# Electrocommunication in a species of weakly electric fish, *Apteronotus leptorhynchus*: Signal patterning and behaviour

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## Dedications

This thesis is dedicated to the fish.

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## Abstract

Weakly electric fish produce and detect electric fields and use their electrosensory modality in a number of behaviours including navigation and communication. They can modulate their electric discharge in frequency and amplitude to produce electrocommunication signals in variable patterns during social interactions. In one model neuroethological species, *Apteronotus leptorhynchus*, the most commonly produced communication signal is the 'small chirp' – a brief 10-30ms modulation. Individuals tend to produce these signals at high rates during agonistic interactions. In this thesis I will explore the social value of chirps, and to a lesser extent other communication behaviours, in *A. leptorhynchus* using a variety of experimental designs involving different staged social contexts. I use time series analysis methods to explore the patterns of chirps produced and accompanying aggressive behaviours.

I first characterize electrocommunication and chirping in pairs of free swimming fish and correlate signal production with aggressive displays. Bursts of echoed, or reciprocated, chirps tend to be produced in the intervals separating aggressive attacks. Behavioural analysis shows that fish respond to conspecific chirps with echoed chirps and decreased aggression in social contexts outside the range in which previous modelling and electrophysiological data predicted that chirps could be encoded effectively.

I then characterize the chirping and aggressive responses to playbacks simulating intruders with different chirping styles to test whether alternative chirp patterns differentially influence conspecific behaviour. In response to simulated intruders producing chirps that echo the real fish's chirps with a short latency, less aggressive fish tend to produce more of their chirps in bursts than more aggressive fish. For randomly chirping intruders, the response of fish

depends on the rate of chirps delivered. Fish respond less aggressively, with fewer chirps, and echo the stimulus chirps at a higher rate when high rates of random chirps are delivered than when responding to simulated intruders with low rates of randomly delivered chirps. Further, across all playback scenarios, fish that produce chirps in response to the playbacks are more aggressive than those that do not chirp. Finally, to better understand the electrosensory inputs during these interactions, I characterize changes in the electric image received by a restrained fish during movements of a free-swimming conspecific and correlate these with chirp production. When one fish is restrained, bursts of chirps tend to be associated with approach behaviours. Communication signals often function to promote individual assessment of potential rivals during agonistic encounters and bursty, antiphonal chirp exchanges may facilitate these assessments and deter potentially costly physical escalations.

**Résumé**

Les poissons faiblement électriques ont la capacité de générer et de percevoir des champs électriques, et ils utilisent cette modalité électrosensoriel dans plusieurs nombres de comportements, tel que la navigation et la communication. Ils peuvent moduler la fréquence et l'amplitude de leur décharge électrique, leur permettant donc de produire une vaste variété de signaux communicatifs électriques lors d'interactions sociales. Chez une des espèce modèle neuroéthologicale, *Apteronotus leptorhynchus*, le signal de communication le plus couramment produit est le «petit chirp» - une courte modulation de 10 à 30ms. Les individus de cette espèce ont tendance à produire, à taux élevés, ces signaux de communication au cours d'interactions agonistiques. Dans cette thèse, je vais explorer la valeur sociale des «chirps» et dans une moindre mesure celle des autres comportements lié à la communication chez *A. leptorhynchus*. Ceci sera effectué avec l'utilisation d'une variété de méthodes expérimentales impliquant des scénarios représentant différents contexte sociaux. J'utilise des méthodes d'analyse de séries temporelles afin d'explorer les différents motifs de chirps produits, et leur relation avec les comportements agressif.

Je caractérise d'abord l'électrocommunication et la production de 'chirp' entre une paire de poissons en nage libre et je corrèle la production de signaux avec la présentation d'actes agressifs. Les rafales de 'chirp', écho ou réciproque, ont tendance à être produits dans les intervalles séparant les attaques de nature agressive. Une analyse comportementale démontre que les poissons répondent aux chirps conspécifiques en produisant des chirps en écho et en diminuant leur niveau d'agressivité. Ceci prend place dans des contextes sociaux en dehors de ceux qui avaient été prédit par des analyses computationnelles et par des expériences électriphysiologiques portant sur l'encodage des chirps. Je décris un nouveau mécanisme

d’encodage de chirp, qui permet de prédire comment les chirps peuvent être décodés à travers tous les contextes qui induisent une réponse comportementale chez un conspécifique.

Je caractérise ensuite les motifs de chirp et les comportements agressifs des poissons en réponse à une multitude de signal enregistré simulant un intrus avec différent motifs de chirp. En réponse aux intrus simulés produisant des chirps en motif écho, où le temps de latence est très court, les poissons moins agressifs ont tendance à produire leurs chirps en éclats (burst) plus fréquemment que les poissons plus agressifs. Pour les intrus simulés produisant des chirps au hasard, la réponse des poissons dépend du taux de chirps livrés. Les poissons répondent avec moins d’agressivité, produisent un faible nombre de chirps, et répondent en écho plus fréquemment lorsqu’un haut nombre de chirps au hasard est livré par l’intrus simulé, que lorsqu’un faible nombre de chirps au hasard est livré par l’intrus. En outre, dans tous les scénarios, les poissons qui produisent des chirps en réponse à l’intrus simulé, sont plus agressif que ceux qui ne produisent aucun chirp. Afin de mieux comprendre les entrées électro-sensorielles durant ces interactions, je caractérise les changements de l’image électrique reçue par un poisson restreint, lorsqu’un conspécifique est en nage libre dans le même aquarium, et puis je corrèle ces changements avec le taux de production de chirps. Lorsqu’un poisson est restreint, les rafales de chirps ont tendance à être associés aux comportements d’approche du poisson en nage libre. Durant les rencontres agonistiques, les signaux de communication servent à promouvoir l’évaluation individuelle de rivaux potentiels. Des échanges de chirp a motif écho et en rafales, peuvent faciliter ces évaluations et en conséquences dissuader les partis à participer dans une échange physique escaladée et couteuse.

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## List of Commonly used Abbreviations

AM – amplitude modulation
DF – difference frequency
E1 – first envelope (beat AM)
E2 – second envelope
EC – echoed chirps (playback stimulus)
EOD – electric organ discharge
EODf – electric organ discharge frequency
FFT – fast Fourier transform
ICI – interchirp interval
JAR – jamming avoidance response
NC – no chirps (playback stimulus)
RC – random chirps (playback stimulus)
RHP – resource holding potential
ROC – Receiver-operator characteristics
xICI – cross-interchirp interval

CLS – central lateral segment of the electrosensory lateral line lobe
CMS – central medial segment of the electrosensory lateral line lobe
CP/PPn – central-posterior prepacemaker nucleus
eGP – eminentia granularis pars posterialis
ELL – electrosensory lateral line lobe
Hl – hypothalamus lateralis
Hv – hypothalamus ventralis
LS – lateral segment of the electrosensory lateral line lobe
nE – nucleus electrosensorius
nP – nucleus praemencaelis
PPn – pre-pacemaker nucleus (diencephalic)
sPPn – sublemniscal prepacemaker nucleus
Pn – pacemaker nucleus (medullary)
PP – preopticus periventricularis
PG – preglomerular nucleus
TeO – optic tectum
TS – torus semicircularis

11KT – 11 ketotestosterone
5HT – serotonin
AVT – arginine vasotocin
CRF – corticotrophin releasing factor
DA – dopamine
NMDA – N-methyl-D-aspartic acid
NA – noradrenalin
SPl-ir – substance P-like immunoreactivity
T – testosterone

## Statement of Contributions

### Chapter 3

Chapter 3 involves an integration of my behavioural characterizations with experimental and modelling neurophysiological interpretations of chirp encoding provided by Dr. Jan Benda from the Department of Biology II: Neuroscience, Ludwig Maximilians University of Munich. Following presentations at the Electrosensory Symposium at the 8$^{th}$ International Congress for Neuroethology, we were invited to submit a manuscript for publication in the Journal of Physiology Paris electrosensory special edition. I performed the analysis of the behavioural data and integrated it with the predictions derived from Dr. Benda's electrophysiological contributions. I was the primary author of the Introduction, Behavioural Descriptions, and Discussion portions of the manuscript. The authors jointly contributed to brainstorming and idea development throughout the collaboration.

### Chapter 5

Dr. Na Yu and Dr. André Longtin, of the Department of Physics model naturalistic stimuli to explore how the electric fields produced by conspecifics interact during social encounters and how these electrosensory stimuli are encoded by electroreceptor afferent populations. I developed a method to record the electric images that are produced when one fish is restrained with one or more fish swimming around it. We describe the relationships between electric field strength and the distances separating interacting fish in a manuscript that will be submitted shortly (Yu *et. al.*, in preparation). To characterize chirping and aggressive behaviours in this context (as presented in Chapter 5), I provided the behavioural characterizations (chirps and fish positions), while Dr. Yu contributed calculations pertaining to the electric fields. All authors contributed to idea development and I was the primary author of the text presented here.

# Chapter 1

## General Introduction

## 1.1 Electrocommunication in Weakly Electric Fish

### *1.1.1 Thesis Overview*

Communication signals are produced during many social interactions and transmit dynamic information about individual differences in ability and motivation (Bradbury and Vehrencamp, 1998; Bohn *et. al.,* 2008; Laidre and Vehrencamp, 2008). The information content can be encoded in signal parameter variability, and/or in the temporal patterns of signals produced, in a manner that varies with social context (Greenfield and Minckley, 1993; Yosida and Okanoya, 2005). Weakly electric fish produce electric discharges, can detect those produced by nearby fish and use the electrosensory modality in electrolocation and electrocommunication (Hopkins, 1988; Moller, 1995; Kawasaki, 2009). Several different types of electrocommunication signals have been identified, varying in the type and pattern of frequency and amplitude modulations of the electric discharge produced during different social interactions (Zakon *et. al.*, 2002; Zupanc, 2002). In one model neuroethological species, *Apteronotus leptorhynchus*, the most commonly produced electrocommunication signal is the 'small chirp' and individuals tend to produce these aggressive signals at high rates during agonistic interactions (Larimer and MacDonald, 1968; Hagedorn and Heiligenberg, 1985; Hupé and Lewis, 2008 (Chapter 2); Triefenbach and Zakon, 2008). The neural mechanisms and hormonal modulating systems that underlie chirp processing have been well characterized (Zupanc, 2002; Benda *et. al.*, 2005, 2006; Marsat and Maler, 2010), however the social function of chirps, and the information content they convey, are not well understood.

In this thesis I will quantify temporal patterns in chirping behaviours, and to a lesser extent other signals, using a variety of experimental designs to examine electrocommunication

behaviours in *A. leptorhynchus* under a variety of staged social contexts. The overarching goal is to better understand the functional significance of chirping in weakly electric fish. Chirping had previously been associated with various social interactions, particularly aggressive displays, but few studies have focused on characterizing these signals and testing hypotheses about their function (Zakon *et. al*., 2002). Here I take several approaches to identify the roles that these signals play during social interactions using observational studies of staged interactions between fish. The results provide insight into how the temporal patterning of chirping correlates with a variety of factors including the distance between fish and aggressive behaviours. The behaviours characterized suggest that chirps are associated with aggression and possibly involved in the assessment of rivals. I also collaborated with other researchers to explore questions about sensory encoding. Throughout the thesis, I incorporate ideas from the literature on communication in other weakly electric fish and other animal communication systems to shed light on the social relevance of the behaviours characterized.

### *1.1.2 The Significance of Examining Communication Behaviours*

Neuroethological research investigates the neural underpinnings of natural behaviours, and it integrates principles from neuroscience, ethology, physiology, ecology, molecular biology, and phylogenetics, among others (Kelley and Bass, 2010). All animals sample and integrate external and internal stimuli using specialized sensory systems to drive adaptive behaviours in response to complex contextual situations (Kelley and Bass, 2010, Seyfarth *et. al.*, 2010). During social interactions, individuals extract information about conspecific identity and behaviour and integrate this information with internal cues (that determine dominance status and motivation, for example) to produce dynamic behavioural responses (Greenfield and Minckley, 1993; Silva *et. al.*, 2008; Salazar and Stoddard, 2009; Kelley and Bass, 2010). Communication behaviours are

ideal for exploring the neural substrates and mechanisms implicated in the sensory, processing, and motor aspects of natural behaviours (Pollack, 2001; Seyfarth and Cheney, 2003). Communication signalling depends on sensorimotor integration of external and internal stimuli during social interaction, and communication signals are often relatively easy to quantify (Marler, 1955; Schleidt, 1973; Heiligenberg, 1973). There is pronounced individual variability in signalling behaviours and context-specific differences among individuals which makes communication systems ideal for exploring the physiological basis of and plasticity in behaviours that permit flexible and adaptive responses (Sih *et. al.*, 2004; Yosida and Okanoya, 2009; Amy *et. al.*, 2010).

## 1.2 Background to Electric Fish

### *1.2.1 Weakly Electric Fish*

There are hundreds of species of electric fish, represented by both marine and freshwater species, and electric fish are classified as either strongly or weakly electric based on the strength of the electric discharges they generate (Moller, 1995; Crampton and Albert, 2006). Strongly electric fish produce electric discharges in the range of a few to several hundred of volts, whereas weakly electric fish produce much lower amplitude electric discharges, on the order of a few millivolts (Stoddard and Markham, 2008). Species of marine electric fish are all strongly electric, and include the stargazer and a few species of rays (Moller, 1995; Rose, 2004). Freshwater strongly electric fish species include the electric eel and a number of species of electric catfishes (Moller, 1995). All weakly electric fish live in freshwater, and two orders, each comprising over a hundred species, have been characterized: the Gymnotiformes and

Mormyriformes native to South America, and Africa, respectively (Moller, 1995, Alves-Gomes, 1999; Crampton and Albert, 2006; Lovejoy *et. al*., 2011).

The electric discharge is produced by means of an electric organ that is composed of specialized, excitable cells called electrocytes (Bennett, 1971). In some species these cells are myogenic (derived from modified muscle cells) whereas in other species the electrocytes are neurogenic (derived from modified neural cells) (Bennett, 1971; Bullock *et. al.,* 2005). The number and arrangement of electrocytes that make up the electric organ is species specific (Bennett, 1972). When electrocytes are stimulated, the channel compositions of the active membranes of individual electrocytes create large ionic currents that cause large action potentials (Markham *et. al.,* 2009). As a consequence of the arrangement of hundreds of electrocytes, these individual ionic currents summate to generate an electric field surrounding the fish (Babineau *et. al.,* 2006; Kelly *et. al.,* 2008; von der Emde *et. al.*, 2010). The electric discharge generated by electric fish is called the electric organ discharge (EOD). The frequency, amplitude and waveform of electric organ discharges (EODs) are species-specific. Within a species, there are often pronounced individual EOD differences, and individual fish can vary parameters of their EODs over time (Knudsen 1975B; Carlson and Hopkins, 2004; Crampton and Albert, 2006).

Weakly electric fish are classified as either pulse-type or wave-type species (Moller, 1995). Pulse-type fish emit discrete EOD pulses that are separated by periods of time in which they do not emit EODs. The length of a single EOD pulse varies between different species, as does the duration between successive EOD pulses (McGregor and Westby, 1992). Wave-type fish, on the other hand, emit EODs continuously with a large range in the frequency at which EODs are produced in different species (from ~25Hz to more than 2100Hz) (Crampton and Albert, 2006). Presumably because of the energetic costs associated with the production of high

amplitude EODs, strongly electric fish are all pulse type (Stoddard, 2008; Stoddard and Salazar, 2011). Both the gymnotiformes and mormyriformes include pulse and wave type species.

Within the gymnotiformes, wave-type electric fish emit in species-specific EOD frequency (EODf) ranges (Crampton and Albert, 2006); the myogenic wave-type Sternopygidae (which include *Eigenmannia sp*.) emit in the ranges of 70-150Hz and 200-600Hz, for *Sternopygus sp.* and *Eigenmannia sp.* respectively, whereas the neurogenic wave-type Apteronotidae have EODfs of 600-1500 Hz (Crampton and Albert, 2006). Although pronounced species differences exist, the EODf of individual, isolated fish tend to remain remarkably stable over time (Moortgat *et. al.*, 1998). EODf functions in species recognition (Dunlap *et. al.*, 2010; Fugère and Krahe, 2010) and individual identification within a species (Dunlap, 2002; Harvey-Girard *et. al.*, 2010).

Electric fish use their electric sensorimotor modality to navigate through often poorly lit, cluttered environments, to find and capture prey (Caputi and Budelli, 2006; von der Emde, 2006), and in conspecific communication (Hupé and Lewis, 2008 (Chapter 2); Triefenbach and Zakon, 2008). A description of the nature of the electric fields is useful for understanding how the EODs of nearby individuals interact and for interpreting the role of different signal modulations produced during social encounters.

### *1.2.2 Description of the Electric Fields Produced and Their Interactions*

Measured at a given point in space, the electric potential produced by the EOD of a single wave-type fish varies in time in a quasisinusoidal fashion, with the anterior and posterior regions alternating between positive and negative potentials (at a rate determined by the EODf) (Assad 1997; Assad *et. al.*, 1999; Kelly *et. al.*, 2008). The spatial geometries and temporal aspects of the

electric field are complex and dynamic and are influenced both by objects in the nearby environment and by the electric signals produced by nearby fish (Kelly *et. al.,* 2008; von der Emde *et. al.*, 2010).

When a fish is in isolation, the electric signal available to electroreceptors for encoding is the field produced by the EOD, and any perturbations imposed by features of its surroundings (Babineau *et. al.,* 2006; von der Emde, 2006). Objects with different conductivities influence the near-field electric field properties, causing localized spatial variations in current densities (von der Emde *et. al.,* 2010). Objects with the same conductance as the water will not perturb the electric field, whereas objects that are more or less conductive than the water will respectively induce local increases, or decreases, in electric field potentials (Caputi and Budelli, 2006; Knudsen, 1975B). These distortions, or perturbations, create amplitude modulations in the electric potentials received at the surface of the fish's skin (the 'electric image') which are encoded by electroreceptors, and are used to navigation and orientation behaviours collectively refer to as electrolocation (Nelson and MacIver, 1999).

When two fish interact, their electric fields interact and produce a combined electric potential with an amplitude that oscillates at a frequency equal to the difference in the EODfs of the interacting fish (the difference frequency, Df). This first order amplitude modulation (AM), is also called the beat, and the magnitude of the beat AM relative to the amplitude of the mean peak-to-peak voltage, is called the contrast (or the first envelope, E1) (Benda *et. al.*, 2005, 2006). Electric field strength falls off as the distance between the fish and receiver increases, thus the magnitude of the contrast in electric signal created by the combination of two EODs depends on the distance separating the interacting fish (Benda *et. al.,* 2005, 2006; Kelly *et. al.,* 2008). Consequently, as fish swim around relative to one another during social interactions the

magnitude of the beat AM, changes yielding a contrast modulation or second envelope, E2. These movement and position related E1 modulations are likely encoded by the electroreceptor afferent population and propagated to central electrosensory processing nuclei (Yu *et. al.*, in preparation). When more than two fish interact, multiple beats arise and more complicated E2 profiles result (Middleton *et. al.*, 2006, 2011; Stamper *et. al*., 2010).

In addition to encoding the diversity of amplitude modulations imposed on the EODs by environmental objects and the position and movement of nearby electrogenic individuals, electroreceptor afferents are also sensitive to temporal changes in E1 and E2 that occur if one or more fish modulate their EODf (Benda *et. al.*, 2005, 2006; Gussin *et. al.*, 2007). During social situations *A. leptorhynchus* produce EOD frequency and amplitude modulations and these are encoded by the electroreceptors of neighbouring fish and are believed to serve in communication (Zupanc, 2002; Zakon *et. al.*, 2002). This system provides a ideal opportunity to characterize the neural mechanisms that encode electric image perturbations caused by the movements and EOD modulations (communication signals) of interacting conspecifics during social encounters.

## 1.3 Electrocommunication in a Wave-type Weakly Electric Fish, *Apteronotus leptorhynchus*

### *1.3.1 Apteronotus leptorhynchus - The Brown Ghost Knifefish*

The brown ghost knifefish, *Apteronotus leptorhynchus*, is a Gymnotiform wave-type weakly electric fish that has remained a neuroethological model for decades (Larimer and Macdonald, 1968; Hopkins, 1988; Krahe, 2008). In the wild, Apteronotids tend to live alone or in small groups (Tan *et. al.*, 2005; Stamper *et. al.*, 2010, personal observation) and males defend territories from potential rival conspecifics (Fugère *et. al.*, 2011). *A. leptorhynchus* electrosensory, processing, and electromotor neural pathways have been well characterized

(Maler *et. al*., 1991; Zupanc, 2002; Bullock *et. al.*, 2005; Marsat and Maler, 2010) as have the neuromodulators and hormones that influence different aspects of its natural behaviours (*e.g.* Maler and Ellis, 1987; Dulka and Maler, 1987; Dunlap *et. al.,* 2002; Telgkamp *et. al.,* 2007; Kolodziejski *et. al.,* 2007; Dunlap *et. al.,* 2011).

*A. leptorhynchus* produce a continuous EOD with a sexually dimorphic EODf; females emit in the range of 600-850Hz, whereas mature males emit in the range of 800-1100Hz (Maler and Ellis, 1987; Zakon *et. al.,* 2002). During development the EODfs of both sexes increase (Meyer *et. al.* 1987). EODf is also positively correlated with water temperature (Dunlap *et. al.,* 2000), and shows diurnal fluctuations, tending to be highest at night when these nocturnal fish are most active (Zupanc *et. al.,* 2001). When individual fish are in isolation, their EODf remains very stable in time and it has been suggested that in males, a higher EODf may act as a badge of status with higher frequencies indicative of a more dominant status (Dunlap *et. al.,* 2002; Triefenbach, 2005). It has also been suggested that EODf may be used in mate selection and conspecific recognition (Hagedorn and Heiligenberg, 1985; Harvey-Girard *et. al.,* 2010; Fugère and Krahe, 2010).

### *1.3.2 Electrocommunication Signals - Chirps*

The electrocommunication modulations produced by *A. leptorhynchus* have been classified into two broad categories: rises and chirps (Zakon *et. al.,* 2002; Zupanc, 2002). As the name suggests, rises describe increases in the EODf and these are produced with a large variability in the duration and profile of the frequency excursion (Tallarovic and Zakon, 2002, 2005). Because they are variable in the duration and magnitude of frequency modulations, it is not known whether there are distinct classes of rises, or whether they exist along a continuum

(Zakon *et. al.,* 2002; Zupanc, 2002; Hupé and Lewis, 2008 (Chapter 2); Triefenbach and Zakon, 2008). The shortest duration rises are usually produced as a series of successive rises and are hence called 'abrupt frequency rises' (AFRs) (Zakon *et. al*., 2002).

Chirps are a type of frequency modulation commonly produced by *A. leptorhynchus* males during social interactions and they are much more stereotyped in duration, and amplitude and frequency modulation than rises (Zakon *et. al.,* 2002). Zupanc *et. al.* (2006) classified six distinct types of chirps, two with relatively short durations (10-15ms; Type 1 and Type 2 chirps, or 'large' and 'small' chirps respectively) and four with similarly long durations (~100ms; Types 3-6, or 'long' chirps). Short duration chirp types are produced predominantly during chirp chamber experiments and during staged interactions between conspecifics (Zakon *et. al.* 2002; Dunlap and Larkins-Ford 2003B; Trifenbach and Zakon, 2008). 'Small' (Type 2) chirps are associated with smaller EODf excursions (50-150Hz) and are the most common type of chirp produced during aggressive conspecific interactions and in response to playbacks with low Dfs (Engler and Zupanc, 2001; Fugère and Krahe 2010). 'Large' (Type 1) chirps are associated with large EODf excursions (200-400Hz) and are produced less commonly during staged conspecific interactions, although produced at higher rates in response to larger Df stimuli. Because chirps are produced as discrete and easy to quantify signals, they are ideal for characterizing interactions between individuals and the factors that influence signal production rates and patterns.

### *1.3.3 Experimental Characterizations of Chirping Behaviours*

Initial research on weakly electric fish centered on a behaviour called the jamming avoidance response (JAR) (Heiligenberg and Bastian, 1984; Heiligenberg *et. al.,* 1996). In many

species of wave-type weakly electric fish, if two interacting fish have similar EODfs, the fish with higher frequency will often increase its EODf to prevent ”jamming” of electrosensory processing that can result when Dfs are small (Matsubara and Heiligenberg, 1978; Westby, 1981; Bastian and Yuthas, 1984; Metzner *et. al.*, 1999; Tallarovic and Zakon, 2005). The neural circuitry implicated in this behaviour, the JAR, was a topic of intensive research in the early days of neuroethology and it was one of the first behaviours in which the neural mechanisms underlying an entire sensorimotor loop were characterized (Heiligenberg, 1991).

In addition to the JAR, other electrical behaviours of fish have also been characterized. The chirping behaviours of *A. leptorhynchus* were first described by Larimer and MacDonald (1968). In isolation, fish produce low rates of chirps, the majority of which are large chirps (Engler *et. al.*, 2000), however chirps of both types are produced at higher rates during reproductive and aggressive contexts (Hagedorn and Heiligenberg, 1985), and in response to electrical playbacks of sine waves or conspecific EODs (*e.g.* Maler and Ellis, 1987; Fugère and Krahe, 2010). The chirping behaviours of fish restrained in an apparatus similar to the one used to assay JAR behaviours (a ‘chirp chamber’), have been characterized in response to sine wave stimuli delivered through stimulating electrodes. Males tend to chirp at significantly higher rates than do females in chirp chambers and during paired interactions (Maler and Ellis, 1987; Tallarovic and Zakon, 2002). Short duration small chirps are produced at higher rates when the Df is small (i.e. the difference between the stimulus EODf and the real fish’s EODf are similar), and large chirps are produced preferentially when the Dfs is large (Engler and Zupanc, 2001). Additionally, the chirp rates of fish responding to conspecific EODs correlate significantly with the EODf of the chirping fish (Dunlap, 2002). *A. leptorhynchus* males also produce chirps in

response to heterospecific pulse-type, and low frequency wave-type (~100Hz) electrical stimuli (Dunlap *et. al*., 2010).

Free-swimming male *A. leptorhynchus* respond with elevated chirp rates and aggression to EOD playbacks with frequencies in the conspecific frequency range (Fugère and Krahe, 2010). The extent of aggression directed towards playback electrodes in free-swimming male *A. leptorhynchus* responding to simulated conspecific EOD stimulus, correlates positively with small chirp production rates, EODf, and body size (Triefenbach, 2005).

Male *A. leptorhynchus* chirping behaviours characterized in chirp chambers and in response to playbacks, show many similarities with chirping behaviours produced under increasingly realistic conditions (Dunlap and Larkins-Ford, 2003B; Zupanc *et. al*., 2006; Triefenbach and Zakon, 2008; Gama Salgado and Zupanc, 2011). Chirp production rates habituate less quickly in pairs of males communicating through electronically coupled tanks, and between pairs of males in visual and electrical contact but physically separated by a partition, than when tested in a chirp chamber (Dunlap and Larkins-Ford, 2003B). When two fish are restrained in separate PVC tubes in the same tank, fish produce up to six types of chirps (Types 1-6, classified based on multiple factor clustering analysis), with small chirps occurring most frequently (Zupanc *et. al*., 2006). Fish tend to produce small chirps in bursts (clusters), and tend to echo one another's chirps more often than expected by chance, a behaviour termed the 'echo response' (Zupanc *et. al*., 2006).

During aggressive paired interactions, *A. leptorhynchus* males tend to produce bursts of often echoed chirps in between physical contact behaviours (Triefenbach and Zakon, 2008). Non-contact aggressive behaviours include jaw-quivering and rapid approaches and contact

aggressive behaviours include mouth wrestling, tail nipping, head clamping, and tail flicks (Triefenbach and Zakon, 2008). Chirping behaviours vary between individuals and chirp rates change tremendously over time, and may signal information about a chirping fish's fighting ability and aggressive motivations, however it is not known what information is contained in these chirp patterns and under what conditions they influence the escalation or resolution of conflicts (Enquist *et. al.*, 1990; Triefenbach and Zakon, 2008; Fugère and Krahe, 2010).

## 1.4 Principles of Chirp Behaviour Paralleled in other Fish Communication Systems

### *1.4.1 Communication in Other Species of Electric Fish*

Many species of electric fish produce stereotyped electric signals and both pulse and wavetype weakly electric fish produce these signals in agonistic contexts (Moller, 1995; Zupanc, 2002). Hopkins (1974) temporally correlated the observable behaviours of *Eigenmannia* (a wave-type Gymnotiform) with the patterns of electric signals they produce. *Eigenmannia* produce electrocommunication signals called interruptions, brief cessations of the EOD, that are produced in variable patterns (Hopkins, 1974; Wong, 2000). Short duration interruptions correlate with aggressive attacks and threat behaviours directed at a conspecific mimic, while longer duration interruptions are associated with submissive behaviours (Hopkins, 1974). Short interruptions tend to be produced in bursts and the number of interruptions produced in a single burst correlates with the signalling fish's propensity to attack (Hopkins, 1974).

Pulse species of weakly electric fish produce stereotyped patterns of EOD pulses which act as communication signals during reproductive and agonistic contexts, and also during a variety of other behavioural contexts (Westby, 1974, 1975A-C; Kramer, 1976, 1978, 1979; Carlson, 2002A; Carlson *et. al*., 2011). The gymnotid pulse species *Gymnotus carapo* patterns its

EOD with its aggressive behaviours such that it stops producing pulses for a short time preceding an attack and remains electrically silent for an extended period if defeated (Black-Cleworth, 1970). Mormyrids are a group of African weakly electric freshwater teleosts and as all weakly electric fish, mormyrids produce species-specific electric organ discharges (EODs) which they use in navigation, prey detection and communicative signalling (Moller, 1995; Zupanc, 2002; Werneyer and Kramer, 2002). In the wild, mormyrids form stable hunting packs and coordinate their EODs during hunting; and it is thought that this echoing behaviour functions in individual identification and group cohesion (Arnegard and Carlson, 2005).

There are many parallels in the behaviours and neural encoding mechanisms that underlie mormyrid and gymnotid electrocommunication, and many of these are analogous to principles of behaviour and the underlying physiological bases of acoustic signalling systems (Hopkins, 1988; Crampton and Albert, 2006; Carlson and Kawasaki, 2008; Kelley and Bass, 2010). In addition to producing electric signals, a number of mormyrid species also produce complex acoustic vocalizations during agonistic displays (Crawford *et. al.,* 1986; Crawford *et. al.,* 1997; Crawford and Huang, 1999). One well-studied species of mormyrid, *Pollimyrus isidori*, produces at least five distinct acoustic calls to advertise its territory and attract mates (Crawford *et. al.,* 1986). Territorial males produce 'grunts' (a series of approximately 15 discrete 5ms pulses produced at 16-25ms intervals), 'moans' (bursts of continuous complex tonal sounds with a clear fundamental and harmonic frequencies in the range of a couple to a few hundred hertz and lasting from 250ms to 3s), and 'growls' (series of broad spectrum pulses with a repetition rate about half that of grunts) during courtship and mating (Crawford *et. al*., 1997; Crawford and Huang, 1999). These signals tend to be produced when females move into and out of a male's territory (Crawford *et. al.,* 1986). Conversely, males produce "pops" and "hoots" during

agonistic encounters. Pops are brief grunt-like signals; whereas hoots are tone-like but with variable and rapid frequency modulations (Crawford *et. al.,* 1986). Playback studies suggest that these signals are produced with identifiable intraspecific individual variation and that signal production types, rates and patterns are important for individual recognition during courtship and territorial social interactions (Crawford *et. al.,* 1997; Crawford and Huang, 1999).

### *1.4.2 Communication in Other Species of Fish*

Bass and McKibben (2003) present a thorough and comparative review of the courtship and territorial signalling behaviours across many groups of fish and the parallels in the neural and hormonal mechanisms that regulate these communication behaviours. Fish use a variety of modalities, including visual, chemical, and electrical signalling systems to advertise and defend territories. Similar selection pressures have helped shape these territorial signalling behaviours and associated neural systems and have led to the development of many parallels across fish taxa and across modalities (Bass and McKibben, 2003; Kelley and Bass, 2010).

#### *1.4.2.1 Batrachoidid Vocalizations*

The production of stereotyped vocalizations is necessary for successful reproduction and territorial defence in the midshipman fish and toadfish, members of the family Batrachoididae (Bass and McKibben, 2003; Remage-Healey and Bass, 2005). Toadfish produce signals called ‘grunts’ and ‘boatwhistles’, and these two signals are produced under different contexts suggesting that they serve distinct functions. Playback studies confirm that ‘grunts’ and ‘boatwhistles’ induce a differential response in conspecifics: boatwhistle playbacks evoke positive phonotaxis (approach to sound emitting speaker) whereas grunt trains do not (Winn, 1972). Grunts are produced alone or in repetitive sequences during agonistic encounters, and are

produced at high rates by males when a conspecific intruder swims in close proximity to its defended nest (Bass and McKibben, 2003).

Steroid hormones influence territory defence and vocal signalling behaviours, influencing aggressive ability and motivation (Sih *et. al*., 2004) and in toadfish, steroid levels vary over the time course of natural encounters and in response to playbacks of aggressive signals (Remage-Healey and Bass, 2005; Kelley and Bass, 2010). Steroid hormone levels change on a moment to moment basis during social interactions and there is a conserved effect of steroids in the regulation of signalling, aggression and other social behaviours across vertebrates (Goodson, 2005; Remage and Healey, 2010). Steroids exert an influence on signalling and aggressive behaviours by acting on various forebrain and midbrain centres collectively termed the 'social behaviour network' with a high degree of structural and functional conservation across diverse vertebrate groups (Newman, 1999; Goodson, 2005). In weakly electric fish, electrocommunication behaviours are extensively modulated by steroid systems and other interacting neuromodulatory systems (Maler and Ellis, 1987; Salazar and Stoddard, 2009; Dunlap *et. al*., 2011). In *A. leptorhynchus*, EODf, chirp rate and body size correlate with circulating levels of 11-ketotestosterone (11KT) (Maler and Ellis, 1987; Dunlap, 2002; Dunlap *et. al*., 2002).

*1.4.2.2 Vocalizations in Gobies and Damselfish*

Many species of both freshwater and marine gobies produce both tonal and pulsatile sounds as part of their territorial breeding behaviour, and pulses with different acoustic qualities are produced context-specifically (Torricelli *et. al.*, 1990; Lugli *et. al.,* 1997, Bass and McKibben, 2003). Male gobies emit trains of pulses, and high frequency pulses, in particular, are used to

attract females to the male's nest. Males emit lower frequency pulses once the female has entered the nesting area (Torricelli *et. al.,* 1990). Playback studies confirm the differential roles of different pulse train rates on conspecific signalling and aggressive behavioural responses (Lugli *et. al.*, 1997).

Damselfish are territorial coral reef dwellers and about a dozen species are known to produce sounds (Bass and McKibben, 2003). Similar to other acoustic fish, damselfish produce acoustic signals during courtship and agonistic encounters (Lobel and Mann, 1995). "Chirps" are one type of acoustic signal produced and their production is associated with courtship swimming displays. Chirp pulse number and pulse interval length are individually specific; hence they can be used to identify neighbours and influence female preference (Lobel and Mann, 1995). Playback studies have demonstrated that female damselfish prefer males who produce chirps at high frequencies and that males can discriminate between chirps of individual neighbours (Myrberg Jr. and Riggio, 1985). Similarly, *A. leptorhynchus* chirps are thought to be involved in individual recognition during reproductive and agonistic contexts (Triefenbach and Zakon, 2008; Harvey-Girard *et. al*., 2010).

## 1.5 Comparisons to Communication Behaviours in Other Territorial Animals

### *1.5.1 Signalling Behaviours Convey Individual Differences in Aggressive Capacity and Motivation*

Territorial signals are communication signals used to indicate, demarcate, or defend the presence of a territory (Bradbury and Vehrencamp, 1998). A territory was initially defined as an area defended by its owner (Noble, 1939), but the definition has been since expanded to include that the territory owner has exclusive access to the resources in this area (Mumby and Wabnitz,

2002) including preferred nest sites. The widespread use of territorial signals throughout the animal kingdom is likely a consequence of the strong adaptive advantages that are conferred through advertising and defending a territory (Parker, 1974; Greenfield and Minckley, 1993). Apteronotid species aggressively defend territories in natural habitats (Fugère *et. al*,, 2011), respond aggressively to potential rivals (Dunlap and Larkins-Ford, 2003B; Fugère and Krahe, 2010) and defend shelters (PVC tubes) during staged dyadic interactions (Triefenbach and Zakon, 2008).

During territorial contests, potential rivals often perform signalling displays prior to engaging in physical contact and these signals permit the assessment of rival dominance status, fighting ability, and aggressive motivation without physical altercation (Parker, 1974; Enquist *et. al.*, 1990; Yosida and Okanoya, 2005). Dominant and subordinate individuals are identified by variability in signalling behaviours, whereby they may produce signals at different rates, or produce signals characterized by distinct signal parameter profiles. (McGregor and Westby, 1992; Triefenbach, 2005) Signal patterns may indicate differences in an individual's capacity and willingness to defend a contested resource, such as a preferred territory or limited space, defined as its resource holding potential, RHP (Greenfield and Minckley, 1993; Triefenbach and Zakon, 2008; Amy *et. al*., 2010). Physical combats can be very costly, and by assessing rival fitness and motivation using signals, individuals can weigh potential outcomes associated with different behavioural choices depending on the perceived threat imposed by potential rivals (*i.e.* the assessment of consequences associated with escalation compared to retreat) (Greenfield and Minckley, 1993).

*A. leptorhynchus* chirping behaviours may allow potential rivals to assess one another's physical strength and aggressive motivations (Dunlap *et. al*., 2002; Triefenbach and Zakon,

2008). Characteristics that correlate with dominance status such as hormone levels, influence the rates and types of chirps produced by individual fish suggesting that chirping behaviours signal aggressive motivation during conspecific interactions (Maler and Ellis, 1987; Dunlap *et. al*., 2002; Triefenbach and Zakon, 2008). However, the functional significance of different chirp production patterns (bursts and echoes, for example) are not known and a comparison to signalling in other territorial systems suggests that different rates and patterns of chirps may serve multiple, context-dependent functions.

### *1.5.2 Communication Signals are often Produced in Clusters*

Many types of communication signals are produced in sequences of rapidly repeated signals and aggressive signals are produced in clusters of repeated signals, often with variability in signal number and rate within a cluster (Schleidt, 1973; Ursprung *et. al.,* 2009). The ubiquity of repetitive, or bursty, signalling suggests many advantages, including to increase signal-to-noise ratios, induce tonic effects on receiver physiology, and encode information via temporal rate modulations (Schleidt, 1973; Forrest, 1994; Brumm and Slater, 2007). Repeated signalling behaviour may induce tonic and graded effects on receivers through temporal rate modulations in signal production patterns, with the responses of receivers to successive signals temporally summated to induce cumulative influences on receiver physiology and behaviour (Schleidt, 1973; Healey and Bass, 2010).

Bursts of signals produced during conflict situations may be associated with particular physical behaviours (Bohn *et. al.,* 2008; Ursprung *et. al.,* 2009; Amy *et. al.,* 2010) and the information exchanged during signalling behaviours may be used by receivers to assess the potential costs and benefits associated with alternate behavioural choices (McGregor and Peake,

2000; Chittka *et. al.,* 2009). In *A. leptorhynchus*, bursts of chirps tend to be patterned with aggression in a manner that depends on the context under with they are produced: bursts of chirps tend to be produced between aggressive interactions during dyadic free-swimming interactions (Triefenbach and Zakon, 2008), whereas they tend to be associated with aggressive approaches in response to EOD playbacks (Fugère and Krahe, 2010).

*1.5.3 Aggressive Signals are often Reciprocated by Interacting Conspecifics*

Antiphonal signalling is defined as the reciprocated exchange of signals between individuals echoing one another's signals with a short latency and without temporal overlap (Yosida and Okanoya, 2005). Antiphony occurs throughout the animal kingdom, and the behaviours and physiological processes regulating it have been characterized in insects (Greenfield and Minckley, 1993; Ronacher *et. al.,* 2008), fish (Bass and McKibben, 2003), frogs (Ursprung *et. al.,* 2009), birds (Amy *et. al.,* 2010) and mammals (Bohn *et. al.,* 2008; Yosida and Okanoya, 2009). Antiphonal signals are produced in a variety of contexts and are believed to serve a diversity of functions. One advantage of antiphonal signalling behaviours is that it confers reciprocally, to the senders, confirmation that signals have been received, and antiphonal communication behaviours are common in noisy conditions, or when visual cues are unreliable (Greenfield, 1994; Yosida and Okanoya, 2005).

The rates and patterns of echoed signals produced and the acoustic properties of antiphonal signals are individually specific, with significant differences in the signalling behaviour and signal structure between individuals (Parker, 1974; Yosida *et. al.,* 2007). In naked mole rats, bursts of antiphonal calls are produced when blind individuals come into physical contact with one another and these are believed to prevent aggressive escalation, convey

information about sender identity, and help guide conspecific movements (Yosida *et. al.*, 2007, Yosida and Okanoya, 2009). Frogs tend to produce bursts of antiphonal signals during phonotaxic approach behaviours in natural and simulated territory disputes, and their approach behaviours depend on the signalling behaviour of the intruder (Ursprung *et. al.*, 2008). During territorial defence behaviours, male frogs approach intruders when the potential rival is signalling, but cease to approach when the intruder (whether real or simulated) stops signalling (Ursprung *et. al.*, 2009).

In grasshoppers, bursts of territorial signals are produced antiphonally during territorial contests and the number of signals produced per burst correlates with an individual's RHP and motivation (Greenfield, 1994). In interactions with unmatched signalling rates, the individual with the lower number of signals per burst tends to stop signalling first and retreat without physical escalation (Greenfield and Minckley, 1993). If antiphonal burst signalling rates are matched, interactions are more likely to escalate resulting in physical combat (Greenfield and Minckley, 1993). Signalling continues during the physical escalation, and when one rival finally retreats (loses), his last burst contains considerably fewer signals than does the last burst of the winning male (Greenfield and Minckley, 1993). In *A. leptorhynchus*, when two fish compete over a limited shelter, the fish that wins the contest and obtains residency produces more small chirps than does the losing fish, especially nearing the end of the contest (Triefenbach and Zakon, 2008).

### *1.5.4 Leaders and Followers in Animal Signalling Systems*

In addition to the information conveyed by signal production rates, the timing of signals produced during antiphonal exchanges associated with physical escalations often carries

information about the strength and motivation of the signaller (Parker, 1974; Laidre and Vehrencamp, 2008). In signalling systems, including during antiphonal duets and during choral singing, 'leaders', individuals that produce the first signals in a sequence of exchanged signals most often, tend to be dominant over 'followers' or 'echoers', individuals that tend to echo signals produced by other individuals more often (Sneddon and Greenfield, 1998). Leaders also tend to have higher signalling rates (Greenfield and Minckley, 1993; Yosida *et. al.* 2007). In many coordinated behaviours, including in paired foraging behaviours, fish that lead concerted behaviours more often tend to be the larger, and bolder individuals (Harcourt *et. al.,* 2010) and 'leading' males are preferred in female mate choice experiments (Sneddon and Greenfield, 1998).

*1.5.5 Echoing Permits Accurate Discrimination of Signal Timing and Quality*

During antiphonal calling, individuals reciprocate each other's signals within a relatively narrow time window and these echoed signals overlap one another infrequently (Kelley and Bass, 2010; Amy *et. al.*, 2010). The timing and acoustic parameters of signals convey information about individual status and motivation and the production of overlapping signals obscures accurate discrimination of potentially meaningful signal parameters (Parker, 1974; Naguib, 1999; Brumm, 2006). In some signalling systems, the production of overlapping signals conveys aggressive intent whereas the production of signals echoed within a relatively short latency conveys intent to negotiate (Brumm and Slater, 2007; Amy *et. al.*, 2010).

The sensitivity of a signalling individual's sensory system may be temporarily suppressed due to intrinsic mechanisms, and may not effectively encode conspecific signals during or immediately following signal production (Yosida and Okanoya, 2005; Kelley and Bass, 2010). The neural mechanisms underlying the inhibition of calling while conspecifics signal during

antiphonal behaviours have been characterized in a variety of acoustic systems (Eliades and Wang, 2008; Kelley and Bass, 2010). In *A. leptorhynchus*, males tend to echo one another's small chirps with a low incidence of simultaneous chirps between interacting conspecifics, and this may enable accurate discrimination of chirp timing and quality (Zupanc *et. al.*, 2006; Hupé and Lewis, 2008 (Chapter 2)).

### *1.5.6 Neuroethological Implications of Communication Behaviour Characterizations*

The information content of animal signals can be examined by observing the dynamic influences produced on the nervous systems, and consequently the behaviour, of interacting individuals (Seyfarth *et. al*., 2010). Sender signals, and the information they transmit, may induce a variety of effects on receiver behaviour and these responses are dependent on a multitude of interacting factors including, for example, the social context under which the signals are produced and the motivational state of both sender and receiver (Goodson, 2005; Kelley and Bass, 2010; Dunlap *et. al*., 2011).

Chirp behaviours in *A. leptorhynchus* are regulated by intricate and extensively modulated neural networks and neuromodulatory systems that permit flexible integration of dynamic extrinsic and intrinsic stimuli (Maler and Ellis, 1987; Maler *et. al.*, 1991; Zupanc, 2002; Dunlap *et. al*., 2011). Chirps are encoded by changes in electroreceptor afferent population activity at the peripheral level, and centrally by chirp selective cells in electrosensory nuclei (*Marsat et. al*., 2009; Marsat and Maler, 2010). Electrosensory pathways are extensively integrated with electromotor pathways (Rose, 2004) and many of the physiological processes underlying chirp sensorimotor integration remain to be elucidated. A characterization of chirping behaviours is central to an understanding of how these physiological systems regulate chirp

production rates, burst patterns and echo responses (temporally integrate chirping behaviours with conspecific signals), pattern chirps with physical behaviours, and permit assessment of the relative differences in aggressive predisposition and motivation between interacting individuals.

### 1.6 Specific Thesis Objectives

In the following chapters I describe the communication behaviours of *A. leptorhynchu*s produced under a variety of experimental conditions using temporal characterizations to examine individual and context dependent differences in chirping behaviours. I quantify chirp rates, chirp burst parameters, echo responses, the relationship between chirping and aggression and the interactions between conspecific chirping and aggressive behaviours, and characterize individual and context dependent differences. Despite the wealth of evidence that particular signal rates, patterns, and timing convey important information during agonistic encounters, few studies have examined the temporal relationships between signal patterning and aggressive behaviours in a neuroethological model species on subsecond time scales.

Chapter 2 examines the electrocommunication behaviour of pairs of freely interacting brown ghost knifefish. Here I characterize individual and contextual differences in signalling behaviour and correlate the temporal patterning of chirps with physical aggression. I examine how males temporally pattern chirps with their aggressive movements, and with chirps produced by, and with movements of, the other fish.

Chapter 3 discusses collaborative efforts with Dr. Jan Benda (Department of Biology II: Neurobiology, Ludwig-Maximilian University of Munich) in which we combine our behavioural results with his electrophysiological considerations of chirp encoding. Previous electrosensory models predicted that the electrosensory system of receiving individuals can encode chirps

produced by signalling conspecifics only when Dfs are small (Benda *et. al.* 2005, 2006). Through additional analyses of the behavioural data presented in Chapter 2, I found that fish responded to conspecific chirps at Dfs greater than predicted by these models. By reconsidering model parameters and interpretations we revealed a novel mechanism by which chirps can be encoded across all behaviourally relevant Dfs through relative changes in electroreceptor afferent population synchrony.

In Chapter 4, I characterize male *A. leptorhynchus* chirping and aggressive response to conspecific intruder playbacks. By changing the rate and pattern of chirps delivered through a simulated intruder, I examine whether different chirp patterns induce differential chirping and aggressive responses. Chirp rates, patterns, and their temporal relationships with aggression depend significantly on the rate and pattern of playback chirps, and individual differences in chirping responses correlate with aggressive behaviours. These results suggest some chirp sequences are perceived as more threatening than others and that specific chirping behaviours are used to assess the costs associated with escalation during agonistic contexts.

In Chapter 5, I investigate the influence of movement-based electrosensory stimuli on chirping behaviour. This work was motivated by a collaboration with Dr. Na Yu and Dr. André Longtin (Department of Physics, University of Ottawa). We recorded the electrical image received by a restrained fish as a second fish swims freely around it and temporally correlate parameters of the electric image with the electrocommunication behaviours produced. Both free-swimming and restrained fish produce more chirps during more aggressive trials. Bursts of chirps produced by the free-swimming and restrained fish are timed with approach and retreat behaviours of the free-swimming fish.

Chapter 6 summarizes and provides a general discussion of the main findings, and outlines the implications and significance of these results through a comparative approach. The sensory and motor pathways implicated in *A. leptorhynchus* chirp encoding and production are reviewed, as are the neuromodulatory systems that regulate them. These physiological systems provide the basis for the individual differences in chirping and aggression, sensorimotor integration involved in echoing behaviours, experience-dependent flexibility of chirp rates and temporal patterns, and the context-dependency of these relationships.

# Chapter 2

## Electrocommunication Signals in Free Swimming Brown Ghost Knifefish, *Apteronotus leptorhynchus*

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## 2.1 Introduction

Many animals produce signals in a context-dependent fashion (Bradbury and Vehrencamp, 1998). A central goal of ethologists is to uncover why animals produce signals and reveal what functional significance they serve. We can examine the conditions under which an animal produces a given signal, or particular combination of signals, to shed light on factors that may influence or induce particular signalling behaviour (causes). In studying behaviours that may reflect signalling motivation, we can also observe any behavioural consequences of signal production in order to reveal the social relevance of different signal types (effects). Characterization of the actions associated with signal production has provided a means of evaluating whether communication occurs and has helped reveal the functions of signals in many animal systems (e.g. Hopkins, 1974; Crawford *et. al*., 1986; Seyfarth and Cheney, 2003; Partan and Marler, 2005; Wong and Hopkins, 2007). Here, we take this approach to study electrocommunication signals in a species of weakly electric fish.

Brown ghost knifefish, *Apteronotus leptorhynchus*, are native to freshwater systems of South America and like all weakly electric fish, they both produce and detect electric signals (Moller, 1995). The strength of the generated electric signal is in the range of a few millivolts and is produced in a species-specific manner by specialized electrocytes that make up the electric organ. Because of its origin, the electric discharge produced by these fish is called the electric organ discharge, EOD. The EOD of *A. leptorhynchus* is emitted as a continuous quasi-sinusoidal wave. The EOD frequency (EODf) of *A. leptorhynchus* is sexually dimorphic and individually specific; males emit in the range of 800–1100Hz, whereas females emit in the range of 600–800Hz (Zakon *et. al*., 2002; Zupanc, 2002; Dunlap and Larkins-Ford, 2003A). The fish are able to sense this self-generated signal and other electric signals in their environment *via*

electroreceptors distributed over their skin surface. They use this combined motor-sensory system to navigate through their surroundings and find prey, a behaviour termed electrolocation (Nelson and MacIver, 1999; von der Emde, 2006). Weakly electric fish are also thought to use their electric sense for communication, specifically electrocommunication (Hagedorn and Heiligenberg, 1985; Zakon *et. al*., 2002; Zupanc, 2002; Turner *et. al*., 2007), the focus of this study.

Although the EOD is highly regular over time (Moortgat *et. al*., 1998), stereotyped amplitude and frequency modulations are common in social situations. It is believed that these modulations serve as communication signals (Larimer and MacDonald, 1968). These modulations have classically been categorized into two broad categories: rises and chirps. Rises are characterized by an increase in the fish's EODf followed by an eventual decrease back to the baseline frequency, lasting from tens of milliseconds to minutes (Tallarovic and Zakon, 2002; Tallarovic and Zakon, 2005). Chirps are a second type of EOD modulation that tend to be shorter in duration (~20ms), and are the most commonly studied signal type (Zakon *et. al*., 2002).

Chirps have traditionally been defined as brief frequency excursions, and several subtypes have been identified. Although there remains controversy surrounding the categorization of chirps, we will be using the categorization scheme outlined by Zupanc and colleagues (Engler *et. al*., 2000; Engler and Zupanc, 2001; Zupanc *et. al*., 2006). Type 2 chirps are the most common type of chirp produced by *A. leptorhynchus*. They are 15–20ms in duration and have frequency excursions of about 50–100Hz. Type 1 chirps occur much less often than Type 2 chirps and they are characterized by a larger frequency excursion than Type 2 chirps, and a similarly short duration. Other longer duration chirp types have been described, called Types 3–6; however, these were found to be produced at very low rates (Engler *et. al*., 2000; Engler

and Zupanc, 2001; Zupanc *et al*., 2006) and were not observed in the current study. Although chirps were first described in *A. leptorhynchus* by Larimer and MacDonald (Larimer and MacDonald, 1968), 40 years later very little is known about the social significance of these or other electrocommunication signals. Various experimental paradigms, involving both isolated fish ('chirp chamber' studies) and artificially interacting fish, have led to a number of conclusions regarding chirping behaviours (e.g. Zupanc and Maler, 1993; Triefenbach and Zakon, 2003; Kolodziejski *et. al*., 2007). Although fundamental to our current understanding of chirping behaviour, there is a need to test if these behavioural patterns persist in naturally interacting fish (Dunlap and Larkins-Ford, 2003B).

The objective of the current study is to examine chirping in pairs of freely interacting male and female *A. leptorhynchus* and characterize the behaviours associated with the production of these signals. In addition to chirps, we observed that the fish commonly produce a type of frequency rise known as an abrupt frequency rise (AFR), a signal type that was first described by Engler and Zupanc (Engler and Zupanc, 2001) and also documented by Tallarovic and Zakon (Tallarovic and Zakon, 2005). This signal consists of a series of brief events, each with a frequency increase and subsequent decrease, produced in rapid succession, with variable duration and repetition number. Our observations of freely swimming fish allow us to analyze the behaviours that are associated with the production of different signal types. We characterized the behaviours associated with chirp and AFR production in *A. leptorhynchus* using three approaches. First, we examine the temporal sequence of signal production in order to reveal potential patterning, both within a fish and between fish. Second, we examine the relationship between signal production and aggressive encounters (attacks). Finally, we relate signalling with

inter-fish distance to characterize further the behaviours associated with signal production in free-swimming *A. leptorhynchus*.

## 2.2 Methods

### 2.2.1 The Fish

We received our fish from a tropical fish supplier. A total of 13 mature *A. leptorhynchus* were assayed, including seven males and six females (mean length: 12.6±0.5cm). Sex was determined by post-mortem gonadal inspection. The fish were housed individually or with up to three other tank mates prior to test trials. Tanks were kept at a temperature of 27–28°C and at a conductivity of 100–200μS/cm. A light:dark cycle of 12:12h was maintained. The fish were fed frozen bloodworms three times weekly. All experimental protocols were approved by the University of Ottawa Animal Care Committee (Protocol BL-192). Table 1 presents the individual characteristics of each of the fish tested and includes, for each fish, the sex, body length, mean EODf, the number of trials it was used in, and mean (±s.e.m.) Type 1 chirp, Type 2 chirp, AFR, and attack rates, averaged across all trials.

### 2.2.2 Experimental Regime

We examined signal production in 21 different pairs of fish. For each trial only novel pairings were used; the two fish selected were housed in separate tanks and had not met following shipment. Table 2 presents, for each of the 21 trials, the identity of the two fish used, the difference in EODf between the interacting fish (the difference frequency, Df), the type of sex pairing, and Type 1 chirp, Type 2 chirp, AFR and attack counts for both of the fish examined.

Trials were performed in the dark in a 9.5L tank measuring 30.0x17.0x13.5cm. The water in the test tank was replaced with heated (26–27°C) water with a conductivity of 100–120uS, between every trial or every second trial. To begin each trial, one fish (‘fish 1’ as listed in Table 2) was transferred from its home tank into the test tank. After 20min a second fish (‘fish

2') was added to the tank. Immediately upon introduction of the second fish into the test tank, 5min electrical and video recordings of the interaction were taken. After 5min of interaction both fish were returned to their respective home tanks. No effects of ordering on chirp or attack rates were found (paired t-test: $p=0.72$ and $p=0.81$, respectively). Fish were identified based upon their anatomical differences and EODf.

### 2.2.3 Video and Electrical Recordings

The EODs were recorded using two pairs of Teflon-coated silverwire electrodes (diameter: 0.38mm, insulated to the tip; WPI, Inc., Sarasota, FL, USA) positioned in opposite corners of the tank (Fig.2.1A) and an AM Systems (Carlsborg, WA, USA) differential amplifier, model 1700 (amplified 10X, low frequency cut-off of 10 Hz, high frequency cut-off of 5kHz). The signals were acquired at a sampling rate of 10kHz using custom programs in Igor Pro (Wavemetrics, Inc., Portland, OR, USA). A grounded Teflon-coated silver-wire electrode (insulated to the tip) was attached to one corner of the test tank. The trials were recorded from above using a Sony video camera (model DCR-TRV 260) equipped with infrared illumination.

### 2.2.4 Signal Characterization and Identification of the Signalling Fish

For each of the 21 trials we created spectrograms using Matlab (window size 700pts, NFFT 700, and overlap 600) and from these, by visual inspection, we recorded the times of all chirps and abrupt frequency rises (AFRs), and the fish producing each. In cases where the fundamental frequencies of the two fish were similar, the higher harmonics were used to identify the signalling fish.

Chirps were categorized as either Type 1 or Type 2 by visual inspection. A given fish produced at most two clearly distinct chirp types, which were distinguishable based upon their

frequency excursion. Type 1 chirps had large frequency excursions whereas Type 2 chirps had notably smaller frequency excursions, comparable to the descriptions outlined by Zupanc and colleagues (Zupanc *et. al*., 2006). All AFRs were lumped into one category and these were variable in terms of frequency excursion, repetition number, and duration. The electrical recordings from each trial were converted into an audio format. These audio files were played and chirps were counted (Dulka and Maler, 1994; Dunlap, 2002) and the timing of each recorded for each of the first five trials. These counts were compared to the chirp counts obtained from spectrogram analysis to assure the reliability of our method ($R^2$=0.9998, $p<0.001$).

### 2.2.5 Behavioural Analysis

From the recorded videos of the interactions we noted the times of all observable attacks. Attacks included all open jawed biting behaviours and all high-speed lunges directed at a conspecific (Heiligenberg, 1973). For each trial we also tracked the position of each fish in the tank, again as viewed from above, using Videopoint software (Lenox, MA, USA). Frames of every 200ms were analyzed. The distance from the head of one fish to the head of the other fish was used as a functional indication of the distance between the two fish, because most bites and lunges were initiated by, and largely directed at, this region.

### 2.2.6 Characterization of Signal Patterning

To characterize the temporal patterning of signal production we created correlograms relating the production of one signal with the production of a second signal type. For each trial, we created signal-centered histograms in which the counts pertaining to a second signal type were plotted in 200ms bins, for 4s prior to and following the production of the first reference signal. The histograms were then averaged over all trials to create correlograms for each

comparison. Those that differed significantly from a flat distribution (the null distribution expected if signals are not temporally correlated), determined using repeated measures ANOVA (RM ANOVA) on ranks ($p<0.05$), were analyzed on a bin by bin basis (see Statistical analysis section). Trials in which fish produced less than 10 of a particular signal were omitted from the correlogram analysis. Although some fish were used in multiple trials, we are assuming independence between trials because all trials involve novel pairings.

To assure that the patterns we observed between two time series were not due to patterning within a given signal time series, we created 'reversed' time series for one of the two signals being considered for each correlogram, and recalculated the averaged correlograms in the same fashion as for the unmodified time series. To create reversed time series, we simply switched the first half of one trial (time=0–150s) with the second half of the same trial (time=150–300s). This reversed time series has the same autocorrelation function as the original, but is not temporally linked to the production of any other signal. In all cases, the reversed-timeseries correlograms were not significantly different than the ones expected if all events occur randomly (RM ANOVA on ranks: $p>0.05$ for all cases), thus verifying that all patterns we observed in the correlograms were not artifacts of non-random patterning in the individual signals.

In a similar way, we created correlograms plotting the attack rate of one fish centered at a given signal type, for 4s before and after, again in 200ms bins. The same statistical analyses were performed to identify relationships between attack rate and signal production.

### 2.2.7 Statistical Analyses

Summary data are expressed as means ± s.e.m., unless otherwise indicated. Linear regressions were used to characterize different parameters (EODf, Df and time) associated with average signal production rates. In all cases, an *F*-test was performed on the slope with $p<0.05$ as the threshold for statistical significance. With respect to the correlograms, to determine significance on a bin-by-bin basis, we calculated a P-value for each bin in each correlogram. To do this, for each correlogram we randomly shuffled one of the two signal (or attack) time series being considered and created a histogram relating this shuffled time series with the unshuffled one. We repeated this procedure one thousand times and used the resulting distributions of counts in each bin to directly calculate a P-value for any particular bin count in a given comparison. We then determined, for each bin, the fraction of all fish in all trials having significant P-values (less than 0.05 or greater than 0.95) and plotted these below the corresponding averaged correlogram.

## 2.3 Results

### 2.3.1 Description of Signals

A total of 3409 chirps were recorded during the 21, 5min trials. Chirps were classified as Type 1 (N=118) or Type 2 (N=3291) as described in the Methods section. For illustrative purposes, we show a typical Type 2 chirp, recorded from an isolated fish, as an oscillogram (Fig. 2.1B), an instantaneous frequency plot (Fig. 2.1C) and a spectrogram (Fig. 2.1D). In addition, a typical Type 1 and Type 2 chirp, recorded during a dyadic interaction, are shown in Fig. 2.1E (a small Type 2 chirp first, followed by a larger Type 1 chirp; both produced by the higher frequency of the two interacting fish). Based upon the oscillograms and spectrograms of chirp recordings, we propose that these two signal types recorded from our free swimming males and females are the same as those defined by Engler and Zupanc (Engler and Zupanc, 2001). They show comparable amplitude decreases and frequency excursions. We did not observe any chirps with a duration greater than 15-20ms. In the spectrogram, they may appear to last longer than this because of time smearing resulting from the spectrogram overlap parameters (compare for example the oscillogram, instantaneous frequency plot, and spectrogram of the Type 2 chirp shown in Fig. 2.1B-D); these parameters were optimized to provide sufficient resolution of both time and frequency.

In addition to these chirps, AFRs were produced in abundance by the fish under these experimental conditions. Two representative AFRs are shown in Fig. 2.1F; both are produced by the lower frequency of the two interacting fish (the third harmonics are shown on the spectrogram). At the time of the AFRs, the higher frequency fish's EOD signal is weak, allowing us to better illustrate the lower frequency fish's frequency modulations. The AFRs shown here

consist of distinct and consecutive frequency rises produced in rapid succession. We recorded a total of 1046 AFRs during the dyadic interactions. They are of variable duration, lasting from tens to hundreds of milliseconds (mean duration: 404±4.3ms). Although this signal type has been described as an AFR in only one study prior to this (Engler and Zupanc, 2001), we believe that it is the same signal type that Tallarovic and Zakon (Tallarovic and Zakon, 2005) describe as a short duration rise. We have found that AFRs are produced extensively during *A. leptorhynchus* social interactions.

Furthermore, our results suggest that these signals are produced in a context-dependent fashion (see following sections), lending strength to the proposition that they are a distinct class of electrocommunication signals produced by *A. leptorhynchus*. Long term changes in frequency, as well as frequency jamming behaviours (Tallarovic and Zakon, 2005) were observed infrequently, and were very variable in terms of frequency excursion and duration. We observed 48 frequency rises which, unlike AFRs involved more gradual frequency modulations (over seconds and tens of seconds), and hence fit into the category of a gradual frequency rise (GFR) (Tallarovic and Zakon, 2002; Serrano-Fernández, 2003; Tallarovic and Zakon, 2005). These longer, more gradual frequency rises, were produced infrequently during our studies and thus will not be discussed further.

### 2.3.2 Signalling Rates as a Function of Sex, EODf and Df

Many researchers have pointed out that there is a need to compare the behaviour of fish observed under artificial experimental conditions with that of freely interacting fish (e.g. Dunlap and Larkins-Ford, 2003B). In this section, we compare the mean chirp rates of free swimming fish with those reported previously using chirp chambers and other experimental conditions. We

examine how the chirp rates are influenced by sex, EODf and the difference in EODf of the interacting fish (the difference frequency, Df). For comparison, we analyze AFR production in the same manner.

*2.3.2.1 Effect of Sex*

It is well established that chirping is a sexually dimorphic behaviour in *A. leptorhynchus* (Maler and Ellis, 1987; Dye, 1987; Dulka and Maler, 1994; Dulka *et. al*., 1995; Dunlap and Larkins-Ford, 2003A; Kolodziejski *et. al*., 2004). We found a similar pattern in free-swimming conditions: males produce significantly more chirps (Type 1 and Type 2 chirps) than females (Fig. 2.2A,B; Mann–Whitney rank sum test: $T_{17}$=262, p=0.01; and $T_{17}$=162.5, p<0.001, respectively). Furthermore, we found that under our conditions both sexes predominantly produce Type 2 chirps, similar to previously reported behaviour in isolated fish (Engler *et. al*., 2000), in chirp chamber studies (Engler and Zupanc, 2001) and in fish interacting electrically (Zupanc *et. al*., 2006). Because Type 1 chirps were produced so infrequently, it is difficult to gain any further insight into the factors controlling their production. So, we will focus mainly on Type 2 chirps in the following sections, and restrict this to chirps produced by males because females chirped so infrequently. Contrary to the sexual dimorphism seen with respect to chirping, we found no sexual dimorphism in the average AFR rates of males and females tested under these conditions (Fig. 2.2C; Mann–Whitney rank sum test: $T_{17}$=333.5, p=0.42).

*2.3.2.2 Effect of EOD Frequency and Difference Frequency*

Previous studies have shown that chirp production rates in males are proportional to the EOD frequency (EODf) and inversely proportional to the difference frequency (Df) between a playback signal and EODf (Zupanc and Maler, 1993; Bastian *et. al*., 2001; Dunlap, 2002; Dunlap

and Larkins-Ford, 2003B; Kolodziejski *et al*., 2007). We found that Type 2 chirp rates were not significantly correlated with EODf in males ($R^2$=0.10, p=0.12). However, we found that Type 2 chirp rates were negatively correlated with Df, independent of the sign of Df (Fig. 2.3A; $R^2$=0.20, p=0.03). Interestingly, although males chirped with a comparable mean rate when paired with males or with females (137.8±42.5 *vs* 130.9±42.5 chirps per trial, respectively), the mean Df was smaller for trials in which males were paired with another male (47.1±8.4Hz) than when paired with a female (144.6±24.7 Hz). From Fig. 2.3A we can see that for any given Df, males tend to chirp at lower rates when paired with a male than when paired with a female. A similar analysis of AFR rates revealed no relationship with EODf ($R^2$=0.03, p=0.32). However, when all fish are considered together we find that AFR rates are negatively correlated with the absolute Df (Fig. 2.3B; $R^2$=0.14, p=0.01). In general, in agreement with previous studies (Zupanc and Maler, 1993; Bastian *et. al*., 2001; Dunlap, 2002; Dunlap and Larkins-Ford, 2003B; Kolodziejski *et. al*., 2007), our results suggest that fish tend to produce chirps and AFRs predominantly when interacting with a fish whose EODf is similar to its own.

### 2.3.3 Signalling Rates over Time: Chirp Rates Increase with Time

Many studies have reported that the chirp rates of male and female *A. leptorhynchus* tend to habituate over time, both in chirp chambers (Dunlap, 2002; Dunlap and Larkins-Ford, 2003B) and in fish communicating through a perforated barrier (Dunlap and Larkins-Ford, 2003B). Contrary to this, over a similar time period, we found that the Type 2 chirp rates of free-swimming male fish did not decrease; instead, they increased significantly as the trial progressed (Fig. 2.4A; $R^2$=0.71, p=0.002). During this same time period, attack counts decreased significantly (Fig. 2.4B; $R^2$=0.63, p=0.05); although the greatest decrease in attack rate occurred over the first minute of the interaction. This suggests a negative relationship between Type 2

chirp rates and attack rates; as chirp rates increase, attack rates decrease. We will investigate this relationship further in a following section. In contrast to Type 2 chirp rates, Type 1 chirp and AFR rates did not change significantly with time ($R^2$=0.08, p=0.44; $R^2$=0.29, p=0.11, respectively).

### 2.3.4 Non-Random Signal Production Rates by Individual Fish

It has been reported that male *A. leptorhynchus* tested under a variety of conditions tend to produce chirps in a non-random, 'bursty' fashion (Bullock, 1969; Zupanc and Maler, 1993; Engler and Zupanc, 2001; Zupanc *et. al*., 2006). Zupanc *et. al*. (Zupanc *et. al*., 2006) quantified this bursty chirping behaviour in male fish, interacting between adjacent PVC tubes, using inter-chirp interval histograms. They found that chirps tend to follow one another at a preferred interchirp interval of approximately 500ms.

We performed a similar analysis on the signals produced by male and female free-swimming interacting *A. leptorhynchus* in order to reveal patterns in the signalling of individual fishes. With respect to male Type 2 chirps, we found a pattern of 'burstiness' and a preferred interchirp interval period comparable to those reported by Zupanc and colleagues (Zupanc *et. al*., 2006). Fig. 2.5 shows an interchirp interval histogram for all males across all trials. It suggests that males tend to produce chirps in a bursty fashion with a preferred interchirp interval of 400–600ms. Fig. 2.6A (top panel) shows the averaged auto-correlogram corresponding to the male Type 2 chirp sequences (unlike the interchirp interval histogram, this analysis considers both first order and higher order inter-chirp intervals). Because the pattern in the auto-correlogram deviates from a flat (null or random) distribution, it clearly indicates that males chirp in a non-random fashion (RM ANOVA: $X^2_{40}$=266.2, p<0.001). The lower panel shows the fraction of

comparisons in which a given bin count (in the correlogram) is significantly greater than (black line $p>0.95$), or less than (grey line $p<0.05$) that expected by chance. In a large number of cases the chirp rates are much less than expected in the period immediately following a chirp event (grey line). This is followed by a period with an increased tendency to chirp, indicated by the peaks in the auto-correlogram and bottom panel (black line). AFRs are also produced in a non-random fashion. The AFR autocorrelograms and bottom panel (Fig. 2.6B) show that the probability of AFR production is reduced for a short time following the first AFR and increases to a peak at a preferred latency of 400–800ms (RM ANOVA: $X^2_{40}=241.7$, $p<0.001$). This suggests that AFRs, like chirps, tend to be produced in bursts, with a similar preferred latency. Interestingly, not only are signals correlated in time with themselves, the patterns of two distinct signal types produced by a given fish are also correlated. The probability that an individual fish produced an AFR just before or after it produced a Type 2 chirp is significantly lower than chance (Fig. 2.6C; RM ANOVA: $X^2_{40}=169.1$, $p<0.001$) and this trend is significant in about 40% of individuals (Fig. 2.6C lower panel, grey line). Overall, this patterning suggests that the fish tend to produce these different signals under different conditions, at different times during an interaction. In the following sections we show that there are different contextual factors associated with the production of the different signal types.

### 2.3.5 Signal Production between Two Interacting Fish is not Independent

A previous study of *A. leptorhynchus* males interacting electrically but confined to separate tubes, has provided evidence for a so-called 'echo response' (Zupanc *et. al*., 2006), such that chirps produced by one fish followed chirps produced by another with a preferred latency. We investigate the relationship between chirp rates in physically interacting fish by means of an inter-signal cross-correlogram, a histogram describing Type 2 chirp production in one fish at

different times before and after another fish's Type 2 chirp. The cross-correlogram for male–male interactions is significantly different from that expected if the two fish chirped independently (Fig. 2.7A; RM ANOVA: $X^2_{40}$=100.4, p<0.001). The fish tend not to chirp at the same time: the chirp rate of one fish at the time when the other is chirping (t=0) is much less than expected by chance in more than 70% of trials (Fig. 2.7A lower panel, grey line). In addition, the chirp rates in adjacent time bins are significantly greater than expected by chance (illustrated by the peaks in the black line, Fig. 2.7A lower panel), reflecting an 'echo response' at a 200–600ms latency.

We performed similar analyses on AFRs. Cross-correlograms for AFR production of one fish relative to that of the other fish show that AFRs are not produced independently (Fig. 2.7B; RM ANOVA: $X^2_{40}$=114.1, p<0.001), but rather, are produced concurrently. This suggests that either the two fish are responding at a very short latency with AFRs in response to their partner's AFRs, or that shared conditions or features of the interaction trigger AFR production in both fish at the same time (both fish may produce AFRs during aggressive situations for example).

In addition to interactions with signals of the same type, we also found a relationship between the patterning of AFRs of one fish relative to Type 2 chirps produced by the other fish. As indicated by the trend in the averaged correlogram (Fig. 2.7C; upper panel), one fish tends not to produce AFRs at the same time as the other fish is producing Type 2 chirps (RM ANOVA: $X^2_{40}$=105.7, p<0.001). Although this trend is significant in a smaller proportion of fish considered, the rate of AFR production in one fish was consistently lower than expected at the time the other fish produces chirps.

Overall, this signal patterning analysis provides a quantitative measure of the temporal interactions in chirp behaviour. Further, it reinforces the idea that Type 2 chirps and AFRs are true communication signals that are produced in bursty temporal patterns, influence the signalling behaviour of interacting conspecifics, and tend to be produced at different times during social interaction.

### 2.3.6 Attack Rates are Correlated with Signalling

Many researchers have referred to chirps as aggressive signals (Bullock, 1969; Maler and Ellis, 1987; Dunlap and Larkins-Ford, 2003B; Triefenbach and Zakon, 2003). In order to investigate this possibility, we have quantified the temporal relationships between attack behaviours and signal production using cross-correlograms. Fig. 2.8A (top panel) shows that a fish's own attack rate is decreased near the time it produces a Type 2 chirp (RM ANOVA: $X^2_{40}=166.5$, $p<0.001$). When we look at the bin-by-bin analysis (bottom panel), it is clear that in more than 40% of fish, attack rates are significantly lower than expected just prior to and following a Type 2 chirp (grey line).

When we consider interactions between fish, we see that chirping in one fish is associated with a decreased attack rate by the other fish (Fig. 2.8B; RM ANOVA: $X^2_{40}=76.8$, $p<0.001$) and the trend is significant in over half of the trials considered. Fish tend to chirp after periods of low aggression, when both its own attack rate and that of the interacting conspecific are lower than expected.

Contrary to the relationships observed for chirps, attack rate is highest at the time of AFR production in both the signalling fish (Fig. 2.9A; RM ANOVA: $X^2_{40}=138.1$, $p<0.001$) and in the fish with which it was interacting (Fig. 2.9B; RM ANOVA: $X^2_{40}=115.4$, $p<0.001$). In other

words, a fish tends to produce AFRs at the same time that it attacks (significant in about three-quarters of fish considered, Fig. 2.9A; black line, lower panel), but also when it is being attacked (Fig. 2.9B), suggesting that AFRs may be aggressive signals used frequently during agonistic encounters.

### 2.3.7 Signal Rates are Correlated with Inter-fish Distances

As would be expected from our analysis of attack rates, the average distance separating the interacting fish also was related to the time of production of both Type 2 chirps and AFRs. The head-to-head distance increases prior to, and decreases immediately following, chirp production, with a maximum close to the time of the chirp (Fig. 2.10A). Alternatively, AFRs tend to be produced when the fish are nearest each other (Fig. 2.10B). These distance trends reinforce the conclusions of the attack correlogram analyses, and further suggest that chirps may be produced at a distance to deter aggressive behaviours whereas AFRs serve as proximity signals that accompany aggressive behaviours.

## 2.4 Discussion

Neuroethological approaches have been very successfully applied towards the understanding of electrosensory-mediated behaviour in weakly electric fish (e.g. Heiligenberg, 1991). Because appropriate context is critical for the production of natural behaviours, such studies must be undertaken in the most natural context that is feasible under the given experimental constraints. In *A. leptorhynchus*, various experimental settings have been used to investigate putative electrocommunication signals, but none before the present study has provided a detailed temporal analysis of signalling between two freely interacting wave-type fish. Using spectrograms to separate the signals of each individual fish and cross-correlation analyses, we have provided further quantitative evidence for the existence of electrocommunication, as well as a first step towards understanding the meaning of the underlying signals. A complete neuroethological description of any physiological process requires both a neural and behavioural description of the phenomenon. Descriptions of the behavioural relevance of the electrocommunication signals produced by *A. leptorhynchus*, such as we are presenting here, will be necessary for elucidating the roles of neural circuits and modulators involved in electrosensory processing and signal production.

### 2.4.1 Signalling Behaviour: Free swimming versus Constrained Fish

Our study in free swimming *A. leptorhynchus* showed that some features of chirp behaviour are preserved between these and other experimental conditions. One feature of chirping that we confirm, and is consistent across many experimental regimes, is the sexual dimorphism in chirp rates (Maler and Ellis, 1987; Dye, 1987; Dulka and Maler, 1994; Dulka *et. al*., 1995; Dunlap and Larkins-Ford, 2003A). Male chirp rates are much higher than female chirp

rates (Fig. 2.2). A second relationship, previously described and confirmed in our study, is the effect of difference frequency, Df, on chirp rates (Zupanc and Maler, 1993; Bastian *et. al*., 2001; Dunlap and Larkins-Ford, 2003B; Kolodziejski *et. al*., 2007). There is an inverse relationship between Type 2 chirp rates of freely interacting male *A. leptorhynchus* and the difference in frequency between its own EODf and that of the fish with which it is paired (Fig. 2.3). This relationship persists when absolute Df is considered (Bastian *et. al*., 2001). This implies that in any given pairing the lower and higher frequency fish tend to chirp at approximately the same rate; a rate influenced by the magnitude of the difference in the EODf of the two fish. This strong relationship between Df and Type 2 chirp rate can explain why the relationship between EODf and chirp rate is less apparent. Another point to consider with respect to the relationship between Df and chirp rates is how these signals are detected or perceived by conspecifics. Type 2 chirps and AFRs are produced most often when Dfs are small, and interestingly, this is also the range in which electroreceptor afferents most effectively encode small Type 2-like chirps (Benda *et. al*., 2006). Our results clearly indicate that although fish chirp at the highest rate to fish whose EODf is similar to its own, they also respond to chirps when the Df is much greater. Thus there are likely to be additional mechanisms involved in the detection and encoding of chirps in cases when the Df is large (Hupé *et. al*., 2008 (Chapter 3)).

An important difference between the chirping behaviour of fish tested under previous experimental conditions and our results involves how chirp rates change with time. Fig. 2.4 shows that the rate of Type 2 chirp production increases significantly with time. This increase is contrary to what has been reported for chirp production in the past (Dunlap and Larkins-Ford, 2003B). It is possible that the decrease in chirp rates, reported in such studies, is at least in part a result of the fish habituating to unrealistic stimuli. In our experiments, the fish do not decrease

chirp rates over time, presumably because multimodal sensory cues are present because of a dynamic interaction involving a real fish. Importantly, Dunlap and Larkins-Ford (2003B) report that chirp rates actually decrease in free-swimming fish. We suggest that this difference may be attributable to their use of a much larger tank in which the fish could separate themselves (Dunlap and Larkins-Ford, 2003B), whereas we used a relatively small tank which forced interaction.

Differences in chirping behaviour with that observed in previous studies suggest that simply being able to physically interact changes the chirping behaviour of *A. leptorhynchus*. Bullock (Bullock, 1969) used different stimulation techniques and fish models to evoke chirping in *A. leptorhynchus* and found that visual cues influenced chirping behaviour. Our study provides further evidence that interactions are an important aspect of shaping the natural signalling behaviour.

Perhaps the most important difference between the electrocommunication behaviour of freely interacting fish compared to those tested using previous experimental designs is the abundance of AFRs that we observed. It appears that features of natural, aggressive physical interactions are necessary to motivate AFR production in this species. Thus, these behavioural considerations will be necessary in future studies aimed at understanding the meaning of AFRs and the biophysical basis of their generation.

### 2.4.2 Behavioural Correlates

Communication signals, by definition, must transfer some form of information from the sender to the receiver (Bradbury and Vehrencamp, 1998; Griffin, 2001). Evaluating this transfer of information is an obvious difficulty confronting ethologists because we are limited to drawing

conclusions based upon an animal's observable behaviours. Thus, communication can be evaluated in terms of how an individual's behaviour is affected by signals emitted by another. It is important to ask what behaviours can be monitored in freely swimming fish that may also be related to signal production. As a step towards solving this problem, we have characterized signal production, attacks and inter-fish distance using correlation analyses.

### 2.2.3 Signal Patterning

Our study has shown that in *A. leptorhynchus*, both chirps and AFRs are produced in a nonrandom, bursty fashion (Figs 2.5, 2.6). Also, through cross-correlation analysis, we provide evidence that the signals produced by one fish influence signal production in an interacting conspecific (Fig. 2.7). The echo response reported here has been observed in both pulse (Moller, 1995) and in wave-type weakly electric fish (Zupanc *et. al*., 2006); in both cases, the fish tend to respond with a species-specific preferred latency. In *A. leptorhynchus*, the latency is much longer than processing by a reflexive sensory-to-motor neural pathway would suggest (Heiligenberg, 1991), so it is possible that higher level decision making is involved in shaping this electrocommunicatory behaviour. In future studies, experimental design can be controlled more specifically to determine the nature of this decision-making process.

### 4.2.4 Signalling and Attack Behaviours

Additionally, our results suggest that different types of signals are produced under different contexts and hence probably serve distinct social roles. In the *A. leptorhynchus* literature, chirps are often referred to as aggressive signals. These assertions are based on experiments which show that males tend to chirp more in response to EOD mimics similar in frequency to their own (Maler and Ellis, 1987; Bastian *et. al*., 2001), and thus more

representative of male–male interactions (because of the sexual dimorphism in EODfs). Thus, because male–male interactions tend to be aggressive in nature, it has been suggested that Type 2 chirps are agonistic signals (Zupanc *et. al*., 2002). Although chirps do occur during aggressive encounters, here we show that on a smaller time scale, chirps do not often occur during attack behaviours. Our analyses of attack rates (Figs 2.8, 2.9) suggest that Type 2 chirps are produced when fish are not attacking, and may be used at a distance by fish to deter aggressive behaviours.

Contrary to chirps, it appears that AFRs are aggressive signals, given that they are specifically produced during attacks when the fish are in close proximity. Aggressive signals are produced by a number of weakly electric fish species. For example, in 1974, Hopkins temporally correlated the observable behaviours of *Eigenmannia* (a related wave-type gymnotiform) with the patterns of electric signals they produce. He found that short duration interruptions were correlated with aggressive attack and threat behaviours. In this species, the number of interruptions contained in a bout is a reliable predictor of the likeliness that the animal will attack; the more interruptions produced the greater the probability of attack (Hopkins, 1974). Additionally, in a number of pulse species, transient pulse accelerations are associated with attacks and other aggressive behaviours (Carlson, 2002).

Our results agree with a previous study by Bullock (Bullock, 1969) who reported that *A. leptorhynchus* were often observed chirping in between bouts of attacks. In addition, a very recent study by Triefenbach and Zakon (Triefenbach and Zakon, 2008) has shown that gradual frequency rises and chirps tend to be produced under different contexts. When two fish are competing for a single tube shelter, they found that chirps tend to be produced when fish are not actively engaged, whereas gradual rises tend to be produced when fish are actively engaged in

contact behaviours (Triefenbach and Zakon, 2008). Our results corroborate these and further show that these relationships are preserved at time scales as small as 200ms.

### 2.4.5 Signalling and Inter-fish Distance

Fish confined to PVC tubes will only chirp in response to a conspecific when their PVC tubes are within 10–15cm of each other (Zupanc *et. al*., 2006). Further, they speculate that this limited communication distance is a consequence of a fish's limited ability to detect conspecific signals. Similarly, we found that freely interacting fish tend to chirp when they are on average 12.5cm apart (head-to-head distance; Fig. 2.10A) but found many chirps are produced at distances even greater than this. It is not clear, however, that such a comparison is straight-forward because the distances reported under our conditions were dynamic whereas those of Zupanc *et al*. (Zupanc *et. al*., 2006) were static. Nonetheless, these distances are within the range of those found to be behaviourally relevant for electrocommunication (Knudsen, 1975A,B).

### 2.4.6 Conclusions

In this study, we found that allowing fish to freely interact changed their chirping behaviour, suggesting that many cues are involved in shaping electrocommunication signalling. It also allowed us to relate chirp production with features of social interaction, such as attack rates and inter-fish distances, not accessible using previous more-constrained methods. Furthermore, observing chirping in freely interacting fish revealed that AFRs, a relatively uncharacterized type of frequency rise, are produced in abundance. We found that both chirps and AFRs are produced in a non-random fashion and that production rates are influenced by the signalling behaviour of interacting conspecifics. Moreover, chirps and AFRs are also produced under different behavioural contexts: chirps tend to be produced in the time between attacks,

whereas AFRs tend to be produced while the fish are in close proximity, during attacks. These results emphasize the importance of experimental context in studying communication signals. In addition, they provide more clues to guide future studies aimed at understanding the physiological mechanisms underlying the detection, interpretation and production of electrocommunication signals.

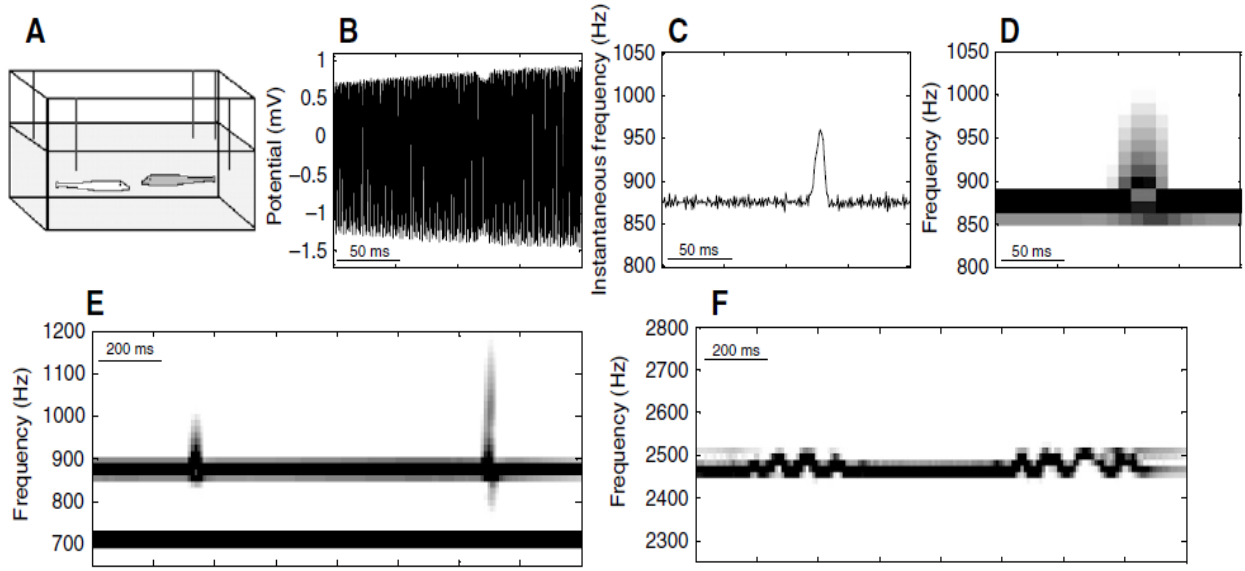



**Figure 2.1** Experimental design and description of signal modulations. **(A)** Experimental design; the short, vertical lines indicate the position of the recording electrodes in the 9.5L tank, the additional short, vertical line in the back right corner indicates the position of the ground electrode. **(B)** Oscillogram of a representative Type 2 chirp recorded from an isolated fish. Note that its duration, indicated by the period of amplitude modulation, is 15–20ms. **(C)** An instantaneous frequency plot corresponding to the same Type 2 chirp as illustrated in B. Instantaneous frequency values were derived by taking the inverse of the cycle length, calculated as the duration between consecutive downstroke zero-crossings. **(D)** A spectrogram displaying the same Type 2 chirp as illustrated in B,C). **(E)** A spectrogram showing a representative Type 2 chirp (on the left) and a Type 1 chirp (on the right) recorded during a dyadic interaction. The higher frequency of two fish (877Hz) is modulating its electric organ discharge frequency (EODf) to produce these chirps, whereas the lower frequency fish (714Hz) does not modulate its EODf during this segment of the interaction. Note that the Type 2 chirp is associated with a much smaller frequency excursion than is the Type 1 chirp. **(F)** Spectrogram showing two abrupt frequency rises (AFRs) produced in succession by the lower frequency of two interacting fish;

the third harmonics are shown and during this segment of the recording the EOD of the higher frequency fish is relatively weak, allowing for a clearer representation of the EOD modulations of the other. Both of the AFRs shown consist of multiple distinct and consecutive small frequency rises. For display purposes, the low amplitude components of each spectrogram were removed and only the strongest (10–20%) amplitude components are shown.

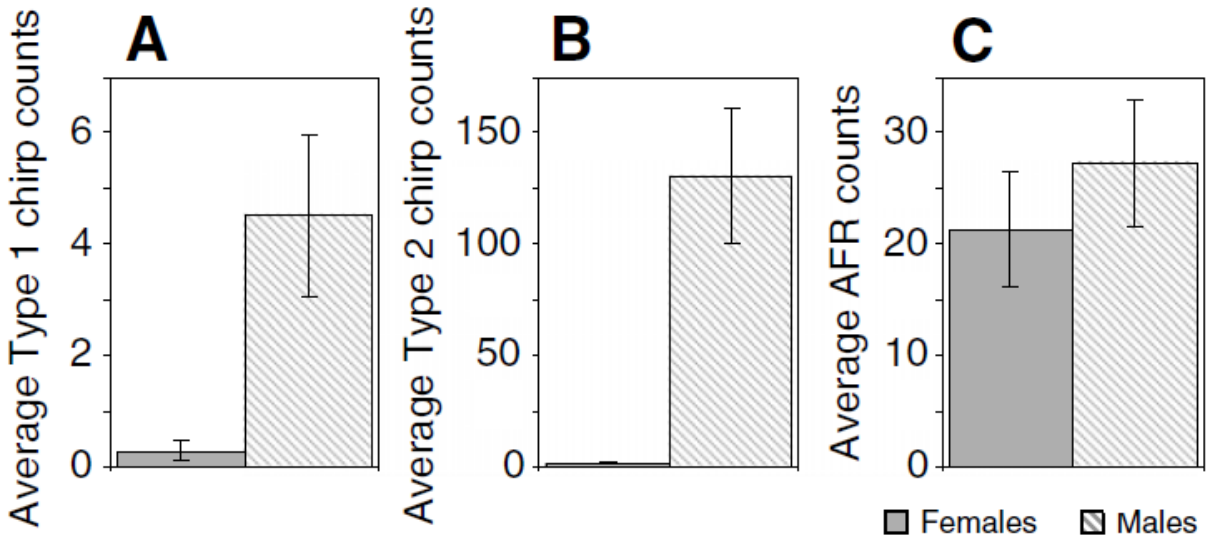



**Figure 2.2** Sexual dimorphism in signal production rates. Type 1 chirps **(A)** and Type 2 chirps **(B)** are produced at sexually dimorphic rates ($p=0.01$ and $p<0.001$, respectively). AFRs **(C)** are not produced at sexually dimorphic rates ($p=0.46$). Values are means (± s.e.m.) for males and females, averaged across all trials (N=42).

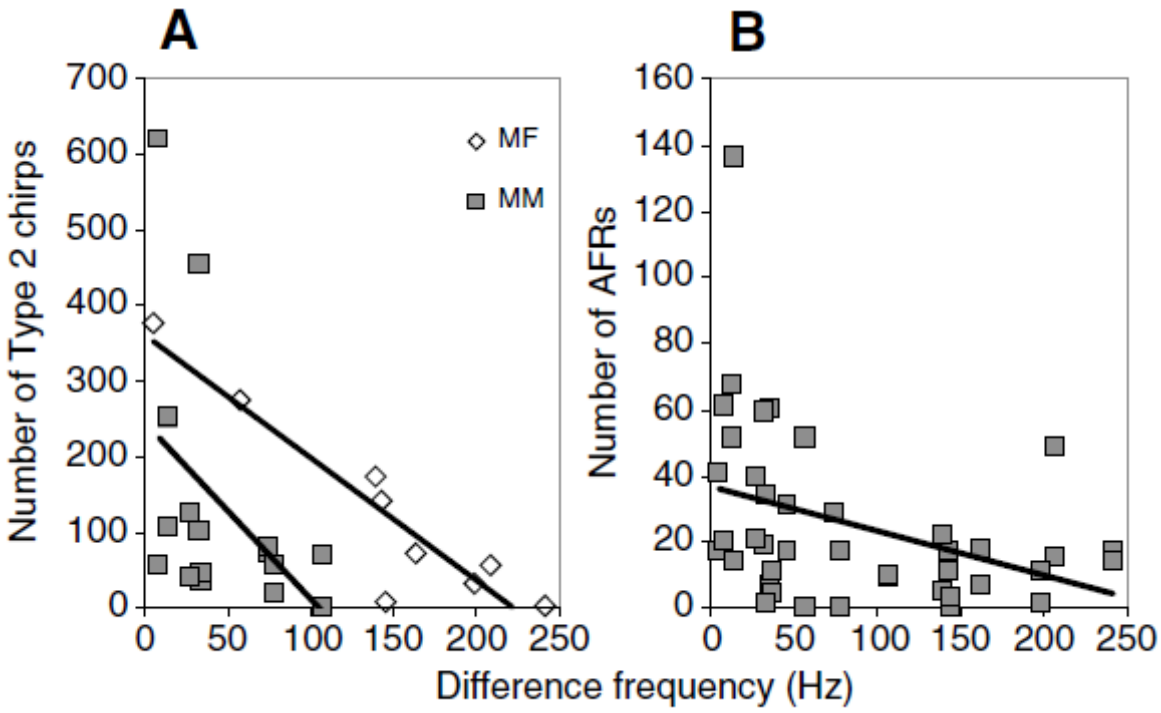



**Figure 2.3** The effect of the absolute difference frequency on signal production rates. **(A)** Type 2 chirps are classified by sex pairing; the Type 2 chirp rates of males paired with a second male (squares; N=16, $R^2$=0.21, p=0.07), and of males paired with a female (diamonds; N=9, $R^2$=0.860, p<0.001). **(B)** AFRs in which all fish were pooled because there were no differences across the different sex pairings (N=42, $R^2$=0.14, p=0.01). Linear regressions are shown.

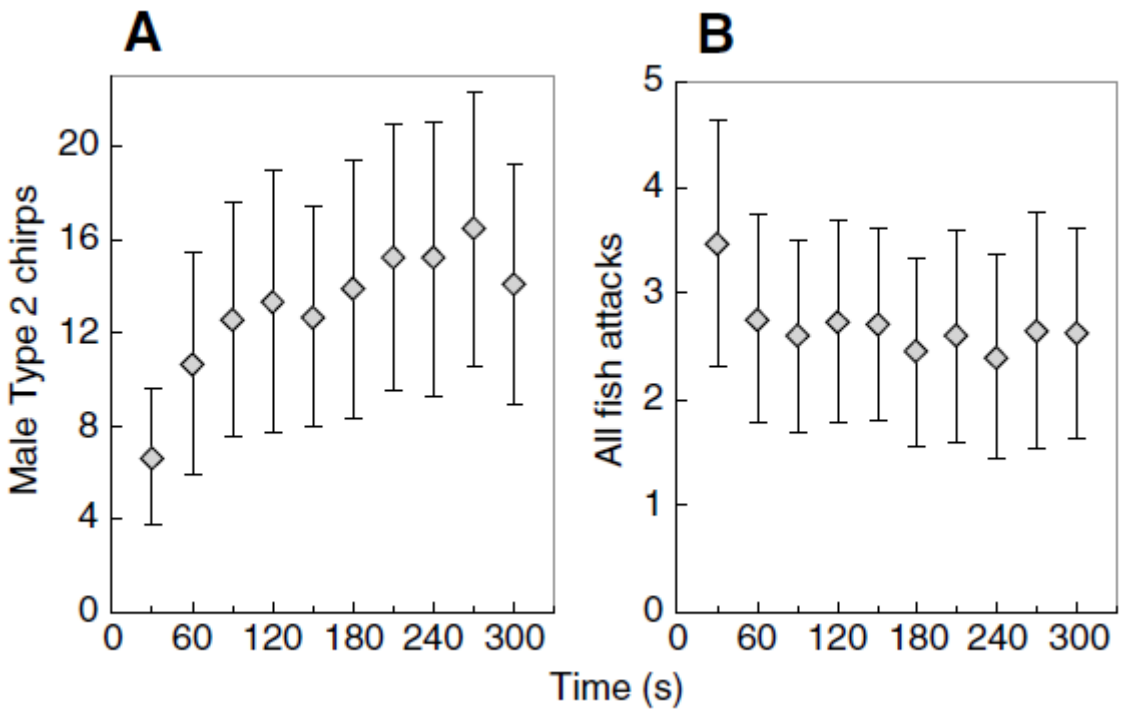



**Figure 2.4** Chirp and attack rates change with time. Mean (± s.e.m) **(A)** Type 2 chirp and **(B)** attack counts, binned into 30s intervals, are shown. With respect to Type 2 chirps, only male counts were included (N=25, p=0.002); whereas for attacks, all fish were pooled (N=42, p=0.05).

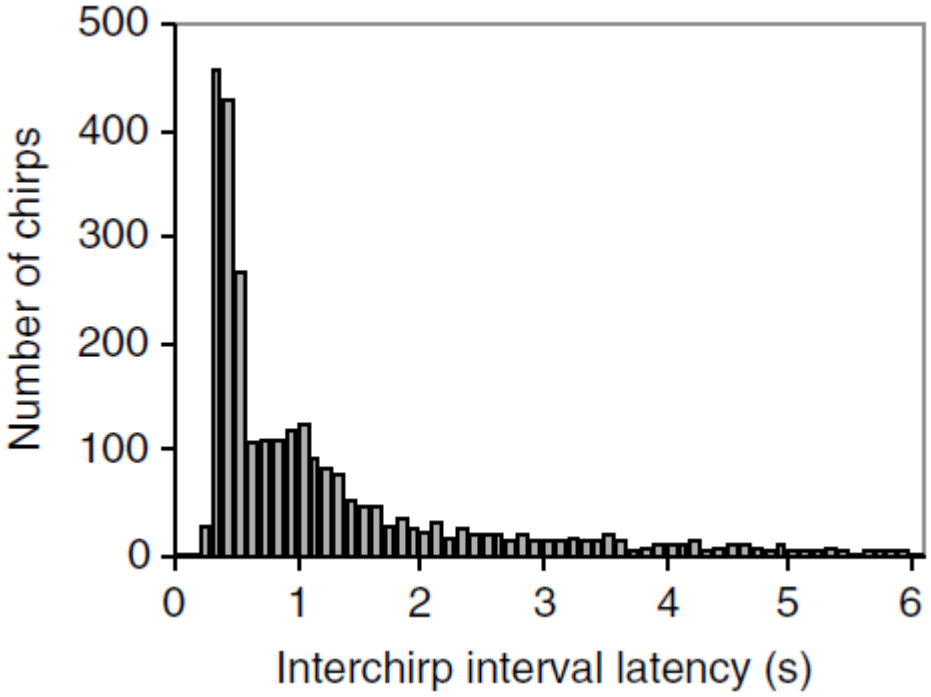



**Figure 2.5** Interchirp interval histogram demonstrates bursty chirp production patterns. Interchirp interval histogram with totals for all males tabulated over all trials (N=24, one male was excluded because he only produced one chirp). The preferred interchirp interval is 400–600s.

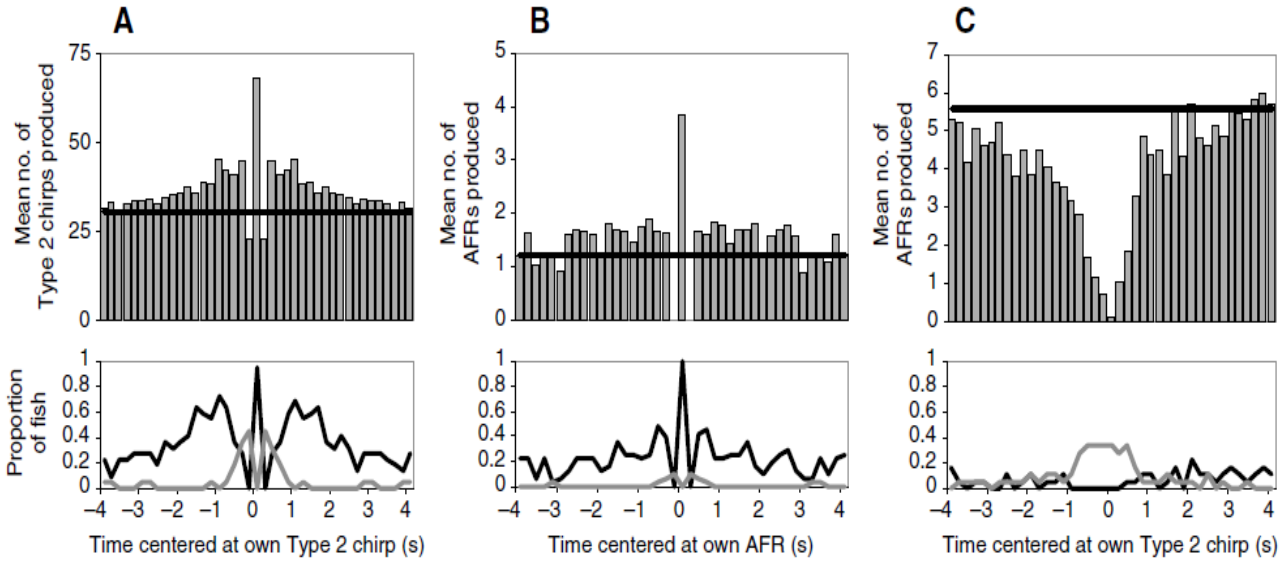



**Figure 2.6** Chirp and AFR autocorrelograms and a negative relationship between chirp and AFRs production times. Upper panels: auto-correlograms illustrate signal patterning of a single fish for **(A)** Type 2 chirps (N=22), **(B)** AFRs (N=31) and **(C)** Type 2 chirps and AFRs (N=18). Means for all fish for all trials in which fish produced 10 or more signals are plotted, except in the case of Type 2 chirps where only male counts are used in the analysis. The horizontal line denotes the null distribution: the distribution expected if signals are produced at random. Lower panels: bin-by-bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance ($p>0.95$, black line) or less than expected by chance ($p<0.05$, grey line).

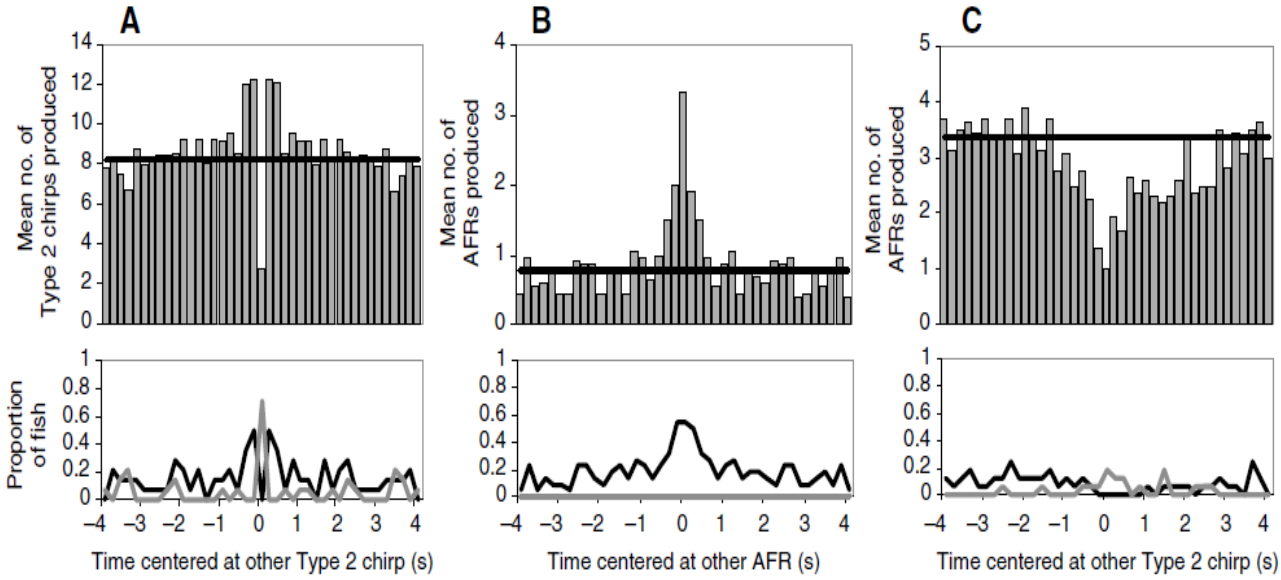



**Figure 2.7** Cross-correlograms show males pattern signals with one another in time. Upper panels: cross-correlograms illustrate signal relationships between fish for **(A)** Type 2 chirps (N=14), **(B)** AFRs (N=22), and **(C)** Type 2 chirps and AFRs (N=16). Means for all fish for all trials in which fish produced 10 or more signals are plotted, except in the case of Type 2 chirps where only male counts are used in the analysis. Horizontal line denotes the null distribution. Lower panels: bin-by-bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance ($p>0.95$, black line) or less than expected by chance ($p<0.05$, grey line).

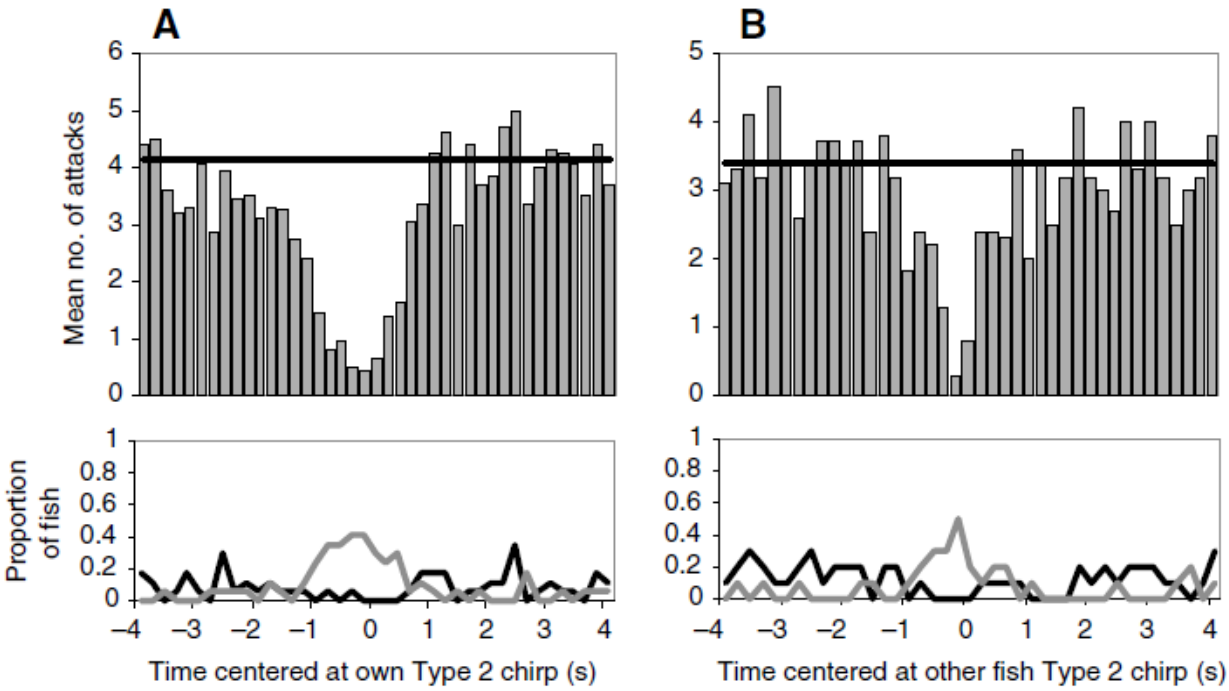



**Figure 2.8** Temporal relationships between Type 2 chirps and attacks. Upper panel: cross-correlogram relating Type 2 chirp production with attack counts are given for **(A)** a single fish (N=16) and **(B)** between fish (N=10). Only male fish, and trials involving 10 or more chirps and attacks are used in the chirp-attack analysis. Horizontal line denotes the null distribution. Lower panels: bin-by bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance ($p>0.95$, black line) or less than expected by chance ($p<0.05$, grey line).

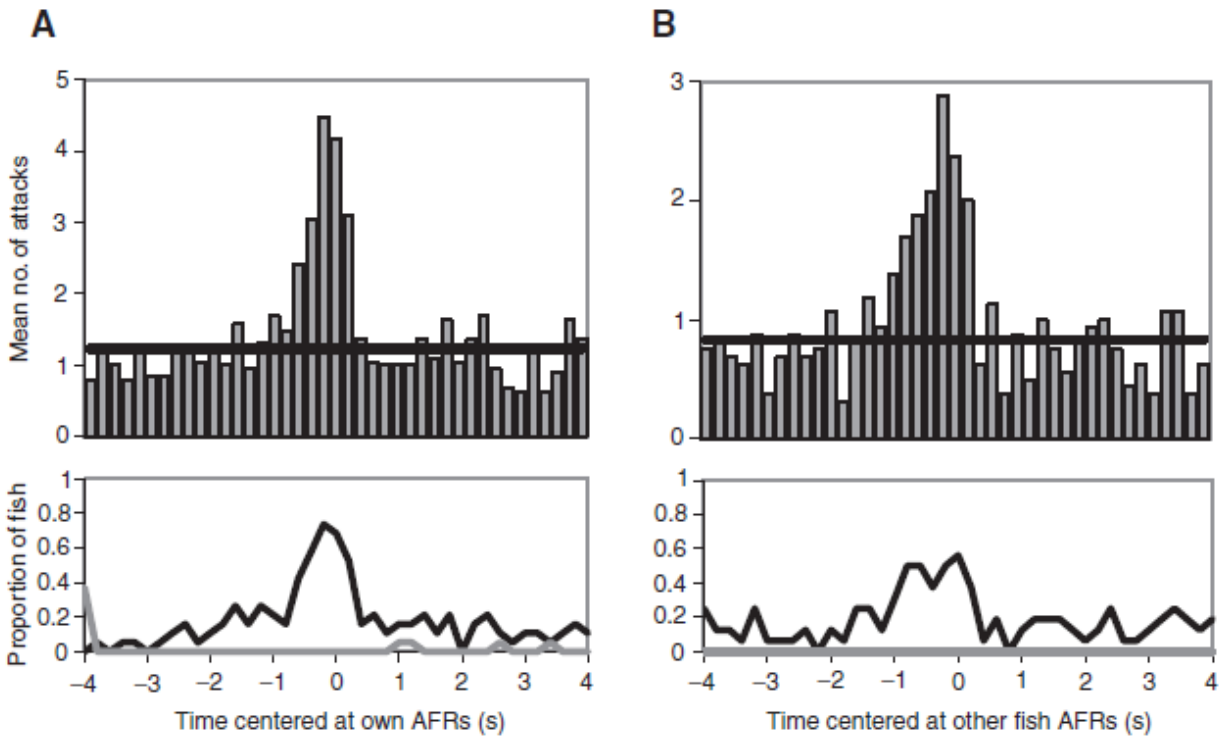



**Figure 2.9** Temporal relationships between AFRs and attacks. Upper panel: cross-correlogram relating AFR production with attack counts are given for **(A)** a single fish (N=19) and **(B)** between fish (N=16) for all trials involving 10 or more AFRs and attacks. Horizontal line denotes the null distribution. Lower panels: bin-by-bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance ($p>0.95$, black line) or less than expected by chance ($p<0.05$, grey line).

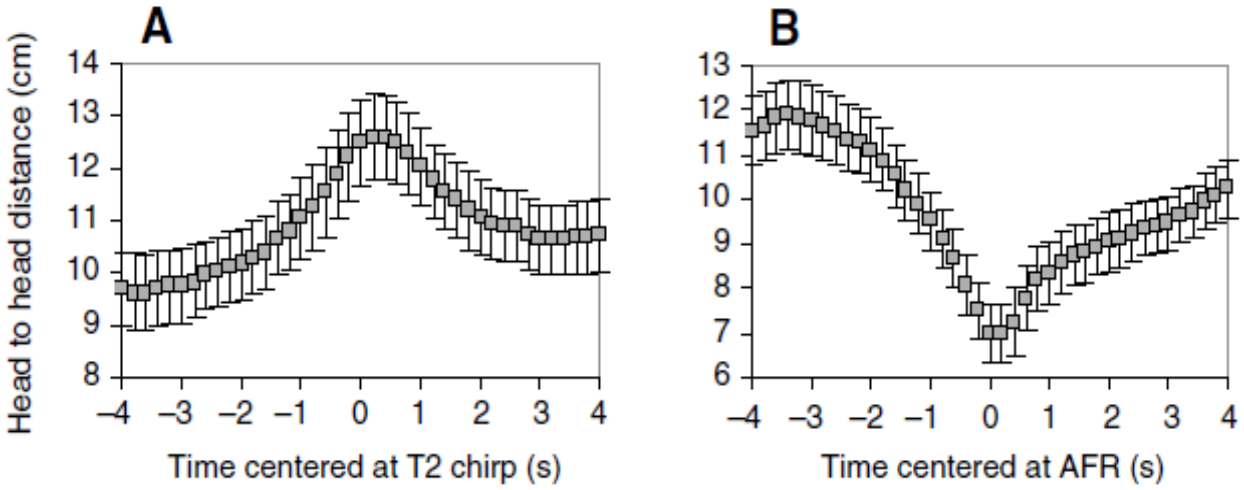



**Figure 2.10** Temporal relationships between signal production and inter-fish distances. Inter-fish distance centred at the time of signal production for **(A)** Type 2 chirps (N=25) and **(B)** AFRs (N=39). The inter-fish distances represent the distance from one fish's snout to the other fish's snout. Means ± s.e.m. are plotted for males only in the case of Type 2 chirps, and all fish in the case of AFRs.

Table 1. Characteristics of individual fish used

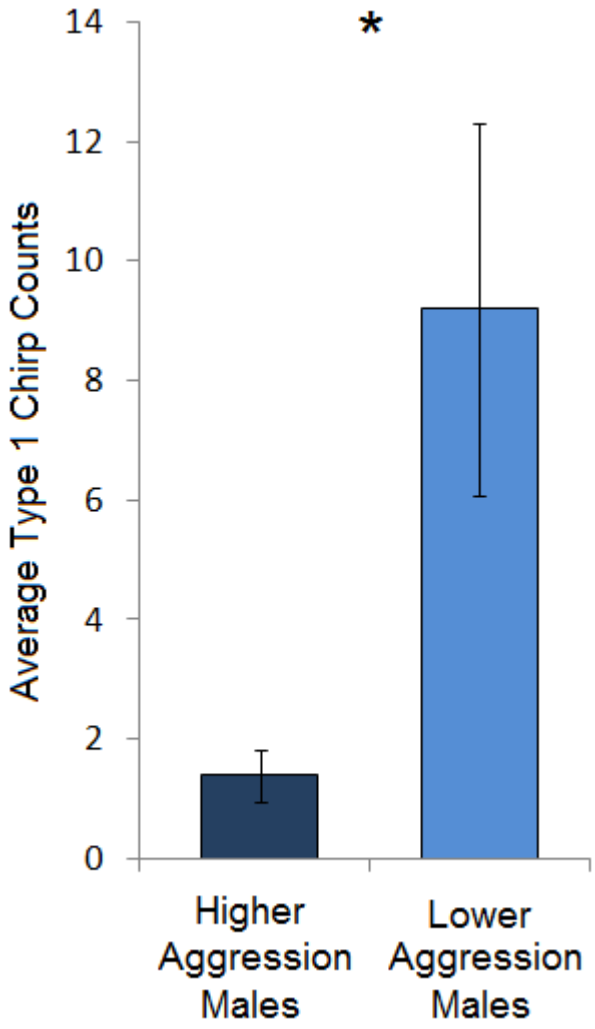
| Fish | Sex | Length (cm) | Mean EODf (Hz) | No. trials | Mean T1 chirp rate | Mean T2 chirp rate | Mean AFR rate | Mean attack rate |
|---|---|---|---|---|---|---|---|---|
| A | F | 12.7 | 732±15.9 | 3 | 1.3±0.9 | 0.7±0.3 | 12.0±5.5 | 3.3±1.0 |
| B | M | 10.6 | 895±17.5 | 7 | 2.3±1.5 | 165.3±78.2 | 26.1±7.5 | 15±5.0 |
| D | M | 14.5 | 880±5.7 | 6 | 5.0±3.7 | 73.3±16.1 | 20.3±6.6 | 63.8±11 |
| E | M | 17.1 | 965±20.6 | 3 | 0 | 34.3±16.5 | 16.0±9.9 | 62.7±13.9 |
| G | F | 12.0 | 723±9.8 | 5 | 0 | 1.0±0.8 | 22.2±8.6 | 23.6±4.2 |
| H | F | 12.3 | 678 | 1 | 0 | 4 | 17 | 5 |
| I | M | 13.6 | 933 | 1 | 2 | 55 | 20 | 50 |
| J | F | 12.2 | 746±6.1 | 3 | 0.3±0.3 | 1.3±1.3 | 21.3±19.4 | 3.7±1.8 |
| K | M | 12.8 | 781±3.0 | 2 | 0 | 188.5±188 | 13.5±3.5 | 5.0±2.0 |
| L | F | 10.3 | 779 | 1 | 0 | 0 | 41 | 6 |
| M | M | 11.7 | 862±15.6 | 3 | 18±3.8 | 51.7±28.6 | 12.7±5.2 | 4.0±0.6 |
| N | F | 12.6 | 701±13.2 | 4 | 0 | 1.5±1.5 | 23.2±14.9 | 18.8±5.4 |
| O | M | 11.5 | 809±9.0 | 3 | 3.7±0.7 | 327.3±64.2 | 82.0±27.1 | 54±15.5 |

EODf, electric organ discharge frequency; AFR, abrupt frequency rise.

Table 2. Details of the 21 trials analyzed in this study

| Trial | Fish 1 | Fish 2 | Df (Hz) | Pairing type | T1 chirps* | T2 chirps* | AFRs* | Attacks* |
|---|---|---|---|---|---|---|---|---|
| 1 | D | E | –78 | MM | 23, 0 | 20, 56 | 17, 0 | 16, 79 |
| 2 | E | A | 242 | MF | 0, 1 | 2, 2 | 14, 17 | 74, 2 |
| 3 | B | D | 74 | MM | 2, 0 | 71, 80 | 29, 29 | 1, 62 |
| 4 | E | B | 34 | MM | 0, 0 | 45, 34 | 34, 1 | 35, 1 |
| 5 | D | A | 163 | MF | 2, 3 | 73, 1 | 7, 18 | 91, 5 |
| 6 | A | B | –199 | FM | 0, 1 | 0, 31 | 1, 11 | 3, 38 |
| 7 | G | D | –143 | FM | 0, 1 | 4, 141 | 17, 11 | 9, 71 |
| 8 | H | G | –46 | FF | 0, 0 | 4, 0 | 17, 31 | 5, 24 |
| 9 | I | B | 8 | MM | 2, 2 | 55, 620 | 20, 61 | 50, 6 |
| 10 | G | J | –36 | FF | 0, 0 | 0, 0 | 7, 60 | 25, 7 |
| 11 | K | L | 5 | MF | 0, 0 | 376, 0 | 17, 41 | 7, 6 |
| 12 | M | B | 28 | MM | 11, 0 | 40, 126 | 21, 40 | 4, 29 |
| 13 | D | N | 208 | MF | 4, 0 | 56, 6 | 49, 15 | 90, 4 |
| 14 | O | B | –33 | MM | 5, 0 | 455, 101 | 59, 19 | 46, 13 |
| 15 | J | N | 38 | FF | 1, 0 | 4, 0 | 4, 11 | 3, 22 |
| 16 | J | O | –57 | FM | 0, 3 | 0, 275 | 0, 51 | 1, 32 |
| 17 | M | N | 145 | MF | 19, 0 | 9, 0 | 3, 0 | 3, 19 |
| 18 | G | B | –139 | FM | 0, 11 | 0, 174 | 5, 22 | 35, 17 |
| 19 | K | D | –107 | MM | 0, 0 | 1, 70 | 10, 9 | 3, 52 |
| 20 | N | G | 13 | FF | 0, 0 | 0, 1 | 67, 51 | 30, 25 |
| 21 | O | M | –15 | MM | 3, 24 | 252, 106 | 136, 14 | 84, 5 |

Df, difference frequency (fish 1 – fish 2); AFR, abrupt frequency rise.
*(fish 1, fish 2).




**Addendum. Figure 2A.1** Type 1 Chirp counts of males from male-male trials. The mean Type 1 chirp counts of the less aggressive males (those with higher attack counts considered within all pairs, N=8) are significantly higher than that of the more aggressive males ($p<0.001$).

# Chapter 3

## The Effect of Difference Frequency on Electrocommunication: Chirp Production and Encoding in a Species of Weakly Electric Fish, *Apteronotus leptorhynchus*

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## 3.1 Introduction

*Apteronotus leptorhynchus* are a species of weakly electric fish native to South America, and like all species of weakly electric fish they can both produce and detect electric signals. *A. leptorhynchus* emit a continuous wave-type electric organ discharge (EOD) which they use in navigation, prey localization (Moller, 1995; MacIver *et. al.*, 2001) and in communication, specifically electrocommunication (Hagedorn and Heiligenberg, 1985). They detect these signals with specialized electroreceptors distributed over the skin. These receptors, in turn, excite sensory afferents that encode the signals and transmit them to the brain.

An animal's perception of the world is limited by the types of information that can be encoded by its sensory receptors and sensory systems. Just as our eyes and ears are responsive to a narrow range of frequencies within the visible and audible range, in weakly electric fish, electroreceptors are tuned to specific stimulus features (Hopkins, 1976; Keller *et. al.*, 1986; Chacron *et. al.*, 2005; Benda *et. al.*, 2006). This limited ability to encode sensory stimuli shapes the detection and perception of conspecific electrocommunication signals and hence is likely to play a central role in shaping behaviour during social interactions.

The EOD frequency (EODf) is sexually dimorphic in *A. leptorhynchus*; female EODfs range from 600–800Hz, whereas males emit in the range of 800–1100Hz (Meyer *et. al.*, 1987). For each fish, the frequency of discharge is very regular over time (Moortgat *et. al.*, 1998); however, stereotyped frequency and amplitude modulations are common in social situations and are believed to serve as communication signals (Hagedorn and Heiligenberg, 1985; Zakon *et. al.*, 2002; Zupanc, 2002). Of these modulations, the most commonly studied is the Type 2 or 'small' chirp (Engler *et. al.*, 2000; Engler and Zupanc, 2001; Zupanc *et. al.*, 2006), defined as a transient (10–20ms) frequency excursion associated with a small amplitude decrease (Fig. 3.1).

When two or more fish are in close proximity, their EODs interact and a beat results – a periodic amplitude modulation (AM) of the EOD with a frequency equal to the difference between the EODfs of the interacting fish, the difference frequency, Df (Fig. 3.2). When one fish chirps, it results in a transient and rapid AM, and imposes a phase shift of the beat cycle (Fig. 3.2C). AMs are detected by tuberous electroreceptors and electroreceptor afferents (P-units) propagate these signals to an electrosensory structure in the hindbrain called the electrosensory lateral line lobe (ELL). From here, signals are sent to a variety of electrosensory and electromotor processing areas, including an indirect pathway to the diencephalic central posterior/prepacemaker nucleus, CP/PPn (Zupanc and Heiligenberg, 1992). The CP/PPn, in turn, influences the medullary pacemaker nucleus (Pn), an endogenous oscillator whose activity determines the frequency of the EOD (Zupanc, 2002). One particular region of the CP/PPn, the PPn-C, is responsible for generating chirps. Activation of the PPn-C induces a frequency shift in the pacemaker which is propagated through relay cell axons to the electrogenic cells of the electric organ, where it results in a brief modulation of the EOD: a chirp (Kawasaki *et. al.*, 1988).

In this paper, we will discuss how Type 2 chirp behaviour and chirp sensory encoding are influenced by the beat frequency, Df. It is well established that chirp production rates of *A. leptorhynchus* are influenced by Df (Zupanc and Maler, 1993; Bastian *et. al.*, 2001; Dunlap, 2002; Dunlap and Larkins-Ford, 2003B; Hupé and Lewis, 2008 (Chapter 2)), and here, for the first time, we show that this is also true for attack counts, and for the strengths of the relationships between chirping in one fish and chirp and attack behaviours of an interacting conspecific. Secondly, we will discuss how Type 2 chirps are encoded in P-units by an enhanced firing rate response that also synchronizes the electroreceptor population at low Dfs (Benda *et. al.*, 2005; 2006). We provide new evidence that at even higher Dfs, Type 2 chirps exert a

behavioural influence on conspecifics, and suggest that they may be encoded by a desynchronization of the P-unit population response under these conditions.

### 3.2 The Effects of Difference Frequency on Chirp Behaviour

In order to understand a particular behaviour from a neuroethological perspective we must understand it on both neurophysiological and behavioural levels. A great deal of work has revealed many of the mechanisms used to encode electric signals in *A. leptorhynchus* (Wessel *et. al.*, 1996; Chacron *et. al.*, 2005; Benda *et. al.*, 2005, 2006). Similarly, much work has been done on characterizing chirp production patterns (Bullock, 1969; Zupanc and Maler, 1993; Engler and Zupanc, 2001; Dunlap and Larkins-Ford, 2003; Zupanc *et. al.*, 2006); however, although chirps were first described almost 40 years ago, we are only now beginning to understand their social significance (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)).

#### 3.2.1 Chirp Production in Free Swimming Dyads

In a recent study (Hupé and Lewis, 2008 (Chapter 2)), we examined chirp production by *A. leptorhynchus* during 5 min dyadic interactions in a small test arena. The fish (N=13) were randomly paired (mean ± sd body length: 12.6 ± 1.7cm), and further details about individual fish and pairings can be found in Chapter 2. Using spectrograms from the electrical recordings for each trial, we noted the times of all chirps produced by each fish. *A. leptorhynchus* produce short and long duration chirps, termed Types 1 and 2 and Types 3–6, respectively (Engler *et. al.*, 2000; Engler and Zupanc, 2001; Zupanc *et. al.*, 2006). Only short duration (10–20ms) chirps were observed in this study, and among these, large Type 1 chirps and smaller Type 2 chirps were differentiated based on the size of the frequency excursion associated with each. Type 1 chirps were produced at very low rates. Type 2 chirps were produced at much higher rates and are the

predominant chirp type produced by *A. leptorhynchus* under a variety of experimental contexts (Engler *et. al.*, 2000; Engler and Zupanc 2001; Zupanc *et. al.*, 2006; Smith and Combs, 2008; Hupé and Lewis, 2008 (Chapter 2)). From video recordings of the interactions, we recorded the times of all attacks, defined as any lunge or bite directed at a conspecific. These chirp and attack counts were used to examine how chirp production rates in free swimming fish are influenced by sex, EODf, Df, and the time course of the interaction (Hupé and Lewis, 2008 (Chapter 2)). We also quantified chirp production patterns and the temporal relationship between chirping and attack behaviours using correlation analyses and showed that chirping in one fish is correlated with both chirping and attack behaviours of the interacting conspecific (Hupé and Lewis, 2008 (Chapter 2)). Although both males and females were tested in Chapter 2, here we will only be considering Type 2 chirps produced by males because of the sexual dimorphism in chirp rates; males produce significantly more chirps than females (Maler and Ellis, 1987; Zupanc and Maler, 1993; Kolodziejski *et. al.*, 2004; Hupé and Lewis, 2008 (Chapter 2)). In our new analyses, we examine the influence of the difference in the EOD frequencies of the interacting fish (Df) on the temporal relationships between chirping and conspecific behaviours. This is motivated by the strong Df dependence of chirp encoding by electroreceptor afferents (Benda *et. al.*, 2005, 2006).

### 3.2.2 Chirp and Attack Rates are Dependent on the Difference Frequency, Df

In Chapter 2, we reported that freely interacting *A. leptorhynchus* males produce Type 2 chirps at the highest rates when the difference in EODf between the two interacting fish is small, and that the chirp rates tend to decrease as the absolute Df increases (the relationship between chirp rates and Df persists for both positive and negative Dfs). Figure 3.3A shows the Type 2 chirp counts of male fish, summed over an entire trial, plotted against Df, and it is evident that chirp rates are highest when the discharge frequencies of the two fish are similar. This trend

persists regardless of whether males are paired with a second male or with a female, thus we have pooled male–male and male–female trials here. The inverse relationship between chirping and Df has been observed in *A. leptorhynchus* tested under a variety of experimental conditions (Zupanc and Maler, 1993; Engler and Zupanc, 2001; Bastian *et. al.*, 2001; Dunlap, 2002; Dunlap and Larkins-Ford, 2003B). In contrast, however, male attack counts tend to increase as Df increases (Fig. 3.3B), regardless of whether they are paired with another male or with a female.

### 3.2.3 Chirping Influences Chirp and Attack Behaviours in an Interacting Conspecific

Communication signals, by definition, transmit information from a sender to a receiver (Bradbury and Vehrencamp, 1998) and our detection of this information transfer is limited to changes in subsequent, observable behaviours. Chirps have long been thought to play a role in communication, but direct evidence for this has been conspicuously absent until recently (Zupanc *et. al.*, 2006; Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)).

To quantify patterning in chirp production between interacting fish, we created chirp-centered cross-correlograms, in which we plot counts of one fish's chirps centered at the time of chirp production by the other fish (Hupé and Lewis, 2008 (Chapter 2)). In the same way, to temporally relate one fish's chirp production with the other fish's attack counts, we created chirp-centered cross-correlograms for one fish's attacks centered at the time of chirp production by the other fish. Only male–male pairings were considered in the correlogram analysis because female chirp and attack counts are much lower than male counts, and too low to represent using correlation analysis. For both correlograms presented, significance was assessed by comparing the calculated cross-correlograms to the null distribution (a flat line) expected if all events occur at random (Hupé and Lewis, 2008).

Fig. 3.4A depicts the averaged cross-correlogram relating chirp production in one fish with that of the other fish (N=14 fish in 7 trials). From the peaks in the correlogram, occurring at 200–600ms, we can see that a chirp produced by one fish is often followed by a chirp produced by the other fish, with a preferred latency of 200–600ms (Hupé and Lewis, 2008 (Chapter 2)). Due to averaging over all fish, this correlogram is symmetrical about t=0s (because for a given trial, the correlograms for the two interacting fish will be mirror-images of each other); the level of symmetry in individual trials actually varies somewhat. Males tend to echo one another reciprocally, thus chirps often occur in bouts with the two fish alternately producing chirps. An 'echo response' has also been reported in electrically interacting male fish confined to separate PVC tubes, with a similar preferred latency of 500ms (Zupanc *et. al.*, 2006), despite being tested under very different experimental conditions.

Observing chirp production in freely swimming, interacting fish allowed us to relate chirp production with aggressive behaviours directly (Hupé and Lewis, 2008 (Chapter 2)). We reported that the attack rates of both the chirping fish and the fish with which it is interacting (Fig. 3.4B) are lower than expected at the time of chirp production; a similar trend was recently reported by Triefenbach and Zakon (2008). The attack counts of one fish tend to be less than expected within about 1s of chirp production in the fish with which it is interacting. The relevance of this negative relationship remains to be determined. Triefenbach and Zakon (2008) suggest that chirps may be used to signal attack motivation and aggression; however, our preliminary results using interactive playbacks suggest that Type 2 chirps may be used to deter aggression (Hupé and Lewis, 2008 (Chapter 2)). The relationships shown in Fig. 3.4 demonstrate that chirp production in one fish influences signalling and attack behaviours in other fish. Benda and colleagues (2006, 2005) have demonstrated that the encoding of chirps by electroreceptor

afferents is dependent on Df, so in the next section we will examine whether the relationships revealed in Chapter 2, namely the magnitude of chirp influence on conspecific chirp and attack behaviour, are also dependent on Df.

### 3.2.4 Chirp Influence on Conspecific Behaviour is affected by Df

As a novel approach taken in this paper, we test if the magnitude of chirp influence on conspecific behaviour (echo response and attack inhibition, reported in Chapter 2 is affected by Df. In the last section, we saw that chirps produced by one fish are often echoed by the interacting conspecific with a preferred latency of 200–600ms. In addition to influencing a conspecific's chirp production patterns, we have also seen that chirping is inversely related to conspecific attack counts. We predict that the influence of chirps on conspecific behaviour will vary along with the efficiency with which they are encoded at different Dfs by electroreceptor afferents (Benda *et. al.*, 2005, 2006) and hence we ask whether the magnitudes of the 'echo response' and 'attack inhibition' are influenced by Df. To quantify the magnitude of the chirp and attack relationships we calculated the relative difference in the correlogram bin counts within one second of t=0 to their expected counts, for each trial. These values were then plotted against Df (Fig. 3.5). The 7 male–male trials considered spanned a range of Dfs from 8 to 78Hz (one male–male trial with a Df of 107Hz was omitted because one of the fish produced only one chirp during the interaction). For comparison, male–female trials spanned a range of Dfs from 5 to 242Hz.

Figure 3.5A shows that the magnitude of the echo response increases significantly with Df (Regression, p=0.035). As will be discussed in the following section, Benda *et. al.* (2005, 2006) suggest that Type 2 chirps are encoded in electroreceptor afferents by increases in

synchrony only when Dfs are small (<30Hz). However, the results presented here suggest that the magnitude of the echo response is actually greatest in trials with a large Df. Because chirps must be detected to induce an echo, this implies that even at large Dfs, chirps are being effectively encoded. Fig. 3.5B shows that the strength of the relationship between chirping and low conspecific attack counts also tends to increase as Df increases, although the increase is not significant (Regression, p=0.16). Even at large Dfs, chirps are associated with lower than expected attack rates. Both the chirp and attack results presented here suggest that Type 2 chirps are detectable even when produced at Dfs greater than the threshold for detectability suggested by Benda *et. al* (2005, 2006). In the next sections, we will review how chirps are encoded by electroreceptor afferents (P-units) at low Dfs and present a novel mechanism by which Type 2 (small) chirps are encoded at higher Dfs.

### 3.3 P-unit Encoding of the EOD and Type 2 Chirps

The amplitude and phase modulations of the EOD that result during the interaction of two fish are encoded by three different types of electrosensory receptors in gymnotid fish. Only information that is contained in the activity patterns of these receptor neurons can be further processed by higher brain areas. The ampullary receptors of the passive electrosensory system are sensitive to slow changes of the electric field and thus likely do not play a role in encoding chirps in *A. leptorhynchus*. A closely related species (*Eigenmannia*), however, produces chirps with a DC offset that has been shown to be encoded by ampullary receptors and higher brain areas (Metzner and Heiligenberg, 1991; Heiligenberg *et. al.*, 1991). The tuberous receptor afferents of the active electrosensory system can be categorized into two groups: T-units and P-units. T-units fire a spike at each cycle of the EOD and thus can measure the phase of the EOD (Heiligenberg and Partridge, 1981). P-units fire with a lower rate than T-units and encode EOD

amplitude (Scheich *et. al.*, 1973; Bastian, 1981). In the following discussion we focus on P-units, the predominant type of tuberous electroreceptor in *A. leptorhynchus*.

Based on in vivo single unit recordings from P-type afferents (Punits), Benda *et. al.* (2005) concluded that Type 2 chirps evoke transiently enhanced firing rate responses, if the chirps occur on Dfs less than about 23Hz. At Df=60Hz the firing rate response to a Type 2 chirp was on average indistinguishable from the response to the beat. Higher Dfs were not used in this study. The study by Benda *et. al.* (2006) focuses on Type 1 chirps, but also shows the summed population activity that has been recorded with silver wire hook electrodes from the posterior branch of the anterior lateral line nerve in response to Type 2 chirps, using Dfs up to 100Hz. The results confirmed that Type 2 chirps evoke an enhanced response only at Dfs below about 23Hz. These findings are in strong discrepancy to the behavioural data introduced above where we demonstrated that Type 2 chirps have a significant behavioural effect at Dfs much greater than this. In the following sections we first repeat our findings for low Dfs and then present a novel analysis of the population activity data at high Dfs revealing a possible code for Type 2 chirps in this context.

### 3.3.1 Type 2 Chirps Synchronize P-unit Population at Low Beat Frequencies

The response of P-units to Type 2 chirps strongly depends on the beat frequency (Df). An example is shown in Fig. 3.6. At low beat frequencies (about Df<30Hz, Fig. 3.6A left column) the firing rate of a single neuron (Fig. 3.6B,C) as well as the summed activity of the whole population of P-units (Fig. 3.6D) is modulated by the beat AM. Individual spikes between trials (Fig. 3.6B) but also between different units (not shown, see Benda *et. al.*, 2006) are not well synchronized. A Type 2 chirp, however, induces a strong, transient increase in firing rate, since

the resulting AM during a chirp is faster than the adaptation processes known in P-units (Benda *et. al.*, 2005). This increase in firing rate also transiently synchronizes the P-units, as can be seen in the spike raster (Fig. 3.6B) as well as in the population response (Fig. 3.6D, Benda *et. al.*, 2006). The population activity is the voltage resulting from the summed activity of all P-unit fibers in the lateral line nerve that are picked up by the hook electrode. This population response will increase when more spikes in the population of P-units are synchronized.

### 3.3.2 At High Beat Frequencies larger Type 2 Chirps Desynchronize the Population

At higher beat frequencies (Df>30Hz, Fig. 3.6 right column), on the other hand, the P-units phase lock to the beat and the population becomes synchronized. The AM induced by the chirp is still faster than the beat, but it fails to induce a clearly visible response. At a first glance this seems to be surprising, since at such a Df the fish clearly showed a behavioural response to Type 2 chirps (Fig. 3.5). However, careful inspection of the spike raster (Fig. 3.6B) suggests that the firing response might actually be reduced by the chirp at these higher Dfs.

In Fig. 3.7 we compare the population response to a moderately fast beat (Df=60Hz as in Fig. 3.6 right column) and four chirps that differ in their size (i.e. in the maximum elevation of the EODf) and thus in the resulting phase shift of the AM. This example indicates that larger Type 2 chirps reduce the P-unit population response more drastically than smaller chirps (compare Fig. 3.7C,D with Fig. 3.7A,B) and thus desynchronize the population. This suggests that the P-unit population can indeed encode Type 2 chirps in trials involving relatively large Dfs.

### 3.3.3 Dependence of Synchrony on Stimulus Frequency Explains Chirp Response Patterns

How can it be that one and the same chirp in the context of a low beat frequency enhances the P-unit population response, whereas at a high beat frequency reduces it? A possible explanation for these contradictory results is based on the analysis shown in Fig. 3.8A. There, the degree of synchrony of the P-unit population is quantified as the standard deviation of the population activity for many different beat frequencies ranging from Df=5 to 300Hz (averaged over N=4 fish). The data show that the level of synchrony increases for Dfs up to about 60Hz. For higher Dfs the degree of synchrony is then gradually reduced again, resulting in a unimodal relationship (Benda *et. al.*, 2006). A chirp briefly increases the EODf and therefore also increases the beat frequency by exactly this frequency for the short duration of the chirp (if the chirp emitting fish has the higher EODf, as was the case in the electrophysiological recordings). Consequently, a rough estimate of the population response during the chirp would be that it equals the response to a beat with a frequency Df plus the size of the frequency excursion associated with the chirp. At low beat frequencies a chirp therefore synchronizes the P-unit population, since the population response increases with small increases in beat frequency. If, on the other hand, the beat frequency is at 60Hz or higher, then the population is already synchronized and the chirp causes a desynchronization, since the population response now falls off for small increases in frequency. We have, therefore, two ranges of beat frequencies that are roughly separated by the maximum in the population response shown in Fig. 3.8A near Df=60Hz.

Unfortunately, no electrophysiological data exist for the complementary scenario where the chirp emitting fish has the lower EODf. When the higher frequency of two fish chirps, it causes a transient increase in the Df, or beat frequency; whereas, if the lower frequency of two fish chirps, it causes a transient decrease in the beat frequency. We propose that when the lower

frequency fish chirps at Dfs below about 60Hz (where P-units are maximally synchronized), it will transiently decrease the Df and hence synchrony, following the above arguments. At higher Dfs, however, Type 2 chirps might synchronize the P-unit population since they reduce the beat frequency into a range where the P-units are better synchronized.

Single unit recordings confirm that at low beat frequencies (Df<30Hz), the synchrony (the correlation between pairs of spike trains) during the chirp is higher relative to the one during the beat (Fig. 3.8B, one tailed Wilcoxon test, $p<0.001$, N=409). The expected reversal of the relative correlation at Df=60Hz is, however, absent. This could be attributed to the fact that only chirps of size 100Hz and less (with many 30Hz chirps) were used in the few experiments where Df=60Hz was tested (Benda *et. al.*, 2005).

The population activity, on the other hand, shows exactly the expected effect (Fig. 3.8C,D). At Dfs below 30Hz the population is clearly more synchronized during the chirp than during the beat (one tailed Wilcoxon test, $p<0.001$, N=72), as in the single unit recordings (Fig. 3.8B). At higher beat frequencies (Df=60Hz) the chirps indeed desynchronize the response (one tailed Wilcoxon test, $p<0.05$, N=65).

### 3.3.4 Only Larger Type 2 Chirps Efficiently Change Population Synchrony at High Dfs

This synchronization and desynchronization of the population by the chirps depends strongly on the chirp size (Fig. 3.8C,D). Very small chirps (30Hz increase in EODf) fail to synchronize or desynchronize the population (two tailed Wilcoxon test, $p>0.1$, N=8). Larger chirps easily synchronize the population at beat frequencies below 30Hz (two tailed Wilcoxon test, $p<0.01$, N=16). These larger chirps are also able to desynchronize the P-unit population at beat frequencies Df=60 and 100Hz (two tailed Wilcoxon test, $p<0.05$, N=57).

In summary, the reanalysis of the electrophysiological data clearly shows that the P-unit population can respond to Type 2 chirps in high beat frequency contexts, however this response is determined by chirp size.

## 3.4 Discussion

### 3.4.1 Comparison of Electrophysiological and Behavioural Data

It is through a combination of behavioural observations and neurophysiological recordings that we can best explore the relationship between sensory encoding ability and social signalling in this species of weakly electric fish. From Fig. 3.3A, it is evident that fish produce most chirps when Dfs are optimal for chirp encoding through transient increases in P-unit firing rate and thus synchrony (i.e. when Dfs are small), as was shown by Benda *et. al.* (2005, 2006). Through correlation analysis, it is now clear that Type 2 chirps are authentic communication signals that influence both signal production and aggression in interacting conspecifics (Hupé and Lewis, 2008 (Chapter 2)). Here we have shown that the influence of chirps on conspecific behaviour depends on Df (Fig. 3.5). Interestingly, the magnitude of chirp influence on conspecifics chirping and attack behaviours is largest for Dfs much higher than the aforementioned optimal range of beat frequencies in which chirps can be coded by increases in synchrony in the P-unit population (Fig. 3.6). Here we provide evidence that the electrosensory system may use a different code for chirps at high Dfs. A reanalysis of the electrophysiological data (Figs. 3.6–3.8), suggests that mechanisms other than increases in P-unit synchrony encode Type 2 chirps at large Dfs, namely that Type 2 chirps with large frequency excursions transiently desynchronize the P-unit population activity that is already synchronized by the beat at large Dfs. Type 1 chirps that are of similar duration as the Type 2 chirps, but increase the EODf much more (by about 500Hz) and decrease the EOD amplitude more, have also been shown to desynchronize the P-unit population (Benda *et. al.*, 2006). Whereas this desynchronization is probably induced by a strong reduction of the amplitude of the AM, the desynchronization due to Type 2 chirps is due to a shift in AM frequency into a range that is less efficient in synchronizing

the P-unit population. Note that Type 2 chirps also induce a sudden phase shift in the beat that could be detected by higher order neurons independently of cues like the synchronization or desynchronization.

Revealing the mechanisms by which stereotyped communication signals, like chirps, are extracted from often noisy sensory stimuli is a behaviourally relevant neuroethological problem (e.g. Carlson and Hopkins, 2004). In *A. leptorhynchus*, it is not completely understood how chirps and other electrocommunication signals are extracted from sensory inputs, and how these signals are processed in higher brain centers based on the activity of the electroreceptor afferent neurons. It is clear that chirp detection pathways must exert a strong influence on the chirp production pathway, demonstrated here by the chirp echo response seen in a number of trials. Future experiments should address how this sensory motor pathway is influenced by Dfs. Our data also show that chirping is related to aggression. This is not surprising, given that chirp production, as well as electroreceptor tuning, are both influenced by levels of circulating androgens (Keller *et. al.*, 1986; Meyer *et. al.*, 1987; Dulka and Maler, 1994; Dulka *et. al.*, 1995). There is also evidence that serotonin, which often mediates aggressive behaviours, may mediate both chirp production and encoding in *A. leptorhynchus* (Telgkamp *et. al.*, 2007; Smith and Combs, 2008). The physiological basis for the relationship between chirp production and a decreased propensity to attack in interacting conspecifics is not yet understood, and the neural basis for these relationships should be investigated in the future.

### 3.4.2 Conclusions

In this study, we unify two areas of research, electrophysiology and behaviour, in an attempt to better understand and better explain how sensory encoding shapes the associated

behaviours. We have provided evidence that both the behavioural relationships and electrosensory encoding of chirps in *A. leptorhynchus* are dependent on the difference in EOD frequency between interacting conspecifics. Although the behavioural data presented in this paper suggests that chirps exert an influence on conspecific behaviours at Dfs outside the range in which chirps can be encoded by increases in P-unit synchrony (i.e. at low Dfs), we also show evidence that a desynchronization of the population response encodes chirps outside of the range in which they can be encoded by increases in synchronization (i.e. at high Dfs).

Our findings demonstrate that chirps can indeed be encoded by P-unit afferents for all Dfs where chirps also elicit a behavioural response. Information about chirps is thus in principle available to higher brain areas. How this information is analyzed and why chirps at high Dfs have a stronger impact on behaviour is, however, still unknown. Future investigations of the communication behaviours of weakly electric fish will certainly reveal many more interesting and unexpected insights that will guide electrophysiological work on the computations performed by the electrosensory system.

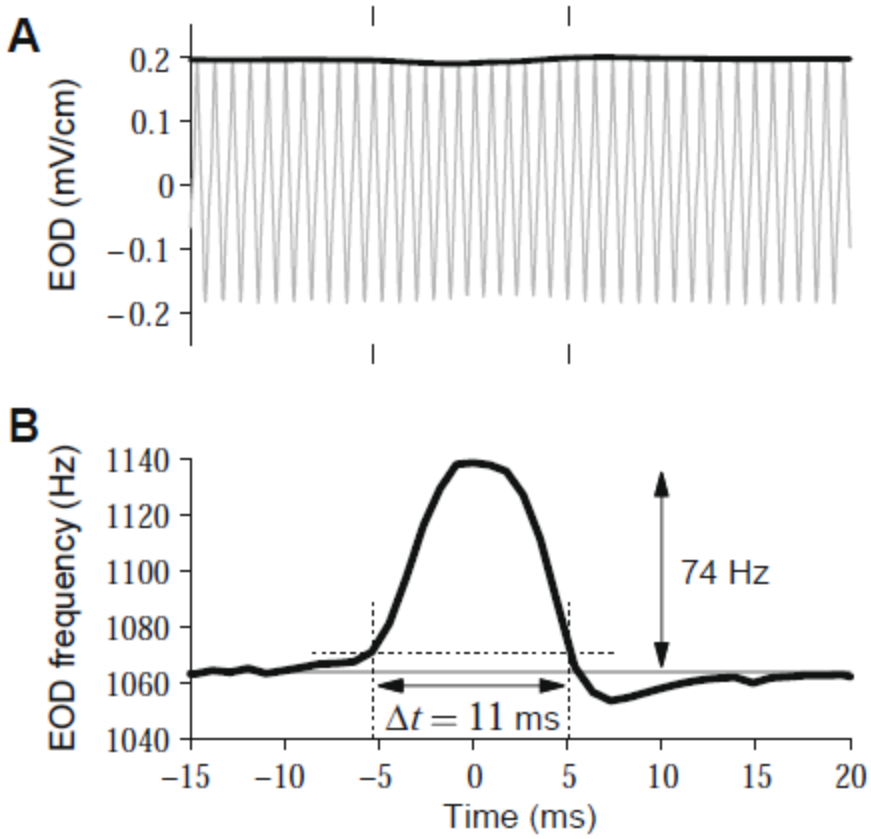



**Figure 3.1** Type 2 chirps are associated with an EOD amplitude decrease and frequency increase. **(A)** Looking at the EOD waveform of an isolated fish (gray line), a Type 2 chirp (within the tic marks) is only visible as a small decrease in EOD amplitude (black line). **(B)** Plotting the frequency of the EOD reveals the chirp: a transient (Δt=11ms) increase in EOD frequency (74Hz) followed sometimes by a much smaller decrease in EOD frequency.

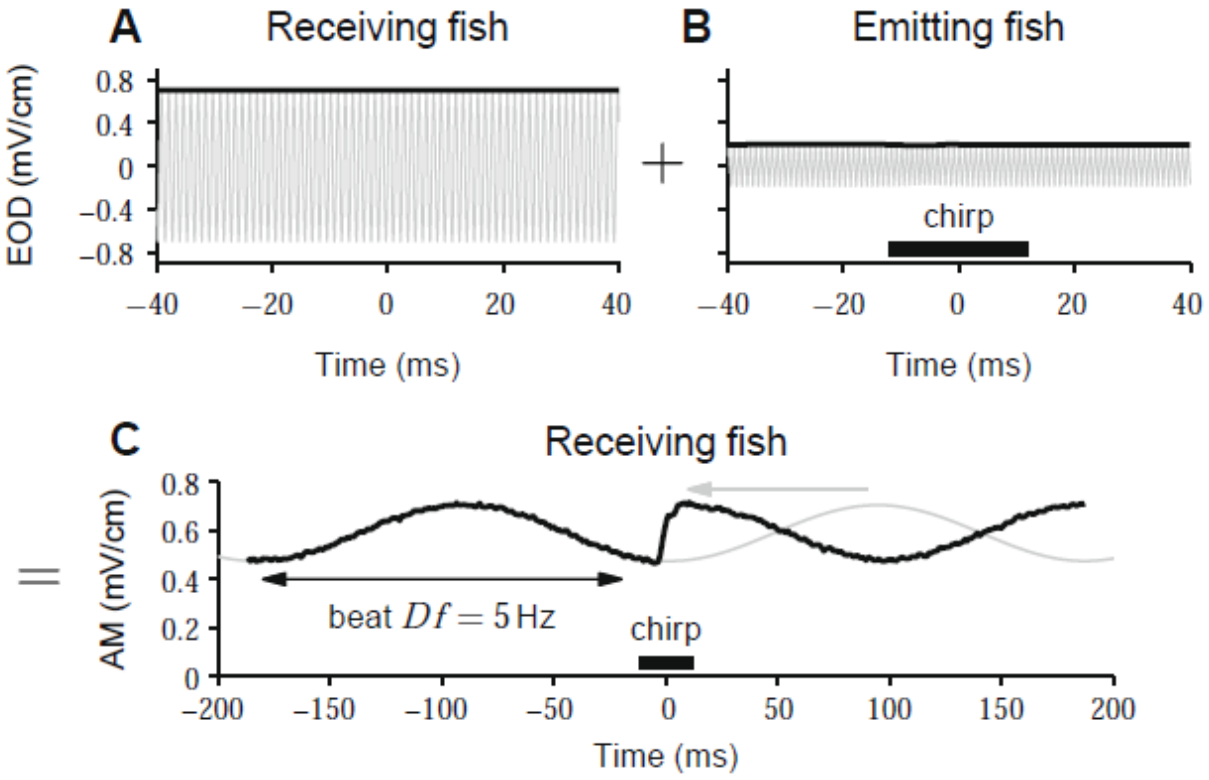



**Figure 3.2** Amplitude modulation evoked by a Type 2 chirp. **(A)** An individual fish experiences its own EOD (gray line) with constant amplitude (black line). **(B)** The EOD of a distant fish has a smaller amplitude at the location of the receiving fish. This distant fish emits a Type 2 chirp (thick horizontal bar). **(C)** The superposition of the two wave forms of the receiving (A) and the emitting fish (B) is the effective electric field that stimulates the electroreceptors of the receiving fish. A slow beat is created (here with frequency Df=5Hz) and the chirp (thick horizontal bar) induces an abrupt upstroke of the amplitude modulation (AM). Half a cycle of the beat (gray line) is briefly accelerated (gray arrow) by the chirp.

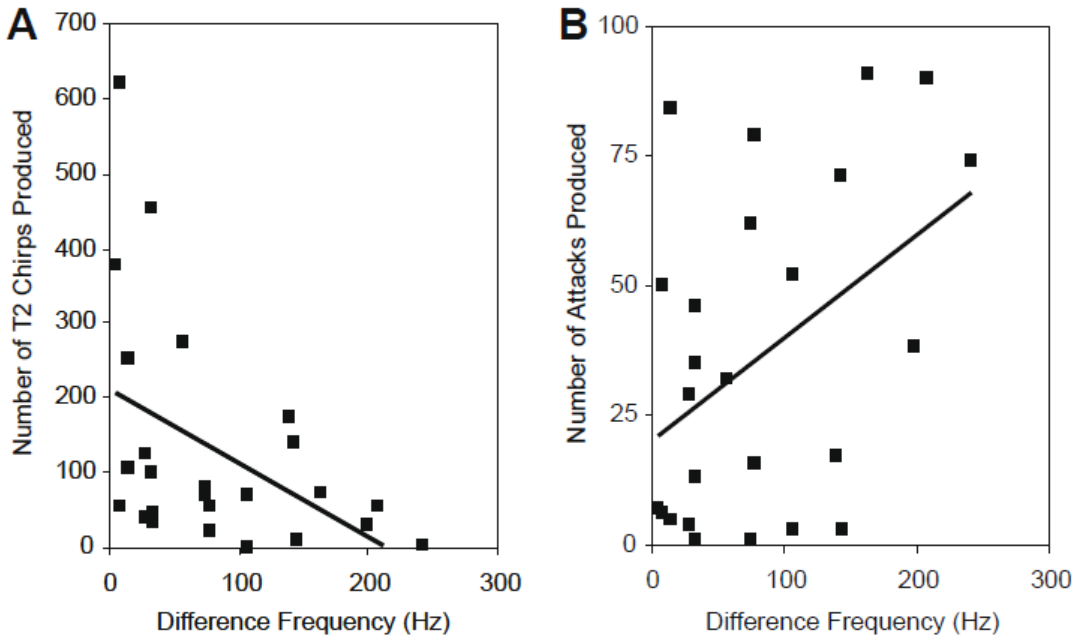



**Figure 3.3** Relationship between Df and chirp and attack rates. Male **(A)** Type 2 chirp and **(B)** attack counts as a function of absolute difference frequency, Df. Chirp rates decrease significantly as Df increases (N=25, F-test, p=0.03); whereas attack counts increase significantly as Df increases (N=25, F-test, p=0.03).

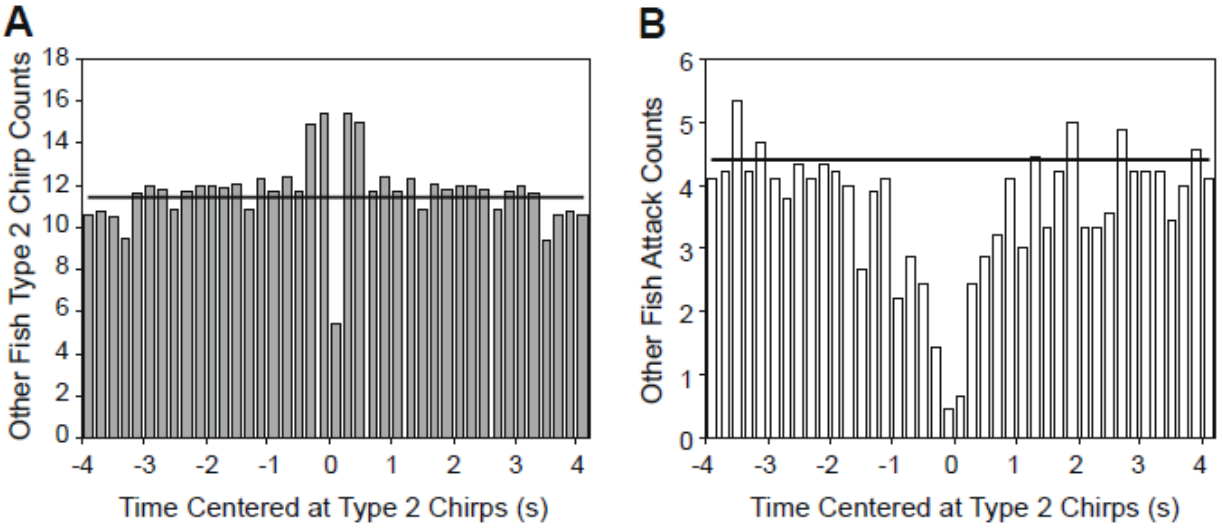



**Figure 3.4** Temporal relationships between conspecific chirps and attacks. Chirp-centered cross-correlograms temporally relating one fish's chirp production with chirp production (**A**; averaged over N=14 individuals) and attacks (**B**; averaged over N=9 individuals) in the other fish. Averages for all male–male trials in which the relevant counts (chirps and attacks) within each bin are greater or equal to 10, are plotted. The thick horizontal line denotes the bin counts expected if chirp production was uncorrelated between the two fish (null hypothesis).

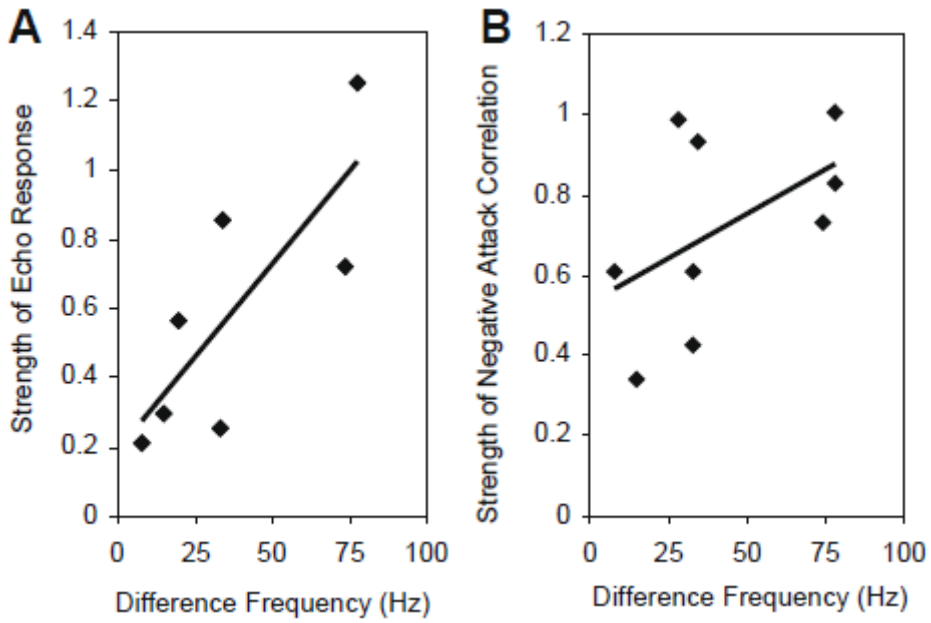



**Figure 3.5** Conspecific responses across a range of Dfs. **(A)** Echo response magnitude (N=7 trials). **(B)** Attack inhibition (N=9 individuals) as a function of Df. These values correspond to the normalized difference in the observed counts within ±1s of center from the values expected based on the null distribution (=abs(observed - expected)/expected), calculated on a fish by fish basis for all fish considered in the correlograms of Fig. 4, and averaged across the bins considered here. The strength of both relationships tend to increase with increases in Df ($p<0.001$ and $p=0.16$ for Type 2 chirps and attacks, respectively).

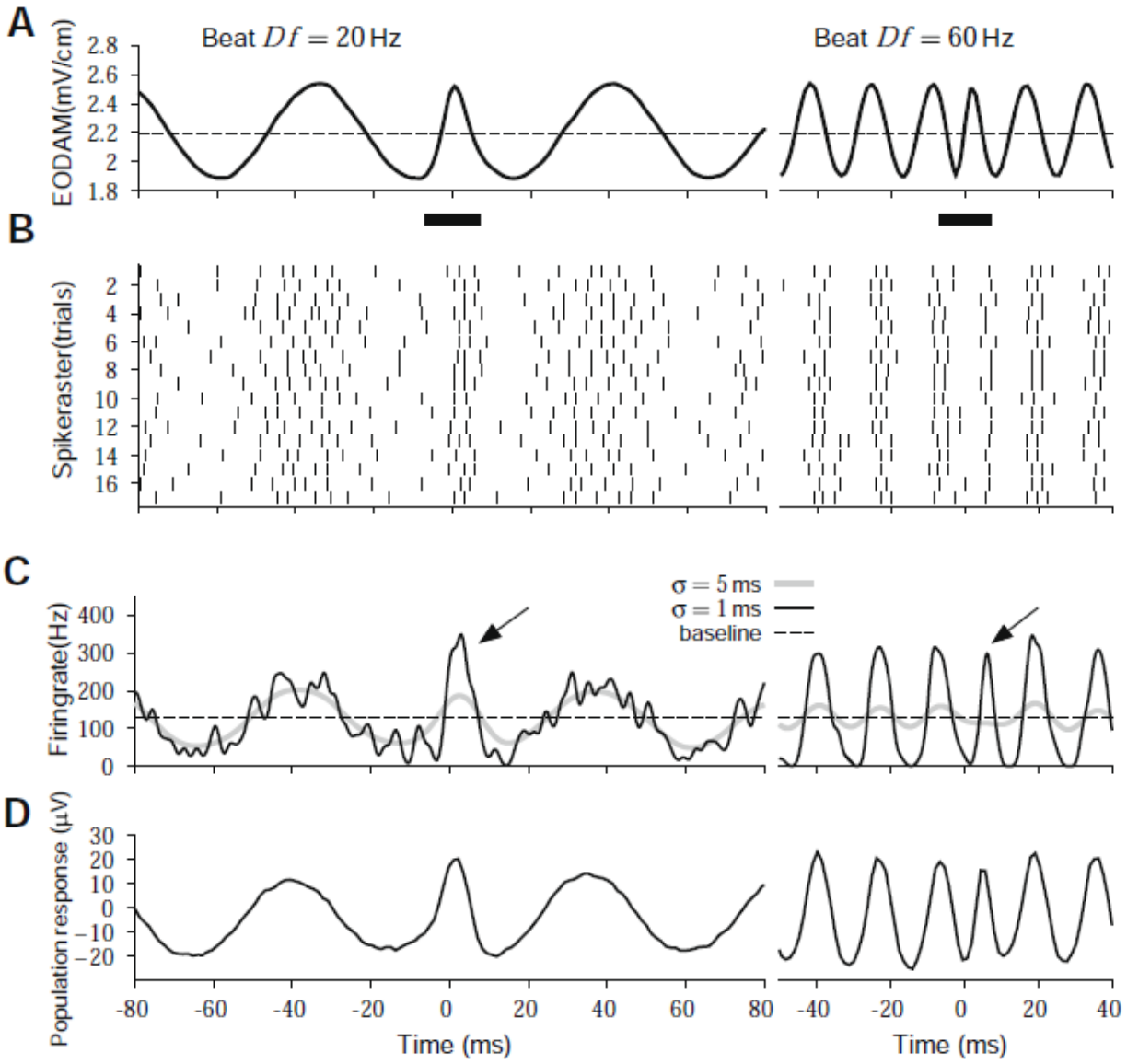



**Figure 3.6** Response of P-units to small, Type 2 chirps. **(A)** The stimulus is a beat AM with frequency Df=20Hz (left column) and Df=60Hz (right column) and Type 2 chirps of size 60Hz at t=0 (thick horizontal bars). The dashed line marks the baseline EOD amplitude. **(B)** Spike raster obtained from a single unit recording. **(C)** Firing rate computed from the spike trains in B using Gaussian kernels with standard deviation 1ms (black line) and 5ms (gray line). The

transiently increased firing rate during the chirp on the 20Hz beat (left column) increases synchrony (arrow). At higher beat frequencies (60Hz, right column) the response to the chirp is slightly weaker than to the beat (arrow). The dashed line is the baseline firing rate. **(D)** The population response resembles the firing rates obtained from single unit recordings using the 1ms kernels. Shown are recordings from the trunk electroreceptor nerve of another fish in response to similar stimuli.

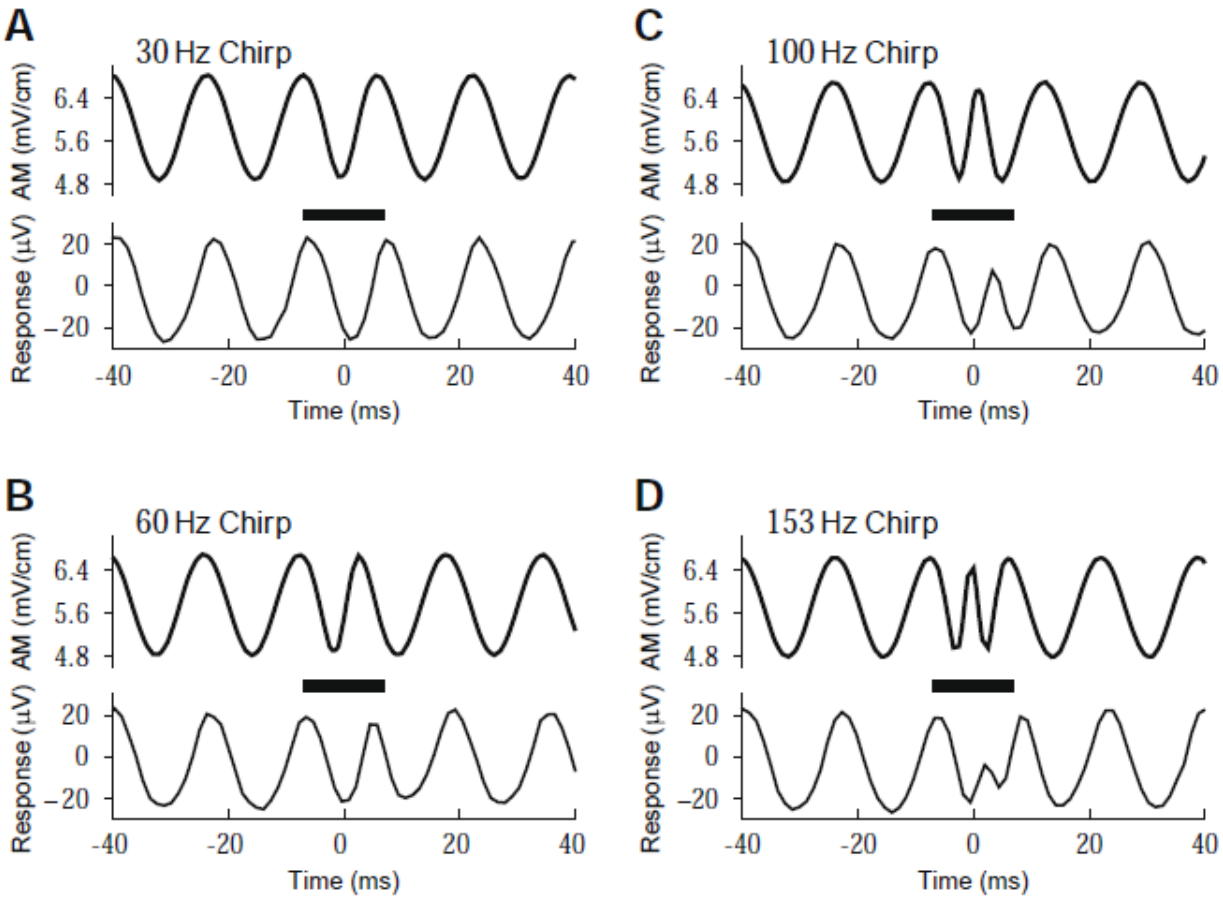



**Figure 3.7** Population response to Type 2 chirps of different sizes. The stimulus is in all four examples a beat with frequency Df=60Hz with a chirp of 14ms duration at time t=0ms (thick horizontal bars, upper panels). The lower panels show the population response recorded from the trunk lateral line nerve (same fish as in Fig. 6). With increasing size of the chirp (30, 60, 100, and 153Hz as indicated) the response to the chirp is reduced.

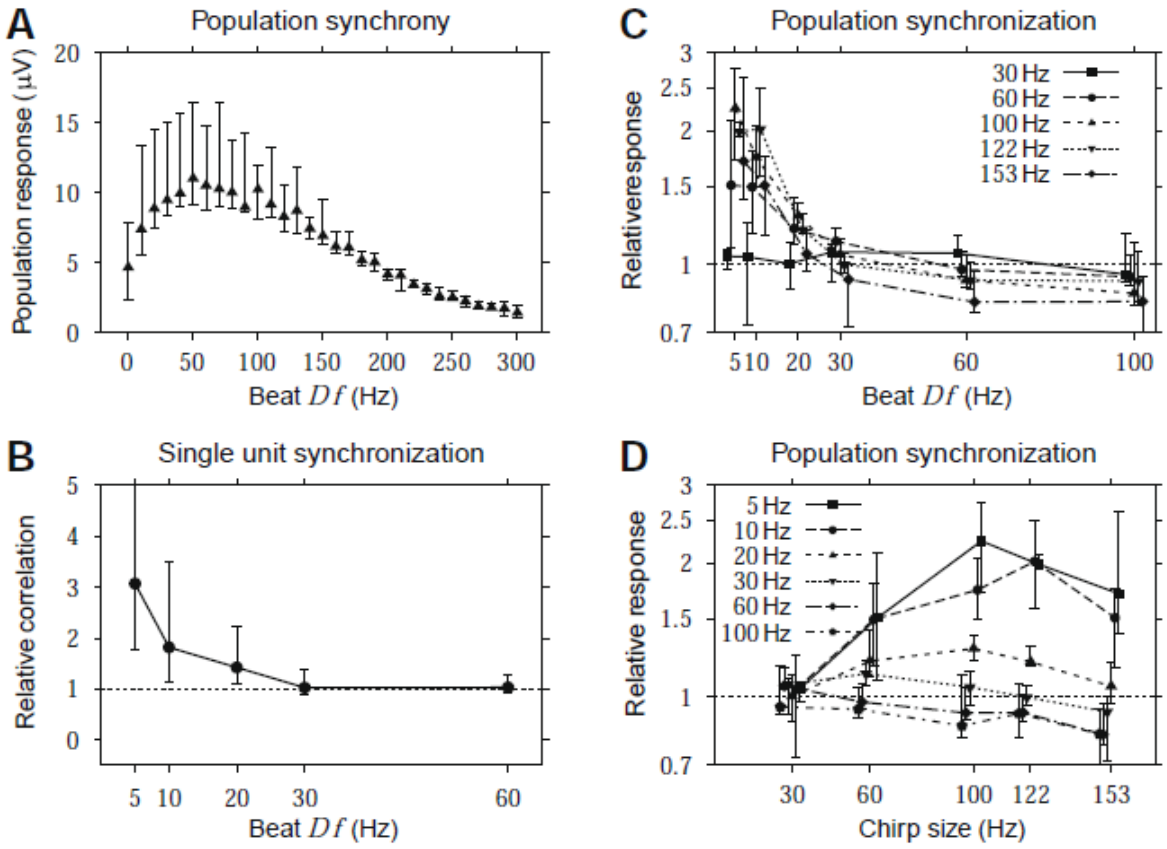



**Figure 3.8** Summary of P-unit response properties to Type 2 chirps. **(A)** The standard deviation of the population activity in response to beats with various frequencies Df ranging from 5 to 300Hz. This measure indicates a high level of synchrony of the P-unit population for beat frequencies between around 60Hz. **(B)** Correlation between pairs of spike trains obtained from a single unit recording during a Type 2 chirp relative to the correlation during the beat. A higher relative correlation indicates that the chirps synchronize the P-unit population. This synchronization by the chirps is possible only at beat frequencies below 30Hz where the population activity is asynchronous as shown in panel A. **(C)** The relative synchronization response, which is the standard deviation of the population activity measured during the chirp divided by the one during the beat, as a function of Df for different chirp sizes as indicated. Whereas most chirps clearly synchronize the population at Dfs below 30Hz, they reduce the level of synchrony at higher beat frequencies. **(D)** Same data as in panel C plotted as a function of chirp size for different beat frequencies as indicated.

# Chapter 4

## Conveying Motivational Tendency Through Signal Patterns: Antiphonal Responses to Electrocommunication Playbacks

## 4.1 Introduction

Many social behaviours central to resource allocation, reproduction, predator avoidance, and conflict resolution, involve flexible and precisely-timed coordination between individuals (Pollack, 2001; Brumm and Slater, 2007; Richardson *et. al.,* 2008; Harcourt *et. al.,* 2010). During social interactions, individuals detect and respond to conspecific behaviours in an individually and context-dependent fashion using intricate and extensively modulated neural networks that permit the integration of dynamic extrinsic and intrinsic stimuli (Remage-Healey and Bass, 2005; Salazar and Stoddard, 2009; Seyfarth *et. al.,* 2010; Kelley and Bass, 2010). In many systems, signalling behaviours produced during agonistic interactions allow potential rivals to assess one another's physical strength and aggressive motivations (Sih *et. al.,* 2004; Kelley and Bass, 2010), and these often involve bursts of reciprocated, or 'antiphonal' signals (Schleidt, 1973; Yosida and Okanoya, 2005). Physical combats often confer large costs, and interacting individuals can weigh the potential outcomes associated with different behavioural strategies by assessing information signalled about rival fitness and motivation (Parker, 1974; Greenfield and Minckley, 1993; Sih *et. al.,* 2004; Chittka *et. al.,* 2009).

*Apteronotus leptorhynchus*, a species of territorial weakly electric fish, engage in social communication behaviours that share many parallels with bursty, antiphonal calling in acoustic territorial communication systems. *A. leptorhynchus* males tend to produce bursts of electrocommunication signals called chirps in response to conspecifics during social interactions, and in response to playback stimuli (Engler *et. al.,* 2001; Zupanc *et. al.,* 2006; Hupé and Lewis, 2008 (Chapter 2)). During both simulated chirp playbacks and authentic conspecific interactions, male *A. leptorhynchus* also tend to reciprocate each other's chirps and playback chirps, echoing with a latency of about half a second (Zupanc *et. al.,* 2006; Hupé and Lewis, 2008 (Chapter 2);

Gama Salgado and Zupanc, 2011). Correlation analyses suggest that chirp exchanges are associated with aggressive interaction and that chirp behaviours are produced with individual, contextual, and time-changing variability (Dunlap *et. al.,* 2002; Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2); Gama Salgado and Zupanc, 2011). Six categories of chirps, each with a stereotyped frequency modulation profile, have been characterized (Zupanc *et. al.*, 2006). Of these, two are produced during agonistic interactions: short duration (10-15ms) 'large' (Type 1) chirps and 'small' (Type 2) chirps distinguished based on the size of their associated frequency excursion. Small chirps are produced at much higher rates during agonistic contexts and here I use 'chirp' synonymously with 'small chirp' unless otherwise specified.

Bursts of antiphonal signals serve many functions including to reaffirm social relations (Ghazanfar *et. al.,* 2001; Miller *et. al.,* 2010), convey information about sender identity (Greenfield and Minckley, 1993, Seyfarth and Cheney, 2003; Yosida and Okanoya, 2005), aid in spatial localization (Ursprung *et. al.,* 2009), and mediate aggressive escalation (Parker, 1974; Amy *et. al.,* 2010). Differences in antiphonal signal parameters (frequency and amplitude modulations), signal rates and signal patterns produced by males during conflict situations indicate individual differences in dominance status, fighting ability, and motivation to defend high quality territories (Parker, 1974; Greenfield and Minckley, 1993; Triefenbach and Zakon, 2008). In many territorial species, males that do not produce signals during territorial contests tend to be less aggressive, lose contests more often, and leave the disputed sites more frequently (Greenfield and Minckley, 1993; Amy *et. al.,* 2010). During agonistic interactions with unmatched signalling rates, individuals with lower signalling rates tend to be less aggressive than individuals with higher signalling rates (Parker, 1974; Greenfield and Minckley, 1993; Triefenbach and Zakon, 2008). Further, in many animal systems, individuals that echo

conspecific signals the most ('followers') tend to be subordinate to the 'leaders' who initiate paired signals or bursts of antiphonal signals (Richardson *et. al.,* 2008; Harcourt *et. al.,* 2010). More dominant 'leaders' also tend to produce signals at higher rates than subordinate 'followers' (Greenfield and Minckley, 1993; Yosida and Okanoya, 2005).

Sender signals, and the information they transmit, can induce a variety of effects on receiver behaviour (Seyfarth *et. al.,* 2010) and these responses are dependent on, for example, the temporal sequence of signals produced, social context (Schleidt, 1973; Arnold and Zuberbühler, 2006; Amrhein and Lerch, 2010) and the motivational states of both sender and receiver (Maynard-Smith and Parker, 1976; Davies, 1978; Seyfarth and Cheney, 2003). Chirp signalling behaviour in weakly electric fish is ideal for exploring the importance of precision and patterning of signals in modulating behaviour during social interaction. The neural substrates and mechanisms responsible for chirp encoding (Benda *et. al.*, 2005, 2006), processing (Marsat *et. al.*, 2009; Marsat and Maler, 2010) and production (Dye, 1987; Zupanc, 2002) have been well characterized. The neuromodulatory systems that influence individually-specific and context-dependent chirp production rates have also been examined extensively (Sas and Maler, 1991; Weld and Maler, 1992; Zupanc, 2002; Kolodziejski *et. al.*, 2007; Dunlap *et. al*., 2011). The conspecific response times characterized in chirp interactions are very rapid (on the order of hundreds of milliseconds), and can involve complex interaction patterns (Hupé and Lewis, 2008 (Chapter 2)) and experience-dependent plasticity (Harvey Girard *et. al.,* 2010), as is seen in acoustic territorial species (Kelley and Bass, 2010).

To determine whether different rates and/or patterns of chirp production induce a differential influence on conspecific behaviour, we use a novel playback system to explore the chirping and aggressive behaviours of male *A. leptorhynchus* in response to intruder mimics with

variable chirp patterning styles. In the first scenario, we present a non-chirping EOD stimulus through a simulated intruder (No Chirps, NC); in the second, chirps were delivered antiphonally echoing those produced by the focal fish (Echoed Chirps, EC); and in the third, chirps were delivered in a random sequence (Random Chirps, RC). Our results indicate that focal fish show pronounced individual differences in chirping and aggression responses and further, that these responses are dependent on the chirping style of the simulated intruder.

## 4.2 Methods

### 4.2.1 The Fish

We characterized the chirping responses of 60 male *A. leptorhynchus* to ten-minute simulated intruder playbacks. The fish were wild caught and delivered by a commercial fish supplier and housed in large flow-through tanks containing 0 to 4 tank mates. Fish were kept on a 12:12 hr dark: light cycle and, because they are nocturnal, we performed all playbacks during the dark phase (within a few hours of dark onset). Fish were fed frozen blood worms at 2-3 day intervals, and were last fed 2-3 days prior to playback presentation.

### 4.2.2 The Experimental Tank

Two days prior to intruder introduction we isolated males in a playback tank (30cm x 40cm x 15cm). The tank contained a filter for aeration and a heater to keep the temperature in the range of 26-28˚C. The tank conductivities were maintained in the range of 150-250μS/cm$^2$. A pair of Teflon-coated silver wires, exposed at the tip, and attached to the tank walls were used to take electrical recordings. Signals were recorded using an AM Systems differential amplifier and acquired using a real-time A/D processor (D1104, dSpace Inc) controlled by Simulink (The Mathworks) and Control Desk software (dSpace Inc). During the playback trial, the heater was unplugged and the filter turned off to decrease environmental noise in the electrical recordings; the temperature decreased only slightly (<1˚C) over the trials. The experiments were video recorded from above, and as the trials were performed in the dark, the tanks were illuminated using an IR light array positioned underneath the tank. Sex was determined by examining the EODf recorded from fish in isolation; only those greater than 850Hz were used in the analysis (Zakon *et. al.*, 2002; Zupanc, 2002).

### 4.2.3 The Intruder Mimic

We created conspecific mimics by embedding two Teflon-coated silver wires in an agar fish-shaped casting (roughly measuring 12cm x 5cm x 1.5cm) The tips of the two electrodes were 2.5cm apart, with one of the ends exposed more than the other (1mm vs 1cm) to reproduce the EOD-generated electric field geometry, that is not symmetrical along the rostral to caudal axis (Assad *et. al.,* 1999; Chen *et. al.,* 2005; Babineau *et. al.,* 2006). We used agar with a conductivity of 35μS, which is considerably less than the surrounding water (as is the case for a real fish, Babineau *et. al.,* 2006). At the onset of each trial, we suspended the mimic near the surface of the water, in the middle of the tank, and delivered artificial stimulus EODs from the mimic containing chirps in one of the three chirp patterns for ten minutes.

### 4.2.4 Playback EOD Stimulus

To create the artificial EODs delivered during the playback experiments, we recorded the EOD of a chirping isolated fish. From this recording, we extracted two segments which we used to create the playback stimulus. The first segment contained an EOD recording during which the fish was stationary and not modulating its EODf (the unmodulated EOD template). The second segment that we extracted contained a small chirp produced while the fish was stationary and at the same position relative to the electrodes as in the first segment (the chirp EOD template). During playbacks, combinations of these two templates were seamlessly spliced together by the real-time system to provide appropriate chirp rates and patterns delivered through the intruder mimic. The frequencies of the template EOD signals were adjusted by resampling at an appropriate rate such that the difference between EODfs of the stimulus and focal fish was between 10 and 30Hz. We maintained the amplitude of the stimulus in the range of 1-2mV

measured one centimetre from the operculum, comparable to the strength of natural EOD (Babineau *et. al.*, 2006; Fugère and Krahe, 2010).

### 4.2.5 Three Playback Stimulus Types with Different Chirp Delivery Patterns

We manipulated the chirp pattern of the stimulus EODs to produce one of three categories of chirp patterns. In one playback scenario (No Chirps, NC), the stimulus EODs contained no chirps, mimicking a non-chirping intruder. The chirping behaviour of each focal fish was characterized in response to a single one of these three simulated intruder chirp pattern scenarios. In the second scenario (Echoed Chirps, EC), chirps were delivered interactively such that delivered chirps echoed the real fish's chirps with a short latency (200ms); similar to the echo delay observed in dyadic interactions (Hupé and Lewis, 2008 (Chapter 2)), and mimicked an antiphonally-chirping intruder. In the third scenario (Random Chirps, RC), stimulus chirps were delivered with a random sequence (intervals larger than 200ms drawn from an exponential distribution, *i.e.*, a Poisson process with 200ms deadtime).

During the No Chirps (NC) scenario, the unmodulated EOD template was played back continuously and no stimulus chirps were delivered. In the Random Chirps (RC) scenario the stimulus EODs contained a variable number of chirps to span the range of chirp rates observed in the EC trials, delivered in a randomly generated sequence.

In the Echoed Chirps (EC) scenario, we detected the focal fish's chirps in real-time and delivered a chirp in response to each of the chirps it produced. We calculated the fast Fourier transform (FFT) of the recorded signal every 512pts (with an overlap of 412pts) in real-time. The median value of the FFT amplitude over a 200Hz bandwidth (the EODf ± 100Hz) was calculated. A chirp results in an abrupt and large increase in this median value and was found to be a reliable

indicator of a chirp. During EC trials, unmodulated EOD segments were played consecutively, except following a chirp detection event, when a chirp segment was spliced into the stimulus EOD and delivered with a fixed latency of 200ms. Due to the length of the chirp segment, we could not respond interactively to all chirps produced when the real fish was chirping at rates greater than ~3 or 4 chirps/sec (these very high chirp rates were uncommon during the playback trials). Chirp response accuracies were calculated and all EC trials had interactive response percentages of greater than 77%.

### 4.2.6 Chirp Response Characterizations

For each playback trial, we determined the times (in 200ms time bins) of all stimulus chirps (Chirps Delivered) and of those produced by the real fish (Chirps Produced), using custom scripts and the spectrograms (Fig. 4.1A) of the electrical recordings (Matlab, The Mathworks).

To characterize the temporal sequence of chirps, we used methods that are standard in spike train analysis (Gabiani and Koch, 1998; Dayan and Abbott, 2001). For every sequence of chirp responses (Chirps Produced), we calculated the time between successive chirps (the 'interchirp interval', ICI, Fig 4.1B). To characterize the patterning of chirp production in each fish, we created a histogram of ICIs and compared it to the distribution of ICIs expected if the chirps were produced in a random pattern (generated using a mean-corrected Poisson process i.e. with an equivalent mean ISI). Specifically, for every fish, we used Matlab scripts to generate 10000 random sequences of the same number of chirps and then determined the associated ICI histograms. Through a standard bootstrapping process, these comparison histograms allowed us to calculate the probability that the counts from the observed ICI histogram were due to a random Poisson process. The significance threshold was set at a probability, $p=0.05$, such that

$p<0.05$ indicated a non-random chirp pattern. We also classified each chirp based on whether it was produced as part of a burst (defined as three or more chirps separated by an ICI less than the mean ICI expected).

We next characterized whether chirp production times are influenced by stimulus chirp delivery to characterize the echo response. For every chirp produced we calculated the time between it and the last stimulus chirp delivered (the cross interchirp interval, xICI, Fig. 4.1C). For every fish we created a histogram of xICIs and compared this to the distribution expected if the chirps occurred independently of the chirp delivery times. We calculated trial-by-trial significance of the xICI histograms as described for the ICI histograms. This permitted us to characterize whether a fish had an increased or decreased propensity to echo stimulus chirps, and whether this response was dependent on the rate and pattern of chirps delivered. Chirps were characterized as 'echoed chirps' if they occurred within the two seconds following a stimulus chirp.

### 4.2.7 Aggressive Response Characterizations

From the video recordings of each playback trial, we tracked the position of the fish in the tank using Video Point Software (Lenox Softworks, Lenox MA). We used these positions to calculate the distance separating the focal fish and the agar fish throughout the course of the playback (in 200ms time bins). The percent of time spent within 10cm of the mimic was used as a measure of aggression. In a subset of trials, we quantified the time spend biting and lunging at the mimic, and it correlated well with the time spent within 10cm ($R^2$=0.58, p=0.003, N=12 trials). We chose to define aggression based on this proximity measure because it was less subjective than attack scoring (a similar measure was used in Fugère and Krahe, 2010).

## 4.3 Results

### 4.3.1 Overview of Chirp Responses

Due to the design of our playback trials, the number and pattern of chirps delivered varies. For non-chirping (NC) and randomly chirping (RC) simulated intruders, the number and timing of stimulus chirps delivered are independent of the response of the focal fish. During Echoed Chirp (EC) playbacks, both the rate and pattern of chirps delivered through the simulated intruder are dependent on the focal fish's chirp response behaviours. Consequentially, EC playback trials can only be performed when fish chirp in response to the playback stimulus ('Chirpers', shown in the shaded rows of Table 1). The physical behavioural responses of the fish that don't produce any chirps, or produce fewer than six chirps ('Nonchirpers', shown in non-shaded rows of Table 1) can be characterized and then compared to those of Chirpers in response to NC and RC playbacks, and we thus consider the chirping and aggressive responses of Chirpers first and then follow with a comparison to the aggressive responses of Nonchirpers, for the NC and RC intruder scenarios.

Three fish produced infrequent large chirps, and one of these fish also produced a few long duration chirps (Zupanc *et. al.*, 2006). The vast majority of chirps produced were small chirps (99.2%) and only small chirps are considered in the following analyses.

### 4.3.2 Response to Simulated Intruders Producing No Chirps (NC)

In many territorial systems, males that are aggressive and motivated to engage in escalation tend to signal their intent (or broadcast their state), and males that do not produce signals during agonistic encounters tend to be less aggressive and less motivated to engage in escalation than males that readily produce signals (Greenfield and Minckley, 1993; Ursprung *et.*

*al.,* 2009; Yosida and Okanoya, 2009). In male *A. leptorhynchus*, chirp rates may correlate with dominance status (Dunlap *et. al.,* 2002) and we predict that non-chirping simulated intruders may represent a conspecific intruder perceived as less threatening.

*4.3.2.1 Chirp Responses to 'No Chirp' Playbacks*

To quantify the response to non-chirping simulated intruders, we determine if small chirps are produced in a bursty pattern by comparing the distribution of interchirp intervals (ICIs) to that expected if chirps were produced in a random sequence. The average interchirp interval (ICI) histogram of Chirpers responding to NC playbacks is shown in Fig. 4.2A (solid line: observed counts, dotted lines: mean-corrected Poisson distribution). The ICI histograms deviate from the Poisson distributions, which indicates that in response to non-chirping simulated intruders, fish tended to produce chirps in bursts (Fig. 4.2B), similar to other aggressive behavioural contexts (Zupanc *et. al.*, 2006; Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)). The preferred intra-burst ICI duration is reflected in the histogram peak at about 400-800ms, similar as is observed during dyadic interactions and in chirp chamber studies (Engler *et. al.*, 2001, Hupé and Lewis, 2008 (Chapter 2)). In response to non-chirping simulated intruders, individual fish produced 70-94% of their chirps in bursts (Table 1) and the percentage of chirps produced in bursts does not correlate with the total number of chirps produced (Fig 4.3A; $R^2$=0.10, p=0.366). Chirp production rates in NC Chirpers correlate positively with EODf ($R^2$=0.58, p=0.011) as has been reported in fish responding to conspecific EODs (Dunlap, 2002).

*4.3.2.2 Aggressive Responses to 'No chirp' Playbacks*

Among Chirpers responding to NC playbacks we found a positive, but non-significant, relationship between chirp production rates and aggression (Fig. 4.3A; $R^2$=0.29, p=0.110). There was no significant relationship between aggression and the percent of chirps produced in bursts in response to non-chirping simulated intruder playbacks ($R^2$=0.13, p=0.306). In response to non-chirping simulated intruders, aggression rates (as defined by the percent of time spent within 10cm of the mimic) of Chirpers were higher although not significantly different from those of Nonchirpers (Chirpers: 32.3±6.8%, Nonchirpers: 23.6±7.3%; t-test, p=0.200). Among Chirpers, aggression did not correlate significantly with EODf ($R^2$=0.12, p=0.334), however, for Non-chirpers there was a negative relationship between EODf and aggression ($R^2$=0.54, p=0.006).

*4.3.2.3 Summary*

In response to non-chirping simulated intruders, there are no consistent correlations between chirp rates and aggression. Chirpers tended to produce chirps in bursts; however burst rates did not correlate with chirp rate or aggression. NC Chirper chirp rates increased with EODf; while in NC Nonchirpers, aggression decreased with EODf.

**4.3.3 Response to Simulated Intruders Producing Echoed Chirps (EC)**

In many communication systems, the antiphonal exchange of aggressive communication signals occurs to mediate aggressive encounters including those involved in territorial contests and it is believed that the exchange of signals may serve to communicate a willingness to settle territorial disputes without physical escalation circumventing costs associated with escalation when possible (Yosida and Okanoya, 2005, Greenfield and Minckley, 1993). A simulated intruder producing echoed chirps may thus represent a 'follower' conspecific rival whose pattern

of chirping behaviour signals the intent to negotiate (Harcourt *et. al.*, 2010). The rate of chirps delivered is dependent on the rate of chirps produced by the focal fish, indicating an ability to rate match, and hence may also represent a case in which two individuals would be matched also in fighting ability and motivation (Parker, 1974).

*4.3.3.1 Chirp Responses to Echoed Chirp Playbacks*

In response to simulated intruders producing echoed chirps, fish produced chirps in bursts as in the NC scenario (Fig 4.2C,D; Table 1). The percent of chirps that individual fish produced in bursts ranged from 54-86%; and there were no significant relationships between the number of chirps in bursts and the total number of chirps produced (Fig. 4.3B, $R^2$=0.15, p=0.189) or delivered ($R^2$=0.07 , p=0.197).

For EC playbacks, we can characterize the temporal relationships between the chirps produced by the focal fish and those delivered via the simulated intruder chirps. For each trial we calculated a cross interchirp interval (xICI) histogram, which describes the distribution of latencies between each of the focal fish's chirps relative to the timing of the most recent playback chirp delivered. We then compared these observed xICI histograms to the distributions expected if chirp production times occurred independently from stimulus chirp delivery times. The mean observed xICI histograms for Chirpers responding to EC playbacks (Fig. 4.4A solid line) differs from the corresponding randomized mean-corrected distribution (Fig. 4.4A dotted line). There are more counts than expected in the 0.4-1.4s following chirp delivery suggesting that on average, focal fish tended to reciprocally echo the chirps produced by simulated echoing intruders. Figure 4.4B shows that the majority of fish reciprocated simulated intruder chirps ($p<0.05$) when they are delivered with a short latency (peak centered at 0.6s). The number of

chirps produced at the same time as the stimulus was less than expected (Fig. 4.4C). This distinct lack of signal overlap is a feature common to many signalling exchange systems to facilitate the discrimination of information encoded in signal parameters and timing (Yosida and Okanoya, 2005).

To explore individual differences in echoing behaviours, for each fish we calculated the percent of echoed chirps (those produced within two seconds of chirp delivery). The tendency to echo EC playbacks varied extensively between individual fish (distributed across a range of 7-94%). As expected, there was a significant positive relationship between the percent of echoed chirps produced and chirp production rates (Fig 4.3B; $R^2$=0.70, p<0.001) and chirp delivery rates (Fig 4.3B; $R^2$=0.72, p<0.001). There was also a significant positive relationship between the percent of chirps produced in bursts and the percent of echoed chirps ($R^2$=0.31, p=0.048) such that fish that produce a higher percent of chirps in bursts also tended to echo delivered chirps more, a behaviour that resulted in a tendency for bouts of antiphonal chirping between the focal fish and simulated intruder.

Contrary to the NC scenario, there was no significant relationship between EODf and chirp rate in response to an echoing simulated intruder, and similarly, no relationship between EODf and bursting or echoing (Fig. 4.3B; p>0.05).

*4.3.3.2 Aggressive Responses to Echoed Chirp Playbacks*

We next asked whether the aggressive responses of focal fish corresponded with their chirping behaviours in response to the echoing chirp simulated intruder. The aggression level of focal fish did not correlate significantly with focal fish chirp production rates (Fig. 4.3B; $R^2$=0.01, p=0.714), echoed chirps delivered ($R^2$=0.01, p=0.699), echoed chirp rate ($R^2$=0.04,

p=0.523), or EODf ($R^2$=0.07, p=0.38). However, aggression did correlate significantly with the percent of chirps produced in bursts (Fig 4.3B; $R^2$=0.38, p=0.024). Fish that produce more chirps in bursts in response to echoing intruder mimics were less aggressive, and indirectly tended to echo more, than fish that produce fewer bursts (Table 1).

*4.3.3.3 Summary*

Taken together with NC results, the EC results suggest that antiphonal chirping is important for the mutual assessment of aggressive intent and willingness to negotiate, such that more bursts are related to less escalation when the signals are echoed. A 'non-signalling" state may indicate an unwillingness to escalate (and presumably a submissive state) and a preference to flee at all costs, whereas echoing may indicate a preference for assessment and negotiation as a means for deterring aggression. To address whether different chirp patterns induce differential responses directly, we now consider the responses to a randomly chirping intruder.

### 4.3.4 Response to Simulated Intruders Producing Random Chirps (RC)

In a number of animal behaviours that involve concerted actions, individuals that behave more aggressively and more boldly tend to lead coordinated behaviours ('leaders'), and less dominant, shyer individuals tend to produce behaviours that follow ('followers'), often reciprocating those of the more dominant individual (Sneddon and Greenfield, 1998; Sih *et. al.*, 2004; Harcourt *et. al.*, 2009). When territories or other resources are contested, it is often the individual that produces signals at higher rates that is victorious (Greenfield and Minckley, 1993), and it has been suggested that when two *A. leptorhynchus* males compete over a shelter (a PVC tube) the winner tends to produce chirps at the highest rates (Triefenbach and Zakon, 2008). The randomly chirping intruder imitates a 'leader' because the timing of the delivered chirps are

independent of the timing of chirps produced by the real fish. Further, across many animal systems leaders with higher signal rates tend to be more aggressive (Harcourt *et. al.,* 2009), thus we predict that the chirping and aggressive behaviours of fish produced in response to a randomly chirping simulated intruder will depend on the rate of chirps delivered.

*4.3.4.1 Chirp Responses to Random Chirp Playbacks*

In response to RC playbacks, the number of chirps produced by Chirpers was inversely correlated with the number of chirps delivered (although with borderline non-significance, Fig. 4.3C; $R^2$=0.38, p=0.058). Fish patterned their chirps in bursts in response to RC playbacks in a similar way as was seen in response to NC and EC playbacks (Fig. 4.2E,F). The majority of individuals produce chirps in bursty sequences (individual range from 44-86%) and the percentage of chirps produced in bursts did not correlate significantly with the total number of chirps produced ($R^2$<0.01, p=0.899) or with the number of random playback chirps delivered ($R^2$=0.05, p=0.522).

Contrary to the pronounced tendency for focal fish to echo a simulated intruder that produces echoed chirps, the xICI histogram for the RC playbacks (Fig. 4.4D) shows that the tendency to echo simulated intruder chirps was greatly diminished when chirps are delivered in a random pattern. Fewer Chirpers showed a significant echo response (Fig. 4.4E) to RC than to EC playbacks (Fig. 4.4B), where several fish demonstrated lower than expected propensity to chirp following random chirp delivery (Fig. 4.4F). In approximately half of the Chirpers responding to randomly chirping simulated intruders, there was a maintained decrease in the likelihood to produce chirps (chirp rate) in the two seconds following chirp delivery.

To discriminate which of the Chirpers respond to random simulated intruder chirps with a pronounced echo response we considered the percentage of echoed chirps produced by each fish. Of the 10 fish who chirped in response to RC playbacks, five focal fish produced 72-86% of their chirps within two seconds of the stimulus chirps, whereas the remaining five focal fish showed a significantly decreased propensity to chirp in the two seconds following chirp delivery; the percentage of echoed chirps they produced ranged from 3-24%. Considered separately, the five fish that preferentially echoed the randomly chirping intruder's chirps (echo >72% of delivered chirps) produced significantly fewer chirps than did the non-echoing fish (echo <24% of delivered chirps) (echoing fish: 144.8±53.0; non-echoing fish: 437.4±145.5, t-test, p=0.05). Fish tended to respond with low rates of mostly echoed chirps when high rates of random chirps were delivered and with high rates of mostly non-echoed chirps when low rates of random chirps were delivered.

The percentage of echoed chirps produced by focal fish correlated negatively, although non-significantly, with the number of chirps produced (Fig. 4.3C; $R^2$=33, p=0.081) and positively with the number of random chirps delivered through the simulated intruder ($R^2$=0.85, p<0.001). Contrary to the EC playbacks, the percentage of chirps produced in bursts did not correlate with the percent of echoed chirps produced ($R^2$=0.01, p=0.827). Similar to the EC scenario, there were no significant relationships between EODf and chirp rates or bursting or echoing in the RC scenario (Fig. 4.3C; p>0.05).

*4.3.4.2 Aggressive Responses to Random Chirp Playbacks*

In response to simulated intruders with random chirp patterns, Chirper aggression did not correlate significantly with the rate of chirps produced (Fig. 4.3C; $R^2$=0.11 p=0.341) or with the

percentage of chirps produced in bursts ($R^2$<0.01, p=0.919), contrary to the response to EC playbacks (where fish that produced more antiphonally exchanged bursts were less aggressive). Chirper aggression did however show a significant negative correlation with the number of random chirps delivered (Fig 4.3C; $R^2$=0.41, p=0.046). Further, Chirper aggression was negatively correlated with echo rate (although non-significantly, $R^2$=0.34, p=0.077) and when considered separately, non-echoing fish were significantly more aggressive than echoing fish (non-echoing fish: 59.2±7.7%; echoing fish: 32.9 ± 10.0%; t-test, p=0.03). Chirpers were significantly more aggressive than Nonchirpers (percent of time within 10cm: Chirpers: 45.6 ± 7.5%; Nonchirpers: 19.5±6.7%, t-test, p=0.009). The average number of chirps delivered during RC playbacks to Chirpers and Nonchirpers was not significantly different (Chirpers: 219.2 ± 65.7; Nonchirpers: 280.6 ± 63.3, p=0.26).

*4.3.4.3 Summary*

The differential relationships between chirping and aggressive responses to EC and RC playbacks show that different chirp rates and patterns selectively influence conspecific behaviours suggesting they convey different messages related to aggressive motivation and ability. These results are also consistent with the observation that in coordinated behaviours, more dominant, aggressive, or bolder, individuals tend to lead concerted behaviours more often, whereas shyer or more subordinate individuals tend to reciprocate or echo behaviours at higher rates (Greenfield and Minckley, 1993, Sneddon and Greenfield, 1998, Yosida *et. al.*, 2007). Non-chirpers may signal a willingness to avoid escalation, echoers may signal intent to negotiate and escalate only if chirp rates are unmatched, and non-echoers signal aggressive intent proportional to chirp rate.

### 4.3.5 Comparisons of Chirp Responses to Simulated Intruders with Different Chirping Styles

We next compared the chirping responses of Chirpers, and differences in Chirper and Nonchirper aggressive responses across the three playback scenarios (and for echoing and non-echoing fish in response to RC playbacks) and examined how these responses change over the course of the trial depending on the style of chirping of the simulated intruder.

#### *4.3.5.1 Chirp Production Rates*

The average total chirp counts of Chirpers responding to NC, EC, and RC playbacks were not significantly different (NC: 257.4±99.9, EC: 165.8±60.8, RC: 291.1±87.8, ANOVA, p=0.48) and in each scenario, the average chirp rates, calculated per minute, did not change significantly over time (ANOVA, NC: p=0.91, EC: p=0.95, RC: p=0.38). Among RC Chirpers, the average chirp rates of non-echoing fish, responding to low rates of random simulated intruder chirps, did not change over the trial (ANOVA, p=0.89), however, the number of chirps produced per minute by echoing fish, responding to high rates of random simulated intruder chirps, decreased significantly over the trial (Fig. 4.5A, ANOVA, p=0.01).

#### *4.3.5.2 Bursty Chirp Production*

The average percent of bursty chirps produced in response to the three playback scenarios did not differ significantly (NC: 76.6±2.5%, EC: 65.9±3.2%, RC: 70.7±3.8%, ANOVA p=0.08) nor did the average number of bursts produced per trial (NC: 29.0±11.0, EC: 16.8±5.5, RC: 30.3±9.8, ANOVA, p=0.46), or the average number of chirps produced per burst (NC: 7.7±1.1, EC: 6.3±1.0, RC: 8.8±0.1, ANOVA, p=0.26).

Across all three playback scenarios there was a decrease in bursting in the last two minutes of the trial compared to the first two minutes of the trial, with the largest difference occurring in response to RC playbacks (Fig.4.6, t-tests, NC: p=0.07, EC: p=0.04; RC: p=0.001). Among RC Chirpers, the average number of bursty chirps produced per minute by RC non-echoing fish did not change significantly over time (ANOVA, p=0.87), however the number of bursty chirps produced by RC echoing fish per minute decreased significantly over the course of the trial (Fig. 4.5B, ANOVA, p=0.01).

*4.3.5.3 Echoing Behaviours*

The percent of echoed chirps produced in response to simulated intruders did not depend on the pattern of stimulus chirps delivered (EC: 45.0±7.9%, RC: 45.7±11.2%, t-test, p=0.48), however the number of echoed chirps produced per minute decreased significantly over the course of a trial in response to randomly chirping, but not echoing, simulated intruders (echoed chirp counts per minute, ANOVA, RC: p=0.003, EC: p=0.98). Among RC Chirpers, the number of echoed chirps produced per minute by non-echoing fish, in response to high rates of randomly delivered chirps, did not change significantly in time (ANOVA, p=0.54), but the number of echoed chirps produced per minute by the echoing fish, in response to high rates of randomly delivered chirps, decreased significantly in time (Fig. 4.5C, ANOVA, p=0.003).

### 4.3.6 Comparisons of Aggressive Responses to Simulated Intruders with Different Chirping Styles

Among all groups of Chirpers and Nonchirpers, average aggression rates did not change significantly in time (ANOVA, NC Chirpers: p=0.88, NC Nonchirpers: p=0.90, RC Chirpers: p=0.99, RC Nonchirpers: p=0.94, EC Chirpers: p=0.69). Among RC chirpers, echoing fish had

lower rates of aggression than non-echoing fish responding to higher rates of random chirps, and these differences in aggression persisted over time, with no change in aggression over the trial period (Fig. 4.5D; non-echoing fish: ANOVA, $p>0.99$; echoing fish: ANOVA, $p=0.87$). The chirp rates of echoing fish decreased over time, but their aggression rates did not change, implying that the temporal patterning relationship between chirping and aggression change over the course of the simulated interaction.

The average aggressive responses of Chirpers and Nonchirpers in response to the three playback scenarios were dependent on both the chirping behaviour of the focal fish and on the pattern of chirps delivered (Fig. 4.7, ANOVA, $p=0.05$). Chirpers responding to randomly chirping simulated intruders were more aggressive than Nonchirpers responding to both randomly chirping and non-chirping simulated intruders ($p=0.01$ and $p=0.04$, respectively). Chirpers responding to echoing playbacks were more aggressive than Nonchirpers responding to randomly chirping intruders ($p=0.02$).

## 4.4 Discussion

### 4.4.1 Summary

During social interactions, individual predispositions and motivations are dynamically integrated with environmental information (for example, conspecific communication signals) to produce and modulate behavioural responses (Seyfarth and Cheney, 2003; Sih *et. al.,* 2004; Kelley and Bass, 2010). Communication signals in many territorial systems are exchanged in bouts of echoed calls (antiphonal exchanges) and individual differences in signal rate and pattern convey different meaning depending on context (Sneddon and Greenfield, 1998; Ursprung *et. al.,* 2009, Amy *et. al.,* 2010). Our results demonstrate that individual and stimulus related differences influence electrocommunication and aggressive response strategies in a species of weakly electric fish, *A. leptorhynchus*, in ways similar to those observed in a number of acoustic territorial signalling systems. As in other territorial systems, our results suggest that specific patterns of chirps signal differentially aggressive intent and function to mediate aggressive escalation by facilitating mutual assessment.

Fish responded to simulated intruders with bursts of chirps (Fig. 4.2), and their tendency to echo playback chirps was dependent on the rate and pattern of chirps delivered through the simulated intruder (Fig. 4.3C; Fig. 4.4). Chirping responses changed over the course of individual trials, with the greatest differences seen in response to randomly chirping simulated intruders, especially when high rates of chirps were delivered (Fig. 4.5, Fig. 4.6). In response to both of the playback scenarios in which the responses of Chirpers and Nonchirpers could be characterized (No Chirps and Random Chirps), Chirpers were more aggressive than Nonchirpers (Fig. 4.7). In response to Echoing Chirp (EC) simulated intruders, the fish that produced higher

rates of bursts and echoed chirps had lower rates of aggression (Fig. 4.3B); suggesting that antiphonal signalling may be used to prevent costs associated with escalation (Parker, 1974, Triefenbach and Zakon, 2005). In response to Random Chirp (RC) playbacks, chirpers aggression was influenced by the rate of random chirps delivered (Fig. 4.3C). In response to high rates of randomly delivered chirps, Chirpers responded with higher echo rates and lower aggression than Chirpers who responded to low rates of randomly delivered chirps with lower echo rates and higher aggression (Fig. 4.8). This suggests that during chirp exchanges, more dominant fish, or fish signalling a more aggressive intent, tend to lead chirp exchanges more often, and produce chirps at higher rates during simulated territorial contests. Chirp exchanges have many parallels with the antiphonal duel ritualized display exchanges characterized in a number of territorial communication systems where males produce signalling behaviours that coincide with predictable aggressive strategy (Greenfield and Minckley, 1993; Seyfarth and Cheney, 2003; Yosida and Okanoya, 2005).

### 4.4.2 Proposed Signal Value of Different Chirp Patterning Behaviours

Based upon these observations we suggest that our non-chirping simulated intruder EOD may have signalled a more subordinate, or less aggressive, fish. This less threatening stimulus failed to evoke a strong motivation to pattern chirps with aggression, and chirp rates may have depended more on intrinsic differences including EODf (Dunlap *et. al.*, 2002; Yosida and Okanoya, 2005; Amrhein and Lerch, 2010; Douglas and Mennill, 2010). These results also suggest that echoing chirps may function to prevent costly escalations by signalling intent to negotiate, as high rates of antiphonal call sequences were also associated with less aggression. Our EC playback would thus represent a conspecific intruder that is signalling intent to negotiate, with a reciprocated rate. Finally, these results suggest that random chirp patterns, especially

when accompanied with high production rates, may signal an aggressive intent. If this is the case, our RC playbacks represent an aggressive conspecific intruder, with the level of the threat imposed dependent on the rate of chirps delivered. Figure 4.8 shows a summary of the three different stimuli presented and the responses characterized.

These results suggest two hypotheses for the signal value of chirp patterning. The first is that antiphonal exchanges (bursts of echoed chirps) between conspecifics function to decrease the likelihood of costly escalations (Parker, 1974; Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)). The second is that both chirp rate and pattern signal aggressive intent or capacity (Triefenbach and Zakon, 2008).

### 4.4.3 Chirping as a Model Antiphonal Signalling System

Production of bouts of calls occurs in insect (Ronacher *et. al.,* 2008), fish (Myrberg and Riggio, 1985; Remage-Healey and Bass, 2005), amphibian (Ursprung *et. al.,* 2008), mammalian (elephant (Leighty *et. al.,* 2008); whale (Schulz *et. al.,* 2008); bat (Bohn et. al., 2008); naked mole rat (Yosida *et. al.,* 2007, Yosida and Okanoya, 2009); primate (Miller *et. al.,* 2001; 2009)), and avian communication systems (Amy *et. al.,* 2010). The ubiquity of repetitive calling is likely a result of the many functional advantages it confers (Greenfield and Minckley, 1993; Yosida and Okanoya, 2005). The potential benefits of producing repeated calls are diverse. One proposed function of repetitive signalling is that it increases the signal-to-noise ratio improving the likelihood that the signal will be detected in noisy conditions (Schleidt, 1973; Brumm and Slabbekoorn, 2005; Yosida and Okanoya, 2005; Bro-Jøergensen, 2010; Einhäupl *et. al.,* 2011). For effective information exchange, signalling behaviours must overcome noise in communication channels induced by many factors including environmental fluctuations,

movements, and objects that impede signal transmission (Potash, 1972; Forrest, 1994; see also Chapter 5). Another function of repetitive signalling is to act as a beacon signal providing interacting individuals information about conspecific position and movement that can be used to make decisions about orientation and manoeuvring strategies (Marler, 1955; Ursprung *et. al.*, 2009; Chittka *et. al.*, 2009; Amy *et. al.*, 2010). A third proposed function of repetitive calling suggests that by modulating the rate of signal production, signallers can dynamically induce graded responses on receiver physiology, and hence behaviour, over short times scales (Schleidt, 1973; Seyfarth *et. al.*, 2010; Kelley and Bass, 2010).

There are also many benefits associated with the production of echoed calls. One advantage of having precisely timed echoing response behaviours is that they may confer reciprocally, to the sender, a confirmation that its signal was received (Parker, 1974; Yosida and Okanoya, 2005); antiphonal signals are especially common in environments were visual cues are uncertain (Marler, 1955; Schleidt, 1973; Yosida *et. al.*, 2007). Additionally, the precise timing of antiphonal signals (response latency) may convey individual and context-specific messages, as when dominance status is indicated by an individual's tendency to lead or follow (Naguib, 1999; Bohn *et. al.*, 2008; Amy *et. al.*, 2010; Harcourt *et. al*., 2010).

Individuals tend to reciprocate each other's signals within a relatively narrow time window, yet signals produced by different individuals overlap one another infrequently (Stokes and Williams, 1968; Brumm, 2006). Because the frequency and amplitude parameters of the signals exchanged can convey information about individual status and motivation, the production of overlapping signals would prevent accurate signal discrimination (Stokes and Williams, 1968; Yosida and Okanoya, 2005; Brumm and Slater, 2007). Further, during signal production, the sensitivity of the signaller's sensory system may be temporarily suppressed due to intrinsic

mechanisms (Greenfield and Minckley, 1993), and may not effectively encode conspecific calls produced during or immediately following signal production. In response to simulated intruders, we found a pronounced inhibition of chirping at the time just after stimulus chirp delivery. Several neural mechanisms that underlie the inhibition of signal production while conspecifics signal during antiphonal behaviours have been proposed in acoustic species (Greenfield and Minckley, 1993; Kelley and Bass, 2010), although it is not known how chirping influences conspecific chirp production rates. At the periphery, chirps are encoded by changes in electroreceptor afferent population synchrony (Hupé *et. al.,* 2008 (Chapter 3)) and by pyramidal cells in primary electrosensory nuclei (Marsat and Maler; 2010). Future studies should examine how these sensory pathways interface with chirp production pathways.

### 4.4.4 Neuroethological Implications

Our findings suggest that chirp processing pathways have memory: fish integrate the patterns of chirps they produce with those from interacting conspecifics in order to facilitate adaptive aggressive responses during social interactions. Specific telencephalic pathways process electrocommunication signals (L. Maler, personal communication) and are likely involved in integrating chirp patterns during chirp exchanges. The neural mechanisms by which conspecific chirps selectively evoke or inhibit chirping in receiving fish are unknown.

Different patterns of chirp playbacks influence receiver behaviour in distinct ways, and there are also individual differences in the tendency of an individual to respond to a given pattern of signals. This suggests that individuals respond differentially to conspecific signals with context appropriate coordination in temporal precision and that the pattern of chirps produced may confer selective information about individual aggressive motivation during territorial

disputes. Despite stimulus-induced differences in averaged responses, there were also individual differences in chirping and aggressive behaviours in response to a given pattern of delivered chirps suggesting that sensory encoding of conspecific chirps influences chirp production but is not sufficient for explaining all of the variability in response characterized. Individual differences in chirping behaviours are likely a result of multiple, interacting factors including the dynamic neurophysiological states of interacting individuals that result from changes in levels of circulating and/or central neuromodulators (Maler and Ellis, 1987; Telgkamp *et. al.,* 2007), influenced by experience-dependent differences in behaviour (Salazar and Stoddard, 2009). Fish respond to given chirp patterns differentially, and these patterns change within an individual over the course of the interaction. The mechanisms that generate this context-dependent plasticity in chirp behaviour over the course of interactions remain to be elucidated. Chirps are patterned with aggressive physical displays over very short time scales and it is not known how this coordination occurs or how signals produced by one fish influence conspecific aggressive behaviours. Given the long history of work in this system, these questions can be readily addressed in future work.

### 4.4.5 Conclusions and Future Directions

#### *4.4.5.1 Conclusions*

Fish show individual differences in responses to playbacks with different chirp patterns. The majority of fish tend to echo the echoed playback chirps, however the echo response varied in response to random playback chirps. In response to randomly chirping playbacks, fish that don't echo tend to be more aggressive and have higher chirp rates. Echoing fish tend to be less aggressive and have lower chirp rates. They chirp and echo less over time. This is consistent with

principles of communication characterized in a number of territorial systems in which ritualized signal exchange behaviours are involved in individual assessment and in which dominant individuals tend to produce more signals be leaders and followers or echoers tend to be more subordinate (Greenfield and Minckley, 1993; Kelley and Bass, 2010). By signalling aggressive intent through patterned exchanges of chirps, individuals can dynamically assess the costs associated with different behavioural strategies (chirp patterns, flee or fight) during agonistic interactions based on the threat conveyed through the chirping behaviour of conspecifics.

*4.4.5.2 Future Directions*

There are intricate, context-specific temporal correlations between chirping and aggressive behaviours, and these relationships change over time. We are examining these relationships and are characterizing persistent individual differences in how fish pattern chirps with aggression (see Chapter 5). Future studies should deliver different modulation types (*e.g.* Type 1 chirps and AFRs) using a similar playback design. Ideally, playbacks should incorporate moving, multimodal stimuli (Amrhein and Lerch, 2010; Douglas and Mennill, 2010).

All behavioural responses are mediated by the underlying neural processing systems and characterizations of behavioural responses are necessary to guide questions and experiments aimed at exploring the neural computations underlying chirp reception and processing. Behavioural and electrophysiological studies could benefit from using such assays to characterize individual differences in chirping behaviours and to determine the neurophysiological mechanisms that underlie individual, experience, and context-dependent influences on chirp behaviour (Cox-Fernandes *et. al.*, 2010). Carefully designed chirp playback experiments should be used as a behavioural probe to explore the cognitive processes (memory,

learning, individual recognition, for example) involved in communication behaviours (Harvey-Girard *et. al.,* 2010). Characterizations of chirp behaviours reinforce the need to better understand the neural mechanisms that facilitate flexibility in behavioural response during social interactions that reinforce adaptive signal patterning behaviours over contextually relevant time scales.

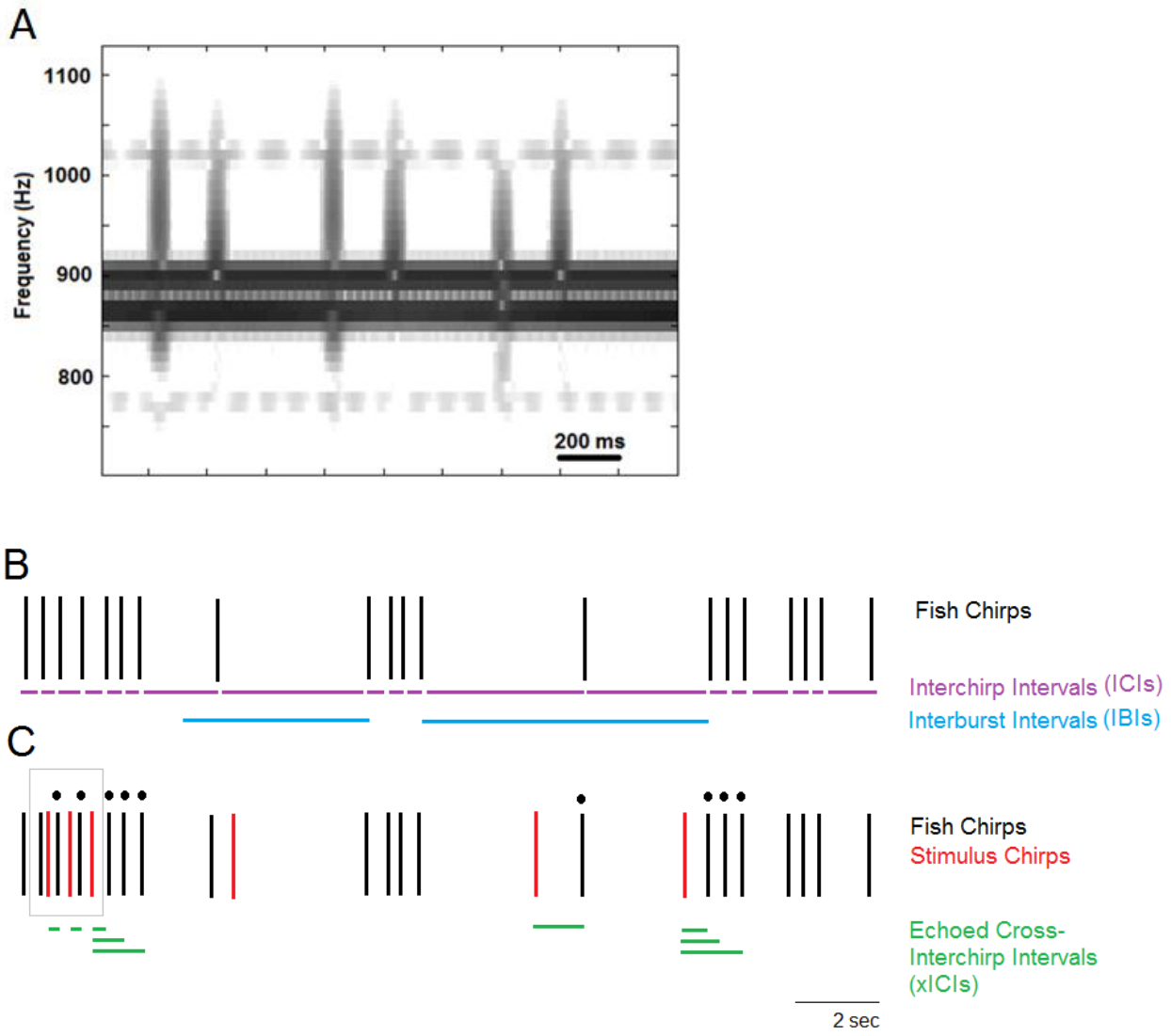



**Figure 4.1** Description of chirp patterning, interchirp intervals (ICIs) and cross-interchirp intervals (xICIs). **(A)** When electrical recordings are displayed as a spectrogram, the fundamental frequency of the real fish and stimulus EODs appear as two horizontal bands over the 2 sec duration shown. The stimulus EOD is the higher frequency of the two signals. Chirps appear as vertical frequency modulations; here three small chirps produced by the real fish, are echoed with a 200ms delay by three stimulus chirps. **(B)** ICIs (purple horizontal lines) characterize patterning within a single chirp time series (vertical black lines) and quantify the extent to which chirps occur in bursts. The intervals separating chirp bursts, interburst intervals (IBIs) are also

demonstrated (blue horizontal lines). **(C)** xICIs characterize patterning between the real fish chirp time series and the stimulus fish chirp time series (red vertical lines). The xICIs are shown (horizontal green lines) for all echoed chirps produced by the real fish (indicated with dots). The grey box shows the sequence of chirps illustrated in (A).

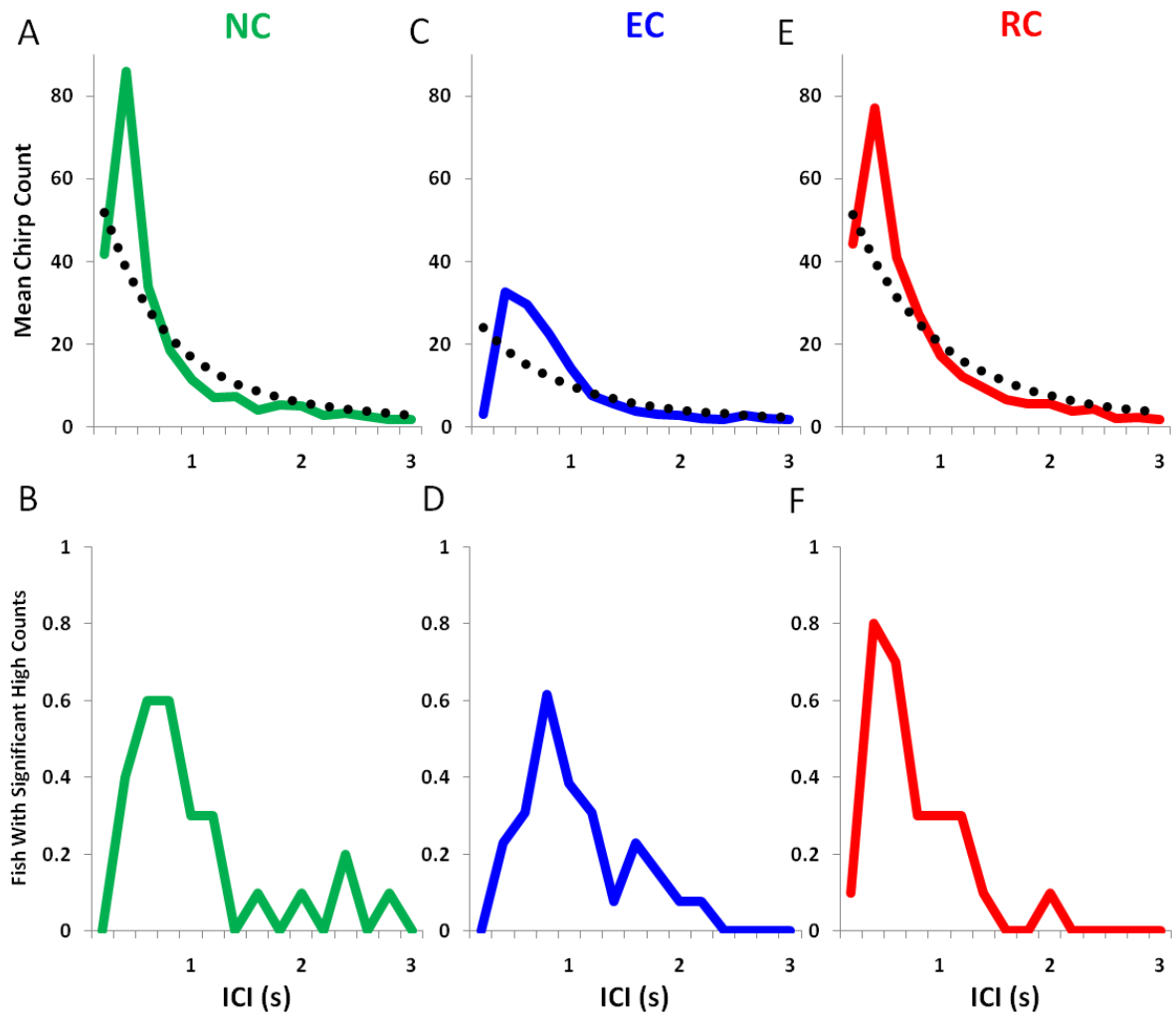



**Figure 4.2** Chirps are produced in bursts in response to simulated intruders. Average interchirp interval (ICI) histograms for **(A)** NC, **(C)** EC, and **(E)** RC playbacks for ICIs of less than three seconds. Observed mean counts are represented by the solid lines with corresponding mean-corrected Poisson distributions represented by the dotted lines. In all three scenarios the higher than expected short duration ICI counts indicate bursty chirp production patterns. The proportion of **(B)** NC, **(D)** EC, and **(F)** RC fish with ICI histogram counts significantly greater than 95% of distributions calculated using reiterative bootstrapping. The peaks denote the preferred ICI.

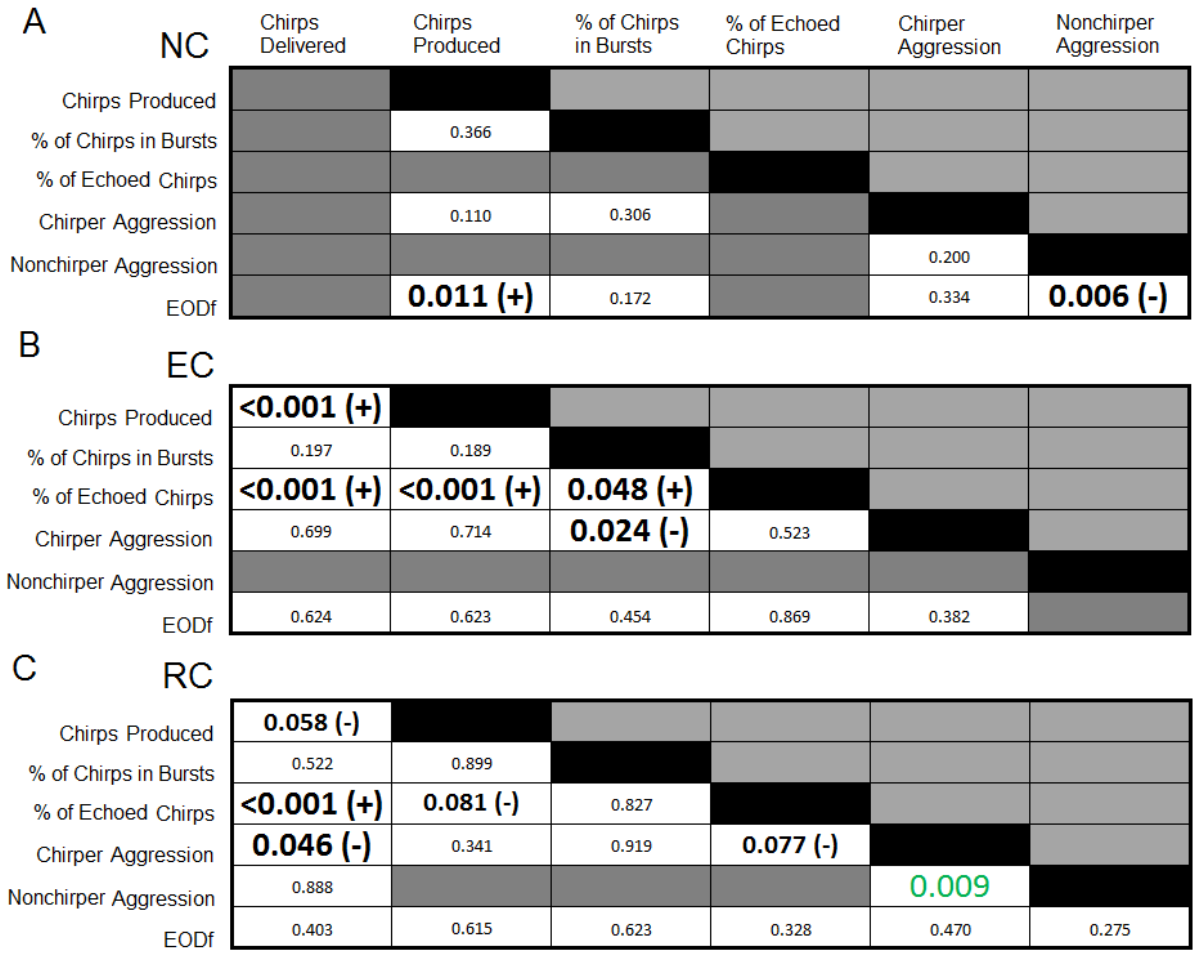



**Figure 4.3** Relationships between chirp delivery and production rates, burst and echo rates, aggression, and EODf. Regression p-values characterizing the relationships for **(A)** NC, **(B)** EC, and **(C)** RC playbacks are shown. Significant relationships ($p<0.05$) are represented in large fonts, and relationships where $0.05<p<0.10$ are shown in medium font. Positive relationships are indicated by (+) and negative relationships by (-). T-test p-values comparing Chirper and Nonchirper aggression are also shown (significant values in green).

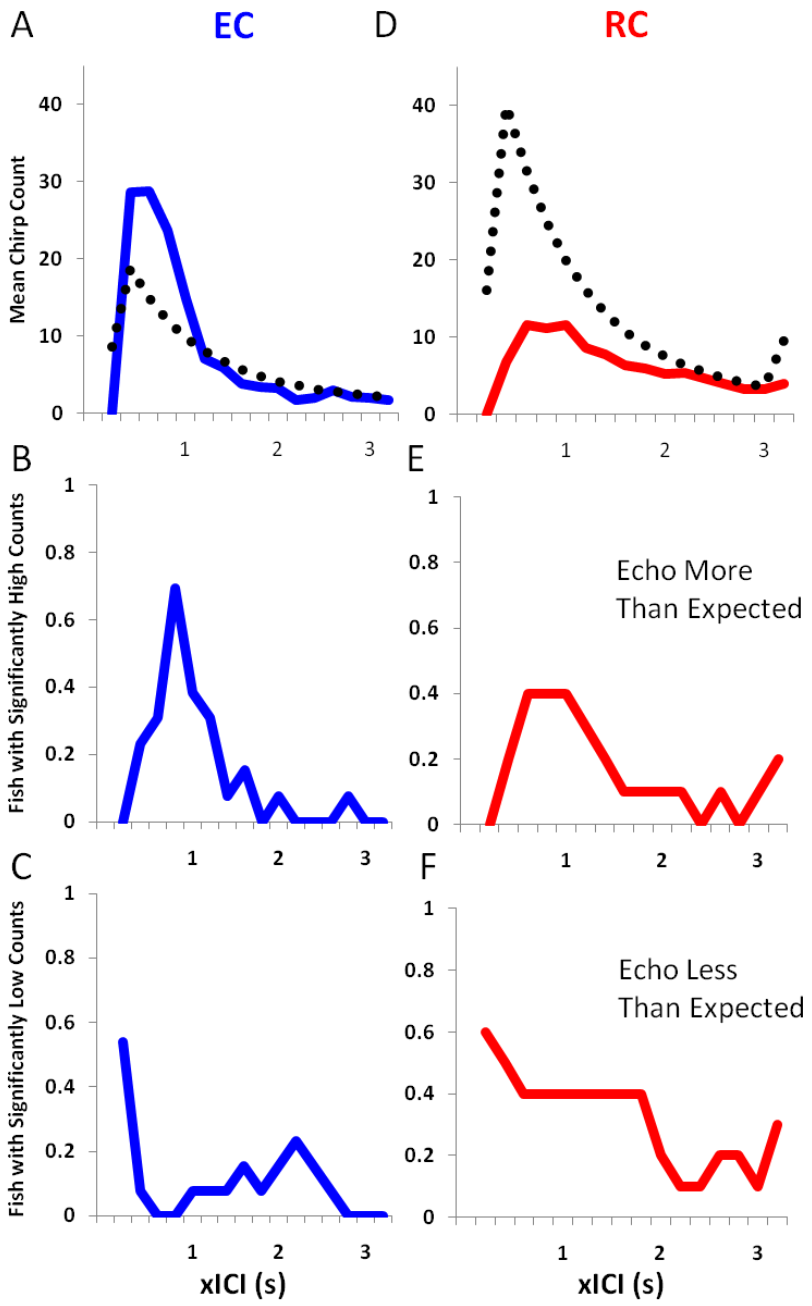



**Figure 4.4** Fish show a differential echo response to RC playbacks. Cross-interchirp interval (xICI) histograms for **(A)** EC and **(D)** RC scenarios for xICI latencies of less than 3 seconds. Observed mean counts are represented by the solid lines with corresponding mean-corrected Poisson distributions represented by the dotted lines (expected if chirps are produced in a random

pattern relative to the timing of the stimulus chirps). The proportion of trials in each scenario with xICI histogram counts significantly greater than 95% of distributions calculated by reiterative bootstrapping for **(B)** EC and **(E)** RC playbacks. The proportion of trials in each scenario with xICI histogram counts significantly less than 5% of distributions calculated by reiterative bootstrapping for **(C)** EC and **(F)** RC playbacks.

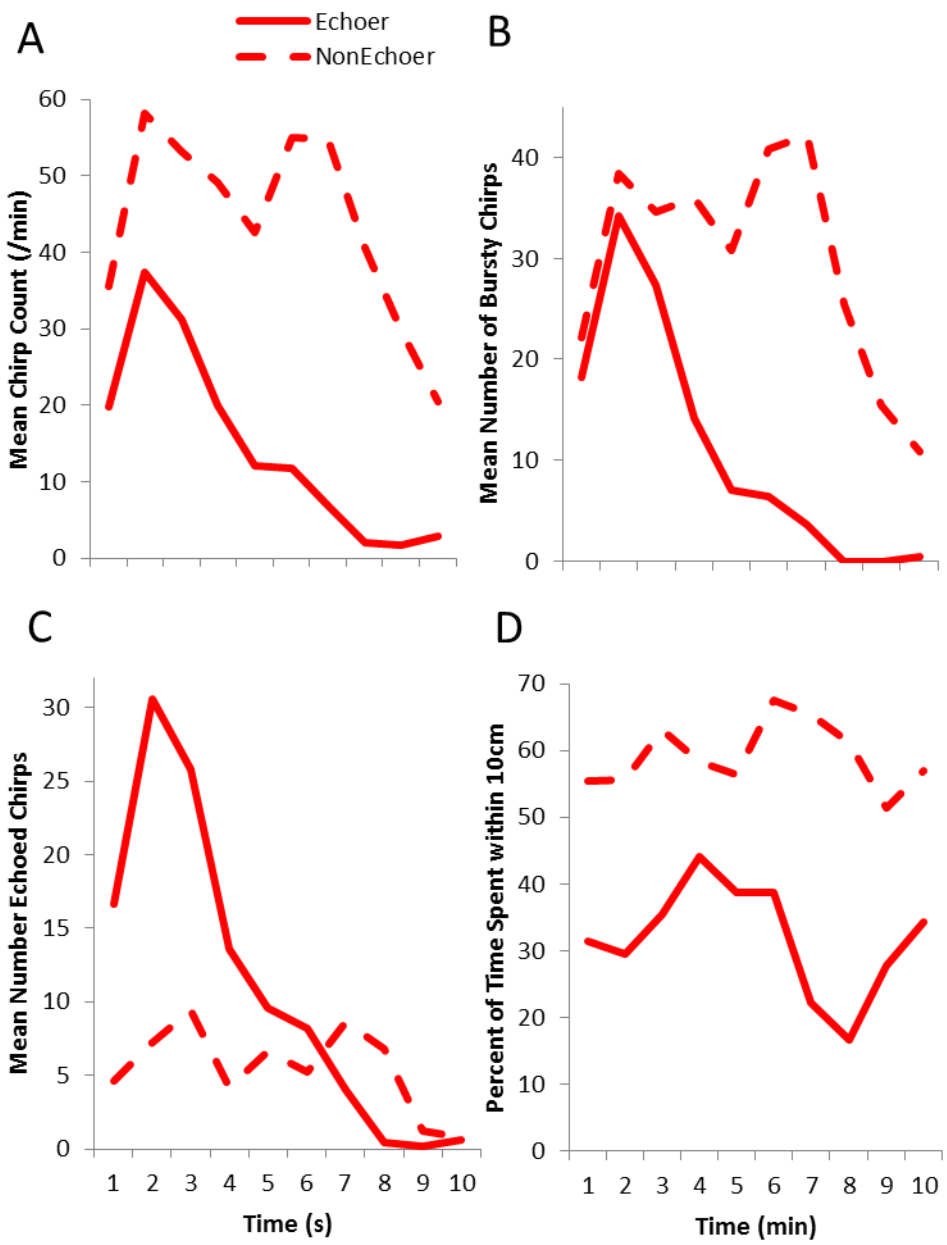



**Figure 4.5** Chirp, bursty chirp, and echoed chirp rates decrease over time in response to high rates of randomly delivered chirps. The average **(A)** chirp rates**, (B)** bursty chirp rates, **(C)** echoed chirp rates, and **(D)** aggression rate of echoing (solid line) and non-echoing (dashed line) fish in response to RC playbacks calculated per minute over the course of the trial. In all cases the chirp counts decrease significantly in the echoing fish.

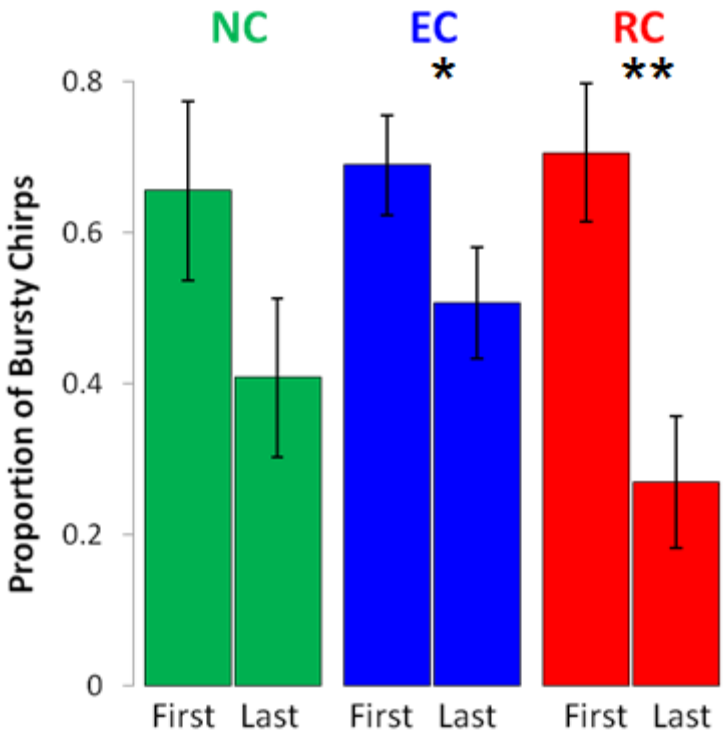



**Figure 4.6** Bursty chirp rates change over time. The mean proportion of chirps produced in bursts calculated in response to NC Chirpers (green), EC Chirpers (blue), and RC Chirpers (red) in the first and last two minutes of each trial are shown. Burst rates decrease in response to every stimulus type with the greatest decrease in response to RC chirps (NC: p=0.07, EC: p=0.04; RC: p=0.001, significant relationships are indicated with an asterisk).

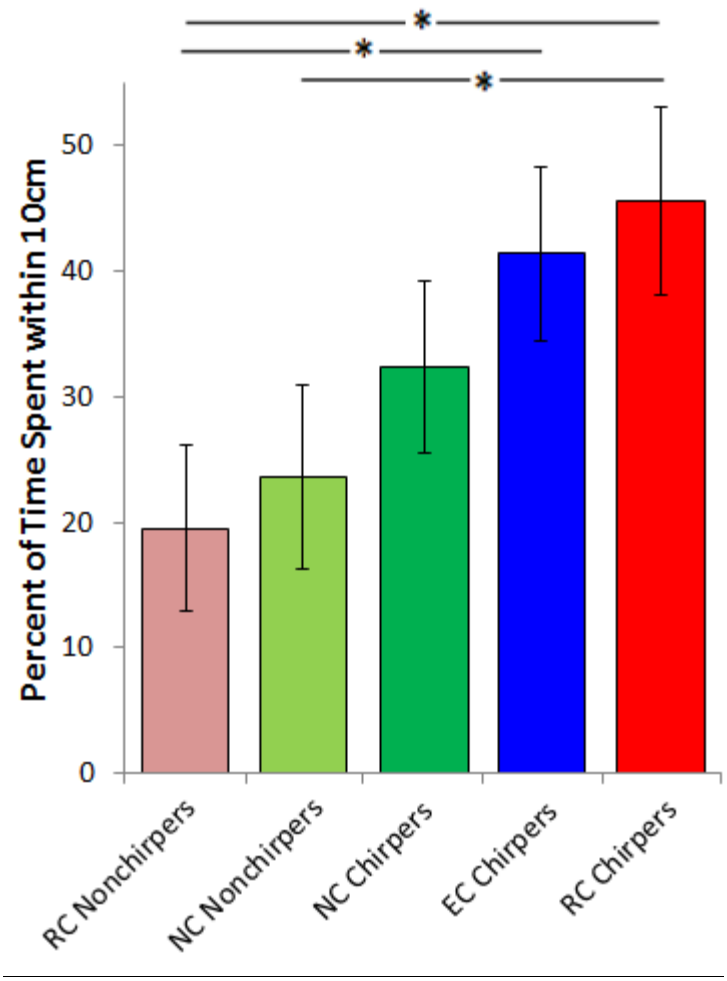



**Figure 4.7** Aggression rates of Chirpers and Nonchirpers. Aggression, as defined by the percent of time spent within 10 cm of the playback mimic, in response to NC (green), RC (red) and EC (blue) playback stimuli. RC Chirpers are significantly more aggressive than both NC Nonchirpers (p=0.04) and RC Nonchirpers (p=0.01). EC Chirpers are significantly more aggressive than RC Nonchirpers (p=0.02).

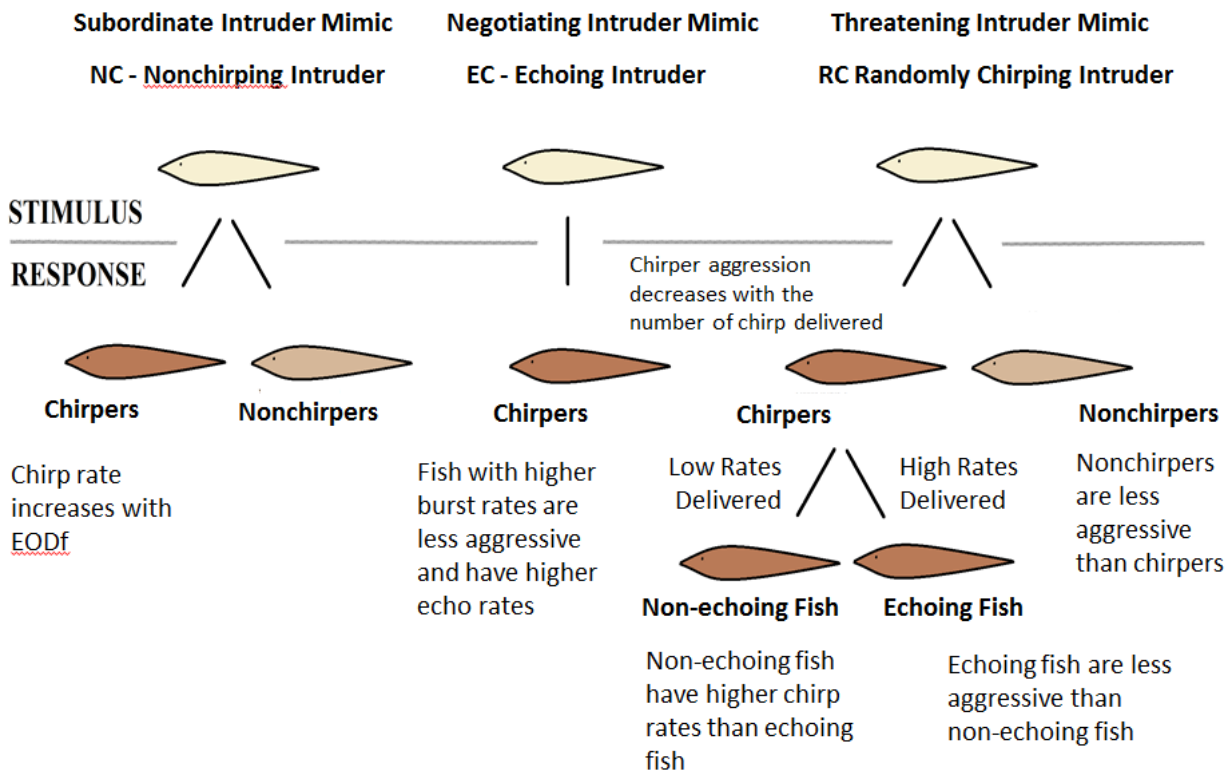



**Figure 4.8** Proposed threat value of different chirp stimuli and a summary of the chirping and aggressive responses they induce. When no chirps are delivered, chirp production rates correlate positively with EODf in Chirpers. In response to an echoing intruder, fish with higher burst rates are less aggressive. In response to randomly chirping playbacks with low rates of chirp delivery, chirpers chirp more, while echoing less than expected by chance, and respond with more aggression. In response to randomly chirping playbacks with high chirp rates, fish chirp less, but echo at a higher rate than expected, and respond with less aggression. We suggest that non-chirping fish signal subordinance, antiphonal signals or echoing fish convey intent to negotiate or a preference for assessment, whereas fish chirping at a high random rate are most threatening/aggressive.

Table 4.1 Individual stimulus and response parameters

| Fish | Scenario | Type 2 Chirps | Chirps Delivered | Burst Percent | Echo Percent | Percent Time Close |
|---|---|---|---|---|---|---|
| 1 | NC | 1003 | 0 | 70 | - | 40 |
| 2 | NC | 549 (15,4) | 0 | 78 | - | 70 |
| 3 | NC | 350 | 0 | 80 | - | 35 |
| 4 | NC | 259 | 0 | 71 | - | 64 |
| 5 | NC | 239 | 0 | 70 | - | 23 |
| 6 | NC | 60 | 0 | 73 | - | 25 |
| 7 | NC | 36 | 0 | 94 | - | 0 |
| 8 | NC | 34 | 0 | 85 | - | 27 |
| 9 | NC | 23 | 0 | 74 | - | 29 |
| 10 | NC | 21 | 0 | 71 | - | 10 |
| 11 | NC | 4 | 0 | - | - | 0 |
| 12 | NC | 2 | 0 | - | - | 50 |
| 13 | NC | 1 | 0 | - | - | 0 |
| 14 | NC | 0 | 0 | - | - | 51 |
| 15 | NC | 0 | 0 | - | - | 29 |
| 16 | NC | 0 | 0 | - | - | 56 |
| 17 | NC | 0 | 0 | - | - | 0 |
| 18 | NC | 0 | 0 | - | - | 59 |
| 19 | NC | 0 | 0 | - | - | 0 |
| 20 | NC | 0 | 0 | - | - | 35 |
| 21 | NC | 0 | 0 | - | - | 0 |
| 22 | NC | 0 | 0 | - | - | 3 |
| 23 | EC | 793 (36) | 705 | 75 | 94 | 44 |
| 24 | EC | 413 | 394 | 78 | 90 | 0 |
| 25 | EC | 233 (4) | 229 | 60 | 61 | 61 |
| 26 | EC | 189 | 183 | 58 | 57 | 73 |
| 27 | EC | 139 | 126 | 86 | 68 | 38 |
| 28 | EC | 90 | 89 | 67 | 48 | 25 |
| 29 | EC | 84 | 62 | 73 | 46 | 34 |
| 30 | EC | 73 | 67 | 63 | 37 | 30 |
| 31 | EC | 39 | 41 | 54 | 28 | 31 |
| 32 | EC | 32 | 24 | 56 | 13 | 62 |
| 33 | EC | 28 | 25 | 79 | 11 | 0 |
| 34 | EC | 28 | 24 | 61 | 25 | 78 |
| 35 | EC | 15 | 13 | 47 | 7 | 62 |

Table 4.1(Cont.) Individual stimulus and response parameters

| Fish | Scenario | Type 2 Chirps | Chirps Delivered | Burst Percent | Echo Percent | Percent Time Close |
|---|---|---|---|---|---|---|
| 36 | RC | 775 | 26 | 61 | 8 | 34 |
| 37 | RC | 763 | 42 | 74 | 14 | 80 |
| 38 | RC | 370 | 87 | 68 | 24 | 69 |
| 39 | RC | 285 | 213 | 80 | 72 | 61 |
| 40 | RC | 258 | 361 | 80 | 76 | 0 |
| 41 | RC | 243 | 13 | 69 | 3 | 59 |
| 42 | RC | 94 | 318 | 66 | 80 | 39 |
| 43 | RC | 62 | 547 | 79 | 86 | 24 |
| 44 | RC | 36 | 61 | 86 | 14 | 54 |
| 45 | RC | 25 | 531 | 44 | 80 | 36 |
| 46 | RC | 6 | 530 | - | - | 77 |
| 47 | RC | 5 | 527 | - | - | 22 |
| 48 | RC | 4 | 25 | - | - | 9 |
| 49 | RC | 1 | 527 | - | - | 1 |
| 50 | RC | 1 | 63 | - | - | 49 |
| 51 | RC | 1 | 61 | - | - | 46 |
| 52 | RC | 1 | 26 | - | - | 0 |
| 53 | RC | 0 | 588 | - | - | 0 |
| 54 | RC | 0 | 497 | - | - | 37 |
| 55 | RC | 0 | 492 | - | - | 0 |
| 56 | RC | 0 | 455 | - | - | 0 |
| 57 | RC | 0 | 365 | - | - | 0 |
| 58 | RC | 0 | 25 | - | - | 0 |
| 59 | RC | 0 | 24 | - | - | 52 |
| 60 | RC | 0 | 4 | - | - | 0 |

# Chapter 5

## Bursts of Chirps are Produced by both Restrained and Free Swimming Fish during Contrast Increases Associated with Approach Behaviours

## 5.1 Introduction

During territorial conflicts, potential rivals often produce bursts of signals that convey individual fighting ability and aggressive motivation (Parker, 1974), and in some cases, signal timing may be temporally correlated with aggressive manoeuvres (Naguib, 1999; Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)). Stereotyped signal patterns provide information for rival assessment and are often produced at high rates during particular signaller behaviours, during specific types of conspecific behaviours (including aggressive approaches and retreats), and/or during coordinated agonistic displays or physical interactions (Yosida and Okanoya, 2005; Amy *et. al.*, 2010). Repeatedly produced signals confer many functional advantages. Information about individual predispositions and motivations can be transmitted through rate modulations of repetitively produced signals (Greenfield and Minckley, 1993; Sih *et. al.*, 2004). Repetitively produced signals increase signal to noise ratios improving signal detectability under noisy conditions (Marler, 1955; Brumm and Slabbekoorn, 2005). Signal sequences can also serve as a 'beacon' signal to guide conspecific manoeuvres during territorial contests and other coordinated actions between pairs or larger groups of individuals (Schleidt, 1973; Richardson *et. al.*, 2008; Ursprung *et. al.*, 2009; Harcourt *et. al.*, 2010). Here we examine communication signal patterning in a model neuroethological species of weakly electric fish when one fish swims around a second, restrained, fish to explore the temporal relationships between bursts of discrete communication signals and aggressive behaviours.

The weakly electric brown ghost knifefish, *Apteronotus leptorhynchus*, produces an electric field (electric organ discharge, EOD) which is emitted continuously at an individually specific frequency. Objects, and other organisms in the fish's environment, perturb the EOD causing spatiotemporal modulations that are detected by an array of electroreceptors distributed

over the fish's body (Bennett, 1972; Caputi and Budelli, 2006). This active electric sense is used both in electrolocation and in electrocommunication. When two fish interact, their EODs combine and produce a complex eletrosensory signal at the skin surface of each fish (Kelly *et. al.*, 2008). The amplitude of this signal oscillates at a frequency equal to the difference in the EOD frequencies (EODfs) of the interacting fish (the 'beat' frequency). Because the strength of an individual fish's EOD decreases with distance (*e.g*. Knudsen, 1975B), this beat modulation also varies with an amplitude, (or "contrast") that is inversely proportional to the distance separating the two fish (Hupé *et. al*., 2008 (Chapter 3)).

Although the EODfs of individual fish remain fairly stable in time, during social interactions, fish modulate the frequency and amplitude of their EOD in stereotyped ways and these modulations are believed to serve as communication signals. The most common signal is called a chirp (Larimer and MacDonald, 1968; Zupanc *et. al.,* 2006) and during male agonistic interactions, one type, the small chirp (a 10-15ms frequency modulation of ~50-100Hz) is the most common (Zakon *et. al.,* 2002). Chirps are produced in individually and context-specific patterns during social interactions, and antiphonal bursts of echoed chirps tend to be produced during aggressive interactions similar to those observed in a number of acoustic territorial species (Greenfield and Minckley, 1993; Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2); Chapter 4). Chirp production rates and patterns are associated with a fish's aggressive behaviours and with the chirping and aggressive behaviours of interacting conspecifics (Hupé and Lewis, 2008 (Chapter 2)). When two male fish are free swimming in a limited space or when competing for a preferred shelter, they tend to produce bursts of echoed chirps at a distance from one another between bouts of aggression (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)). However, the pattern in which chirps are timed

relative to aggression is dependent on the context under which they are produced (Hupé *et. al.*, 2008 (Chapter 3); Fugère and Krahe, 2010; Chapter 4).

Communication signals convey information about sender motivation and influence receiver physiology and behaviour (Seyfarth and Cheney, 2003; Kelley and Bass, 2010), and different patterns of signals induce differential responses implying that successive signals induce cumulative effects in the receiver (Schleidt, 1973; Yosida and Okanoya, 2005). Electroreceptor afferents called P-units carry electrosensory information from electroreceptors to central electrosensory structures (Bennett, 1972). When electroreceptors receive beat stimuli, the activity (spiking rate) of P-units varies according to amplitude of the beat with the level of synchrony between P-units in a population determined by the contrast and beat frequency (Benda *et. al.*, 2005, 2006; Hupé *et. al.*, 2008 (Chapter 3)). When one fish in a pair of interacting fish produces a chirp, it results in a disruption of the beat and this causes a transient change in the P-unit population synchrony relative to its response to the beat. The P-unit response to conspecific chirps also depends on both the beat frequency and contrast (Benda *et. al.*, 2005, 2006; Hupé *et. al.*, 2008 (Chapter 3)). The ability to encode the EODs of conspecifics, their position and movement, and their chirps, may also depend on changes in contrast that are associated with movement.

Here we characterize the EOD contrast changes that are associated with aggressive behaviours (approaches and retreats) when one fish was restrained in an electrically transparent hammock while a second fish swam freely. We then correlated the chirping behaviours of each fish with these contrast changes. Our results show that bursts of chirps in both fish were temporally correlated with contrast changes. Restrained fish preferentially initiated chirp bursts as the free-swimming fish approached and the chirp rates of both fish correlated with the

aggression of the free swimming fish. When aggression levels were highest and the EODf of the free-swimming fish was greater than that of the restrained fish, the restrained fish tended to produce echoed chirps following approach behaviours, which in turn, were followed by retreats of the free-swimming fish. These results corroborate that chirp patterns, and their relationship with aggression, show individual and context-dependent variability as demonstrated in Chapters 2 and 4. Further, it demonstrates that restrained fish pattern bursts of chirps with movements of interacting fish and that the rate of chirps produced depends on conspecific aggression.

## 5.2 Methods

### 5.2.1 The Fish

Mature male and female *A. leptorhynchus* were obtained from a tropical fish supplier. Fish were kept in large flow-through community tanks with 0-4 tank mates, were fed thawed blood worms three times weekly, and were kept on a 12:12 hr light:dark cycle. All experiments were performed within the first few hours of the dark stage in the light cycle in a tank measuring 30x30cm with a depth of either 4cm or 10cm. All experiments were approved by the University of Ottawa Animal Care Committee.

### 5.2.2 Experiments with Two Restrained Fish: Preliminary Measurements of Contrasts

To characterize the contrasts produced while two fish are stationary and positioned at various distances from one another, we restrained two fish in hammocks oriented parallel to one another and systematically modulated the distance separating them. The hammocks were fashioned by creating rectangular tulle holders measuring 15cm long and 6cm deep, closed along the top edge with Velcro. The fish showed no visible discomfort, and as in other restrained conditions, produced chirps readily (Hitschfeld *et. al*., 2009). We recorded the electrical potential using a pair of electrodes attached to the hammock and positioned adjacent to one fish and calculated the contrasts (as described below) produced while the fish were positioned 5, 10, 15, 20, 25, 30 ,40 and 50cm apart and aligned parallel to one another.

### 5.2.3 Experiments with One Restrained Fish and One Free Swimming Fish

In a total of 12 trials (Table 5.1), we restrained one fish in a hammock (the restrained fish, 'RFish') in the center of an experimental tank while a second fish (the free swimming fish,

'FSFish') was allowed to swim freely in the tank around it. In eight of the trials we recorded electrical signals for five minutes and correlated chirp production rates and patterns with contrast means and contrast modulations. In the remaining four trials, we videotaped the interactions for five minutes using an infrared (IR) video camera positioned above the tank (with the tank illuminated from below using an IR light panel) to record physical behaviours of the FSFish over the course of the interaction while simultaneously acquiring electrical recordings.

### 5.2.4 Electrical Recordings

Electrical recordings sampled at 50kHz were acquired using Teflon-coated silver wire electrodes, an AM Systems differential amplifier, model 1700 (Carlsborg, WA, USA), and a real-time A/D processor (D1104, ControlDesk) controlled by Simulink software in Matlab (The Mathworks). Electrical recordings were collected from two sets of recording electrodes each trial. We positioned one pair of recording electrodes near the head of the RFish at the level of the operculum with the tips of the electrodes positioned 1cm apart, perpendicular to the axis of the RFish (with one tip immediately adjacent to the skin). The position of this pair of electrodes was chosen to sample the composite electrical image received near the surface of the RFish's body (*i.e.* available to nearby electroreceptors) during conspecific movements. The electrical signal recorded here was primarily composed of the RFish's EOD, and the influence of the FSFish's EOD increased whenever the FSFish moved closer to the RFish. We positioned the second pair of electrodes on the tank walls (near diagonally-opposite corners) to obtain a signal that was not dominated by the RFish's EOD.

We determined contrasts using methods described in Yu *et. al.* (in preparation) to examine temporal changes in contrast resulting from movements of the FSFish and their

correlations with RFish and FSFish chirp production rates. From the recordings, we calculated the contrast, defined as the depth of the beat modulation relative to the mean amplitude of the EOD, over 100ms bins. We distinguished contrast bumps as any period over which the instantaneous contrasts remained over 0.04 for two or more consecutive 100ms bins.

### 5.2.5 Behavioural Responses

#### *5.2.5.1 Chirping Responses of the RFish and FSFish*

Chirps were characterized using spectrograms of the electrical recording generated in Matlab and were defined as small, large, and long chirps based on their duration and the magnitude of their associated frequency excursion (Zupanc *et. al.*, 2006). The times of chirps produced by the RFish and FSFish were recorded separately by visual inspection of the spectrograms and we created chirp time series for each fish with 100ms resolution. To characterize the pattern of chirps produced by RFish and FSFish and determine whether chirps were produced in bursts as they are in other restrained and free-swimming conditions (Chapter 2, Chapter 4), we calculated interchirp-interval (ICI) histograms for the chirping male RFish and FSFish and compared these to the distributions expected based on Poisson processes of the same mean rate). Further, we determined the number of chirps produced in bursts (defined as any falling within two seconds of another chirp), the mean length of bursts produced, and the total number of bursts produced per trial for male chirping RFish and FSFish. There were only two trials in which both the RFish and FSFish produced more than ten chirps (Table 1), and for these we calculated the number of echoed chirps produced by both the RFish and FSFish. We defined echoed chirps as any that occurs in the two seconds following a chirp produced by the other fish.

*5.2.5.2 Approach Behaviours of the FSFish*

From the four videotaped trials, we tracked the position of the FSFish's head, body centre, and tail with 100ms resolution from the video recordings using Videopoint tracking software. From these positions we calculated the distance separating the first pair of electrodes and each body part and extracted information about the FSFish's position, orientation and movement relative to the RFish over time. We temporally correlated these with the contrasts calculated from the electrical recordings.

### 5.2.6 Correlations between Chirps and Contrast

To determine if the chirp rates of the RFish and FSFish correlate with the amount of time the FSFish spends in close proximity to the RFish, we compared chirp rates with mean contrasts. As is observed in other experimental contexts (Zupanc *et. al.*, 2006; Hupé and Lewis, 2008 (Chapter 2)), when one fish is restrained, fish pattern bursts of chirps with aggression. We used Receiver-operating characteristic (ROC) analysis to examine the robustness of the relationship between the timing of chirps and contrast bumps. ROC is a 'detection' or 'prediction' analysis method that can be used to determine the accuracy of temporal patterning predictions determining the occurrence of one event given the other, and how errors vary with decision criterion (Green and Swets, 1966).

To explore the temporal patterns between chirping and contrast changes further, we calculated the average instantaneous contrasts centered at the time of all chirps produced by the RFish and FSFish. For each fish, we determined whether chirps were produced as part of a burst (occurring within two seconds of another chirp) or produced as an isolated chirp (not occurring with two seconds of any other chirp), and also calculated the mean instantaneous contrast for five

seconds before and after the timing of all bursty and isolated chirps and of all chirps leading and finishing chirp bursts. We calculated the number of trials with observed contrasts significantly greater than expected using bootstrapping methods as described in Chapters 2 and 4.

## 5.3 Results

### 5.3.1 Contrast Characterizations

When two fish are restrained and aligned parallel to one another, the amplitude of the composite electrical signal recorded adjacent to one fish results from the combination of its EOD with that of the nearby fish. The amplitude of this combined electric signal oscillates at a frequency equal to the difference between the EODfs of the two interacting fish, the difference frequency Df, and produces a beat, or first order amplitude modulation, of the composite carrier signal (Fig. 5.1A, red line). When two fish are in fixed positions relative to one another, the magnitude of this oscillation, the contrast (Fig 5.1A, blue line), does not change and it is inversely proportional to the distance separating the two fish (Fig. 5.1B).

When only one fish is restrained in a hammock (RFish), the contrast changed in time as the free-swimming fish (FSFish) swam around and changed its position relative to the recording electrodes positioned next to the RFish. This produced an amplitude modulation of the beat (or a second order amplitude modulation; Fig. 5.1C).

### 5.3.2 Movements Produce Contrast Changes

To explore the temporal relationships between contrast and the distance separating interacting conspecifics, we show in Fig. 5.2A the instantaneous contrast (top) and the inter-fish distances (RFish's head to the FSFish's head (red), body center (green), and tail (blue)). The temporal correlation between distance decreases and contrast increases are pronounced (Fig. 5.2A, Box 2), although some discrepancies exist (Fig. 5.2A, Box 1). The two consecutive contrast bumps in Box 2 occurred simultaneously with the two large decreases in the distance separating the RFish and FSFish. Figure 5.2B (panel 2) shows that these distance minima result

from the FSFish swimming backwards circling the FSFish, and twice passing particularly close to the recording electrodes (denoted in red). The smaller contrast bump in Box 1 was not associated with a pronounced change in the distances separating the RFish and FSFish. Figure 5.2B (panel 1) shows that this smaller contrast increase was associated with a behaviour in which the FSFish turns around and changes direction.

To characterize the contrast increases associated with approaches that occur over time we distinguished unchanging contrasts from large transient increases, or bumps, in contrast (Fig. 5.3A). To characterize the temporal distribution of contrast bumps, we calculated the average autocorrelation of the contrast bumps and found that the distribution matched a Poisson distribution with the same mean (Fig. 5.3B) suggesting that approach behaviours occurred in a random pattern over time. The mean bump duration was 3.2±0.7 seconds. There was no change in the mean contrast calculated per minute over the course of each trial (ANOVA, $p>0.99$, $n=12$).

### 5.3.3 Chirp Characterizations

#### *5.3.3.1 Chirp Types Produced*

Considered over all trials, the RFish produced a total of 817 small chirps and 27 large chirps and the FSFish produced a total of 383 small chirps and 4 large chirps (Table 5.1). Male RFish and FSFish did not differ in their small chirp production rates (Fig. 5.4A; RFish: 68.1±18.9; FSFish: 47.9±24.1, t-test, $p=0.26$) however, RFish produced significantly more large chirps than did FSFish despite low production rates (Fig. 5.4A; RFish: 2.3±0.9; FSFish: 0.5±0.5; t-test, $p=0.05$). This is consistent with the suggestion that large chirps function to signal subordinance during aggressive interactions (Hagedorn and Heiligenberg, 1985; Cuddy *et. al*., 2011; Chapter 2

Addendum). Because large chirps were produced so infrequently, only small chirps will be considered in the following analyses.

The mean chirp rates (calculated per 30 seconds) of RFish and FSFish do not change significantly over the five minute trials (ANOVA, RFish: p=0.86, N=10; FSFish: p=0.92, N=5). However, during the first 30 seconds, male RFish produce significantly more chirps than male FSFish (RFish: 7.0 ± 2.7, N=12; FSFish: 1.6 ± 0.7, N=8, t-test, p=0.03). Calculated over the entire trial, the number of chirps produced by male RFish and FSFish do not correlate with one another ($R^2$=0.01, p=0.82, N=8).

*5.3.3.2 FSFish Small Chirp Rates depend on the Beat Frequency*

Chirp encoding is influenced by the beat frequency (or difference frequency, Df), or the difference between the EODfs of interacting fish. Here the Df is defined as the EODf of the FSFish relative to that of the RFish. The number of chirps produced by the RFish (N=12) does not depend on the fish's own EODf ($R^2$=0.08, p=0.39), on the EODf of the FSFish ($R^2$=0.05, p=0.48), or on the Df ($R^2$=0.002, p=0.87). The number of chirps produced by male FSFish (N=8) does not depend on the fish's own EODf ($R^2$=0.18, p=0.18) or on the EODf of the RFish ($R^2$=0.07, p=0.42), but it does correlate significantly with the Df (Fig. 5.4B; $R^2$=0.38, p=0.03). FSFish only produced more than five chirps when their EODf was higher than that of the RFish, a behavioural response that indicates fish discriminate the sign of Df even when Dfs are large.

*5.3.3.3 Burst Characterizations*

To characterize whether RFish and FSFish pattern their chirps in discrete clusters during these constrained interactions, we considered the distribution of interchirp intervals (ICIs). The averaged interchirp interval (ICI) histograms of both the RFish and FSFish show that the chirp

ICI distributions differ significantly from the corresponding mean-corrected Poisson distributions (Fig. 5.5A,B) indicating that small chirps are produced in bursts in both male RFish and FSFish.

We defined bursts as sequences of chirps separated by ICIs of less than 2 seconds (this corresponds to the time at which the observed ICI distribution crossed the mean-corrected Poisson distribution in the majority of trials). We recorded the times of all chirps that occur in bursts ('bursty chirps'), and of those that don't occur within two seconds of any other chirp ('isolated chirps'). The mean number of bursts produced (RFish: 18.4 ± 1.3, FSFish: 16.8 ± 5.1; t-test, p=0.39), number of chirps per burst (RFish: 5.1 ± 0.8, FSFish: 4.0 ± 1.0; t-test, p=0.21), and burst duration (RFish: 4.0 ± 0.6sec, FSFish: 3.0 ± 0.6; t-test, p=0.14) do not differ between RFish and FSFish. Calculated over all fish, the percent of chirps produced in bursts increases with the number of chirps produced ($R^2$=0.64, p=0.003).

To determine whether the sequence of chirp bursts is itself patterned, we calculated the intervals between bursts (the inter-burst intervals, IBIs) and compared the distribution of IBIs to the Poisson mean corrected distribution. Chirp bursts tend to be produced in a distribution that deviates from the distribution of a Poisson process with the same mean number of bursts (Fig. 5.5C; $X^2$=154.6, N=9, p<0.001) suggesting that chirp bursts tend to occur in bursts throughout the interaction between RFish and FSFish. The mean number of chirps produced in bursts does not change over time for both the RFish and FSFish (ANOVA, RFish: p=0.43, N=7, FSFish: p=0.89, N=4). As was the case with the number of chirps produced, the percent of chirps produced in bursts is not significantly correlated with the Df (RFish: $R^2$=0.005, p=0.88, N=12, FSFish: $R^2$=0.25, p=0.50, N=12)

*5.3.3.4 Echoed Chirp Responses*

In only two of the 12 twelve trials (or 8 male-male trials) did both the RFish and FSFish produce more than ten chirps each. For these two trials we calculated the percent of echoed chirps (those following a chirp by the other fish with a latency of less than two seconds) produced by both the RFish and FSFish. In the first trial (Trial 1), 81.7% of the RFish's chirps echoed those of the FSFish, and 64.7% of the FSFish's chirps echoed those of the RFish. In the second trial (Trial 2), the RFish echoed only 25.6% of the FSFish's chirps, and the FSFish echoed 31.0% of the RFish's chirps. The difference in the mean percent of echoed chirps for the RFish and FSFish in these two trials is borderline non-significant (t-test, p=0.06). In the following section we will explore how these differences in chirping and echoing behaviours are associated with differences in the mean trial contrast and temporally with different instantaneous contrast changes.

**5.3.4 Comparisons of Chirps and Contrast**

During dyadic interactions bursts of chirps are produced between bouts of aggression when the fish are at a distance from one another (Chapter 2). In response to playbacks, fish pattern their chirps with aggression depending on the stimulus received (Chapter 4). Chirps appear as pronounced and transient modulations of the contrast; this is due to the amplitude and frequency changes that occur in the chirping fish's EOD (Yu *et. al.*, in preparation). In the following sections, we will examine the relationship between chirping and contrast when one fish is restrained and a second is freely swimming. The saliency of the electric signal modulation resulting from a chirp depends on the dynamic contrast and this experimental setup presents an

ideal situation for exploring how chirps received during conspecific movements influence behaviour.

*5.3.4.1 Chirp Rates and Contrast Means*

RFish and FSFish fish tend to produce more chirps during trials when the FSFish is closer, in trials with higher mean contrasts (Fig. 5.6, $R^2$=0.64, p=0.002). Although the relationship between chirp rate and mean contrast is not-significant for RFish chirp counts ($R^2$=0.27, p=0.08), the chirp rates of the FSFish correlate significantly with mean contrast ($R^2$=0.39, p=0.03).

*5.3.4.2 Bursting and Contrast Means*

There are no significant relationships between the percent of chirps produced in bursts and the mean contrast (RFish: $R^2$=0.08, p=0.53; FSFish: $R^2$=0.79, p=0.11; pooled: $R^2$=0.28, p=0.09). However there is a positive correlation between the total number of bursty chirps produced by the RFish and FSFish combined and the mean contrast (RFish: $R^2$=0.23, p=0.27; FSFish: $R^2$=0.78, p=0.12; pooled: $R^2$=0.54, p=0.03) and a significant relationship between the total number of bursts produced and the mean contrast (RFish: $R^2$=0.42, p=0.12; FSFish: $R^2$=0.86, p=0.07; pooled: $R^2$=0.41, p=0.03). The mean burst duration and mean number of chirps produced per burst by RFish and FSFish do not correlate significantly with mean contrast (p>0.05).

### 5.3.5 Chirp and Contrast Temporal Correlations

During dyadic interactions, and in response to playbacks, the timing and patterns of chirps produced are temporally associated with aggression and these relationships depend on the

context. Free-swimming, male *A. leptorhynchus* tend to produce chirps in between bouts of aggression (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)). In response to simulated intruder playbacks, fish tend to pattern their aggressive behaviours and approaches with bursts of chirps in a manner that is dependent on the chirping style of the simulated intruder (Chapter 4). When one fish is restrained, RFish and FSFish chirps and chirp bursts tend to be timed with contrast bumps, consistent with the observations during playback studies (Fig. 5.7A). Chirp bursts are temporally correlated with instantaneous contrasts increases.

We characterized the sensitivity of the temporal relationship between chirp burst and contrast bump timing using ROC (Receiver-operating characteristics) analysis with conditions defined in Fig. 5.7B. The rate of true positives, or the probability of having a chirp at the same time as a contrast bump less the probability of a chirp in the absence of a contrast bump (calculated over 100ms bins), is plotted against the rate of true negatives, or the probability of having no chirp when there is no contrast bump less the probability of having no chirp in the presence of a contrast bump. When points fall above the diagonal it indicates that chirps (and chirp bursts) are temporally correlated with contrast bumps. Chirps are produced during contrast bumps in both RFish (Fig. 5.7C) and FSFish (Fig. 5.7D) at rates higher than expected based on chance.

To investigate if the timing of bursty and isolated chirps produced by the RFish and FSFish are correlated with particular contrast changes we calculated ”chirp-triggered average contrasts” to determine the average instantaneous contrasts preceding and following each chirp for five seconds in each direction. Figure 5.8 shows the average contrasts for five seconds before and after the production of all bursty chirps (Fig. 5.8A), isolated chirps (Fig. 5.8D), and chirps starting (Fig. 5.8B) and ending (Fig. 5.8C) bursts, produced by the RFish (black lines) and FSFish (grey lines). The percentage of male trials (RFish n=12, FSFish n=8) with observed

average contrasts significantly greater than (Fig. 5.8E-H), or less than (Fig. 5.8I-L) iterations were also calculated for the four types of chirps characterized. The average instantaneous contrast centered at the time of RFish bursty chirp production shows a bump with a peak around the time of chirp production (Fig. 5.8A). This trend is significant in 4 of the 12 RFish males tested (or 4 or the 7 RFish males that produced chirps in bursts; Fig. 5.8E). The mean contrast centered at the chirps that start and end bursts suggest that bursts of RFish chirps are initiated during FSFish approaches, when contrast is increasing (Fig. 5.8B), and terminate during FSFish retreats, when contrast is decreasing (Fig. 5.8C). The mean contrast is significantly greater than expected at the time of chirps that start bursts and in the time following their production (Fig. 5.8F). Further, the mean contrast tends to be greater than expected in the time preceding the timing of the last chirp in a burst (Fig. 5.8G), and significantly less than expected for a short time afterwards (Fig. 5.8J).

For the two trials with sufficient counts to examine the echo response, we calculated the average contrast for five seconds before and after each echoed chirp for the RFish and FSFish separately (Fig. 5.9A,B). The trial in which the RFish produced the highest percent of echoed chirps (87.1%, Trial 1) was the trial with the highest mean contrast, and the EODf of the FSFish was 88Hz higher than that of the RFish. The echoed chirp-centered averaged contrasts for the RFish and FSFish show variability in how the RFish and FSFish echoed chirps are patterned with contrast. We calculated whether the observed counts varied significantly for each of these echoing fish. For both of the restrained fish, echoed chirps tended to be produced following greater than expected contrasts (Fig. 5.9C). The pattern was not as strong for FSFish (Fig. 5.9D) suggesting that RFish echoes are patterned more with FSFish aggressive movements than are echoed chirps produced by the FSFish.

## 5.4 Discussion

### 5.4.1 Summary

*A. leptorhynchus* males produce bursts of chirps in a variety of social contexts and in response to playbacks of unmodulated EODs and EODs containing frequency and amplitude modulations (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2); Gama Salgado and Zupanc, 2011). During aggressive interactions, conspecifics often engage in rapid circling and chasing behaviours (Triefenbach and Zakon, 2008), and these movements may impede conspecific detection and localization (Yu *et. al.*, in preparation). Here we show that contrasts are influenced by the distance separating two fish (Fig. 5.1) and that changes in contrast are produced by swimming movements that modulate the distance separating the two fish (Fig. 5.2). Contrast bumps are associated with approach behaviours and these occur in a sequence approximated by a Poisson distribution suggesting that approaches by the FSFish tend to occur in a random sequence, potentially influenced by the chirping behaviour of the RFish (Fig. 5.3). Both RFish and FSFish preferentially produce small chirps, and RFish produce more large chirps than FSFish reinforcing the hypothesis that large chirps function to signal subordinance and that small chirps are produced most commonly during aggressive interactions (Fig. 5.4). As observed in a number of other experimental contexts, fish tend to produce small chirps in discrete bursts (Fig. 5.5). Higher chirp counts are associated with higher mean contrast (Fig. 5.6). Further, bursts of chirps tend to be temporally associated with an increase in contrast, suggesting that both FSFish and RFish chirp rates increase when the FSFish approaches (Fig. 5.7). During aggressive trials with high chirp rates, and when the FSFish have higher EODfs than the RFish, the RFish tend to pattern bursts of chirps with FSFish approaches. The result is that the chirps at the start of chirp bursts tend to be produced during contrast increases and chirps at the end of

bursts tend to be produced during contrast decreases (Fig. 5.8). Individual differences in echo responses were also pronounced and echoing RFish responding to the trials with the highest contrasts also produced the greatest number of echoed chirps and produced them preferentially in the time following contrast increases and preceding contrast decreases (Fig. 5.9).

### 5.4.2 Comparison to Other Studies on Chirping

Both chirp rates and patterns may convey aggressive information and these are influenced by individual differences in behavioural tendency and the context under which they are produced (Dunlap *et. al.*, 2002; Sih *et. al.*, 2004; Chapter 4). In dyadic situations, fish tend to produce bursts of echoed chirps while they are at a distance from one another, between attacks (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)). During EOD playbacks, free-swimming males produce chirps preferentially when they are in close proximity to the stimulating electrodes (Fugère and Krahe, 2010). During chirp playback experiments, fish pattern their approaches with chirps, bursts of chirps, and echoed chirps in a fashion that is dependent on the chirping strategy of the simulated intruder (Chapter 4). When one fish is restrained, both the RFish and the FSFish produce bursts of chirps preferentially when the mean contrast is higher. Higher contrast signals occur when the distance separating two fish are smaller, producing combined signals with larger signal intensities. In response to chirp chamber presentation of sine waves delivered at different stimulus intensities, chirp rates correlated strongly with signal intensity, producing chirps at the highest rate in response to signal intensities corresponding to small inter-fish distances (Zupanc and Maler, 1993; Engler and Zupanc, 2001).

FSFish tend to produce more chirps and be more aggressive when they have a higher EODf than the RFish. A reanalysis of the dyadic data presented in Chapter 2 provides additional

evidence that fish discriminate the sign of the Df (Fig. 5A.1). When paired with a second male, chirp rates are correlated with the Df only when the chirping fish has the lower of the two EODfs (Positive Dfs: $R^2$=0.05, p=0.61; Negative Dfs: $R^2$=0.48, p=0.06), These sign-selective responses have important implications for both the behavioural interpretations of the social relevance of individual EODf and chirp rates in signalling aggressive differences, and for interpretations of how sensory systems encoded the sign of beat frequency when Dfs are large.

Determining sign of Df requires information regarding the amplitude of the beat (P units) and phase of the beat (T-units) within each cycle. Rotations of amplitude modulation and differential phase plots uniquely determine the sign of the Df (Heiligenberg and Partridge, 1981; Heilegenberg and Bastian, 1984). For high frequency beats (`100Hz+) circular trajectory of amplitude and differential phases planes are predicted to be too small for Df sign discrimination. It is not known how this computation could be carried out by electrosensory circuitry. As discussed in Chapter 3 (Hupé *et. al*., 2008)) one function of chirps could be to help discriminate the sign of the Df because chirp production results in a transient beat acceleration or deceleration depending on the sign of the Df.

These individual and context-dependent differences in the relationships between pattern of chirps and physical behaviours suggest that chirping behaviours, and their relationship with aggression, are flexible and dependent on the context under which they are produced. Further it suggests that fish dynamically modulate their chirping behaviours according to the degree of threat perceived during real or simulated interactions.

### 5.4.3 Potential Interpretations of the Association of Chirp Bursts with Contrast Increases

The extent to which individuals produce communication signals and pattern them with specific aggressive behaviours depends on the context under which they are produced and with differences in individual motivations (Amrhein *et. al.*, 2010; Douglas and Mennill, 2010). The differences in the precision with which the RFish and FSFish pattern their chirps with particular behaviours may reflect both the differences in threat perceived by the RFish and FSFish, and the differential ability of the RFish and FSFish to exert an influence on the other fish's movement. The RFish is more vulnerable to threat than the FSFish and the RFish can potentially influence the FSFish's movement; whereas the RFish poses no threat to the FSFish and FSFish chirps cannot influence the RFish aggression because it is unable to move. We suggest that the RFish may increase its chirp rate when contrasts increase during aggressive trials to attempt to exert an influence on FSFish aggressive behaviours by indicating a willingness to negotiate or to attempt to appear more threatening (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2); Chapter 4). An alternative, but not mutually exclusive, interpretation of these results is that by increasing its chirp rate, the RFish may be attempting to induce an antiphonal response in the FSFish to promote a reciprocated negotiation to mitigate potentially costly aggressive escalations (Greenfield and Minckley, 1993; Yosida and Okanoya, 2005). Further, the increase in RFish chirp rate in response to perceived threats, may function not only to communicate aggressive capability and an attempt to negotiate, but to induce a tonic or graded influence on the receiver (FSFish) physiology, and potentially modify aggressive behaviour (Schleidt, 1973; Kelley and Bass, 2010).

Increases in signalling rates may also function to overcome noise induced by movements to promote effective conspecific localization during dynamic interactions (Marler, 1955; Schleidt,

1973; Brumm and Slabbekoorn, 2005; Ruxton, 2009). Animals often modify signalling behaviours to increase detectability of relevant social signals amongst noisy fluctuations. Sources of noise include environmental fluctuations (for example induced by air and water flow patterns), perturbations by objects in the environment, movement-generated distortions or noise, and interference produced by other conspecific and heterospecific signals (Potash, 1972, Forrest, 1994). Movement-induced noise may make conspecific localization more difficult (Ruxton, 2009) and it is not known whether repetitively produced signals may also serve to overcome movement imposed noise in the cues available for conspecific localizability. While interacting *A. leptorhynchus* move relative to another, there is a decrease in the effectiveness by which electroreceptors encode information about conspecific EODf and position, and hence their responsiveness to information about the position, orientation, identity, and motivations of interacting conspecifics (Yu *et. al.*, in preparation). We suggest that bursts of chirps may be produced to increase the electrosensory system's ability to encode and extract information about conspecific behaviour during interactions.

When two fish interact freely, bursts of echoed chirps tend to be produced when fish are at a distance from one another (Hupé and Lewis, 2008 (Chapter 2)) and these may function to increase their ability to detect, identify and/or localize one another when contrasts are small. Contrary to this, when one fish is restrained, both RFish and FSFish tend to produce chirps during aggressive approach behaviours, although a larger number of RFish pattern chirps with approaches than FSFish (Chapter 5). The position of the RFish doesn't change and the RFish poses no threat to the FSFish, hence RFish chirps may be patterned more tightly with approaches than are FSFish chirps because RFish have more motivation to localize the FSFish than vice versa.

### 5.4.4 Future Directions: Chirps and Contrast Encoding

Social interaction in *A. leptorhynchus* likely requires the integration of beat and contrast information with communication signals produced over the short term. It is not known how the electrosensory system integrates chirp sequences over time, with contrast information and internal motivations and proprioceptive information to produce appropriate behavioural chirp and physical responses to changing interactive contexts.

Chirps induce transient modulations in the contrast, or in the second order amplitude modulation, over time scales that are much shorter than movement related modulations. It is not known how repeated sequences of chirps produced in rapid succession influence the contrasts effectively encoded by receiver sensory systems. In many animal systems, bursts of reciprocated signals, are produced by interacting individuals and these can aid in localization (Greenfield and Minckley, 1993; Ursprung *et. al.*, 2009). One advantage conferred by producing bursts of signals is that it can increase the probability that they are detected by receivers, especially under noisy conditions (Schleidt, 1973). During interactions, movements of individual *A. leptorhynchus* may decrease the information available to conspecifics about its position and physical behaviours by decreasing the ability of P-units to encode contrast changes reliably (Yu *et. al.,* in preparation). Future work should address how single chirps and bursts of chirps influence the contrast during movement and how these chirp-induced contrast modulations are encoded.

We also propose that fish may modulate the rates of chirps in a manner that is dependent on the threat imposed to attempt to negotiate by inducing an echo response. Individuals may also increase their signalling rates to induce larger cumulative effects on receiver, and RFish may have been especially motivated to do so when threatened because they were unable to defend

themselves or swim away. We suggest that the functions of chirps are likely multifaceted where the benefits conferred by producing chirps in different rates and patterns depend on a number of factors including individual and context-dependent differences.

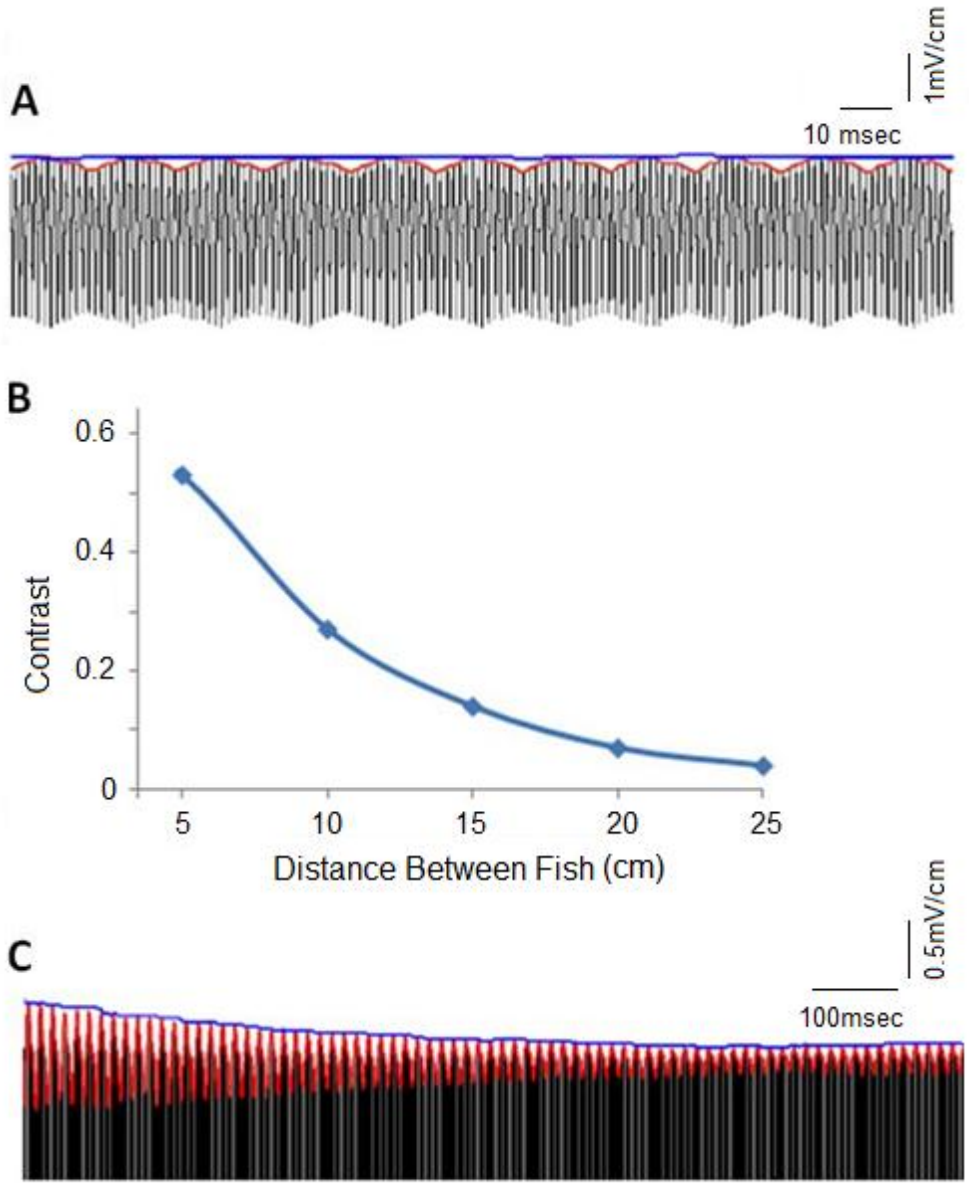



**Figure 5.1** Description of beats, contrasts and contrast modulations. **(A)** Recording of two restrained fish showing beat (AM, red line) oscillating at the difference frequency (Df), with the contrast defined by the magnitude the beat relative to the composite signal. **(B)** When two fish are restrained, an electrical recording sampled adjacent to the skin of one of the fish, shows the contrast increases as the distance between the fish decreases. **(C)** When one fish is free to swim, amplitude modulations of the beat arise (blue line).

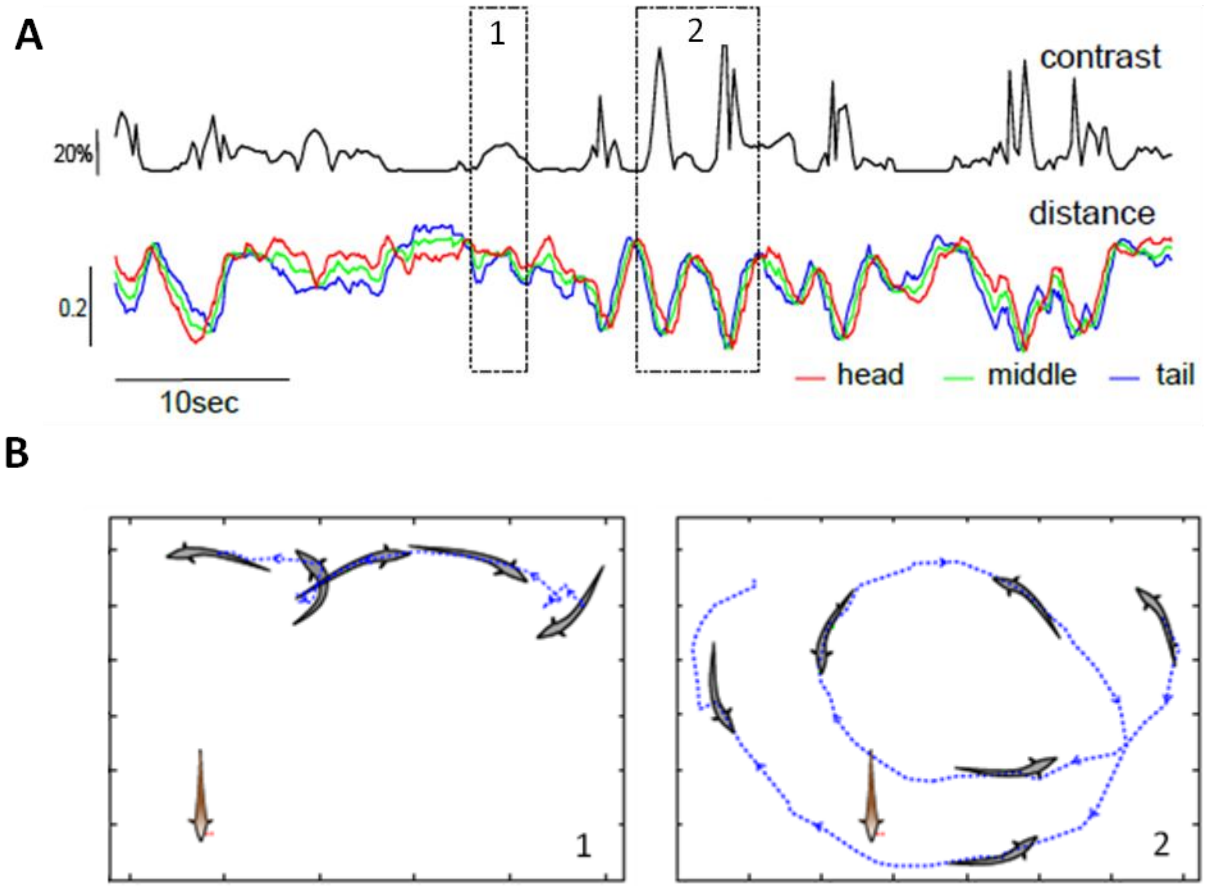



**Figure 5.2** The temporal correlation between contrast and distance. **(A)** Shown are traces of instantaneous contrast (top) and the distances (bottom) separating the recording electrodes and the head (red), middle (blue) and tail (green) of the FSFish over time. Contrast increases are tightly correlated with distance decreases. **(B)** The movements associated with the contrast bumps demarked in the Box 1 and Box 2 in (A). During time associated with the paired contrast bumps labelled in Box 2 the FSFish loops around the RFish twice, each time passing close to the recording electrodes as indicated by the paired dips in the distance trace. In the times associated with the contrast bump in Box 1, there is no noticeable change in the distance trace, however, from the position plots in (B) we can see that this contrast modulation is associated with a turning behaviour during which the fish changes direction quickly.

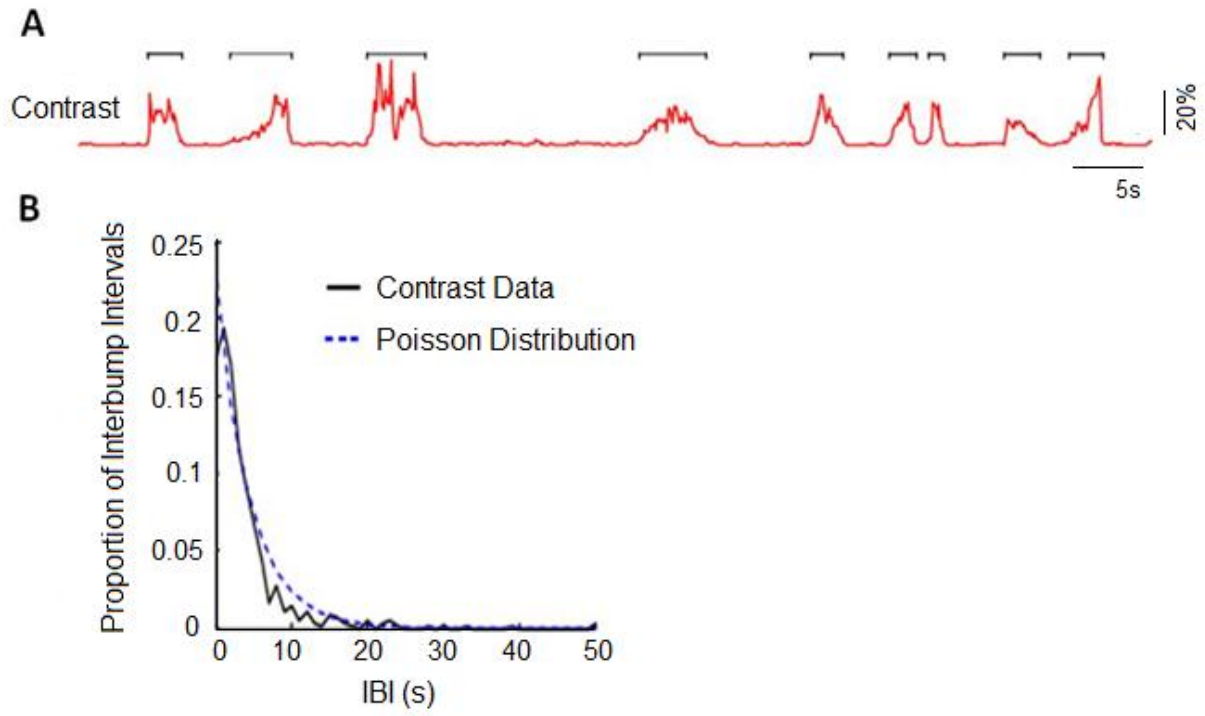



**Figure 5.3** Discrete contrasts bumps occur at random intervals. **(A)** A trace of instantaneous contrast plotted over shows that discrete contrast bumps occur (indicated by the horizontal brackets above). We calculated the intervals separating adjacent bumps (inter-bump intervals). **(B)** A histogram of inter-bump intervals shows that the distribution of observed inter-bump intervals is approximated by a Poisson distribution of the same mean.

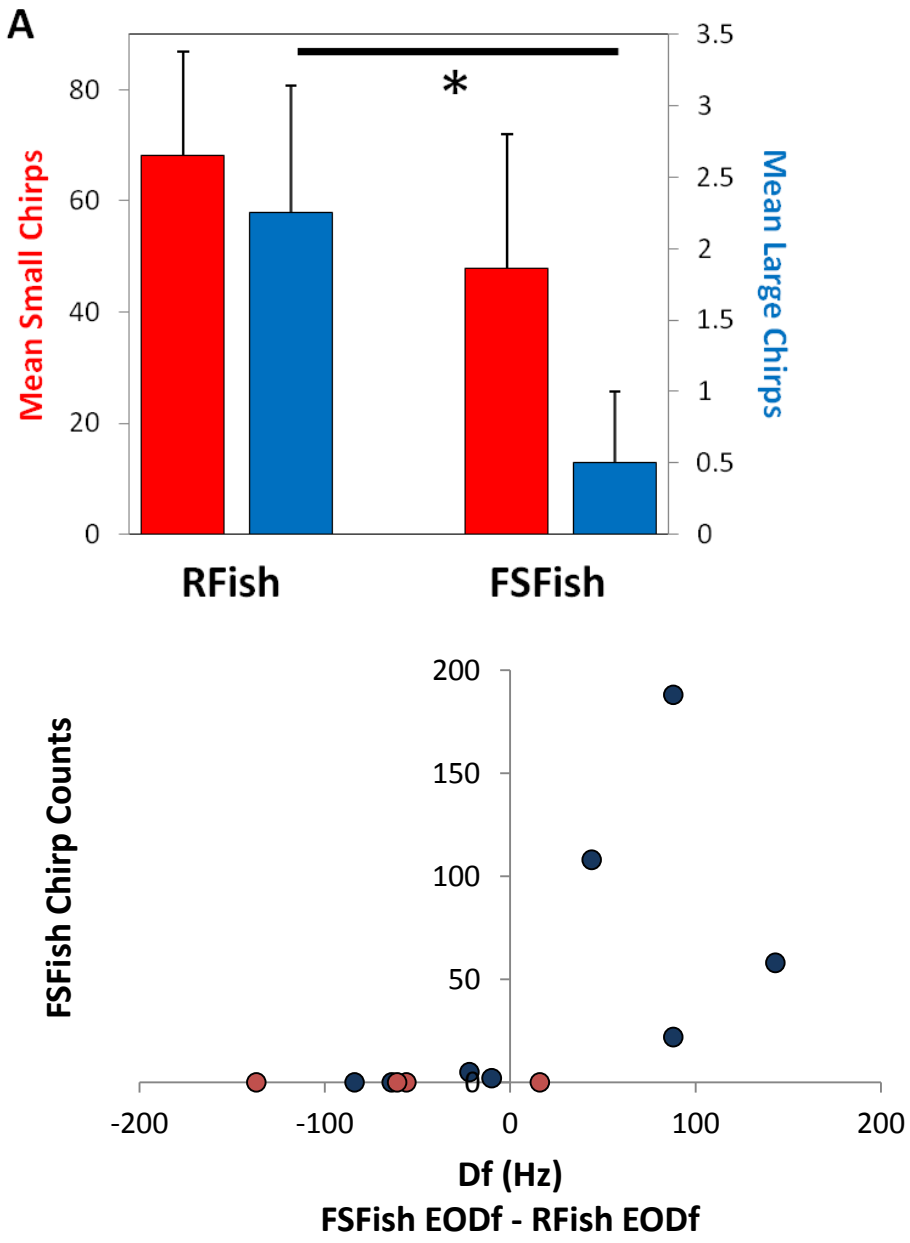



**Figure 5.4** RFish produce more large chirps than FSFish and FSFish small chirp rates depend on Df. **(A)** Mean small (red) and large (blue) chirp rates for RFish and FSFish. There is no significant difference in the mean number of small chirps produced by the RFish and FSFish ($p=0.26$), whereas RFish produce significantly more large chirps than do FSFish ($p=0.05$). **(B)** FSFish small chirp rate increases with the difference between the EODf of the FSFish and RFish ($p=0.03$). Male FSFish (dark blue symbols) only produce more than five chirps per trial when their EODf is higher than that of the RFish; female FSFish (magenta symbols) do not chirp.

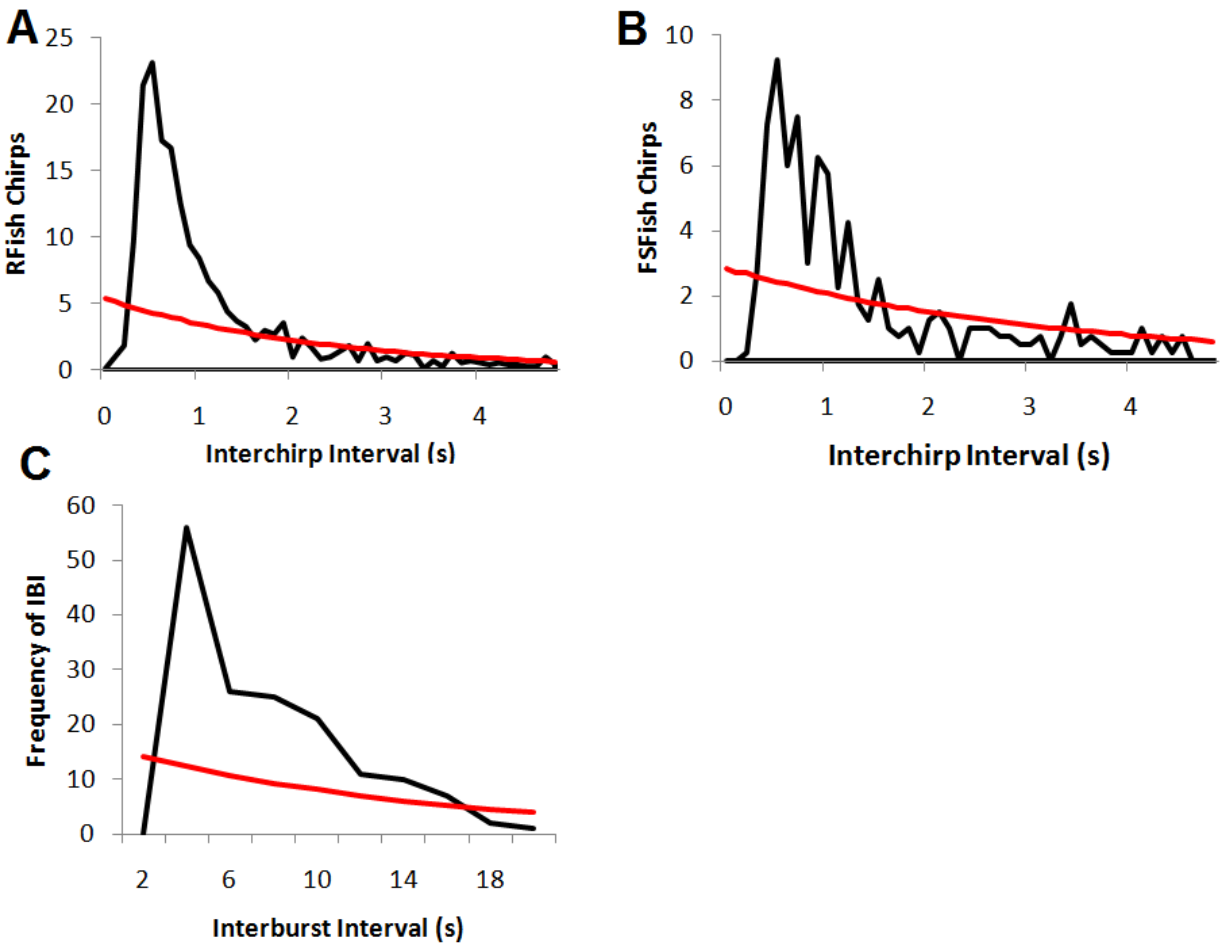



**Figure 5.5** Chirps tend to be produced in bursts and bursts occur at randomly spaced intervals. Histograms of inter-chirp intervals (ICIs) for both **(A)** RFish and **(B)** FSFish show that chirps tend to be produced in a burst fashion. There are fewer short latency ICIs than expected if chirps were produced at random (mean corrected Poisson distributions represented by the red lines) indicating that chirps tend to be produced in clusters. **(C)** A histogram of inter-burst intervals shows that the distribution of inter-burst intervals deviates from a Poisson distribution of the same mean suggesting that bursts of chirps tend to be produced in bursts.

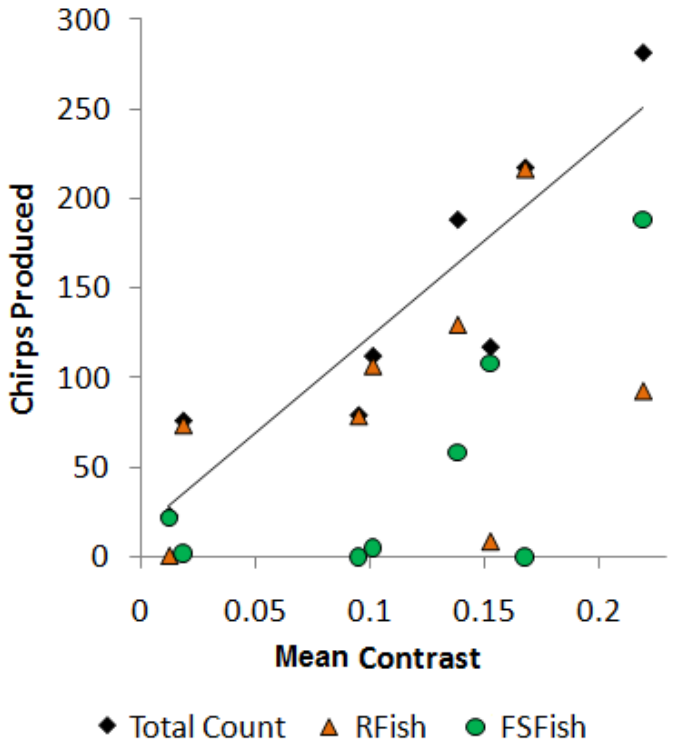
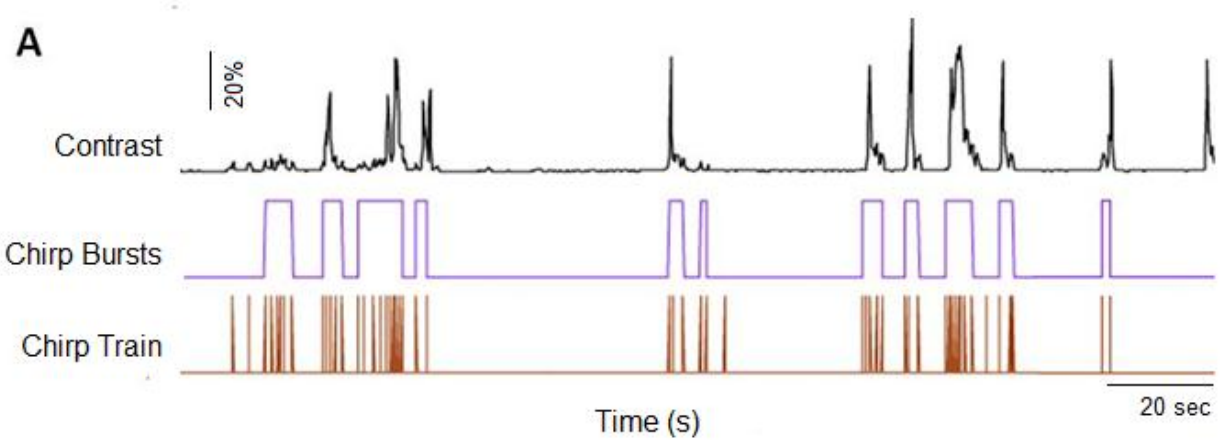



**Figure 5.6** Chirp production rates are correlated with mean contrast. The combined chirp rate of the RFish and FSFish is correlated with trial mean contrast (diamonds, p=0.002). The chirp rates of RFish (triangles) and FSFish (circles) are also shown.




B

| Contrast bump | Chirp: Present | Chirp: Absence |
|---|---|---|
| Yes | True Positive (TP) | False Positive (FP) |
| No | False Negative (FN) | True Negative (TN) |
| Sensitivity | TPR=TP/(TP+FN) | |
| 1-Specificity | | FPR=FP/(FP+TN) |

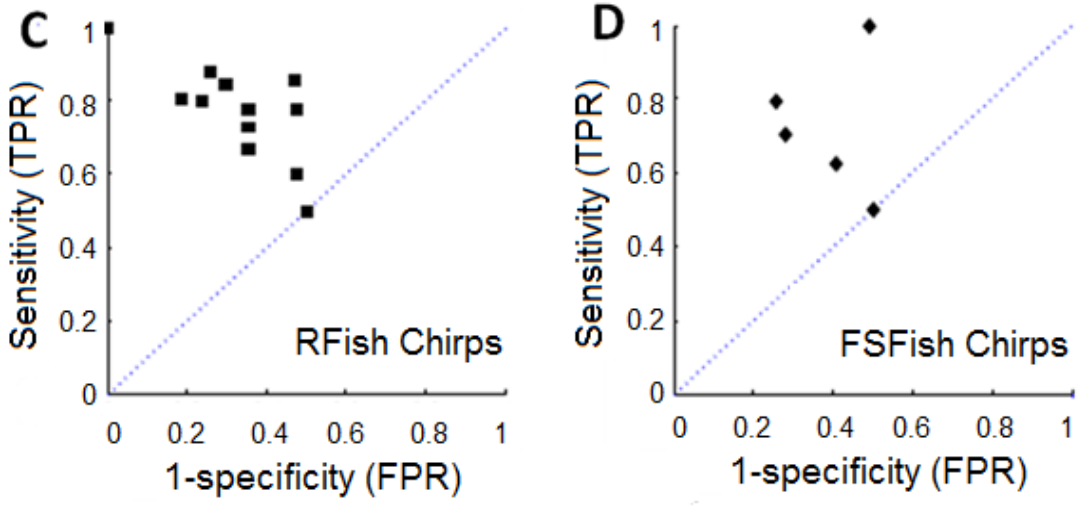



**Figure 5.7** Chirp bursts are temporally correlated with contrast bumps. **(A)** A trace of instantaneous contrast in time (top), chirp bursts (middle), and individual chirps (bottom) (bursts of chirps were distinguished as chirps occurring within two seconds of each other). ROC analysis (conditions as outlined in **(B)**) shows that in both **(C)** RFish and **(D)** FSFish the timing of contrast bumps tend to be associated with the production of bursts of chirps.

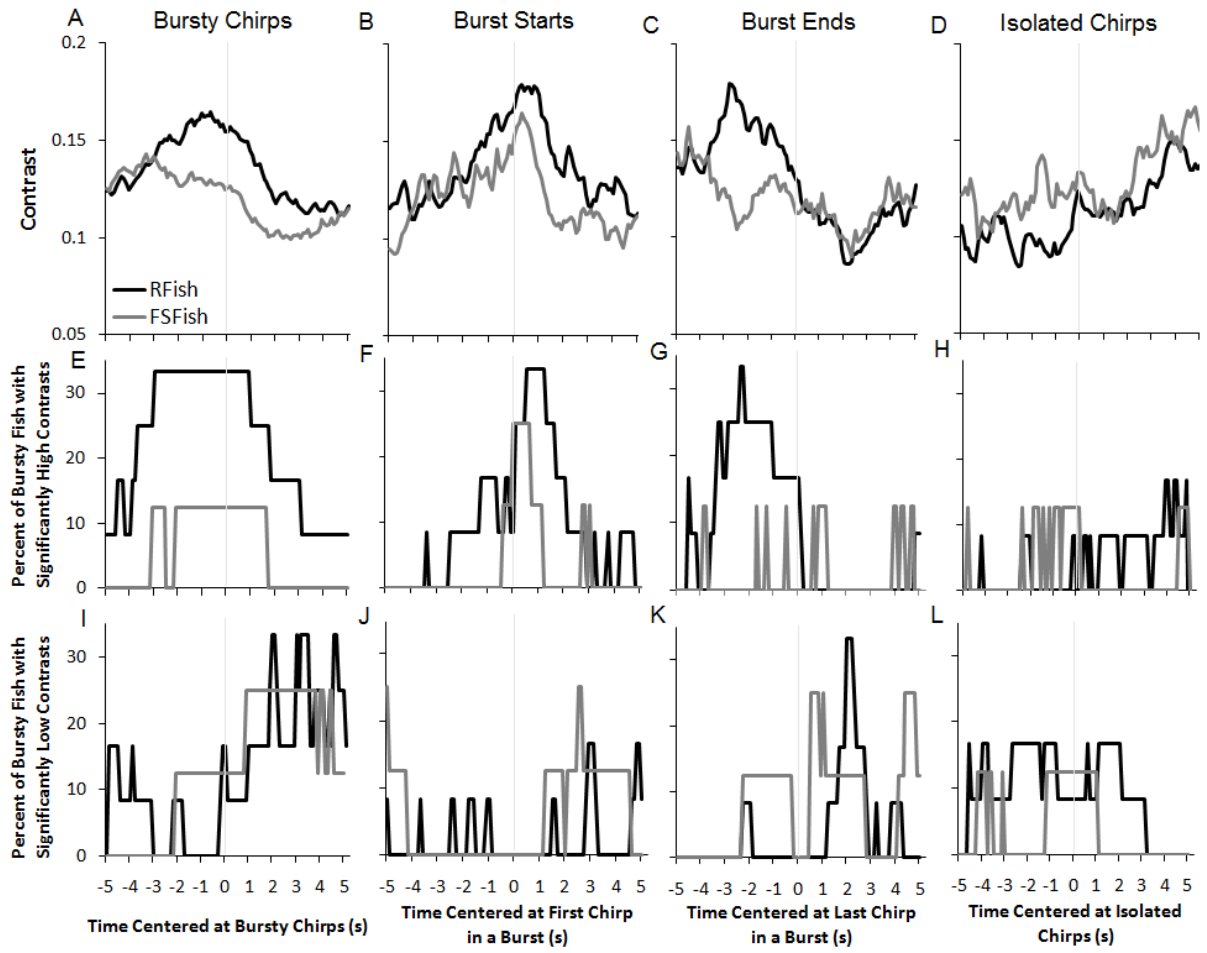



**Figure 5.8** The temporal correlation between chirps of different types and contrast. Shown for **(A)** bursty chirps and **(D)** isolated chirps, and for chirps occurring at the **(B)** start and **(C)** end of bursts for RFish (black lines) and FSFish (grey lines). The percent of fish with mean contrasts significantly greater **(E-H)** or less **(I-L)** than expected by chance are shown in the second and third rows for each chirp type (as specified in the top row). In the population of RFish bursty chirps tend to be produced when the contrast is high, where chirp burst onsets are associated with contrast increases, and burst terminations are associated with contrast decreases.

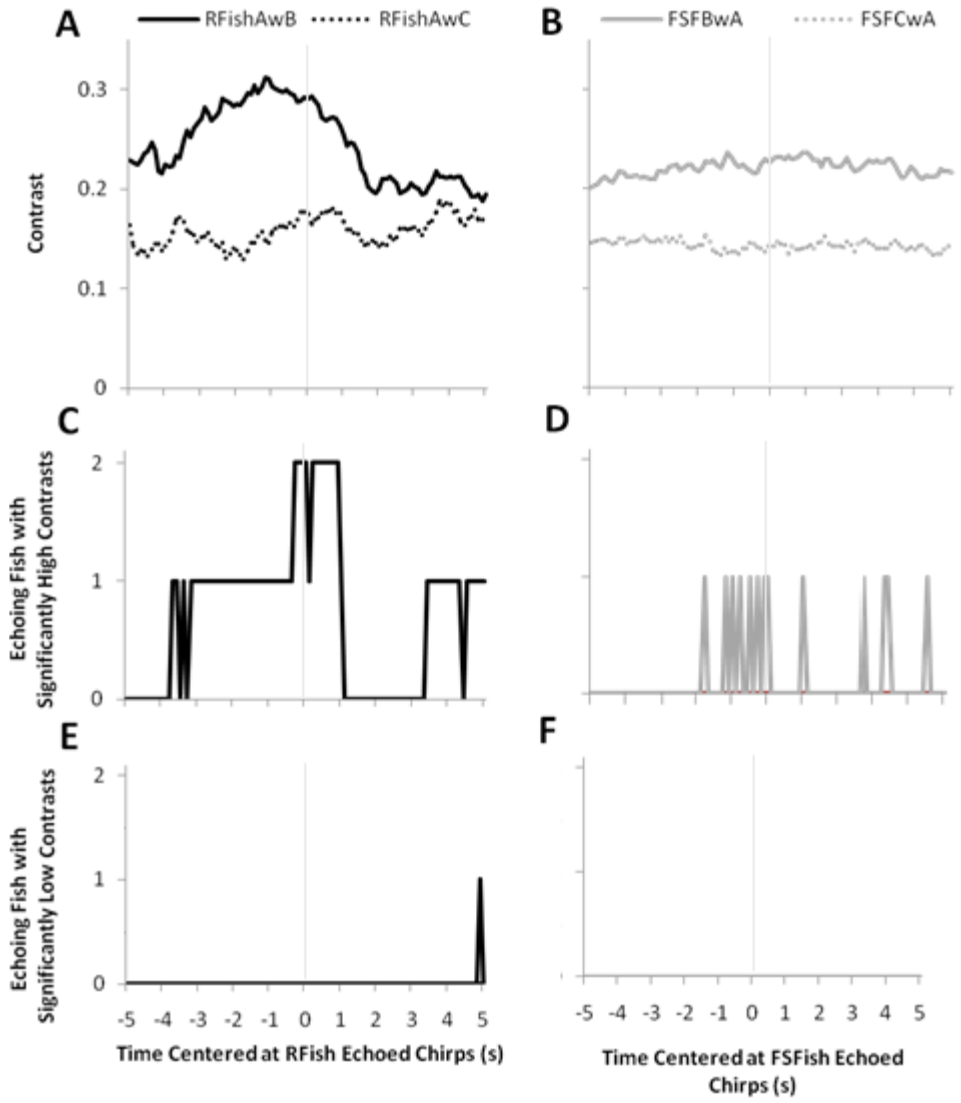
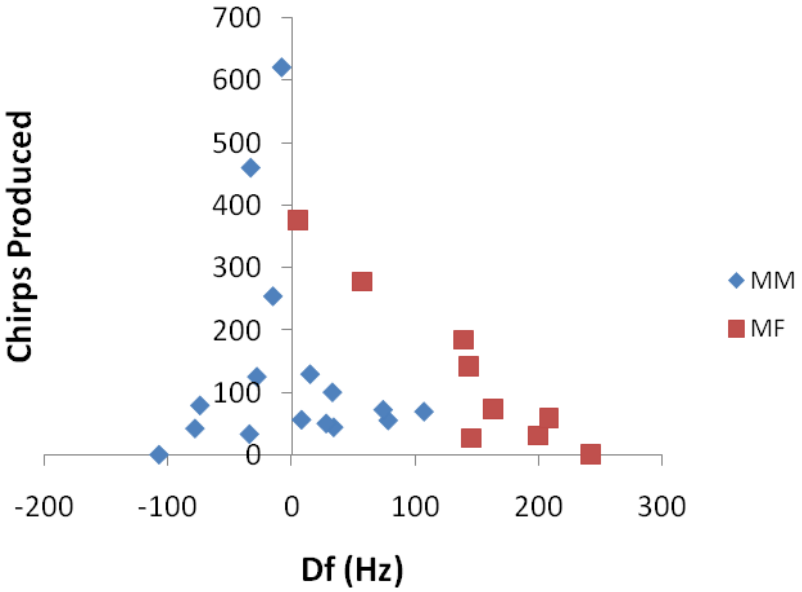



**Figure 5.9** Echoed chirp-centered contrasts. The mean instantaneous contrast centered at **(A)** RFish echoed chirps and **(B)** FSFish echoed chirps. In both trials characterized, the RFish was the same individual ('Fish A') paired with two different FSFish (designated 'Fish B' and 'Fish C'). For each comparison, the bins with mean contrasts significantly greater **(C,D)** or less **(E,F)** than expected by chance are shown for the RFish (left column) and FSFish (right column).

Table 5.1 Trial EODf, Df, chirp and contrast summaries

| Trial | RF EODf | FSF EODf | DF | RF Small Chirps | FSF Small Chirps | RF Big Chirps | FSF Big Chirps | Mean Contrast |
|---|---|---|---|---|---|---|---|---|
| 1 | 787 | 875 | -88 | 93 | 188 | 3 | 4 | 0.22 |
| 2 | 780 | 923 | -143 | 130 | 58 | 4 | 0 | 0.14 |
| 3 | 767 | 783 | -16 | 79 | 0 | 9 | 0 | 0.09 |
| 4 | 756 | 700 | 56 | 10 | 0 | 7 | 0 | 0.13 |
| 5 | 840 | 776 | 64 | 1 | 0 | 0 | 0 | 0.01 |
| 6 | 847 | 891 | -44 | 9 | 108 | 0 | 0 | 0.15 |
| 7 | 828 | 767 | 61 | 80 | 0 | 1 | 0 | 0.13 |
| 8 | 817 | 680 | 137 | 16 | 0 | 0 | 0 | 0.06 |
| 9 | 850 | 938 | -88 | 1 | 22 | 0 | 0 | 0.02 |
| 10 | 919 | 897 | 22 | 107 | 5 | 0 | 0 | 0.06 |
| 11 | 910 | 900 | 10 | 74 | 2 | 0 | 0 | 0.02 |
| 12 | 896 | 812 | 84 | 217 | 0 | 3 | 0 | 0.19 |

*Red values indicate female fish




**Addendum Figure 5A.1** Chirp rates as a function of relative difference frequency (DF) for males paired with a second male (MM), or paired with a female (MF). In MM trials, the relationship between chirp rate and Df is more pronounced in response to negative Dfs (MM: $R^2$=0.47, p=0.058; MF: $R^2$=0.04, p=0.610). Males paired with females chirp significantly more in response to small (positive) Dfs ($R^2$=0.88, p<0.001).

# Chapter 6

# General Discussion

## 6.1 Summary

*Apteronotus leptorhynchus*, a territorial wave-type weakly electric fish, produces electrocommunication signals called chirps during agonistic interactions (Zakon *et. al.*, 2002; Hupé and Lewis, 2008 (Chapter 2)). In territorial systems, males defend territories from potential rivals, and during agonistic encounters males often perform antiphonal signalling displays that permit the assessment of rival dominance status, fighting ability, and aggressive motivation (Parker, 1974; Greenfield, 1994). Intrinsic differences in chirp propensity are integrated with sensory information about conspecific chirp behaviours to dictate a fish's response strategy in a given social context (Triefenbach, 2005; Hupé and Lewis, 2008 (Chapter 2); Chittka *et. al.*, 2009; Chapter 4; Chapter 5). During social encounters individuals make sequences of choices that depend on the perceived threat imposed by potential rivals as indicated through signalling behaviours, assessing, for example, the costs and/or benefits associated with signalling an aggressive or subordinate motivational state depending on the perceived threat during a territorial contest (Chapter 4; Chapter 5). There are many similarities between the signalling behaviours and neuroethological principles of electric communication with those observed in acoustic systems (Chapter 1; Chapter 4; Chapter 5). Although often costly to produce, territorial signals serve to deter aggression, and advertise defended resources (Parker, 1974, Greenfield and Minckley, 1993). Similar selection pressures acting on different territorial signalling systems have led to the development of a number of parallels across signalling systems in diverse taxa (Yosida and Okanoya, 2005; Kelley and Bass, 2010).

*6.1.1 Chirping Behaviours are Individually Specific and Context Dependent*

The behavioural repertoires displayed by animals during social interactions are complex, dynamic, and often involve precisely timed coordination between individuals (Pollack, 2001; Bohn *et. al.,* 2008). Many behaviours central to reproduction, predator avoidance, conflict resolution, and resource allocation involve flexible and coordinated efforts between pairs of individuals, or between larger groups (Yosida and Okanoya, 2005; Eliades and Wang, 2008; Miller *et. al.,* 2009; Harcourt *et. al.,* 2010). Across a variety of experimental contexts, male *A. leptorhynchus* produce chirps in variable patterns and associate the timing of their chirps and aggressive displays with the signals produced by interacting conspecifics (Zupanc *et. al.,* 2006; Hupé and Lewis, 2008, (Chapter 2); Chapter 5) or simulated conspecifics (Triefenbach, 2005; Gama Salgado and Zupanc, 2011; Chapter 4). Antiphonal exchanges often mediate individual recognition and may signal intent to negotiate during agonistic encounters (Greenfield and Minckley, 1993; Richardson *et. al.,* 2008).

Non-signalling individuals often are subordinate, less-threatening, and/or less willing to engage in physical combat than are their signalling counterparts (Greenfield and Minckley, 1993; Yosida and Okanoya, 2005; Chapter 4). In response to playbacks, non-chirping male *A. leptorhynchus* are less aggressive than are chirping fish regardless of the chirp playback sequence (Chapter 4). Chirp rates are associated with individual resource holding potential (RHP) and aggressive motivation (Dunlap *et. al.,* 2002; Triefenbach and Zakon, 2008), where less aggressive individuals tend to produce lower rates of chirps (Maler and Ellis, 1987; Dunlap *et. al.,* 2002), although these relationships are dependent on the context (Chapter 4; Chapter 5).

Bursts of chirps were produced by male *A. leptorhynchus* under every context examined, and repetitive signal production may confer many advantages; including to increase the saliency of signals to improve detection, and/or enhance information transmission through temporally integrated rate modulations (Schleidt, 1973; Carlson and Hopkins, 2004). Bursts of chirps are produced between attacks when two conspecifics are free-swimming and able to engage one another (Chapter 2); however, in response to a stationary simulated intruder and when one of two fish is restrained, bursts of chirps tend to be produced during approach behaviours (Chapter 4; Chapter 5).

Antiphonal signalling occurs in many territorial communication systems during negotiations in an exchange of information about sender aggressive ability and motivation. Signal rates, patterns and the 'quality' of signals (e.g., frequency and amplitude modulation profiles) produced can be used to assess the costs associated with escalation relative to alternate behavioural strategies (Greenfield, 1994; Chittka *et. al.,* 2009). In a number of ritualized territorial communication displays, less aggressive individuals tend to echo chirps produced by more aggressive conspecifics more often than aggressive individuals echo those of less aggressive individuals (Parker, 1974; Greenfield and Minckley, 1993; Sneddon and Greenfield, 1998). In response to high rates of randomly delivered chirps, fish echo (follow) at higher rates and are less aggressive than those that respond to low rates of randomly delivered chirps with higher chirp rates, lower echo rates (leaders) and more aggression (Chapter 4). The signalling pattern conveys information about individual aggressive ability and intent and implies that there is integration of multiple signals over time during coordinated social behaviours (Laidre and Vehrencamp, 2008; Kelley and Bass, 2010).

*6.1.2 Bursts of Chirps May Overcome Noise Including that Induced by Movements*

During social interactions, behavioural responses depend on a dynamic integration of extrinsic and intrinsic information, and thus selection pressures shape natural behaviours and sensory systems to promote the reliable transmission of stimuli, including communication signals (Miller *et al.*, 2009, 2010; Kelley and Bass, 2010). The signalling behaviours of individuals are greatly influenced by a noisy background. To overcome the constraints imposed by noise, an individual may change their signalling behaviours by, for example, increasing signal amplitude, modulating the frequency profile or increasing signalling rate (Bohn *et. al.*, 2008; Ursprung *et. al.*, 2009; Einhäupl *et. al.*, 2011).

Many communication signals involve rapid amplitude and frequency modulations and these enhance the detectability of the signals produced and function to enhance localization of the signaller (Marler, 1955; Schleidt, 1973; Bro-Jørgensen, 2010). When one fish is restrained and another is swimming freely in a tank around it, bursts of chirps produced by both of the fish tend to be associated with aggressive behaviours (Chapter 5). Burst onsets tend to be associated with approaches, while burst offsets are timed with retreats, with relationships more prevalent for the restrained fish's chirps. Changes in the distance separating the fish are paralleled by changes in the contrast of the composite signal (the electric image) received by either fish. Movement may decrease the ability of the electrosensory system to encode relevant social signals (*e.g.*, the contrast which provides information about relative position (Yu *et. al,* in preparation); and we hypothesize that under some conditions chirps may function to modulate conspecific tracking during movements, especially those associated with aggressive behaviours.

## 6.2 Behavioural Descriptions Complement Neural Characterizations

The neural pathways implicated in *A. leptorhynchus* chirp production, encoding, and processing are well characterized and provide an avenue from which to explore the physiological basis of individual and context specific differences in aggressive signalling behaviours (Zakon *et. al.,* 2002; Zupanc, 2002). Individual differences in chirping behaviours and experience-dependent plasticity in chirping behaviours may be explained based upon differences in the physiological systems that regulate chirping (Dunlap *et. al.,* 2002; Zupanc, 2002).

### *6.2.1 Overview of the Electrosensory and Electromotor System*

Electric potential differences across the skin arising from properties of the fish's environment and passively and actively induced modulations are transduced at the skin surface by electroreceptors tuned to encode specific stimulus features (Bullock *et. al.,* 2005). One type of electroreceptor, ampullary receptors, encode passive electrosensory signals (for example, the bioelectric fields produced by prey) and also respond to low frequency stimuli signals (Heiligenberg and Bastian 1984; Dunlap 2010). A second class of electroreceptors, tuberous receptors, encode elements of the active electrosense, including electrocommunication EOD modulations and beat modulations induced by movement (Bullock, 1969; Benda *et. al.,* 2005, 2006). Tuberous electroreceptors innervate primary electrosensory afferents (called P-units and T-units) and these transmit peripheral electrosensory information centrally (information from tuberous and ampullary receptor pathways converge in higher processing centers). During dyadic interactions, P-unit firing rates are phase locked to the beat that results from the interaction of two nearby EODs and chirps (which disrupt the beat cycle) result in changes in P-unit population

activity, increasing or decreasing the population synchrony response to the beat (Benda *et. al.*, 2005, 2006; Hupé *et. al.*, 2008 (Chapter 3)).

P-units ascend and converge onto the primary electrosensory area of the brain, the electrosensory lateral line lobe (ELL). In the lateral segment (LS) of the ELL, two populations of neurons, E cells and I cells, respond to small and large chirp stimuli, respectively (Marsat *et. al.*, 2009; Marsat and Maler, 2010). The ELL projects to higher processing areas including the nucleus praeeminentialis (nP) and torus semicircularis (TS, an inferior colliculus homologue) (Metzner and Heiligenberg, 1991; Maler *et. al.*, 1991; Rose, 2004). The nP provides direct and indirect (via the eminentia granularis pars posterior, EGp) feedback to the ELL; this feedback is involved in reafference suppression and enhanced feature detection (Berman and Maler, 1999; Bastian *et. al.*, 2004; Lewis *et. al.*, 2007). The torus projects to the tectum, to the diencephalic nucleus electrosensorius (nE), as well as back to nP (Maler *et. al.*, 1991; Heiligenberg *et. al.*, 1991; Rose, 2004). The sensorimotor nE integrates convergent electrosensory information and sends projections to two prepacemaker nuclei: the sublemniscal prepacemaker nucleus (sPPn) and the diencephalic prepacemaker nucleus (PPn) that are responsible for controlling the frequency of the emitted EOD set by the medullary pacemaker nucleus (Pn). Spatially specific stimulation of the nE by glutamate iontophoresis results in EODf modulations (rises and chirps) via distinct inputs to the PPn (Rose, 2004). The sPPn and PPn project to the medullary pacemaker nucleus (Pn). The Pn contains electrotonically-coupled pacemaker neurons whose endogenously oscillating membrane potential sets the EODf, and relay cells which propagate these signals to the electric organ (Zupanc, 2002).

The electric organ in the Apteronotidae is composed of modified spinal motorneurons adapted to generate large potential differences that result in the EOD (Chapter 1). Modulations of

EODf (including those that occur during the JAR and during chirp production) occur as a consequence of specific sPPn and PPn inputs to the Pn (Dye, 1987, 1988; Kawasaki *et. al.,* 1988).

### *6.2.2 The 'Chirp Nucleus' – The Central Posterior Prepacemaker Nucleus*

#### *6.2.2.1 Anatomical Organization of the Diencephalic CP/PPn*

The prepacemaker nuclei were identified based on retrograde labelling techniques from the Pn (Heiligenberg *et. al.,* 1981; Zupanc and Heiligenberg, 1992). The sublemniscal prepacemaker (sPPn) is one of the two prepacemaker nuclei, and is a bilateral cluster of neurons, located about 500-600μm caudal to the diencephalic PPn (Keller *et. al.,* 1991). Heiligenberg *et. al.,* (1996) suggest that the sPPn in *Eigenmannia* mediates EOD decelerations and perhaps interruptions (Zupanc and Maler, 1997), and its role in electrocommunication behaviours is unclear and requires further investigation.

The diencephalic PPn was first described as a bilateral cluster of neurons in the thalamus positioned about 400μm laterally from the third ventricle and connecting at its medial edge with the central posterior nucleus (CP) (Heiligenberg *et. al.,* 1981; Zupanc and Heiligenberg, 1992). However, detailed tracer experiments later revealed no morphological separation between the CP of the dorsal thalamus and neurons of the PPn, and the entire complex was subsequently renamed the central posterior/prepacemaker nucleus (CP/PPn) (Zupanc and Heiligenberg, 1992; Zupanc and Maler, 1997). Neurogenesis occurs in the ventricular zone of the CP throughout adulthood and some of these newborn neurons migrate laterally towards the PPn (Zupanc and Zupanc, 1992). There are approximately 10000 cells in the CP/PPn of *A. leptorhynchus*, only a few hundred of which project to the Pn and comprise the PPn proper (Zupanc and Maler, 1997).

Electrical stimulation of the PPn induces two distinct types of EOD modulations: gradual frequency accelerations (rises) and brief frequency modulations (chirps) (Bastian and Yuthas, 1984; Kawasaki *et. al.,* 1988; Kawasaki and Heiligenberg, 1988). The PPn, in both *Eigenmannia* and *Apteronotus*, is made up of two subnuclei: two anatomically and functionally distinct populations of neurons (Kawasaki *et. al.,* 1988). The dorsomedial part of the PPn is made up of small, ovoid neurons (granule cells) and is called the PPn-G because electrical stimulation and glutamate iontophoresis onto these neurons induces a 'gradual' frequency acceleration of Pn neurons and hence EODf. The ventrolateral region of the PPn is made up of large, multipolar neurons and is termed the PPn-C because stimulation to this area induces chirp production (Dye, 1987; Kawasaki *et. al.,* 1988). A single action potential in a PPn-C neuron is sufficient for chirp generation, and current injection-induced depolarization of the PPn neurons induces bursts of chirps in *Eigenmannia* (Dye, 1987; Kawasaki *et. al.,* 1988; Kawasaki and Heiligenberg, 1988). This suggests that chirp production can be tightly regulated to produce specific chirp patterns in response to conspecific chirping and aggressive behaviours.

PPn-G neurons synapse onto pacemaker neurons via NMDA receptors (Dye *et. al.,* 1989; Kawasaki and Heiligenberg, 1990). PPn-C neurons depolarize relay cells, perhaps via glutamatergic non-NMDA, quisqualate/kainite receptors, and subsequently PPn cells (Dye, 1988; Kawasaki *et. al.,* 1988; Zupanc and Heiligenberg, 1992). The small ovoid cells in PPn-G are tightly packed and the juxtapositions of neighbouring cell membranes suggests electrotonic gap junction coupling for synchronization within the subpopulation (Zupanc, 1991A). The larger multipolar neurons in the PPn-C are much more scattered in distribution and immunohistochemical observations suggest that electrotonic coupling occurs at specialized dendritic arborization bundles (Zupanc, 1991A; Zupanc and Maler, 1997). The dendritic

arborization of PPn-C neurons changes with sexual maturation and in adults the dendritic arbors of PPn-C neurons form distinct ventral, lateral, and dorsomedial dendritic fields, with extensive and diverse connections within and outside of the PPn (Kawasaki *et. al.*, 1988; Zupanc, 1991B; Zupanc and Maler, 1997; Dulka and Ebling, 1999).

*6.2.2.2 CP/PPn Afferent and Efferent Connections*

Behavioural observations have shown that during dyadic interactions, fish tend to echo one another with a preferred latency of about 500ms during bouts of chirping (Zupanc *et. al.*, 2006; Hupé and Lewis, 2008 (Chapter 2); Chapter 4; Chapter 5). This is longer than a direct sensory motor pathway would suggest (Zupanc and Maler, 1997; Harvey Girard *et. al.*, 2010) and the involvement of higher processing areas in the reciprocation of these behaviourally relevant electrocommunication signals is supported by the complexity of neural pathways associated with processing chirp signals. The CP/PPn receives extensive afferent and efferent connections from a variety of brain areas presumably to provide precise, yet flexible, regulation of chirping behaviours.

Wong (1997) provided a detailed characterization of CP/PPn afferent and efferent connections in *Eigenmannia virescens* using retrograde and anterograde Neurobiotin labelling. Retrograde transport shows that the CP/PPn receives inputs from the ventral telencephalon, the hypothalamus (involved in neuroendocrine control), and the pretectal nE (Wong, 1997). Anterograde labelling demonstrates that the CP/PPn sends projection fibers to the ventral telencephalon, the hypothalamus, regions adjacent to the nE, the optic tectum, and to the Pn (Wong, 1997). Many of these areas are associated with reproductive and neuroendocrine control

in other fish and the efferent projections of the gymnotiform CP/PPn may be homologous to those of the tetrapod auditory thalamus pathways (Wong, 1997).

Zupanc and Maler (1997) and Zupanc (2002) provide detailed reviews of CP/PPn connectivity and of the neurotransmitters and neuromodulators involved in chirp production. The CP/PPn receives classical glutamatergic inputs (as demonstrated by L-glutamate iontophoresis studies), GABAergic innervation (Kawasaki and Heiligenberg, 1990), noradrenergic input (likely from the locus coeruleus), serotonergic input (from the raphe nuclei and paraventricular nucleus of the hypothalamus), and shows dopaminergic immunoreactivity (reviewed in Zupanc and Maler, 1997). A number of neuropeptides have been implemented in chirp control including galanin (Yamamoto *et. al*., 1992), metenkephalin (Richards and Maler, 1996), substance P (Dulka *et. al.,* 1995), somatostatin (Sas and Maler, 1991), arginine vasotocin (Bastian *et. al.,* 2001), and corticotropin releasing factor (Zupanc *et. al.,* 1999; Zupanc, 2002; Dunlap *et. al.,* 2011); most of these originate from the hypothalamus and project to innervate the PPn and its dendritic arborization (Zupanc and Maler, 1997). The CP/PPn also receives projections from the optic tectum (TeO) which may mediate light-dark changes in chirp rates (Zupanc, 2002; Dunlap and Oliveri, 2002). In addition to receiving inputs from the nE and optic tectum, the CP/PPn also receives inputs from the preglomerular nucleus (PG), which serves as a relay for reciprocal connections between the CP/PPn and the telencephalon (Tel), and from the nucleus preopticus periventricularis (PP), which relays inputs from the hypothalamic nuclei.

The CP/PPn receives inputs from two areas of the hypothalamus: the hypothalamus ventralis (Hv) and the hypothalamus lateralis (Hl), received indirectly through a relay in the preglomerular nucleus (Correa and Zupanc, 2002). There is also a reciprocal connection between the CP/PPn and a nucleus with unknown homology, and no detectable connections to the

hypothalamus, called “nucleus G” (previously suggested as a glomerular nucleus) (Zupanc and Correa, 2005).

*6.2.3 Neurogenesis in the Adult CP/PPn*

An additional dimension to the functional complexity of chirp regulation is that neurogenesis occurs throughout adulthood in the CP/PPn, especially accompanying seasonal changes in reproductive behaviour (Zupanc and Zupanc, 1992; Zupanc, 2006B). Neurogenesis occurs in many regions of the brain in mature *A. leptorhynchus* including regions in the telencephalon (including areas in the dorsal telencephalon likely homologous to the hippocampus), diencephalon, mesencephalon (in the optic tectum for example), and cerebellum (where neurogenesis predominates) (Zupanc, 2006B). Neurogenesis occurs in the ventricular zone of the CP/PPn and newly born cells begin to migrate laterally along radial glial fibers soon thereafter (Zupanc, 2006B). The density of these radial glial fibers changes with seasonal alterations in gonadal maturation in male *A. leptorhynchus.* This occurs concurrently with a decrease in the volume of CP/PPn in gonadally mature males and is thought to be a result of interplay between enhanced rates of activity-induced apoptosis and unmatched replenishment from laterally migrating neurons (Zupanc, 2006B).

Male *A. leptorhynchus* demonstrate experience dependent changes in chirping behaviour where the chirp rates of males paired with another male increase significantly over the course of a week (control fish kept in isolation continually produce low rates of spontaneous chirps) (Dunlap and Larkins-Ford, 2003B; Dunlap *et. al.*, 2006; 2011). Measured after one week, paired males also show significantly enhanced neurogenesis in the paraventricular zone, specifically in

a region adjacent to the PPn (Dunlap *et. al*., 2006; 2011). This socially induced enhancement of neurogenesis is mediated in part by glucocorticoid receptors (Dunlap *et. al*., 2011).

## 6.3 Neuromodulatory Systems Implicated in the Regulation of Chirping Behaviours

Studying chirp production in *A. leptorhynchus* provides an ideal system for examining how specific neuromodulatory experimental manipulations influence an easily induced and quantifiable, socially-relevant behaviour (Zakon *et. al.*, 2002). The CP/PPn exerts control over chirp production on multiple time-scales (Zupanc, 2002). Individual action potentials in PPn-C neurons elicit chirp production and this circuitry enables subsecond antiphonal echo responses. Over longer periods of time, mirroring developmental and seasonal changes in chirp rates for example, the CP/PPn is also modulated to mediate changes in chirp behaviours. Networks of interacting neuromodulatory systems maintain individual predispositions and experience-dependent differences in electrocommunication behaviours in many species of weakly electric fish (Silva *et. al.*, 2008; Perrone *et. al.*, 2010) and in other territorial animals (Newman, 1999; Goodson, 2005; Conrad *et. al.*, 2011).

### *6.3.1 Monoamines and Chirping Behaviour*

Monoamines regulate aggressive behaviours and dominance status in fish and a variety of other animal taxa (Winberg and Nilsson, 1993; Haller *et. al*., 1997; Korzan *et. al*., 2000; Libersat and Pflueger, 2004; DiBattista *et. al.*, 2005). Male *A. leptorhynchus* treated with ventricular injections of the monoamines noradrenaline (NA), serotonin (5HT), or dopamine (DA) demonstrate changes in playback induced chirp behaviour (Maler and Ellis, 1987). NA administration increases chirp production rates for approximately 30min, whereas 5HT causes a significant decrease in chirp rates following treatment over the same time frame (Maler and Ellis,

1987). DA treatment has inconsistent effects on chirping, causing increases in chirp rates in some individuals, and decreases in others (Maler and Ellis, 1987).

5HT regulates aggressive and reproductive behaviours (Edwards and Kravitz 1997; Huber *et. al*., 1997; Summers *et. al*., 2001; McDonald *et. al*., 2011) and is implicated in regulating the sexual dimorphism of chirping in *A. leptorhynchus* and mediating individual differences in chirping and aggressive responses (Maler and Ellis, 1987; Telgkamp *et. al.,* 2007; Smith and Combs, 2008). Chirp rates are sexually dimorphic in *A. leptorhynchus*: males chirp significantly more than females (Maler and Ellis, 1987; Hupé and Lewis, 2008 (Chapter 2); Chapter 5) and the expression of serotonin immunoreactive fibers in the CP/PPn is also sexually dimorphic and likely contributes to the sexual dimorphism in chirp behaviours (Telgkamp *et. al.,* 2007). The CP/PPn of female *A. leptorhynchus* receives significantly greater serotonergic innervation than does the CP/PPn of males, and further, the extent of serotonergic innervation correlates negatively with indicators of dominance including body size and EODf (Telgkamp *et. al.,* 2007).

Smith and Combs (2008) use intramuscular injections of selective serotonin receptor agonists and antagonists to observe the influence of different 5HT receptor types on serotonin mediated changes in chirp behaviour, characterizing the effects of pharmacological manipulation on the production rates of small and large chirps. Treatment with selective $5HT_{1B/1D}$ receptor agonists and antagonists has no effect on chirping. $5HT_{1A}$ receptor agonists increase the rate of large chirp production specifically, whereas $5HT_2$ receptor agonists decrease overall chirp rates (Smith and Combs, 2008). Serotonin may mediate decreases in chirp production via $5HT_2$ receptors, whereas $5HT_{1A}$ receptors are thought to interact with the cortisol and arginine

vasotocin systems to mediate changes in chirp quality, namely to regulate relative small and large chirp production rates (Smith and Combs, 2008).

### *6.3.2 Androgens and Chirping Behaviour*

During dyadic interactions male chirp rates correlate positively with endogenous 11-ketotestosterone (11KT) levels and with other indicators of dominance (including body size and EODf) (Dunlap, 2002). Dunlap *et. al*, (2002) examined 11KT, testosterone, and cortisol levels in fish exposed to isolated, paired, or playback conditions and compared these levels with body size, chirping, and aggression. After one week, paired individuals chirped more than isolated individuals and had significant differences in cortisol, but no difference in 11KT levels.

Female *A. leptorhynchus* rarely chirp in response to EOD playbacks; however, females treated with testosterone produce more chirps and respond to playbacks with chirp behaviour similar to that of male conspecifics (Dulka and Maler, 1994). Androgen treatment changes not only the number of chirps produced, but also the quality of chirps produced: chirps produced post-treatment were associated with larger frequency excursions than those produced prior to treatment (Dulka and Maler, 1994).

Testosterone mediates chirping through changes in substance P immunoreactive fibers that innervate the CP/PPn (Dulka *et. al.*, 1995; Dulka and Ebling, 1999; Kolodziejski *et. al.*, 2005). Substance P-like immunoreactivity (SPl-ir) is distributed throughout diencephalic regions including the CP/PPn in males, but not in females (Weld and Maler, 1992). Androgen treatment not only enhances chirping and changes the quality of chirps produced, it also masculinizes the pattern of SPl-ir in diencephalic regions of treated females (Dulka *et. al.*, 1995). Testosterone treatment did not change SPl-ir in telencephalic regions, but increased SPl-ir expression in a

number of diencephalic regions implicated in chirp control including the CP/PPn, Hv, Hl, and PP, regions (Dulka *et. al.,* 1995). The posterior region of the Hl, specifically, projects SPl-ir fibers to the PPn, and this neuromodulatory pathway may be involved in the upregulation of chirp production in females that is associated with spawning behaviours (Dulka and Ebling, 1999). SPl-ir fibers connect to the ventral dendritic fields of PPn-C neurons and testosterone may act in this pathway by activating an androgen receptor that leads to an enhanced synthesis of a substance P-like peptide in Hl neurons that project to the CP/PPn. This may function to upregulate female chirping by increasing the presynaptic inputs of SPl-ir fibers from Hl (Weld *et. al.,* 1994; Dulka and Ebling, 1999).

*6.3.3 Cortisol Influences Chirp Production*

Cortisol is a circulating steroid hormone produced by interrenal cells, implicated in stress responses and in dominance and aggressive behaviours, and regulated by corticotropin releasing factor (CRF) (Hennessy *et. al*., 1991; Dunlap *et. al*., 2006). CRF is produced in the hypothalamus and plays a fundamental role in regulating a number of behaviourally relevant responses, including a prominent role in mediating stress responses in vertebrates (Takahashi *et. al*., 1989; Hennessy *et. al*. 1991; Francis *et. al*., 1999; Dunlap *et. al.,* 2011). CRF acts centrally, for example in the CP/PPn of *A. leptorhynchus* (Zupanc *et. al.,* 1999; Zupanc, 2002) and peripherally, it regulates circulating levels of cortisol via its actions on the hypothalamic-pituitary-interrenal axis (or hypothalamic-pituitary adrenal axis in non-fish). Dunlap *et. al.,* (2002) demonstrate that chirp rates of paired *A. leptorhynchus* correlate positively with endogenous cortisol levels. Silastic cortisol implantation increases chirp production rates in males over those of sham implanted males, without elevating 11KT levels suggesting that male-male interactions result in increased circulating cortisol levels which enhance chirp production

during aggressive social contexts, in a testosterone independent mechanism (Dunlap *et. al.*, 2002). Cortisol provides central feedback, for example, to neurons that produce CRF in the hypothalamus, that themselves project to various areas including to the CP/PPn (Zupanc *et. al.*, 1999; Dunlap *et. al.*, 2002).

Glucocorticoids also influence catecholaminergic systems and socially induced increases in cortisol can influence chirping behaviour through interactions with other neuromodulatory systems. For example, cortisol influences the expression of NA receptors (Dunlap *et. al.*, 2002), and NA treatment increases chirping in *A. leptorhynchus* (Maler and Ellis, 1987). Chirp rates and glucocorticoid levels are higher in paired fish than in isolated fish (Dunlap *et. al.,* 2006). Long-term social interaction increases plasma cortisol levels, neuronal proliferation, and enhances chirp production rates (Dunlap *et. al.*, 2006; Dunlap *et. al*., 2011). These socially-mediated changes are inhibited by blocking glucocorticoid receptors, suggesting that cortisol regulates experience-dependent changes in chirp production over the course of natural interactions (Dunlap *et. al.,* 2011)

### *6.3.4 Arginine Vasotocin Modulates Chirping Behaviours*

Arginine vasotocin (AVT) regulates reproductive and territorial communication behaviours in aquatic and terrestrial vertebrates (Marler *et. al*., 1995; Maney *et. al*., 1997; Goodson and Bass, 2000). Bastian *et. al.* (2001) examined the effects of intraperitoneal injections of AVT on chirp production in male and female *A. leptorhynchus*. AVT treatment decreases the production of small chirps in males, whereas it increases the production of less common, large chirps in some of the treated males, and has no effects on the chirp rates of tested females (Bastian *et. al.,* 2001). Neurons in the preoptic area produce AVT and project to the

CP/PPn (Bastian *et. al.,* 2001). In trout these preoptic areas express glucocorticoid receptors (Teitsma *et. al*., 1998), and stressors that increase plasma cortisol levels also result in increased expression of AVT gene transcripts in the preoptic area (Dunlap *et. al.,* 2002). Thus, socially induced changes in cortisol may also influence chirp production rates via AVT projections between the preoptic area and the CP/PPn (Bastian *et. al.,* 2001; Dunlap *et. al.,* 2002). Small chirp and large chirp rates increase under both courtship and aggressive contexts, but the precise social roles of the different chirp types and their production patterns may be regulated by modulators such as cortisol and AVT that influence the relative production rates of distinct chirp types depending on the social context and perceived threat (Bastian *et. al.,* 2001; Chapter 5).

## 6.4 Integrating Behaviour and Physiology

### *6.4.1 Individual and Context Specific Differences in Chirping Behaviours: Neural Considerations*

Individual and experience-dependent differences in electrocommunication behaviours are maintained through interacting neuromodulatory systems (Perrone *et. al.,* 2009, 2010; Pouso *et. al.,* 2010). Fish differ in their chirp responses to specific behavioural contexts (Chapter 4; Gama Salgado and Zupanc, 2011) and it is not known how these individual differences are maintained: how internal predispositions are integrated with external sensory stimuli to assess threats associated with alternate chirp and aggressive responses (Hopkins, 1974; Goodson, 2005).

The chirping and aggressive behaviours characterized in *A. leptorhynchus*, for example, the echo response, and the context dependent differences in the temporal patterning between bursts and aggressive behaviours, require precise sensorimotor integration (Hopkins, 1999, Zupanc, 2006A; Hupé and Lewis, 2008 (Chapter 2); Gama Salgado and Zupanc, 2011; Chapter

4). Small and large chirps are encoded in ELL pyramidal cells (Marsat and Maler, 2010); however it is not known how this information influences chirp production pathways during the echo response, or during other chirp patterning behaviours. Greenfield (1994) provides a review of the neural mechanisms that underlie rhythmic and alternating signalling behaviours, revealing many similarities in the sensorimotor mechanisms that underlie these communication exchanges in diverse taxa.

In many systems, the temporal aspects of the territorial signals produced have important behavioural roles (Bohn *et. al.,* 2008; Miller *et. al.,* 2009). Playback studies involving both acoustic and electric signals have demonstrated that signal duration, modulation rate, repetition number, and other temporal signalling features are important for a signal's communicative value (Hopkins, 1974; Greenfield and Minckley, 1993; Sneddon and Greenfield, 1998). For example, territorial signal rates and patterns are important in many types of social fish and other animals for species, sex and individual recognition (Bass and McKibben, 2003; Dunlap *et. al.*, 2011). The mechanisms by which *A. leptorhynchus* compare their own chirp rates and patterns with those of potential rivals are unknown, as are the mechanisms by which animals pattern their signal production with their physical behaviours and with those of interacting conspecifics. Further, chirps and AFRs were associated with different behaviours during conspecific interactions and future studies should examine how AFRs are encoded, how they are produced, and how they are integrated with chirping and aggressive behaviours.

*6.4.2 Electrocommunication Behaviours and their Neural and Hormonal Substrates show many Parallels with Those of Other Territorial Signalling Systems*

There are a number of parallels across the territorial signalling behaviours of different species of weakly electric fish and across signalling systems in a diversity of taxa, and similarities in the neural systems and mechanisms involved in processing territorial signals across different modalities (Bass and McKibben, 2003; Kelley and Bass, 2010). Sensory systems efficiently encode communication signals, with examples characterized in a number of modalities (Bass and McKibben, 2003; Kelley and Bass, 2010). Temporal variations in signal production mirror temporal encoding sensitivities of sensory systems, thereby promoting efficient signal transmission. In midshipman fish, females become sensitive to male calls only when they are primed for reproduction (Sisneros *et. al.*, 2004). Cichlids that use UV colouration patterns to signal aggressive intent are sensitive to wavelengths in the UV range, unlike most species of fish (Siebeck, 2004). In weakly electric fish, electroreceptors are tuned to the species specific frequency range, and are specialized for efficient encoding of EOD modulations including chirps (Benda *et. al.*, 2006; Hupé *et. al.*, 2008 (Chapter 3)).

In acoustic fish there is an extensive interface between auditory and vocal pathways for integration of auditory sensory inputs with signal production patterns (Bass and McKibben, 2003; Kelley and Bass, 2010). Territorial vocalizations in fish are encoded by the eighth cranial nerve (it also encodes sound in terrestrial vertebrates), and auditory encoding occurs in a similar fashion across vertebrate taxa: by frequency coding and phase locking (Bass and McKibben, 2003). Throughout the electrosensory system, electric image information pertaining to conspecific position and electric signalling behaviours is encoded through frequency coding and phase locking, among other mechanisms (Hupé *et. al.*, 2008 (Chapter 3); Marsat and Maler,

2010; Savard *et. al.*, 2011; Deemyad *et. al.*, 2011). The midbrain auditory site in teleosts is in the torus semicircularis (the fish homologue to the mammalian inferior colliculus) and is called the nucleus centralis. Interestingly, this brain region is also associated with processing electrical signals in the brains of non-vocal weakly electric fish (Moller, 1995).

In many territorial species (both fish and others) there is strong sexual dimorphism in the sensory pathways and motor circuits involved in territorial signalling systems (Maler *et. al.*, 1991; Zupanc, 2002; Bass and McKibben, 2003). In midshipman fish and toadfish, vocal pacemakers are sexually dimorphic in size and in the composition of neuropeptides they contain (Bass and McKibben, 2003). Neuromodulators shape territorial behaviour across many systems, for example, arginine vasotocin (AVT) and isotocin (IT) play a role in regulating vocal signalling seasonal plasticity in acoustic sensitivity and territorial behaviours of fish (Ferguson *et. al.*, 2002). AVT and IT are homologues of the mammalian neuron peptides arginine vasopressin and oxytocin, respectively, which are also involved in the regulation of mammalian territorial signalling behaviours (Wersinger *et. al.*, 2004). The role of vasotocin in regulating electrocommunication in a species of pulse gymnotiform has also been demonstrated where individual differences in electrocommunication and aggressive behaviours correlate with body size and vasotocin levels (Perrone *et. al.*, 2010).

### *6.4.3 Communication Behaviours Offer Insights into Cognitive Processes*

The modulatory systems that regulate species, sex, and individual differences in signalling demonstrate how physiological changes mediate behavioural predispositions and strategies (Dunlap *et. al.*, 2002; Sih *et. al.*, 2004; Salazar and Stoddard, 2009; Kelley and Bass, 2010). Electrocommunication behaviours are involved in individual recognition and demonstrate

experience-dependent plasticity implicating that long-term learning and memory mechanisms operate in the pathways that regulate chirping and other electrocommunication behaviours (Silva *et. al.,* 2008; Perrone *et. al.,* 2009; Salazar and Stoddard, 2009; Harvey Girard *et. al.,* 2010). The processes underlying individual and context-dependent differences in chirping behaviour have many parallels with the processing mechanisms in other modalities (Hagedorn *et. al.,* 1987; Maler and Ellis, 1987; Triefenbach and Zakon, 2008; Caputi and Nogueira, 2011). Further, chirping behaviours involve complicated sequences of signals produced by interacting individuals and it is not known if, as is the case in other signalling systems, the unit of perception or categorization is the individual chirp or the pattern of chirps produced (Ghazanfar *et. al.,* 2001; Carlson and Hopkins, 2004)

## 6.5 - Future Directions and Conclusions

We propose that the function of chirps is likely multifaceted. Chirp type, rates and patterns convey individual RHP and motivation (Triefenbach and Zakon, 2008; Hupé and Lewis, 2008 (Chapter 2)). Chirps influence receiver physiology and behaviour and some chirp patterns appear more threatening than others (Chapter 3; Chapter 4). For example, bursts of echoed chirps may induce a reciprocated echo response and deter aggression (Chapter 4). Chirps, especially bursts of chirps, may increase conspecifics' ability to localize one another during aggressive movements (Chapter 5).

### *6.5.1 Playbacks with Different Chirp Types Delivered In Specific Chirp Patterns with a Moving Conspecific Mimic*

Different signal types often convey unique meaning (Crawford *et. al.,* 1986; Seyfarth and Cheney, 2003) as do different temporal patterns of a single signal type (Pollack, 2001; Brumm,

2006; Miller *et. al.,* 2009). Small chirps are produced at high rates during agonistic contexts and signal aggressive motivation whereas large chirps are produced much less often. Large chirps tend to be produced more often by subordinate fish (Cuddy *et. al.*, 2011; Chapter 2 Addendum) and restrained fish (Chapter 5). AFRs are most commonly produced during physical aggression. Future studies should deliver different chirp types, and AFRs and other rises, in different patterns to both males and females under a variety of behavioural contexts, and in conjunction with different neuromodulatory manipulations. Researchers should also incorporate a moving simulated intruder (Laidre and Vehrencamp, 2008) from which to deliver playback chirps to address the responses of conspecifics to movements accompanied by different patterns of delivered chirps. It is important to consider the effect of the experimental manipulations on the behaviours being characterized (Hitschfeld *et. al.,* 2009), especially because chirping behaviours depend on the contexts under which they are produced (Hupé and Lewis, 2008 (Chapter 2); Chapter 4; Chapter 5).

### *6.5.2 Model P-unit Response to Contrast Changes during Different Chirp Patterns*

Different patterns of chirps will likely influence the ability of P-units to encode beat (E1) and beat amplitude (E2) information. Models could be used to characterize how the response of P-units to different contrast changes is affected by different patterns of chirps. In the baseline condition, the P-unit responses to a simulated waveform with an E2 electric image containing bumps and non-bump periods (Chapter 5) similar to those recorded during the movements in natural interaction could be characterized, representing a scenario in which no chirps are produced by either of two interacting fish. To determine how isolated and bursts of chirps influence the encoding of contrasts and contrast changes, the P-unit responses to the baseline E2 electric image with the addition of isolated chirps and bursts of chirps could be compared. Chirps

result in transient acceleration or decelerations of the beat and these can be modelled as phase shift of the beat (E1), represented as either isolated or bursts of beat phase modulations (Hupé *et. al.*, 2008 (Chapter 3)). If bursts of chirps associated with movement-induced contrast changes improve signal detection and conspecific localizability during social interactions over subsecond and second time scales, the P-unit response to contrast modulations should be enhanced in the presence of chirp bursts.

### *6.5.3 Multimodal Interactions in Electrocommunication*

The nature of the information available to individuals during conspecific encounters varies widely across animal systems and depends on the complement of sensory systems involved. Sensitivities to stimulus frequency and amplitude show tremendous differentiation, with receptor response profiles and central processing mechanisms shaped by diverse selection pressures to enhance detection of relevant stimulus features (Hupé *et. al.*, 2008 (Chapter 3); Marsat and Maler, 2010; Savard *et. al.*, 2011). The extent to which different species integrate information from any given modality also varies enormously, with the importance of particular modalities shaped by environmental pressures and evolutionary constraints (Forrest, 1974; Brumm and Slabbekoorn, 2005). Multimodal cues lead to better accuracy in social recognition in damselfish (Ward and Mehner, 2010). Multimodal sensory integration occurs in weakly electric fish (Moller, 2002), for example, both gymnotid and mormyrids produce EOD pulses in response to acoustic stimuli (Kramer *et. al.*, 1981). In *A. leptorhynchus*, we may have observed sound production but have not recorded the signals produced and future studies should examine whether acoustic signals are produced in addition to chirps and the other electrocommunication signals characterized.

### *6.5.4 Conclusions*

Chirping in the weakly electric fish *A. leptorhynchus* provides an ideal system for studying the complex neural processing and physiological modulation that underlies the production of communication signalling behaviours. The diencephalic CP/PPn regulates chirp production via specific efferent connections to the medullary pacemaker nucleus, and is itself regulated by a diversity of afferent connections and neuromodulatory systems. The NE, in conjunction with telencephalic centres, receives behaviourally relevant sensory stimuli, integrates these with motor inputs, and uses this information to generate appropriate chirp production patterns during social interactions through projections to the CP/PPn (Zakon *et. al.*, 2002). Longer term developmental and seasonal changes in chirp production rates (and quality) are induced by neuromodulatory influences that mediate changes in chirp behaviour through physiological changes in the CP/PPn (Zupanc, 2002; Telgkamp *et. al.*, 2007). The regulation of chirp production, controlled by the CP/PPn, is complex and an understanding of its functional regulation will be best explored using an integrative approach as different modulatory systems function in concert for precise and appropriate behavioural control. Advances in physiological and molecular techniques, in conjunction with the development of methods to analyze complex temporal relationships, will continue to provide new directions for exploring the basis of natural behaviours, and the individual and context specific flexibility in signalling characterized. Future work should continue to address how neuromodulatory systems interact during chirp sensorimotor integration to generate contextually relevant electrocommunication and aggressive behaviours during social interactions.

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