndromes and animal personalities (Sih *et al.*, 2004B). Although these concepts are often used synonymously, there are distinct differences between them. Animal personality describes a behavioural type or trait consistently expressed by an individual across varying contexts and time (Bergmüller, 2010; Briffa and Weiss, 2010). A behavioural syndrome represents a group phenomenon where there is not only consistency within an individual's behaviour, but also consistency in the rank order within the population across contexts and time (Sih *et al.*, 2004B; Bergmüller, 2010; Briffa and Weiss, 2010).

Additional terminology that is relevant to behavioural syndromes is the idea of contexts and situations. Contexts are defined as categories of biological functions in which behaviour occurs, such as aggression, feeding, and courtship (Briffa and Weiss, 2010). During an observation within a given context, a situation refers to the environmental or social condition at that specific moment in time, for example aggression in high versus low predation risk, or feeding behaviours when an individual is alone versus in a social group (Briffa and Weiss, 2010). Although the feeding context is the same, an animal might display different behaviours when faced with a social versus non-social situation. Therefore, behaviours may vary at the level of contexts and/or situations.

1.2.2 Types of behavioural syndromes

Aggression is a behavioural context associated with actual attacks, threats of attacks, or signals that suggest potential attacks (Drews, 1993). Consistent individual variation in aggression has been observed in many taxa including arthropods (Riechert and Hedrick, 1993; Wilson *et al.*, 2010), birds (Verbeek *et al.*, 1996), mammals (Svartberg *et al.*, 2005), and fishes (Huntingford, 1976; Brick and Jakobsson, 2002; Bell, 2005; Bell and Sih, 2007; Dingemanse *et al.*, 2007). These studies, however, do not only investigate aggression, but discern individual variability in both aggression and boldness in their target species. Boldness is defined as any risk-taking behaviour, which indirectly implies that aggression represents an escalated form of boldness (Huntingford, 1976; Dingemanse and Réale, 2005; Conrad *et al.*, 2011). Individual co-variability in aggressive and bold behaviours can be characterized as an aggression-boldness behavioural syndrome (Huntingford, 1976; Riechert and Hedrick, 1993; Verbeek *et al.*, 1996; Bell, 2005; Dingemanse *et al.* 2007, Brick and Jakobsson, 2002). For example, in the stickleback *Gasterosteus aculeatus,* bold behaviours in the presence of a potential predator are positively correlated with displays of aggression towards conspecifics (Bell, 2005).

Exploration and activity levels also have been shown to form a syndrome with aggression and/or boldness (Verbeek *et al.*, 1996; Brown *et al.*, 2007; Wilson and Godin, 2009). In great tits, *Parus major*, fast exploring individuals were more aggressive and initiated more fights than shy explorers, and were also more successful in these fights (Verbeek *et al*., 1996). Similarly, strong correlations were found among boldness, exploration and activity in the invasive mosquitofish (*Gambusia affinis*), resulting in

variation in individual tendencies to disperse and invade novel environments (Cote *et al.*, 2010).

As previously mentioned, the presence or absence of correlated behaviours can be affected by the situation in which a subject's behaviour is observed. Predation risk is one of the most common situational factors that can affect behavioural variability. For instance, an aggression-boldness behavioural syndrome was found within a predator-exposed population, but not in a predator-free population of three spined sticklebacks (Bell and Sih, 2007). Behavioural correlates also have been found to be affected by the social environment in which the subject is being observed (Sih and Watters, 2005; Webster *et al.*, 2007). An individual's overall activity and tendency to forage under predation risk correlated positively in sticklebacks tested alone, while no such correlations were found in fish tested in group settings (Webster *et al.*, 2007).

1.2.3 Evolution of behavioural syndromes

Evidently, contexts and situations affect the expression of behavioural consistency. However, the underlying mechanisms by which this consistency and correlativity occur are not well understood. Due to the effects of context and situation, it appears that selection forces play a major role in the evolution of behavioural syndromes as adaptations (Dall *et al.*, 2004; Sih *et al.*, 2004A; Bell, 2005; Dingemanse and Réale, 2005; Bergmüller, 2010). The adaptive hypothesis states that behavioural correlations are a result of selection and therefore the existence of such behavioural correlates is dependent on the behaviours being adaptive (i.e. favours the fitness of an individual) (Bell, 2005; Bergmüller, 2010). Although this hypothesis is valid in many cases, where the outcome of correlation is beneficial, it can also lead to correlated behaviours that are

maladaptive (Sih *et al.*, 2003; Johnson and Sih, 2005). An example of this phenomenon is seen in female fishing spiders *Dolomedes triton*. In this species, selection-favoured voracity in females can lead to unnecessary sexual cannibalism, which consequently reduces the female's potential to successfully mate and can effectively reduce her fitness (Johnson and Sih, 2005). Such contradiction has led to the formulation of an alternate hypothesis.

The 'constraint hypothesis' states that behavioural syndromes originate from underlying proximate mechanisms such as pleiotropic effects of genes and hormonal axes. Consequently, a change in correlated behavioural traits would require genetic mutations or the development of different hormonal axes (Sih *et al.*, 2004A; Bell, 2005; Bell, 2007; Bergmüller, 2010). For example, if a correlation was observed between aggression against conspecifics and predators, and these were under the control of androgens, they could become decoupled if selection for the stress axis overcame selection for the androgen axis to solely act upon behaviour in response to predation. The stress axis would modulate the anti-predatory response so that the behaviours were no longer correlated with conspecific aggressive behaviours. Support for this hypothesis has been obtained in sticklebacks where individual behavioural responses to experimental presentation of environmentally-relevant stressors (i.e. predator and unknown conspecific) were reflected in the neuroendocrine stress response of the individual (Bell *et al.*, 2007). The HPI axes in this case dictated behavioural responses.

Although the adaptive and constraint hypotheses are both supported by evidence, they do not always successfully account for all the behavioural variability within a system (Sih *et al.*, 2004; Bell, 2005; Bergmüller, 2010). It is possible that these limitations are

due to shortcomings in experimental design. However, it seems likely that correlated behaviours occur due to varying combinations of both adaptive and constraint mechanisms. Although mechanisms for the evolution of behavioural syndromes have not been determined, the concept of correlated behaviours advocates the study of different behaviours across varying situations rather than studying isolated behaviours (Sih *et al.* 2004A).

1.2.4. Evaluating behavioural syndromes

In order to assess correlated behaviours, studies of behavioural syndromes devise experimental designs that consist of series of behavioural tests. The choice of tests reflects behavioural contexts where the desired behaviours would be manifested by individuals. There are disagreements in the literature regarding which tests are appropriate assessors of behaviour. Here, I provide examples of tests that are used to assess the most studied behavioural axis: aggression, boldness, and exploration.

Aggression, as previously stated, is defined as behaviours associated with actual attacks, threats of attacks, or signals that suggest potential attacks (Drews, 1993). Assessments of aggression usually involve confrontations with conspecific individuals of the same or opposite sex, heterospecific individuals, or potential prey (Riechert and Hedrick, 1993; Bell, 2005; Johnson and Sih, 2005; Dingemanse *et al.*, 2007; Wilson *et al.,* 2010). Common behavioural measures of aggression are time spent orienting towards target, time spent within a certain distance of target, number of attacks, latency to attack, and conflict outcome (Riechert and Hedrick, 1993; Bell, 2005; Johnson and Sih, 2005; Dingemanse *et al.*, 2007; Wilson *et al,* 2010).

Boldness is often defined as any risk-taking behaviour (Huntingford, 1976; Dingemanse and Réale, 2005, Conrad *et al.*, 2011). Tests aiming to assess bold behaviour therefore present a context that exposes the focal individual to risk. Experimentally, this is usually achieved by the use of open field tests, predatory inspection tests, feeding tests, and/or novel object inspection tests (Riechert and Hedrick, 1993; Gosling, 2001; Bell, 2005; Burns, 2008;Wilson *et al.*, 2010). Boldness is frequently quantified using parameters such as: time spent investigating an object or predator, latency for investigation, latency to exit a refuge, total time spent foraging, etc (Riechert and Hedrick, 1993; Gosling, 2001; Bell, 2005; Burns, 2008; Wilson *et al.*, 2010).

Distinctions between boldness and exploration behaviours are sometimes difficult to discern. Exploration is defined by an individual's willingness to investigate sources of novelty (such as the environment, food, or an object) (Conrad *et al.*, 2011). To avoid overlap with bold behaviours, the element of risk is removed in exploration tests. Therefore, similar tests such as open field tests, feeding tests, and novel object tests are used to assess exploration, but without any obvious risks (Verbeek *et al.*, 1998; Bell, 2005; Dingemanse *et al.*, 2007; Wilson and Godin, 2009; Wilson *et al.*, 2010). Behaviours quantified in exploration tests often include: latency to explore, time spent stationary, area used or covered, and time spent exploring (Verbeek *et al.*, 1998; Bell, 2005; Dingemanse *et al.*, 2007; Wilson and Godin, 2009; Wilson *et al.*, 2010).

Consequently, test designs require thought and consideration in order to ensure that the test properly addresses the target behaviour. Furthermore, it is also very important to properly select behavioural measures that will be noted during these tests. It is sometimes possible that one test with two different sets of measures can assess two

types of behaviour, such as boldness and exploration. It is also important to note that commonly used tests are not always feasible for the species of study and that modifications from the common designs can be made with careful consideration (Bell, 2007).

1.3 Electric fish

In recent years, electric fish have become popular model organisms for neurobiological and ethological studies. Great focus has been placed on investigating their anatomy and physiology owing to their unique electric sense. This extensive work provides an ideal platform to study behaviour, because direct links can be made among neural processes, motor outputs, and behaviour. However, many behavioural studies are experimental subsets to neurobiological studies, and few studies have aimed to solely characterize behaviour.

1.3.1 Electric fish diversity

Aquatic organisms generate weak electric fields because of the uneven distribution of ions between their bodies and the surrounding aqueous environment (Moller, 1995; Caputi *et al.*, 2005). These weak electric fields are generated by biological events such as neurotransmission and muscle contractions. Electric fish have exploited this natural phenomenon via the evolution of a sophisticated mechanism for electroreception and electrogeneration. Through the generation and reception of electric fields, electric fish are capable of achieving complex behaviours such as navigation, prey

capture, defence and communication in the dark or in turbid waters (Hagedorn and Heiligenberg, 1985; Nelson and Maciver, 1999; Von der Emde, 2006).

Although electroreception is not a sensory modality that is unique to electric fish, electrogeneration has evolved independently six times within electric fish lineages (Moller, 1995; Rose, 2004). Consequently, there are four major groups of electric fish: the Mormyriforms, the Gymnotiforms, the Siluriforms and the Rajiformes (Moller, 1995). Within these groups, fish are classified based on the strength of their electric discharge: strongly electric (voltages up to several hundred volts) or weakly electric (voltages in the millivolts range) (Zupanc & Bullock, 2005; Stoddard and Markham, 2008). All species of the Siluriforms (catfish) and the Rajiformes (skates & rays), as well as the *Electrophorus* (electric eel) of the gymnotids, generate intermittent strong electric discharges (Moller, 1995; Rose, 2004). These strongly electric fish are found both in freshwater and marine environments (Westby, 1988; Stoddard, 1999; Moller, 1995; Rose, 2004) . Mormyriforms and Gymnotiforms, indigenous to African and South American freshwaters, respectively, emit their weak electrical field in a more consistent manner (Moller, 1995; Alves-Gomes, 1999; Zupanc & Bullock, 2005; Crampton and Albert, 2006; Lovejoy *et al.*, 2010).

Further compartmentalization occurs within the weakly electric fish regarding the type of discharge with which the electric field is generated. In pulse type discharges, the delay between discharges is longer than the discharge duration, thereby creating non-continuous electrical pulses (fig. 1.1) (Moller, 1995; Zupanc and Bullock, 2005). These pulses can be emitted in a regular or irregular fashion, depending on the species. In contrast, wave-type discharges are characterised by similar discharge intervals and

discharge duration, resulting in a continuous, wave-like electrical signal (fig. 1.1) (Moller, 1995; Zupanc and Bullock, 2005). The generation of the electrical signal from the electric organ in both cases follows a similar mechanism in all electric fish, notwithstanding the type or strength of the discharge.

1.3.2 Electrogeneration

There is great diversity in the properties of the electric discharge within both mormyrids and gymnotids. As can be seen from field recordings by Hopkins (1981), great diversity in the number of phases of the waveforms as well as the duration of the waveform are present in Mormyrids (fig. 1.1). Sexual differences in signals are also present in mormyrids species such as in *Marcusenius senegalensis*, *Petrocephalus bovei*, and multiple species of the *Brienomyrus* group, where males have longer waveform durations (Carlson, 2002). In gymnotids, similar differences are seen within pulse-type fish, as well as in wave-type fish. Diversity in wave-type signals is generated by the shape of the waveform (fig. 1.1), as well by the frequency of the discharge. The frequency range expands from 25 Hz in *Sternopygus branco*, to 2180 Hz in *Sternachella schotti* (Crampton and Albert, 2006). The frequency of the EOD can be used to distinguish conspecifics from other wave-type species, as observed in *Apteronotus leptorhynchus* (Fugère and Krahe, 2010). *Apteronotus leptorhynchus* also displays sexual dimorphism in its frequencies with males emitting from 800-1100 Hz and females emitting in the 600-850 Hz range (Maler and Ellis, 1987; Zakon *et al.*, 2002; Dunlap and Larkins-Ford, 2003). Therefore, shape and frequency of the waveform of the electric discharges can provide information that can be used in social interactions.

The neural pathway that regulates the electric discharge is very specific, and is well known. The most important centre of the pathway is the pacemaker nucleus, which consists of neurons that are electrically- and chemically-coupled and fire endogenously (Metzner, 1999; Rose, 2004). The firing rate of the pacemaker nucleus, located in the medullary region of the hindbrain, determines the frequency of the EOD (Metzner, 1999; Rose, 2004). In the pacemaker nucleus, pacemaker cells innervate relay cells which in turn transmit the signal down the spinal cord and synapse onto motor neurons (Metzner, 1999; Rose, 2004). Depending on the type of electric organ, the motor neurons will either form the organ with their distal ends, or they will innervate the electric organ. The electric organ then generates the EOD, at the frequency set by the pacemaker nucleus (Caputi *et al.*, 2005; Stoddard *et al.*, 2006). It is important to note that there are many more centers in the brain that innervate the pacemaker nucleus and modulate its firing rate (Kawasaki *et al.*, 1988; Caputi et al., 2005).

Electric organs are derived mostly from modified muscle cells; however, Apteronidae species of the Gymnotiform group have electric organs derived from modified spinal neurons (Caputi *et al.*, 2005). The electric organs are composed of electrocytes, long and slender cells that are stacked in rows along the horizontal axis of the body forming columns enclosed in connective tissue (Bennett, 1970). Motor neuron innervation causes a synchronous depolarization of the electrocyte membranes, creating a "parallel" movement of charges inside the cells, thereby creating a net positive charge inside the electric organ and a net negative charge on the outside (Stoddard *et al.*, 2006). This separation of charges creates the electrical field, and changes in these charges create changes in the overall shape of the field (Caputi *et al.*, 2005; Knight, 2008).

Diversity in the kinetic properties of the channels involved in electrocyte depolarization, as well as diversity in the innervation patterns, lead to the previously discussed diversity of EOD waveforms across electric fish species (Bennett, 1961; Caputi *et al.*, 2005). The pacemaker nucleus action potentials drive the waveforms in a one-to-one pattern, giving rise to specific electric organ discharge frequencies (EODf) (Stoddard, 1999; Carlson, 2002; Triefenbach and Zakon, 2003; Stoddard *et al.*, 2006; Crampton and Albert, 2006).

1.3.3 Electroreception

As well as having electrogenic capacity, electric fish have evolved an electroreception system, permitting them not only to sense electric fields in their environment, but also to sense their own electric discharge. At the basis of electroreception are two types of electroreceptors: ampullary and tuberous receptors (Zupanc and Bullock, 2005). Ampullary receptors, widespread amongst non-teleost and teleost fish, including electric fish, respond to AC (alternating current) electric fields, low frequency DCs (direct currents), and also serve to detect low frequency components of electric discharges (Zupanc and Bullock, 2005). Tuberous receptors, found only in weakly electric fish species, respond to EODs within a biologically relevant range of frequencies (Zupanc and Bullock, 2005). T-type tuberous receptors encode the timing of the EOD waveforms, whereas P-type tuberous receptors respond to changes in the EOD amplitude (Zupanc and Bullock, 2005). The combination of these two types of tuberous receptors, as well as the ampullary receptors, permits a complete assimilation of the electric signal, thereby permitting the use of the signal for complex behaviours.

Encoding of the electroreceptor response is initiated by the transmission of the signal to the electrosensory lateral line lobe (ELL) via the electroreceptor afferents (p-units) (Zupanc and Heiligenberg, 1992; Rose, 2004). From the ELL, the signal is then passed on to other electrosensory and electromotor processing centers in the brain, until it reaches the nucleus electrosensorius (NE), and finally with projections ending in pacemaker nucleus input (Zupanc and Heiligenberg, 1992; Rose, 2004).

1.3.4 Electrocommunication

Communication is a complex behaviour used in a context-specific fashion to transfer information from one individual to another (Bradbury and Vehrencamp, 1998). More specifically, communication is believed to represent a situation where information, in the form of a signal, is transferred from a sender to a receiver resulting in a behavioural influence upon the receiver (Bradbury and Vehrencamp, 1998). Furthermore, a signal is only considered a true communication signal when it satisfies the following: 1) that the transfer of information benefits the sender and is not accidental, i.e. where the costs of signal production are outweighed by gains of signal generation; 2) the receiver must also benefit from the information encapsulated within the signal (Bradbury and Vehrencamp, 1998).

The combination of electroreception and electrogeneration in electric fish presents an ideal system for the evolution of electrocommunication. Electrocommunication in weakly electric fish species is achieved by the modulation of the EOD (Moller, 1995). Multiple studies have demonstrated that a variety of factors such as circadian rhythms (Stoddard *et al.*, 2006; Perrone *et al.*, 2010), hormones and neurotransmitters (adrenocorticotropic hormone, androgens, serotonin, etc.) (Mills and Zakon, 1991;

Stoddard *et al.*, 2003; Stoddard *et al.*, 2006; Perrone *et al.*, 2010), and social interactions (Zakon and Dunlap, 1999; Carlson *et al.*, 2000; Zakon *et al.*, 2002; Dunlap and Larkins-Ford, 2003), can cause changes in the electric waveforms. Such changes include frequency excursions, modulation of waveform amplitude, specific pattern modulations, and signal interruptions (Mills and Zakon, 1991; Zakon and Dunlap, 1999; Carlson *et al.*, 2000; Zakon *et al.*, 2002; Dunlap and Larkins-Ford, 2003; Stoddard *et al.*, 2003; Stoddard *et al.*, 2006; Perrone *et al.*, 2010). To date, studies have failed to demonstrate that electrocommunication signals satisfy both criteria of true communication, which is why they are deemed to be putative communication signals. Nevertheless, multiple examples of electrocommunication are found within weakly electric fish. A well-developed EOD "dialogue" exists within male-male and male-female interactions of *Brachyhypopomus pinnicaudatus*, punctuated with accelerations, interruptions and chirps (Perrone *et al.*, 2009). Individuals of the mormyrid pulse-type species *Pollimyrus isidori* modify the length and uniformity of their EODs in both aggressive and reproductive contexts (Bratton and Kramer, 1989).

The most widely studied communication system within the weakly electric fish group is that of *A. leptorhynchus*, a South American wave-type species. *Apteronotus leptorhynchys*, commonly known as the brown ghost knifefish, can modulate its EOD into four well characterised behaviours: JARs (jamming avoidance responses), dips, rises, and chirps (Zakon *et al.*, 2002; Zupanc, 2002). Amongst these, only chirps, defined as transient frequency excursions, are considered potential communication signals (Zakon *et al.*, 2002; Zupanc, 2002). Although, chirp production occurs in both males and females of the species, rates of production are sexually dimorphic and are type-

dependent, occurring more in males than females (Zakon *et al.*, 2002). Characterisations of chirps have led to the identification of four to six different types of chirps, with disagreement arising in the characterisation of types 3-6, which are thought to possess social significance in reproductive contexts (Zakon *et al.*, 2002; Zupanc, 2002; Zupanc *et al.*, 2006). Type 1 (~ 20 ms long with frequency excursion of 200-300 Hz) and type 2 chirps (~20 ms long with frequency excursions of 50 Hz) (fig. 1.2) are speculated to act as aggressive signals as their incidence, especially type 2 chirps, increases in male-male interactions (Zakon *et* al., 2002; Zupanc, 2002; Zupanc *et* al., 2006).

Multiple studies have attempted to shed light on the social significance of type 2 chirps and the role they play in mediating conspecific aggression (e.g. Hupé and Lewis, 2008). Correlations among EOD frequency, body size, and chirp rates have been discovered, suggesting that *A. leptorhynchus* form social dominance hierarchies (Hagedorn and Heiligenberg, 1985; Dunlap, 2002; Triefenbach and Zakon, 2003; Triefenbach and Zakon, 2008). Furthermore, certain studies have found that chirps predict attacks and are positively correlated with overall expressed aggression, supporting the dominance hypothesis (Triefenbach and Zakon, 2008).

Other studies, however, contradict this school of thought. Chirps have been found to deter conspecific aggression (Hupé and Lewis, 2008; Fugère *et al.*, 2011). From these observations, it was deduced that chirps are used in opponent assessment and serve to mediate agonistic conflicts in order to avoid costs associated with elevated aggression (Hupé and Lewis, 2008; Fugère *et al.*, 2011; Hupé, 2012).

The discrepancy between these two ideas on the social significance of chirps may be due to the variability in aggressive displays between male individuals observed in

response to chirp playback trials. While some fish respond quite aggressively to the conspecific stimulus (chirp), some respond moderately, while others do not respond at all (Hupé, 2012). Furthermore, the chirp response of these male individuals faced with a conspecific stimulus (chirp), was shown to be greatly variable (Hupé, 2012). For example, fish that exhibit high levels of aggression, showed both high and low chirp rates (Hupé, 2012). These findings have raised questions regarding the manifestation and control of aggression in this species as well as the role of chirps in mediating aggression. A characterization of the variability in aggressive behaviour in this species would provide a stronger platform on which to study variation in electrocommunication strategies. As previously stated, behaviours are varied and multidimensional, requiring in depth characterization in order for social significance to be established.

1.4 Objectives

This study had two major objectives. The first was to determine whether the variability of aggressive and bold behaviours within male *A. leptorhynchus* could be described by a behavioural syndrome. A behavioural syndrome requires that the behaviours of individuals within a population be:

1) Consistent in time, and
2) Correlated across varying contexts.

A repeated assay of five behavioural tests, where boldness or aggression could be manifested (anti-predation behaviour test, object novelty test, feeding test, conspecific intrusion test and novel environment exploration test), was developed in order to assess these criteria within the brown ghost knifefish (fig. 1.3).

The second objective of this study, pertaining to the chirping behaviour of *A. leptorhynchus*, was dependent on the results of the first objective. Therefore, two sets of hypotheses were developed to address 1) the case of the presence of a behavioural syndrome or 2) the case of no behavioural syndrome:

1) In the instance that an aggression-boldness behavioural syndrome was observed across designated contexts and time, it was hypothesized that the respective behavioural type of an individual could act as a predictor of chirping behaviour. Bold-aggressive behavioural types were predicted to show higher chirp rates whereas low or null chirp rates were predicted in shy-non aggressive behavioural types.

2) If a behavioural syndrome did not characterize the aggressive-bold behaviours in male *A. leptorhynchus*, it was hypothesized that the significance of chirping would be reflected in the presence of correlations between exhibited behaviours in one of the specific contexts (anti-predation behaviour test, object novelty test, feeding test, conspecific intrusion test and novel environment exploration test) and chirp rates. Based on the existing school of ideas relating to the purpose of chirps, three hypotheses were outlined:

a) If chirping acts as a dominance and aggressive signal in *A. leptorhynchus,* it was hypothesized that chirp counts will exert an effect on aggressive behaviours. It was predicted that a positive correlation should be present between chirp counts and aggressive behaviours exhibited in the conspecific intrusion test.

b) Alternatively, chirps may act to deter aggression and serve to assess an opponent with minimized costs. Engaging in chirping behaviour still

entails significant risk for an individual as opposed to avoidance, but represents less risk than a direct encounter. Therefore, chirping may reflect innate boldness rather than innate aggression. Thus it was predicted that behaviours exhibited in tests where boldness is prominent (i.e., the object novelty test, the feeding test or/and the anti-predation test) should positively correlate with chirp rates.

c) If chirps are not a communication signal, but instead serve as an electrolocation mechanism (i.e. chirps used to prime electroreceptors and improve the acquisition of sensory information when signal interference results from the EOD of a conspecific), it was predicted that chirp counts and exploration behaviours would correlate positively with one another.

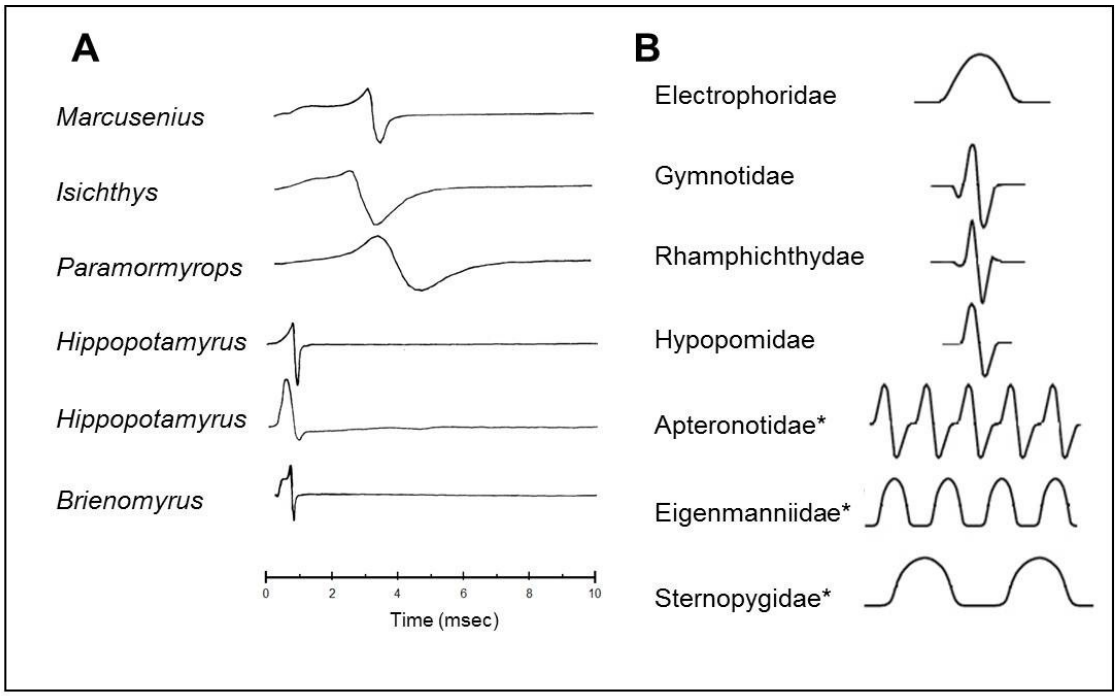

Figure 1.1 Representation of electric waveform diversity in weakly electric fish species. Panel A displays examples of mormyrid waveforms (modified from Hopkins, 1981). Panel B illustrates an assortment of gymnotid waveforms for both wave and pulse type discharges (modified from Stoddard, 1999).
* Wave type electric discharges

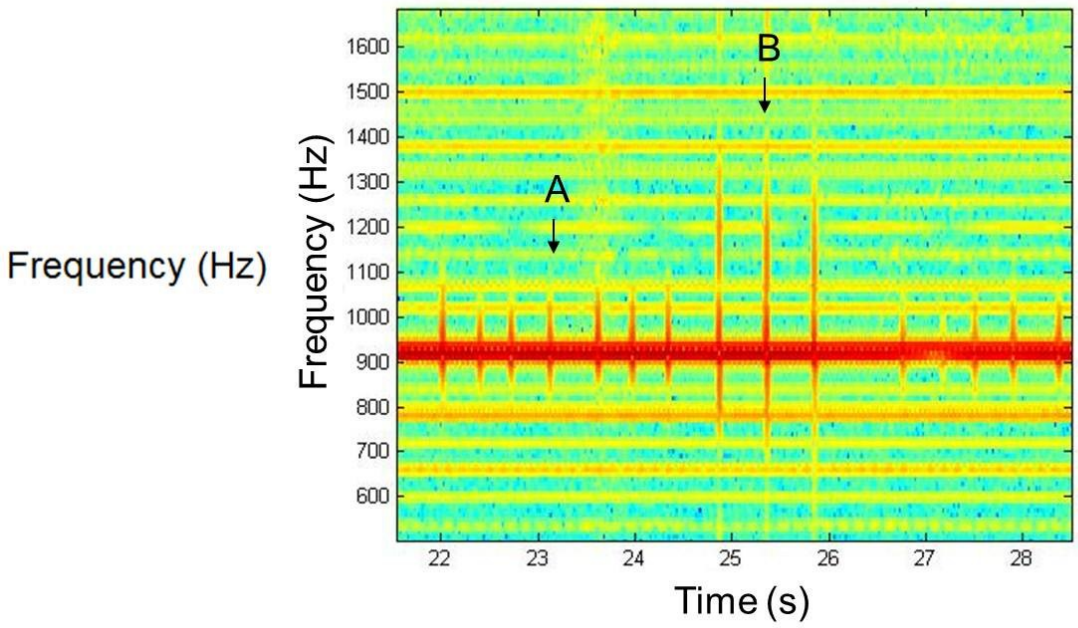

Figure 1.2. Spectrogram displaying the EOD of two *A. leptorhynchus* with a DF of 10 Hz (DF too small to see two separate bands). Distinction of two chirp types, Type 1 (B) and Type 2 (A) is illustrated in this electrical recording.

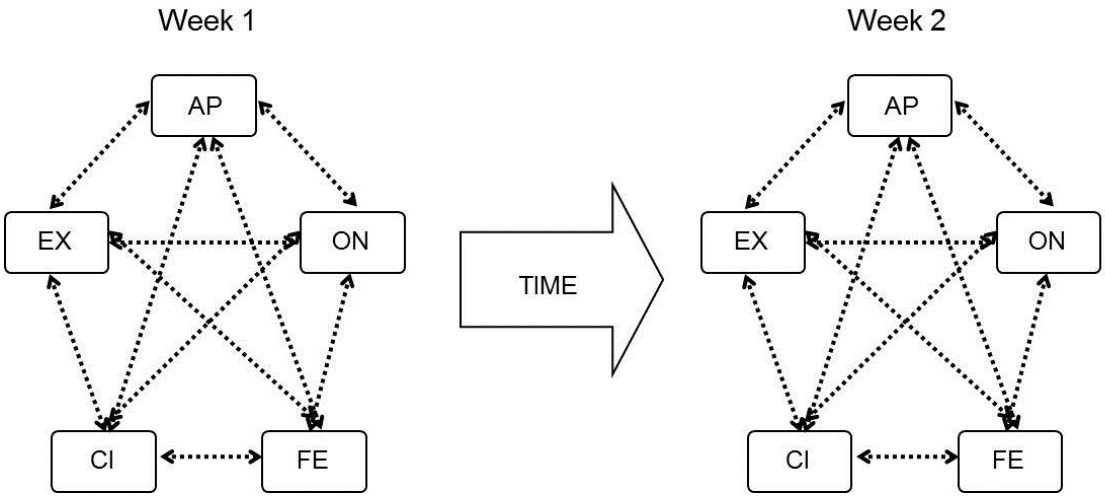

Figure 1.3. Summary figure illustrating the expected correlations across the five behavioural tests if an aggression-boldness behavioural syndrome was present in male *A. leptorhynchus*. Behavioural tests: Anti-predation (AP), Object novelty (ON), Feeding (FE), Conspecific intrusion (CI) & Novel environment exploration (EX).

2. Materials & Methods

2.1 Housing conditions

Wild caught brown ghost knife fish, *Apteronotus leptorhynchus*, were purchased from commercial tropical fish suppliers (AQUAlity, Mississauga, ON; DAP International Ltd., Etobicoke, ON). To ensure optimal temperature conditions during land transport, the animals were placed in insulated containers equipped with heating packs. Upon arrival to the University of Ottawa aquatic care facility, the fish were placed in holding tanks of 115 litres in mixed sex groups of 4 to 7 individuals. Tank conditions were maintained at temperatures of 27 to 28 °C and at conductivities of 100 to 200 μS through the help of a flow-through system supplied by dechloraminated city of Ottawa tap water. Fish were exposed to a reversed12:12 (dark/light) photoperiod to permit nocturnal observations during the day. Feeding occurred three times weekly and consisted of frozen blood worms (~30 bloodworms/fish). All experiments followed protocols approved by the University of Ottawa Animal Care Committee (BL-229). Fish remained in the holding tanks for at least one week before they were exposed to the experimental regime.

2.2 Behavioural observations

Experiments were performed from October to December 2011 and March to October 2012. To standardize feeding motivation, fish were fed at least one hour prior to behavioural observations. Post feeding, fish were selected for sex determination, as only males were chosen for experimental observations. Sex was determined by EOD frequency (EODf) of the fish. As previously mentioned, males emit an EODf in the range of 800-1100 Hz (Maler and Ellis, 1987; Zakon *et al.*, 2002; Dunlap and Larkins-Ford,

2003). To ensure that only males were included in our data set, only fish with EODf higher than 850 Hz were chosen for experiments.

EODs were recorded using a pair of Teflon-coated silver-wire electrodes (diameter 0.25 mm, insulated to the tip; WPI, Inc., Sarasota, FL, USA) and a grounded Teflon-coated silver-wire electrode (insulated to the tip). An AM Systems differential amplifier and a real-time A/D processor (D1104, dSpace Inc) were used to amplify and acquire the electrical signals, respectively. Data acquisition was controlled by Simulink (the Mathworks) and Control Desk (dSpace Inc) software. The EODf was determined by visual inspection of spectrograms (window size 1000 pts, NFFT 1000, and overlap 900; Matlab, The Mathworks) of the recorded EOD signals (eg. see fig. 1.2). Fish were placed into the shelter (W= 30 cm; L= 17 cm; H= 27 cm), a restricted area at one end of the experimental tank (W= 30 cm; L= 60 cm; H= 27 cm) that was closed off by a trapdoor until testing. Further details are provided in the descriptions of the individual behavioural tests. Experimental tank conditions were set to 26.5-29.5 °C and 200-300 μS.

During experimentation, each male *A. leptorhynchus* was exposed to a series of behavioural tests representing different contexts to determine whether measures of aggression and boldness were correlated within and across contexts. The series of tests was: (1) anti-predation test, (2) object novelty test, (3) feeding test, (4) conspecific intrusion test, and (5) novel environment exploration test (descriptions to follow). One trial was performed per day for a total of five consecutive experimental days. The order of the tests was consistent for all fish, i.e. were not randomized, this allowed for better assessment of repeatability (Bell, 2013). All tests were performed during the dark cycle of the photoperiod. After the last test, fish were weighed and their total body lengths were measured.

Following the five behavioural tests, fish were returned to specialized holding tanks with mesh dividers to provide physical isolation, but prevent social isolation from other males. Because social isolation has been shown to have various effects on aggressive and bold behaviours in fish species (Halperin *et al.*, 1992; Gomez-Laplaza and Morgan, 2000), we wanted to avoid such confounds in our experimental design. To assess the robustness of the behavioural correlations, the same series of tests was repeated 9 days after the last test of the first series (fig. 2.1). Therefore, there was a period of 14 days between each given test, which has been shown to be an appropriate delay to assess behavioural repeatability (Bell *et al.*, 2009). Test order remained the same as in the first series. A total of 37 male *A. leptorhynchus* were initially tested, however due to deaths (2 fish) and failed individual tests (6 fish; due to malfunction of electrical equipment), our final sample size was of 29 fish.

2.3 Behavioural tests

(1) Anti-predation behaviour test

The first behavioural test of the series, the anti-predation test, served to observe the tendency of a fish to exit a shelter in the presence of a predation threat (Riechert and Hedrick, 1993; Bell, 2005; Dingemanse *et al.*, 2007; Wilson *et al.*, 2010). The fish was placed inside the shelter area of the experimental tank 30 mins prior to the onset of the trial (Bell, 2005; Brown *et al.*, 2005; Dingemanse *et al.*, 2007; Wilson and Godin, 2009). The trial began at the start of the predation stimulus with the simultaneous opening of the shelter trap door. The fish was then exposed to the experimental tank which only contained a heater (fig. 2.2), and the predation stimulus.

The presented predation stimulus was that of an electrical playback signal mimicking the EOD of an electric eel, *Electrophorus electricus*, a natural predator of *A. leptorhynchus* (Moller, 1995; Stoddard, 1999; Westby, 1988). The eel signal was extracted from an electrical recording of an eel made in the Amazonian waters of Ecuador (courtesy GJ Hupé). The electrical recording was then cropped to obtain a template of a single pulse (eels emit pulse-type signals) (Moller, 1995; Stoddard, 1999; Westby, 1988). The template could then be played back to the male *A. leptorhuchus* at a 10 Hz frequency with an amplitude of 1 volt (reflective of an eel's natural EOD) with the same equipment that was used to record the EODs (Moller, 1995; Stoddard, 1999; Westby, 1988). The stimulus was played back through a pair of Teflon-coated silver-wire electrodes (diameter 0.25 mm, insulated to the tip; WPI, Inc., Sarasota, FL, USA), which were 10 cm apart, with 4 mm each of tip exposed. The electrodes were placed at the opposite end of the tank to the shelter (fig. 2.2). Electrical recording of the tests were performed using the same methods as for sex identification. Recording electrodes were placed centrally on each side of the tank, while the ground electrode was placed adjacent to the stimulus electrodes (fig. 2.2). The behaviour of the fish was observed via video recordings, for the 2 mins that the eel stimulus was delivered, and for an additional 8 mins post predatory event, for a total of 10 mins of observation. Parameters of behaviour that were measured are listed in table 3.1. At the end of the test, re-entry into the shelter was prohibited by closing of the trap door and a floss air filter was added to the experimental tank, permitting the fish to establish residency in the experimental tank.

(2) Object novelty test

The second test presented to our experimental fish was an object novelty test. Introducing non-threatening novelty to a subject's environment is a common way to assess boldness behaviours (Sundstrom *et al.*, 2004; Brown *et al.*, 2005; Burns, 2008; Wilson and Godin, 2009; Wilson *et al*., 2010). As previously mentioned, the fish remained in the experimental tanks after the anti-predation test, giving the fish an opportunity to familiarize itself with its new environment. The shelter at this point was blocked off to the fish, thereby forcing it to remain in the experimental arena. Approximately 24 hours after the previous test, the object novelty test began with the placement of a 250 ml glass beaker (by hand) in the centre of the experimental tank. The subject's behaviour was recorded by video for a period of 5 mins. The object was then removed from the tank, and the fish remained in the experimental tank. Table 3.1 includes the behavioural parameters that were recorded for this test.

(3) Feeding test

In order to respect the regular feeding schedule, the feeding test took place on the third day of experiments. The fish having remained in the experimental tank after the previous test, the feeding test could take place in the experimental arena without any handling being necessary (fig. 2.3). Again, the shelter was not made available to the fish. The test involved delivery frozen bloodworms (20-30) by pipette to the centre of the experimental tank and 5 mins of behavioural observations beginning immediately afterwards. Feeding tests have been shown to reliably assess boldness and aggressive behaviour in various fish species (Bell, 2005, Sundstrom *et al*., 2004, Sih *et al.,* 2004B). Observed behavioural parameters are enumerated in table 3.1.

(4) Conspecific intrusion test

To assess conspecific aggression in male *A. leptorhynchus*, we created a behavioural test that utilised the resident-intruder paradigm (Raab *et al.*, 1986). Because the fish had been in the experimental tank for the previous three days, we assumed it to be the resident of a home territory. An 'intruder' was introduced to the tank in the form of a playback mimic. The mimic was an agar fish, whose morphology (size: W=1.5 cm; L=12 cm; H=5 cm; conductivity= 35 μS) attempted to match that of a "typical" *A. leptorhynchus* (Babineau *et al.*, 2006; Hupé, 2012). A pair of Teflon-coated silver-wire electrodes were embedded in the agar fish (diameter 0.25 mm, insulated to the tip; WPI, Inc., Sarasota, FL, USA). The electrode tips were exposed differentially (1mm at the tail & 1 cm at the head) and were separated by 2.5 cm to reproduce the asymmetrical electric field geometry that occurs along the rostral to caudal axis in real fish (Assad *et al.*, 1999; Chen *et al.*, 2005; Babineau *et al.*, 2006; Hupé, 2012).

The electrical signal delivered through the mimic was an EOD with a frequency that represented another male *A. leptorhychus*. Furthermore, the stimulus EOD was modulated with chirps, thereby mimicking a communicating male conspecific. The playback stimulus originated from recordings that were made of a restrained, isolated fish. Templates of the EOD and a single chirp were then created with the use of the recording. The software Simulink (the Mathworks) and Control Desk (dSpace Inc) were then used during the trials to seamlessly fuse the templates together to generate a continuous EOD discharge with the designated chirp pattern. The templates could be modified by re-sampling at varying rates to create varying EOD frequencies.

To elicit a maximal behavioural response, the frequency of the playback discharge was set to be 5-25 Hz higher than the subject's EODf. According to the literature, a higher EODf signifies a more dominant male (Hagedorn and Heiligenberg, 1985; Dunlap, 2002; Triefenbach and Zakon, 2003; Triefenbach and Zakon, 2008). Furthermore, it has been shown that the difference frequency (DF) between two *A. leptorhychus* influences the numbers of chirps emitted by each fish, where smaller DFs lead to higher chirp counts (Harvey-Girard *et al.*, 2010). In addition to the frequency stimulus, we designed a random chirp sequence to be incorporated into the EOD playback. Although the sequence was random, every experimental fish received the same randomized chirp sequence composed of approximately 310 chirps. Previous work in our lab has hinted that high random chirp rates are usually associated with non-cooperative males (Hupé, 2012). Hence, we were presenting our subject with a potentially threatening intruder.

At the onset of the trial, the EODf of the fish was determined. Recording electrodes were placed in the same position as in the anti-predation test (fig. 2.3). We then adjusted our mimic EODf to match the focal fish as best we could (DF varied from 5-25 Hz as previously mentioned) and the amplitude was also set to a strength comparable to that of the focal fish (Hupé, 2012). The mimic was suspended in the middle of the tank and rested in the middle of the water column. To avoid electrical noise in the recordings, the heater and air filter were turned off. An average temperature decline of -0.3°C for a period of 10 mins was observed, therefore the fish remained in an appropriate temperature range. The trial began as soon as the playback signal was initiated and lasted for 10 minutes. Amplitude adjustments were made if the signal strength of the mimic did not match that of the experimental fish. At the end of the trial,

the filter and heater were turned back on. Listed in table 3.1 are the behavioural parameters that were used to assess aggression & boldness for this behavioural assay.

(5) Novel environment exploration test

In the last test, we observed the fish's behavioural response when faced with a novel environment (Bell, 2005; Wilson & Godin, 2009; Wilson *et al*., 2010, Dingemanse *et al*., 2007). Here the novel environment consisted of a larger experimental tank (W= 48 cm; L= 58 cm; H= 20 cm) that contained a shelter equipped with a trap door (W= 10 cm; L= 15 cm; H= 20 cm) and three plastic plants (fig. 2.4). The shelter and plant locations were the same for all fish. However, the layout of the objects changed from the first round to the second round of tests to ensure that although the tank was the same, the environment was novel (fig. 2.4). Water temperature and conductivity of the larger tank matched those of the smaller experimental tank (26.5-29 °C & 200-300 μS). Temperature was maintained with a heater that was removed just before beginning the test. Again, there was no considerable decline in water temperature. Thirty minutes before the onset of the test, the fish were transferred from their experimental tanks to the shelter. The test start point was defined by the opening of the trap door. The exploratory behaviour of the subject was observed for a period of 10 minutes. The list of observed parameters for this test is found in table 3.1. At the conclusion of the test, the fish were returned to their smaller experimental tanks and were fed (to respect the feeding schedule).

2.4 Video analysis

Owing to the nocturnal nature of *A. leptorhynchus* (Moller, 1995), all tests were performed during the dark cycle of the photoperiod. Infrared lighting was used to record video footage of the fish's behaviour with a Canon FS30 camcorder (Canon Canada Inc.). Video recordings from the object novelty test, the conspecifc intrusion test and the novel environment exploration test were processed with the video analysis software, Videopoint®, which allows one to collect the position coordinates of objects/subjects through time. Using custom scripts in Matlab (The Mathworks), behavioural parameters were extracted from the resulting position coordinates.

2.5 Chirp analysis

For the duration of the conspecific intrusion test, electrical recordings were made of the fish's EOD responses to the conspecific playback. From these electrical recordings, visual inspection of generated spectrograms (Matlab, the Mathworks)(eg. fig. 1.2) permitted the determination of total chirp counts produced by the experimental fish in the 10 minute period. Type 2 chirps were easily identified as outlined in the literature: duration of approximately 20 ms and frequency excursions of approximately 50 Hz (Zakon *et al.*, 2002; Zupanc, 2002; Zupanc *et al.*, 2006). Chirps were counted for both week 1 and week 2 of experiments.

2.6 Statistical analysis

The existence of a behavioural syndrome implies correlations across behaviours. Owing to the high number of behavioural parameters for each test, performing multiple cross correlations across all parameters would increase the probability of obtaining false positives. To reduce data dimensionality without bias, principal component analyses (PCAs) were performed on the data sets of each behavioural test (Bell, 2005; Dingemanse *et al.*, 2007; Wilson and Godin, 2009; Wilson *et al.*, 2010). The PCA method is a non-parametric technique of reducing data variability by identifying the common variance within a data set. Outlined below are the general steps involved in a PCA (Shlens, 2009):

1) The data set is represented in an m x n matrix where m is the number of variables/parameters and n is the number of samples

2) A covariance matrix of the data set is generated

3) From the covariance matrix, axes along which the variance is maximized are characterized by vectors and are termed principal components. This process of axis identification is achieved in a decreasing order until there are as many principal components as there are variables (# of principal components = m). Therefore, principal component 1 explains the highest proportion of the variance, principal component 2 explains the second highest proportion, etc.

4) For each principal component, an eigenvalue or loading factor is determined for each of the variables (m). These values represent the scaling factor, by which the variable deviates from the eigenvector (the principal component).

5) The eigenvalues and eigenvectors are used to transform the multivariate data set into composite scores. Each principal component generates one set of scores (one score value per sample).

Therefore, if only the first principal component is used in analyses, an initial data set with five parameters is efficiently reduced to one variable (the principal component score).

For the present study, the sign (negative or positive) of loadings generated by the PCA were inverted when necessary to ensure that the scores intuitively matched the direction of the behaviour in the appropriate context. For example, parameters involving latencies should have negative loadings to ensure negative scores, which would indicate shy, non-aggressive behaviours. Parameters such as number of attacks should obtain positive loadings, as larger positive behavioural scores indicate bold, aggressive fish (Bell, 2005).

The subsequent analytical step consisted of Spearman rank correlations on the behavioural scores that resulted from the various PCAs. A non-parametric correlation analysis had to be applied since the various sets of data did not comply with the assumption of normality. The first Spearman rank correlation performed assessed the temporal correlation within each behavioural test (week 1 versus week 2). Correlation coefficients that showed a *P*-value below 0.05 were considered significant. The second sets of Spearman rank correlations quantified the relationship among the five behavioural tests for each given week. To address the issue of multiple comparisons in this situation, Bonferroni adjustments were made on the resulting *P*-values (Bell, 2005).

Similar statistical analyses were performed to shed light on the correlations between chirp counts and behavioural scores. Spearman rank correlations were

considered significant if the *P*-value was smaller than 0.05. Finally, Spearman correlations were also applied to the cross correlations between the physiological parameters, the behavioural scores and chirping. For length and mass, a PCA was performed to obtain a single principal component of body size, thereby facilitating future correlations. Tank temperatures varied slightly between experiments, therefore prior to correlation analyses, EOD frequencies were standardized to a temperature of 28°C using a Q_{10} of 1.62, in order to account for the effects of temperature on the EODf (Dunlap *et al.,* 2002).

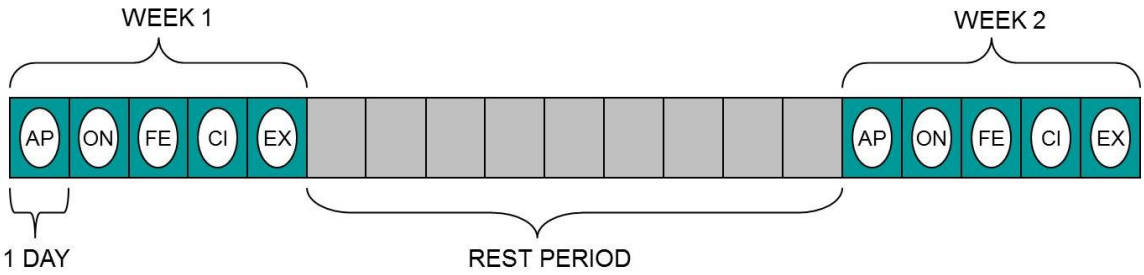

Figure 2.1. Schematic of the experimental regime to assess an aggression-boldness behavioural syndrome. Each box represents a day, for a total of 19 days. Teal boxes represent days where behavioural tests took place (10 in total), while grey boxes represent rest days (9 in total). Behavioural tests: Anti-predation (AP), Object novelty (ON), Feeding (FE), Conspecific intrusion (CI) & Novel environment exploration (EX).

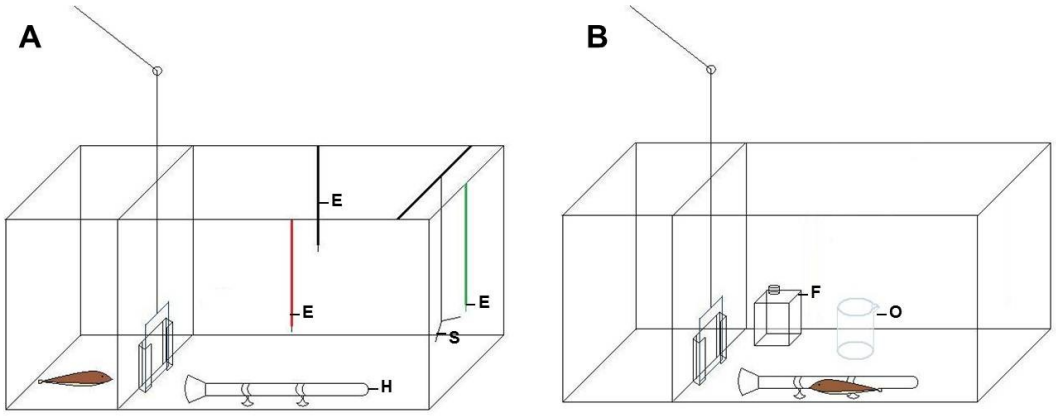

Figure 2.2. Schematics of the experimental tanks for the anti-predation test (Panel A) and the object novelty test (Panel B), E = recording electrodes, S = stimulus electrodes, H = heater, F = air filter, O = 250 ml glass beaker.

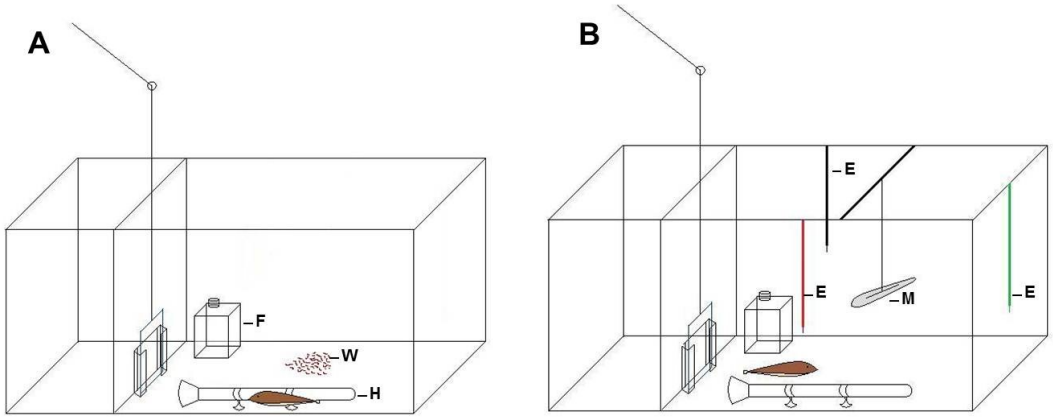

Figure 2.3. Schematics of the experimental tanks for the feeding test (Panel A) and the conspecific intrusion tests (Panel B), F = air filter, W = bloodworms, H = heater, E = recording electrodes, M = conspecific mimic.

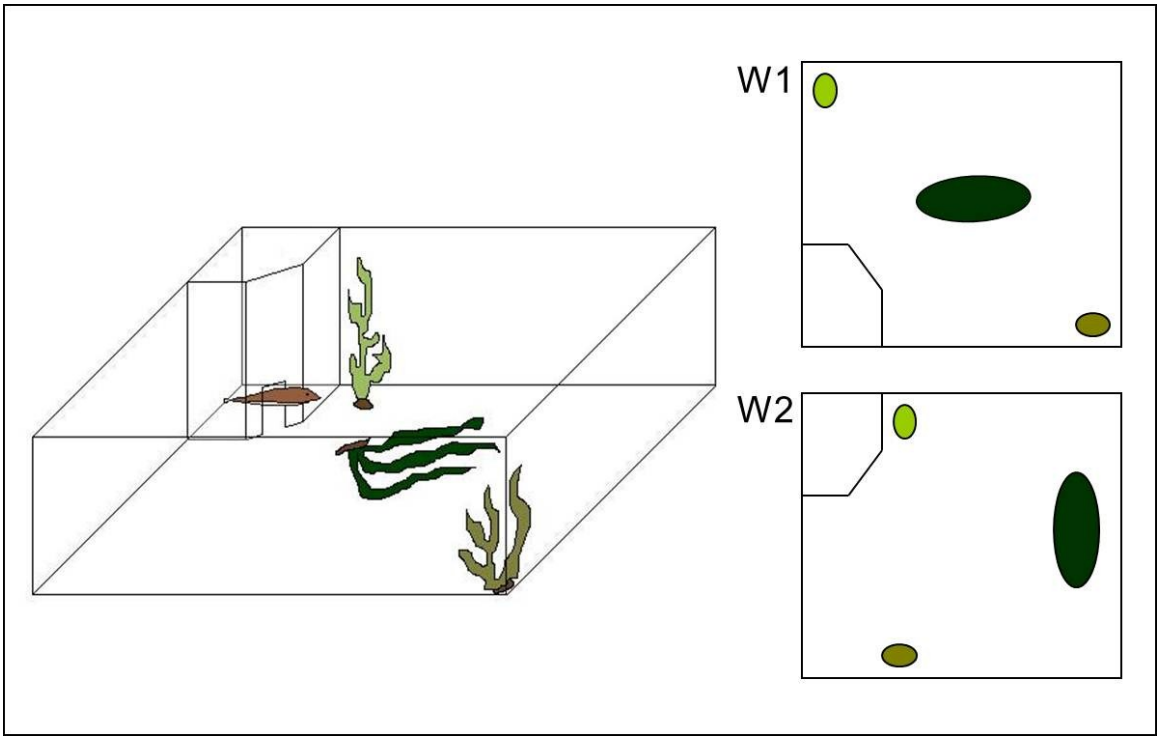

Figure 2.4. Schematics of the experimental tank for the novel environment exploration test. Panel W1 shows the orientation of the shelter and of the three plastic plants for the first week of tests. Panel W2 illustrates the change in tank layout with regards to the shelter and the plants for the second week of test. Although not featured in this schematic, this shelter was also equipped with a trap door.

3. Results

3.1 Behavioural syndrome

As outlined in the objectives, the first analytical step involved the characterization of an aggression-boldness behavioural syndrome in *A. leptorhynchus*. Behavioural analysis began with the production of movement traces of all three behavioural tests for which we had position coordinates. The traces, examples of which are provided in figure 3.1, provided a means to qualitatively analyse the data, i.e. the swimming behaviour depicted in fig. 3.1 suggested that the fish in panels A, C and E are bolder than fish in panels B, D and F. Values obtained for behavioural parameters matched these resulting traces. For example, comparison of the displacement values obtained for the object novelty test for fish A (total displacement = 2576.2 cm) and B (total displacement = 51.85 cm), match the qualitative differences in the traces. For the conspecific test, traces C and D properly display the differences in time spent within 10 cm of the conspecific intruder (471.4 s and 9.6 s, respectively) between an aggressive/bold fish and a non-aggressive/shy fish. The behavioural differences between panels E and F, representing the novel environment exploration test, are less obvious than those among other panels. Here, close attention is required to observe the differences in the fractions of the tank that were visited by the fish, differences that are echoed in the parameter values, 30.7% versus 19.8%, for fish E and F, respectively.

Separate principal components analyses (PCA) were performed for each behavioural test. To effectively reduce the dimensionality of the data set, only the loadings and scores of the first principal component were considered for analysis (table 3.1) (Bell, 2005; Dingemanse *et al.*, 2007; Wilson and Godin, 2009; Wilson *et al.*, 2010). Some of the components only explained approximately 60% of the total variance.

However, it is important to note that although our tests were designed to assess boldness and aggression specifically, it is possible that other aspects of behaviours were assessed with the chosen behavioural parameters. In this situation, additional variability vectors would be generated in the data set, variability that would not be encompassed by the first component, thereby resulting in a lower proportion of total variance explained.

To determine whether the behaviours for each test were consistent in time, Spearman rank correlations were performed on the first component scores across the two measurement periods (fig. 3.2). All behavioural tests, except for the conspecific intrusion test, showed significant temporal correlations. For some tests, the resulting correlation coefficients were not high (i.e., $r = 0.455$ for the object novelty test), which as mentioned above, could be attributed to the variance not accounted for by the first component. However, the positive significant correlations that were found indicated that the behaviours were consistent in time despite the unaccounted variability.

We examined in close detail the results of the conspecific intrusion test to determine what factors might have resulted in temporal behavioural inconsistencies. Inspection of the raw data from the conspecific intrusion test revealed that for a given week, certain fish failed to respond to the behavioural test. When these non-responding fish were removed from the data set and new PCAs were performed for each week of the conspecific intrusion test (fig. 3.3), the conspecific intrusion context was then significantly consistent in time ($r = 0.662$, $P = 0.001$). These results fulfilled the first criterion of a behavioural syndrome, that of repeatability in behaviour over time.

The next step of the analysis focused on the second criterion of behavioural syndromes: that behaviours correlate across contexts. To address this criterion, Spearman

rank correlations were performed across the behavioural tests for each respective week using the first component scores (table 3.2). For the contexts to be considered part of a syndrome, it required that a significant correlation was present in both weeks (criterion 1). Our cross-correlations analysis involved multiple comparisons; therefore, to minimize the probability of false positive results, we adjusted *P*-values with a Bonferroni transformation (Bell, 2005). Among all comparisons, only the object novelty test and the feeding test showed a significant correlation that persisted over time (Week 1: r = 0.525, *P* = 0.003; Week 2: r = 0.685, *P* = <0.001).

The small yield of behavioural correlates lead us to investigate the possibility that variability associated with other behaviours might be affecting our results. To validate the results and analysis, we re-ran our analysis on a reduced data set. It was thought that perhaps they were the sources of variation within the data set that prevented cross-correlations across tests. For each given test, PCAs were carried out with the adjusted parameter sets, and the Spearman rank correlations were redone. These alterations resulted in the same conclusion, that only the object novelty test and the feeding test were significantly correlated (data not shown).

3.2 Chirp analysis

The behavioural syndrome analysis was followed by the investigation of relationships between behavioural scores and exhibited chirp counts. Owing to the lack of an over-arching aggression-boldness behavioural syndrome, we applied our second set of hypotheses for the purpose of the chirp analysis. Outlined in figure 3.4 are the three possible hypotheses for the function of chirps. The boldness hypothesis (B is fig. 3.4)

stated that a positive relationship should arise between chirp counts and bold behaviours if chirps served as a conflict-mediating or cooperating signal. If chirps, however, serve as a communication signal that reflects dominance and predicts aggression, it was hypothesized that the aggressive behavioural scores would associate with chirp counts (A in fig. 3.4). The third alternate hypothesis broached the idea that chirps could be used as a means of electrolocation, rather than electrocommunication, therefore showing a relationship with exploration behaviours rather than bold or aggressive behaviours (E in fig. 3.4).

As with the behavioural analysis, the consistency of chirp counts over time was considered. Chirp counts were significantly correlated in time with an r value of 0.706 ($p<0.01$). Therefore, any correlations between chirp counts and behavioural parameters should be consistent in time.

To test the set of hypotheses, Spearman rank correlations were calculated between chirp counts and first component scores for the outlined behavioural tests for both weeks of testing (table 3.3). Chirp counts correlated significantly with the scores in only one case, that of the second week of the conspecific intrusion test ($r = 0.632$). Therefore, these results do not support the exploration or the boldness hypotheses, as no significant correlations were found between the respective behaviours and chirp counts.

To shed light on the lack of consistent support for the aggression hypothesis, an examination of the correlations between the conspecific intrusion test and chirp counts was implemented. We began firstly by looking at the correlations with the adjusted conspecific intrusion test scores (smaller sample size due to the removal of outliers, see across time correlations section) and chirp counts. Even after the removal of outliers, only

the second round of the conspecific intrusion test correlated significantly with chirp rates (fig. 3.5). Secondly, we attempted to take into account possible effects of the difference frequency. As previously stated, the difference between the EOD frequencies (DF) of two interacting fish can have an effect on chirp rates (Harvey-Girard *et al.*, 2010). To assess the effects of DF, we completed a Spearman correlation with chirp counts for each given week. Results of the non-significant correlations are presented in figure 3.5. Therefore, since DF did not correlate to chirp counts, we concluded that its effects were not strong enough to contribute to the time inconsistency in the conspecific intrusion test-chirp correlations. From these results, we must consequently conclude that expressed aggression is not a reliable predictor of chirp rates, thereby refuting our third hypothesis.

3.3 Morphometric and physiological parameters

Previous studies demonstrated that morphometric and physiological parameters such as length, mass and EOD frequency correlated positively with aggression and chirp counts in electric fish (Hagedorn and Heiligenberg, 1985; Dunlap, 2002; Triefenbach and Zakon, 2003; Triefenbach and Zakon, 2008). Consequently, these parameters were assessed in this study in an attempt to further validate previous results. Because, both length and mass are related to the body size of the animal and that they showed a strong correlation ($r = 0.859$, $P = <0.01$), we performed a principal component analysis on these two traits. From the PCA, we obtained a single score for body size, which simplified any future correlations. Consistent with the literature, we found that the EODf of the fish correlated positively and significantly with the body size of the individual for both week 1 and week 2 (week 1: $r = 0.487$, $P = <0.01$; week 2: $r = 0.654$, $P = <0.01$) (Hagedorn and

Heiligenberg, 1985; Dunlap, 2002; Triefenbach and Zakon, 2003; Triefenbach and Zakon, 2008). Spearman rank correlations between the physiological parameters (EODf and size) and the first component scores of the behavioural contexts are found in table 3.4. From the lack of significant correlations consistent over time, we concluded that neither EODf nor body size could reliably predict behaviour in the current study. A case could be made for the relationship between size and the novel environment exploration test; however, this relationship also could be due to some of the measured parameters of this test being related to body size, i.e. average speed.

The correlations between physiological parameters and chirp counts did not yield significant results that were consistent over time (table 3.4). Therefore, larger fish with higher EOD frequencies did not show a higher propensity to chirp.

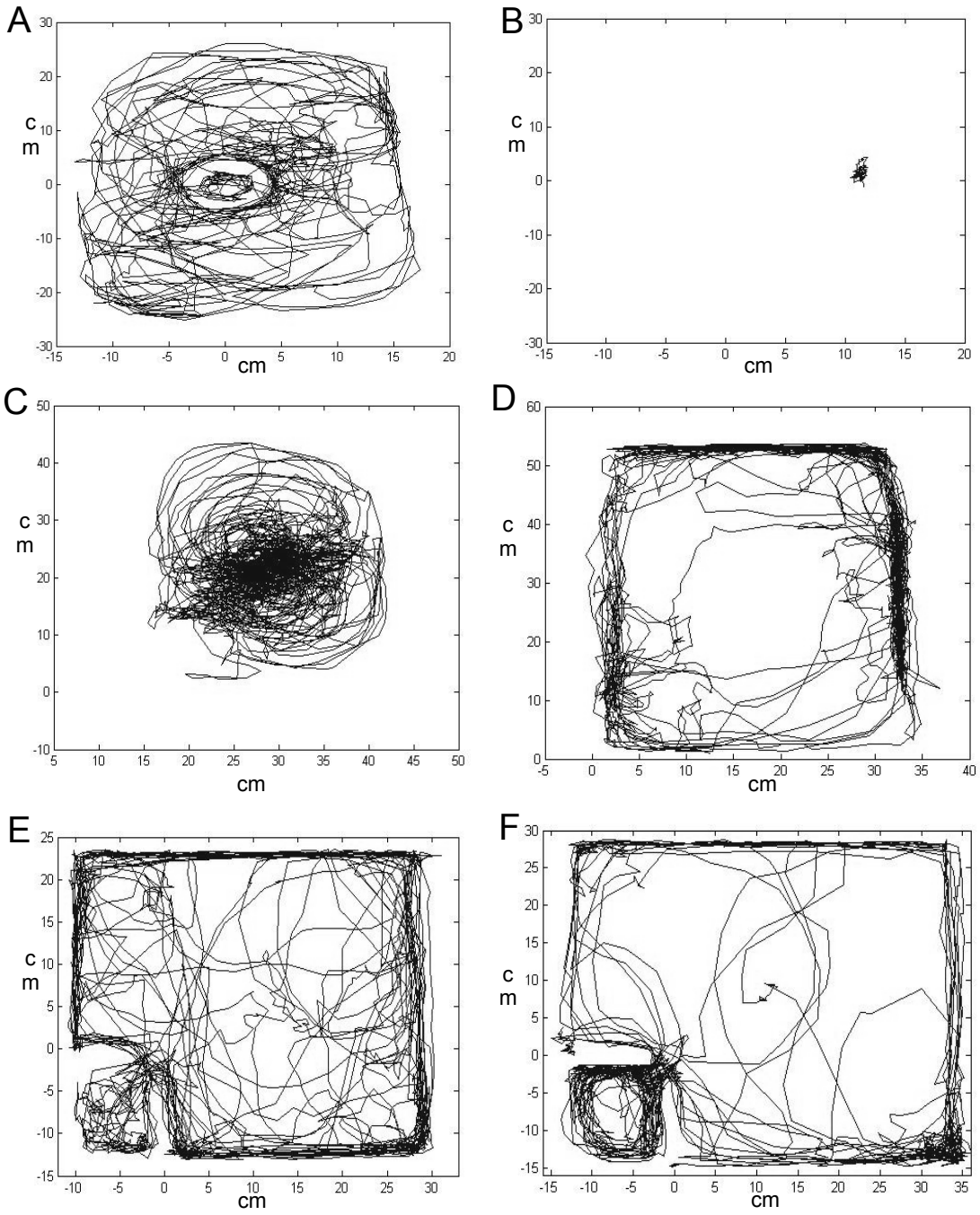

Figure 3.1. Swimming traces of fish for 3 of the behavioural tests: object novelty test (panel A & B), conspecific intrusion test (Panel C & D) and the novel environment exploration test (Panel E & F). Panels A, C & E illustrate a typical aggressive-bold fish, while Panels B, D & F illustrate a typical non-aggressive-shy fish.

Table 3.1. Results for principal component analysis for 1) anti-predation test; 2) object novelty test; 3) feeding test; 4) conspecific intrusion test; 5) novel environment exploration test. Loadings of the first component are shown for both week 1 and week 2 (n=29).

Behavioural parameters	Loading week 1	Loading week 2
1) Anti-predatory test		
Latency for head to exit shelter (s)	-0.465*	-0.488
Latency for body to exit shelter (s)	-0.466*	-0.487
Time spent in shelter (s)	-0.458*	-0.469
Latency to reach predatory zone (s)	-0.458*	-0.483
Time spent in predatory zone (s)	0.385*	0.268
Variance explained	73.46%	77.20%
2) Object novelty test		
Minimum distance from object (cm)	-0.493	-0.462
Latency to reach 5cm from object (s)	-0.475	-0.458
Time spent within 5cm from object (s)	0.430	0.427
Total displacement (cm)	0.484	0.456
Average distance from object (cm)	-0.335	-0.432
Variance explained	69.71%	80.10%
3) Feeding test		
Latency of 1st feeding event (s)	-0.528*	-0.534*
Number of feeding events (s)	0.462*	0.200*
Total feeding time (s)	0.552*	0.614*
Longest feeding event (s)	0.450*	0.545*
Variance explained	68.96%	58.87%
4) Conspecific intrusion test		
Time spent within 10 cm of mimic (s)	0.453*	0.426*
Time spent within 5 cm of mimic (s)	0.439*	0.398*
Latency to reach 10 cm from mimic (s)	-0.292*	-0.406*
Latency to reach 5 cm from mimic (s)	-0.428*	-0.432*
Total displacement fish (cm)	0.410*	0.386*
Total displacement mimic (cm)	0.407*	0.400*
Variance explained	73.51%	77.52%
5) Novel environment exploration test		
Total displacement (cm)	0.496	0.448
Time stationary(s)	-0.443	-0.464
Fractions of tank visited	0.450	0.428
Time spent in shelter (s)	-0.387	-0.454
Average speed (cm/s)	0.453	0.441
Variance explained	70.12%	82.45%

* Loading scales were inverted so that numerical value reflected behaviour (i.e. positive value reflects bold behaviour, negative value reflects shy behaviour

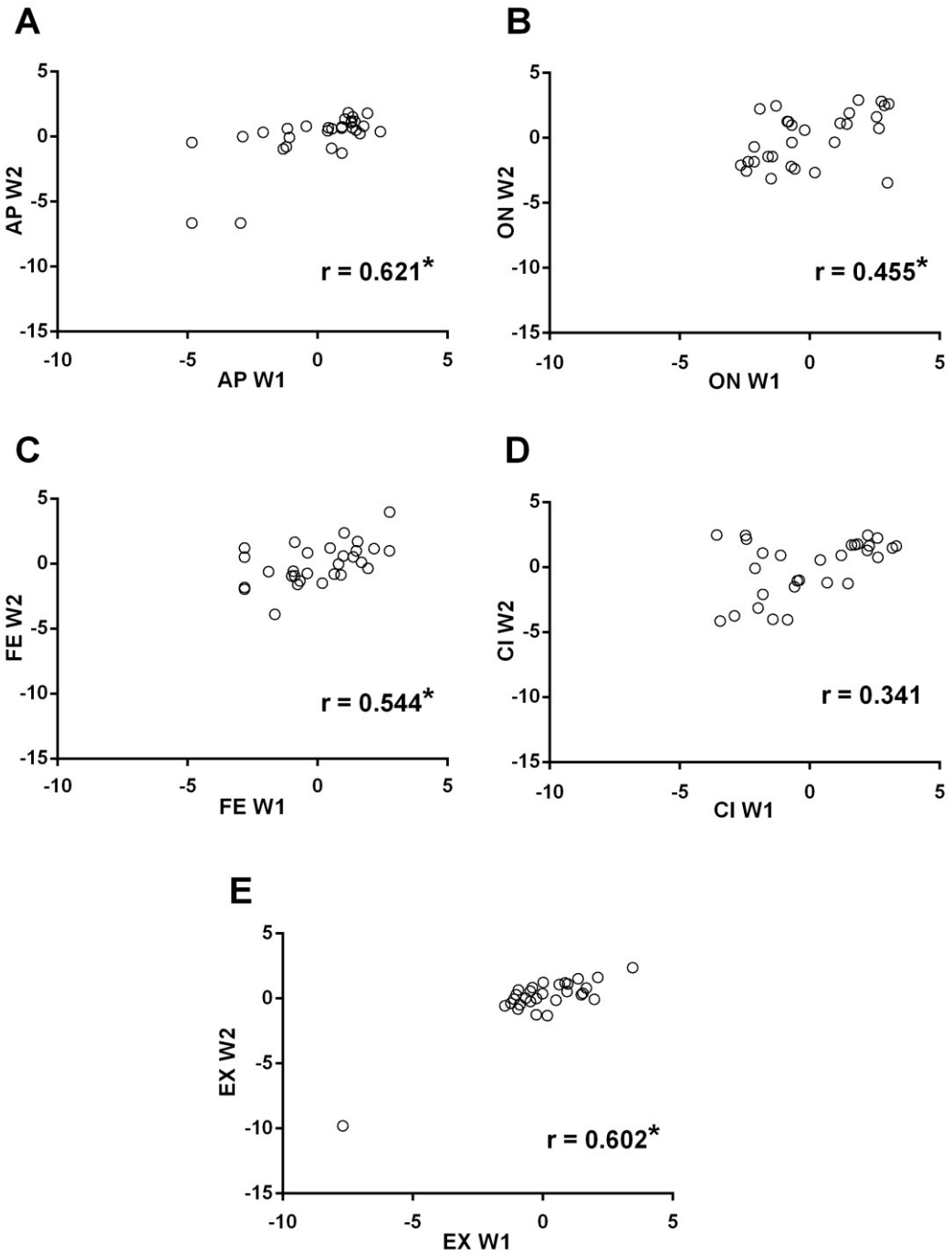

Figure 3.2. Spearman correlations between PCA scores of each individual fish for week 1 (W1) and week 2 (W2), for each behavioural test: the anti-predation (AP) test (Panel A), the object novelty (ON) test (Panel B), the feeding (FE) test (Panel C), the conspecific intrusion (CI) test (Panel D) & the novel environment exploration (EX) test (Panel E) (n=29). Statistically significant correlations ($p<0.05$) are indicated by an asterisk.

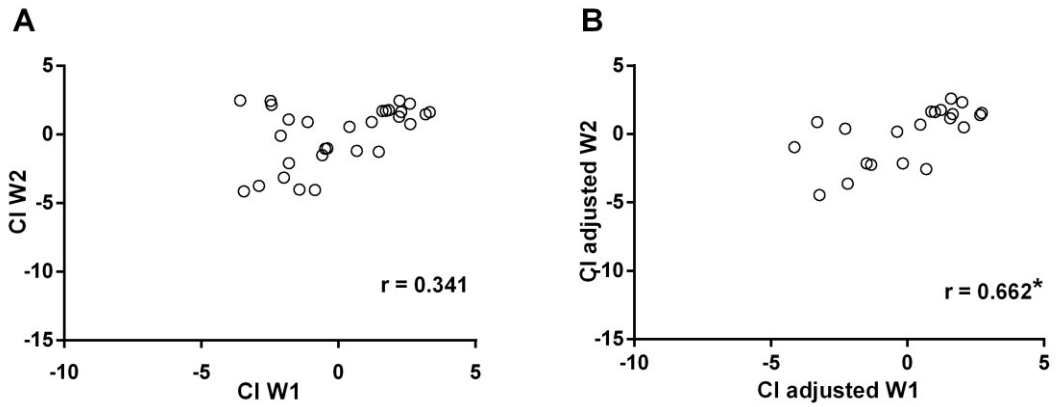

Figure 3.3. Spearman rank correlations between PCA scores of week 1 (W1) and week 2 (W2) for the conspecific intrusion test. Panel A demonstrates the correlation of the initial data set (n=29), while Panel B illustrates the correlation from the adjusted data set (n=21). Statistically significant correlations ($p<0.05$) are indicated by an asterisk.

Table 3.2. Spearman rank correlations (r) between the first component scores for pairwise comparison of the five behavioural tests (n=29).

	Week 1		Week 2	
Behavioural tests pairs	r	*P*	r	*P*
Anti-predation / Object novelty	0.311	0.101	0.385	0.039
Anti-predation/ Feeding	0.200	0.298	0.443	0.016
Anti-predation/ Conspecific intrusion	0.217	0.259	0.035	0.857
Anti-predation/ Novel environ. exploration	0.422	0.023	0.219	0.253
Object Novelty/ Feeding	0.525	0.003*	0.685	<0.001*
Object Novelty/ Conspecific intrusion	0.250	0.190	0.587	0.001*
Object Novelty/ Novel environ. exploration	0.482	0.009	0.087	0.654
Feeding/ Conspecific intrusion	0.405	0.029	0.438	0.018
Feeding/ Novel environment exploration	0.491	0.007	0.044	0.821
Conspecific intrusion/ Novel environ. Exploration	0.371	0.048	0.213	0.267

* Indicates correlations that remained significant after a sequential Bonferroni adjustment

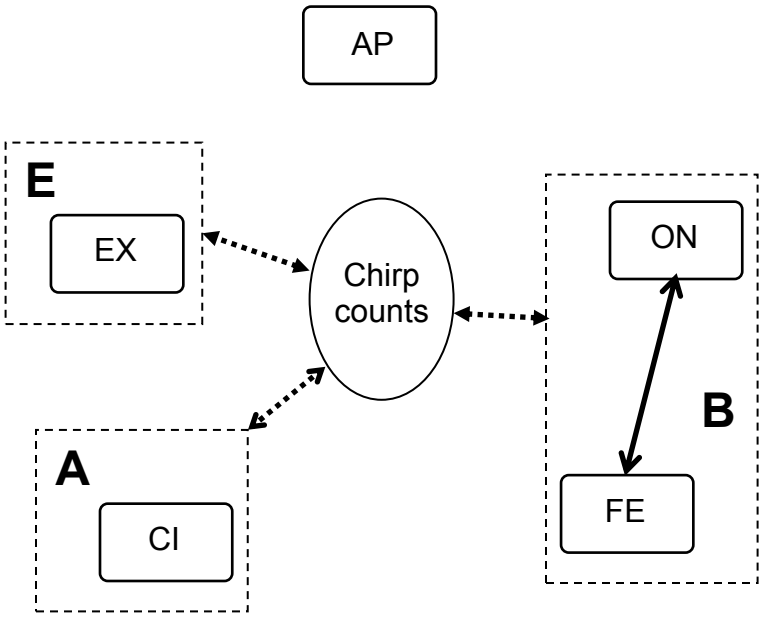

Figure 3.4. Summary diagram outlining the three possible hypotheses relating chirp counts to behaviour (E=exploration hypothesis, A= aggression hypothesis, B= boldness hypothesis) in these behavioural tests: the anti-predation (AP) test, the object novelty (ON) test, the feeding (FE) test, the conspecific intrusion (CI) test & the novel environment exploration (EX) test.

Table 3.3. Spearman rank correlations (r) between chirp counts and behavioural tests PCA scores (n=28).

	Week 1		Week 2	
Behaviour tests	r	*P*	r	*P*
Novel environ. exploration test	0.140	0.477	-0.055	0.779
Object novelty test	0.120	0.544	0.314	0.103
Feeding test	0.228	0.243	0.397	0.036*
Conspecific intrusion test	0.223	0.254	0.632	<0.001*

* *P*-values <0.05

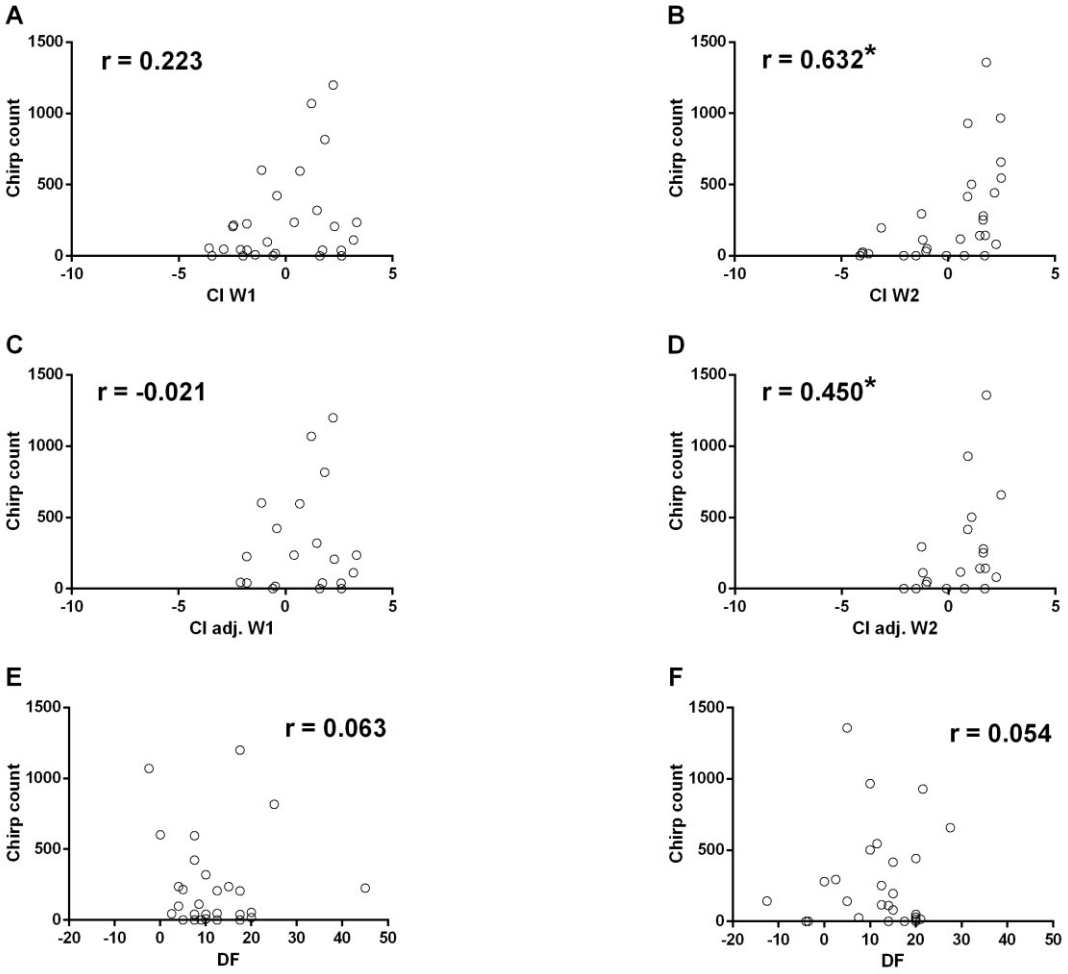

Figure 3.5. Correlations between the initial conspecific intrusion (CI) test scores and chirp counts (week 1 = Panel A & week 2 = B) (n=28), correlations between the adjusted conspecific intrusion (CI adj.) test scores and chirp counts (week 1 = Panel C & week 2 = D) (n=20), and correlations between difference frequency (DF) and chirp count (week 1 = Panel E, week 2 = Panel F), (n=28). Statistically significant correlations are marked with an asterisk.

Table 3.4. Spearman rank correlations (r) between physiological parameters, size and EOD frequency, and the first principal component scores of the five behavioural tests (anti-predation behaviour test (AP), object novelty test (ON), feeding test (FE), conspecific intrusion test (CI) & novel environment exploration test (EX)) (n=29). Correlations with chirp counts are also found below (n=28).

	Size				EOD frequency			
	Week 1		Week 2		Week 1		Week 2	
Behavioural tests	r	*P*	r	*P*	r	*P*	r	*P*
AP	0.268	0.163	0.111	0.566	0.401	0.031*	0.288	0.131
ON	-0.282	0.139	0.228	0.233	-0.077	0.693	0.147	0.446
FE	0.040	0.837	0.257	0.178	0.104	0.590	0.267	0.161
CI	0.175	0.362	0.558	0.002*	0.056	0.772	0.341	0.071
EX	0.336	0.075	0.494	0.007*	0.167	0.387	0.386	0.039*
Chirp counts	0.249	0.202	0.485	0.009*	0.344	0.073	0.372	0.051

* *P*-values <0.05

4. Discussion

4.1 Behavioural syndrome

The presence of a behavioural syndrome within a population is determined by the following criteria: 1) consistency in behaviour across time and 2) consistency of behaviour across contexts (Bell, 2007; Sih *et al.*, 2008A; Sih *et al.*, 2008B; Bergmüller, 2010; Conrad *et al.*, 2011). Our experimental design provided male *A. leptorhynchus* with two repeated series of five behavioural tests each (representing five behavioural contexts), where aggressive and bold behaviours were expected to be exhibited by individuals. Spearman rank correlations performed on the 1st principal component scores of the behavioural test revealed two main findings. First, behaviours were consistent in time for each test, and second, a boldness behavioural syndrome was present across only two of the five tests.

4.1.1 Criterion 1: time consistency

Behaviours exhibited by individuals were found to be consistent over the experimental period (14 days) for each of the five tests (fig. 3.2), thereby satisfying the first criterion for a behavioural syndrome. These correlations suggest a limited plasticity in behaviour over time for a given behavioural context. For the conspecific intrusion test, however, the correlation in time was only found to be significant once non-responding fish were removed from the data set (fig. 3.3). Due to the extreme nature of the values obtained for a non-responding fish, it was expected that they would affect the data in a non representative way, as such, they were removed from the data set. For example, parameters such as "latency to reach 5 cm" would obtain a maximum value of 600 s if a fish failed to approach within 5 cm of the object. Such cut off values did not allow for a continuous representation of the fish's behaviour and could therefore affect the variability

within our conspecific intrusion data set. Indeed, this seemed to have been the case, as removal of these non-responding fish resulted in a significant correlation. Nonetheless, it is important to address the issue of why certain fish altered their responses to the conspecific intruder from one week to another.

Close inspection of these non-responding fish revealed that a majority of them experienced weight loss between the two weeks (-0.66 ± 0.74 g, n=8). A change in weight ultimately can indicate a change in state and motivation, thereby altering the value of territoriality (Bradbury and Vehrencamp, 1998; Elwood *et al.*, 1998; Dall *et al.*, 2004; Kappeler and Kraus, 2010). Maintaining a territory with high food source availability becomes a higher priority for an animal experiencing decreased body condition, consequently increasing the competitive state of the individual (Bradbury and Vehrencamp, 1998; Elwood *et al.*, 1998; Dall *et al.*, 2004). A change in competitive motivation could be at the basis of the variability in aggressive response in these non-responsive brown ghost knifefish. However, weight loss was experienced within the group of responding fish (-0.18 ± 0.44 g, n=21) as well. A Mann-Whitney-Wilcoxon test revealed that there was no significant difference between the weight loss experienced by the non-responding and the responding fish ($P = 0.164$) Therefore, a change in state is not likely a factor that contributed to the change in behavioural responses.

An additional contributor to the variation in behavioural responses in the non-responding fish could be the behavioural effects attributed to the mesh baskets the fish were housed in between test series. While the baskets prevented the unwanted effects of social isolation, they may have created an "overconfidence" effect (Dugatkin, 1997; Bradbury and Vehrencamp, 1998; Peake and McGregor, 2004). Because the fish could

communicate across the mesh and to some extent, interact with one another, it is possible that the lack of physical attack from a conspecific in response to an individual's EODf and emitted chirps could have presented a novel agonistic experience that influenced future behaviour. The alternative to social or physical isolation was a free-swimming group tank. This method would not have been ideal as numerous studies have shown that fish exposed to social groups experience winner-loser effects as well as behavioural type group composition effects (Dugatkin, 1997; Magnhagen and Staffan, 2005; Sih & Watters, 2005). Effects of group composition as such that the behaviour expressed by an individual could vary based on whether this fish was in a group composed only of aggressive fish versus a group composed only of shy fish (Sih and Watters, 2005). Thus, the physical isolation method could have had effects on aggressive behaviours in *A. leptorhynchus*; however, these effects were not as significant as the effects of social isolation or group dynamics.

Although there was variation within the conspecific intrusion test, the overall results suggest that male *A.leptorhynchus* behave in a consistent matter across time. Investigation of behavioural consistencies over longer periods of time would provide a better understanding of this idea of limited time-scaled behavioural plasticity.

4.1.2 Criterion 2: Cross context correlations

The second criterion required of a behavioural syndrome is that behaviours should be correlated across varying contexts. Here, we found that among five different contexts, only the object novelty test and the feeding test were correlated (table 3.2). Since both criteria are satisfied for these two tests, they may be encompassed in a boldness behavioural syndrome. Several studies on fish behaviour have reported correlated

behaviours in contexts involving bold behavioural traits (Murphy and Pitcher, 1991; Sneddon, 2003; Wilson and Stevens, 2005; Bell and Sih, 2007; Piyapong *et al.*, 2009). However, the majority of these studies show significant correlations in boldness behaviours involving anti-predatory responses of individuals. In most cases, inspection and activity levels in the presence of a predator correlated with other aspects of bold behaviours such as feeding strategies, time spent in open areas and shoaling behaviours (Murphy and Pitcher, 1991; Sneddon, 2003; Bell and Sih, 2007; Piyapong *et al.*, 2010). In the present study, however, such correlations were not present.

Two possible explanations for this lack of correlation are proposed. The first deals with the true level of threat provided by the predatory stimulus, while the second deals with the nature of the behaviours that are assessed by the chosen behavioural tests. Predatory threats in laboratory experiments are usually provided in the form of a model or physical mimic of the appropriate predator, or in the form of a simulated predatory attack (ex: using the skull of a predator to mimic biting or pecking behaviours) (Bell and Sih, 2007; Wilson and Godin, 2009). Since the species used in this study has limited eyesight and is predominantly dependent on its electric sense for predator identification and localization, the popular methods were not appropriate, and as such, a novel approach to predatory simulation was necessary (Moller, 1995; Von der Emde, 2006). Using an electrical signal to mimic an electric eel (*Electrophorus electricus*), a natural predator of *A. leptorhynchus*, presented certain limitations. The maximum strength at which the signal could be sent mimicked an eel that was in the vicinity of the fish's territory, but not immediately adjacent. Furthermore, the maximum frequency (10 Hz) of pulses generated represented an eel that was scanning its environment. However, the pulse frequency of

the eel has been shown to increase to much higher frequencies once it has located a prey item (Moller, 1995). Therefore, it is possible that although the simulated signal represented an eel in the vicinity of the male brown ghost's territory, the threat may not have been severe enough for the fish to deviate from its normal behaviour. In nature, the magnitude of an eel's weak signal is approximately 10 V (Moller, 1995). Unfortunately, limitations within our electrical system, only permitted us to send out an eel stimulus with an amplitude of approximately 1 V. Consequently, the attenuated magnitude of the signal may have been perceived as an eel in the vicinity of the fish's territory and not within it. As the fish approached the stimulus, they could potentially sense the electrodes and the lack of a physical manifestation of an eel at the signal source. The absence of a physical presence of a predator may have potentially negated the effects of the mimic.

The alternative explanation for a lack of correlation, as previously mentioned, relates to the nature of the behaviours exhibited in the behavioural tests. There are disagreements in the field as to which types of test properly assesses the various axes of behaviour (boldness, aggression, exploration, etc.) (Réale *et al.*, 2007; Burns, 2008; Toms *et al.*, 2010). Réale *et al.* (2007) argue that true assessments of bold behaviour require sufficient risk, and that tests involving novel objects, feeding strategy, or open field exploration without a predatory threat or a stressor do not properly assess boldness, as they are alternative tests of exploration behaviours. According to this argument, out of the three tests where we presumed that bold behaviours would be exhibited, only the anti-predation test offered a true characterization of the bold behavioural trait. Consequently, the object novelty and feeding tests would fall into assessments of exploration. If this

were in fact the case, we would have expected these last two tests to show a positive correlation with the exploration test, which was not the case.

Counter to this argument, Toms *et al*. (2010) suggest that behaviour under predation risk might not represent innate bold behaviour, but more so reactive or "flight or fight" behaviours, thereby not providing a true representation of the behavioural phenotype. They further argue that it is almost impossible to pinpoint the distinct behaviours that will be manifested in a given test, and that reliability in assessing a behavioural trait can be achieved with the use of multiple tests or measures (Toms *et al.*, 2010). In accordance with this viewpoint, certain studies have illustrated that there could be multiple levels of bold behaviours and that correlations could only be found within a level or within similar levels (Coleman and Wilson, 1998; Wilson and Stevens, 2005). This explanation would be appropriate to explain the lack of overall correlation across the boldness tests used in the present study.

Another approach taken in behavioural syndrome studies relies on the association of behaviours, such as aggression and boldness, with the individual behavioural parameters rather than the different experimental tests (Wilson and Godin, 2009; Wilson *et al.*, 2010). For each behavioural test, sets of parameters for each targeted behaviour are created. For example, in a "feeding post-predatory threat" test, parameters such as latency to reach area of predatory threat and time spent in open area would be associated with boldness. On the other hand, parameters of total displacement and latency to exit refuge would be associated with exploration. Although this method may be ideal because it allows for the identification of various behaviours within a test, it biases the choice of

what parameters properly represent each behaviour. Using this alternative method might have yielded different behavioural correlates within our data set.

As previously mentioned, aggression-boldness syndromes arise quite frequently in nature, especially across fish species. However, our data suggested that no such syndrome was exhibited in *A. leptorhynchus* (table 3.2) in the current study. Arguments similar to those presented above, regarding the nature of the assessed behaviour, can be made in an attempt to explain the lack of correlation between bold and aggressive behaviours. However, there are also more direct factors that could be the cause of this lack of correlation. In the conspecific intrusion test, where aggressive behaviours were exhibited, the 'desperado' effect could have affected the behaviours of shy individuals (Grafen, 1987; Maan *et al.*, 2001; Peake and McGregor, 2004; Elias *et al.*, 2010). The 'desperado' effect occurs in staged laboratory agonistic interactions where shy fish cannot escape their opponent because they are in a confined space. In response to this confinement, the shy fish is forced to maintain prolonged aggressive interactions that are not reflections of its true behavioural nature (Grafen 1987; Maan *et al.*, 2001; Peake and McGregor, 2004; Elias *et al.*, 2010). Furthermore, the use of a conspecific mimic meant that no physical feedback or harm was provided to the experimental fish, which could result in escalation of aggressive behaviour. At the onset of a conflict, an individual assesses its opponent and its own chances of winning and determines the proper behavioural response. Throughout the conflict, the individual is continuously reassessing its opponent and its chances of success (Bradbury and Vehrencamp, 1998; Morrell *et al.*, 2005). Consequently, shy fish that initially do not perceive high success rates and display low levels of aggression could escalate their expressed aggression owing to the lack of

physical feedback and perceptions of increased winning probabilities throughout the conflict.

In summary, the lack of correlation between aggression and boldness in *A. leptorhynchus* could be attributed to heightened levels of aggression in shy individuals, thereby abolishing the possibility of a continuum between these behavioural traits. Furthermore, the plasticity in aggressive and bold behaviours may reflect the need for optimal behaviours in face of a dynamic, changing environment representative of their natural habitat (Moller, 1995; Dall *et al.*, 2004; Kappeler and Kraus, 2010; Bergmüller, 2010). In a dynamic environment, it is more advantageous to display appropriate independent behaviours when faced with varying situations, rather than adopting a fixed response notwithstanding situation variance. For example, always displaying bold feeding behaviours in the presence or absence of predators (variance that is seen in dynamic environments) may not be optimal in terms of energy expenditure. Displaying alternate behaviours, for example bold behaviours in the absence of predators and shy/cautious behaviours in the presence of a predator, is more beneficial. Therefore, behavioural plasticity might serve as an optimal strategy in *A. leptorhynchus*.

4.2 Chirping behaviours

The second objective of this study was to investigate the relationship between behavioural syndromes and chirping behaviours. Based on the results of the behavioural syndrome characterization, two conditional sets of hypotheses were devised. Owing to the lack of an aggressive-boldness behavioural syndrome, the second set of hypotheses were adopted in order to examine the implications for the observed behaviours on

chirping. In summary, the three proposed hypotheses presented three alternative functions to chirping behaviours in electro-communication: an aggressive signal that predicts physical attack, a bold signal used in conflict negotiations, or an electrosensory strategy that increases environmental sampling. In order to investigate each hypothesis, the relationship between chirp counts and the conspecific intrusion test, the object novelty test & feeding test (bold related tests), and the exploration test, respectively, were investigated.

Similar to the behavioural tests, chirping rates were significantly correlated, and thus consistent, across the two observation weeks. Support for each hypothesis required that correlations between particular behaviours and chirping rates be consistent over time. The lack of correlations between the behavioural scores of the object novelty test and the feeding test (table 3.3) does not support the boldness hypothesis. Bold fish did not tend to exhibit high chirp rates, suggesting that chirping is not an expression of the bold behavioural phenotype in conflict scenarios. Similarly, analysis of the correlations between chirp rates and behavioural scores of the exploration test resulted in non-significant results (table 3.3), results that are at odds with our exploration hypothesis.

Interesting results arose from the correlation analysis between the conspecific intruder test and chirp rates. A significant correlation was found between the two factors in the second, but not the first week of experiments (fig. 3.5). It was expected that the lack of temporal correlation would reflect non-responding fish in the conspecific intruder data set. However, the lack of consistency in correlation remained despite the adjusted conspecific intruder data set (fig. 3.5).

To explain this discrepancy, we considered the effects of the difference frequency (DF) between the EODs of the mimic and the experimental fish. However, as illustrated in figure 3.5, a significant relationship between chirp rates and DFs was absent, contrary to reports in the literature (Harvey-Girard *et al.*, 2010; Hupé, 2012). It is important to note, however, that these results were obtained from very different behavioural contexts, a factor that could explain the variance in DF effects. The effects of DFs on behavioural scores were also investigated, yet no significant correlations were found (data not shown). Therefore, even though, DF is likely a small contributor to variability in chirping behaviour, it is possible that an unidentified and far more significant factor is responsible for generating this temporal discrepancy.

We propose multiple means by which variability in signalling and aggressive behaviour occurs in *A. leptorhynchus* males. Firstly, the number of chirps might not be the only salient feature of chirping behaviour. Qualitative observations suggested that fish responded with different temporal patterns of chirping and with varied chirp structures. A recent study of chirp patterns in the brown ghost reported that males usually produce chirps in bursts and sometimes chirp in an antiphonal manner (i.e., echo signal of conspecific) (Hupé, 2012). However, the relationship between chirping patterns and aggressive behaviours remains unclear, with most behavioural variability being explained by chirp counts alone (Hupé, 2012). As previously described, the different types of chirps are characterized based on their duration and the magnitude of their frequency excursions (Zakon *et al.*, 2002; Zupanc, 2002; Zupanc *et al.*, 2006). The most common Type 1 and Type 2 chirps vary mostly in their excursion ranges (~20 ms & 200-300 Hz and 50 Hz, respectively), but are also thought to have two different behavioural significances:

submission and dominance, respectively (Zakon *et al.*, 2002; Zupanc, 2002; Zupanc *et al.*, 2006). Visual inspection of our recorded chirps demonstrated variance in the magnitude of frequency excursions of Type 2 chirps across the different individuals, although this variance was not quantified. Perhaps the magnitude of the excursion reflects aspects of behaviour, where the smaller frequency excursions signify a dominant male, while the larger signify less dominant males and once a certain size is reached, the chirps are then categorized as Type 1 and imply submission. Experimentally, this could be easily assessed by presenting males with chirp sequences that only differ in their frequency excursions and observing differences in aggressive behaviours in response to such stimuli.

Secondly, the lack of a relationship between aggression and chirping suggests that fish do not display a set strategy with regards to their behaviours and communication signals in agonistic interactions. Lack of strong selection pressure on an optimal aggressive signalling strategy could lead to high behavioural variability. In other words, there are multiple ways to resolve an agonistic conflict, where different combinations of aggression and signalling can be used, with no ultimate change in fitness (Bradbury and Vehrencamp, 1998; Searcy *et al.*, 2006; Kappeler and Kraus, 2010). If this were the case here, we would expect no correlations between chirping and aggressive behaviours, which was not the case in our second week of tests.

As a third approach towards interpreting these behavioural inconsistencies, we propose the idea of 'dishonest' signalling. 'Honest' signalling occurs when the cost of signal production outweighs the benefits of generating the signal (Bradbury and Vehrencamp, 1998; Van Staaden *et al.*, 2011; Gavassa *et al.*, 2012). If signal production

bears no significant cost to the sender, deceptive strategies can be adopted by the sender (Bradbury and Vehrencamp, 1998; Van Staaden *et al.*, 2011; Gavassa *et al.*, 2012). In the case of male *A. leptorhynchus*, if chirps are signals associated with aggression and dominance, non-aggressive or submissive individuals would benefit from generating chirp rates that mimic an aggressive dominant individual when faced by a superior opponent. In other words, the subordinate fish could bluff his opponent using chirps. This possibility would suggest that the energetic cost of chirp production is minimal.

The few studies that have looked at the energetic demands of EOD generation, in pulse and wave type species, have yielded equivocal results (e.g. Julian *et al*., 2003; Stoddard and Salazar, 2011). Although they have shown that the EOD does take a significant portion of the energy budget, they have also shown that in certain species, the EOD is unchanged by metabolic stressors such as hypoxia and exercise (Julian *et al*., 2003; Reardon *et al.*, 2011; Moorhead 2010; Stoddard and Salazar, 2011). Due to the involvement of the electric field in the majority of weakly electric fish behaviours, it is possible that although costly, the generation of the EOD is a protected process and is not affected by energy budget tradeoffs until extreme energetic stress is reached (Julian *et al.*, 2003; Moorhead 2010; Reardon *et al.*, 2011; Stoddard and Salazar, 2011). The energetic cost of chirping has yet to be determined. However, since a chirp is produced via a frequency shift in the pacemaker cells of the pacemaker nucleus, and they are extremely brief signals, it is expected that the energetic costs would be minimal in the context of the EOD energy budget (Kawasaki *et al.*, 1988).

High costs of EOD production, but low costs in chirp signalling, would permit male individuals to produce dishonest chirp patterns. This situation would result in

behavioural variation between chirping and aggression. If dishonest signalling was indeed at play in our species, experimentally, we would expect chirps to predict attacks in honest signallers (dominant aggressive males), but chirps would not be reliable predictors of attacks in cheaters (submissive non aggressive males).

4.3 Morphometric and physiological parameters

Contrary to previous studies, we found no significant correlations between body size or EODf and the various behavioural scores (table 3.4) (Hagedorn and Heiligenberg, 1985; Dunlap, 2002; Triefenbach and Zakon, 2003; Triefenbach and Zakon, 2008). Although size and EODf were significantly correlated with one another, we could not state that the larger fish with higher EOD frequencies were bolder or more aggressive individuals. These discrepancies may be due to the relatively small sample sizes of previous studies and/or their different behavioural assessments. The present study was the first to examine multiple aspects of behaviour across time with such a large sample of males. The study design suggests that our results are reliable, and therefore we conclude that EODf does not act as a badge of dominance in male *A. leptorhynchus*.

Furthermore, body size and EODf were not predictors of chirp rates, because no significant correlations were found amongst these factors. Larger fish with higher EOD frequencies did not show a stronger propensity to chirp. Owing to the previously mentioned costs of EOD production, it is likely that EOD frequency and amplitude simply reflect body condition or size, and thus could provide an honest signal of fitness. Our conclusions are limited by the lack of knowledge of the natural history (age, provenance and population) of our sampled fish.

4.4 Future work

The overarching theme that arose from the present study is the number of factors that can modulate and shape behaviour. Our results suggest that the variability in the chirping literature may be a consequence of a lack of understanding of the fundamental natural variations in behaviour in these fish. Future studies aimed at understanding the social significance of chirping in *A. leptorhynchus* should involve experiments that assess the honesty of the signal. The first step would require addressing the energetic cost of chirping behaviour. This measurement could be achieved by presenting a male with a conspecific EOD and evoking chirping, while monitoring the rate of oxygen consumption as an index of metabolism. Secondly, if chirping were found to have minimal cost, assessing signal honesty could be achieved by looking at the chirp responses of individuals when faced with multiple conspecific signals of increasing threat. When faced with increasingly superior intruders, it is expected that the likelihood of deceit and dishonest signalling would increase in submissive individuals, resulting in increased chirp rates. Dominant individuals would be expected not to alter their chirp rates. Results from these studies would greatly influence future endeavours related to understanding chirping and aggressive behaviour in male *A. leptorhynchus*.

4.5 Summary

Characterization of aggressive and bold behaviours was achieved in *A. leptorhynchus* via the application of the behavioural syndrome concept. Using an experimental design of five behavioural tests, each representing a behavioural context where bold and aggressive behaviours could be exhibited, I concluded that a boldness behavioural syndrome exits in males of our species. Fish that investigated more readily a novel object in their environment were more prone to eat in an open area. These findings satisfied the first objective of our study. Our second aim was to shed light on the implications of behavioural syndromes in the varying electrocommunication behaviours in *A. leptorhynchus*. Because correlations among exploration, boldness, aggressive behaviours and chirping were not detected, the function of chirping remains elusive and requires further investigation.

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