Aversive control of *Betta splendens* behaviour using water disturbances: effects of signalled and

unsignalled free-operant avoidance, escape, and punishment contingencies

by

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ABSTRACT

Research on aversive control of behaviour has dramatically declined over the past decades. This trend is primarily a consequence of an over-reliance on shock-based procedures, which have been increasingly criticized on ethical, practical, and ecological validity grounds. The continued study of aversive regulation thus requires the development of viable alternatives. Six preliminary experiments, triggered by serendipitous observations of *Betta splendens*' reactions to unintended water disturbances, allowed for (a) developing a water flows (WFs) experimental paradigm; (b) confirming the aversive function of WFs; and (c) demonstrating the feasibility of the WFs paradigm as an alternative to the use of electric shock, as it does not involve painful stimulation and carries a higher level of inherent ecological validity.

Based on the relevance of free-operant avoidance phenomena (Sidman, 1953a) for the study of aversive control, the fact that these have only been demonstrated in one fish species (goldfish) using shocks, and that the only attempt to show another form of avoidance in *Betta splendens* produced inconclusive results (Otis & Cerf, 1963), the WFs paradigm was implemented in two experiments aimed at addressing these issues. These studies were aligned with a research program on spatiotemporal analysis of behaviour that has demonstrated, over the course of several decades, that a comprehensive understanding of behavioural processes requires an approach that includes, but is not limited to, the study of rates of discrete responses (e.g., key pecks of a pigeon). Accordingly, a more holistic interpretation of experimental data than is typical for behavioural studies was attained through a combined analysis of the frequency and temporal distribution of a target response (crossings in a shuttle-tank), patterns of swimming trajectories, instances and durations of the aversive stimulus, and the occurrence of behaviour related to different features of the experimental tank.

In Experiment 1, *Betta splendens* exposed to a free-operant avoidance procedure reliably escaped WFs but did not develop avoidance behaviour even though escape improved with practice. Moreover, adding a warning stimulus (curtains of air bubbles - CABs) to the free-operant procedure did not produce increments in avoidance behaviour*,* as has been demonstrated in other species. Considering these findings, Experiment 2 maintained the same free-operant avoidance contingencies, but escape responses were now scheduled to produce the WFs (punishment and extinction of escape). The result of this manipulation was not a substantial decrease of escape, but an initial large increase of this response, followed by a progressive

decrease to approximately pre-punishment levels. In addition, punishment did not result in increased avoidance responding as an alternative response.

The explanations for these unexpected findings relate to the duration of the CABs; sign- and goal-tracking effects; uncontrolled stimulation produced by water pump activation/operation; unintended reinforcement (mirror reflections and delay between the pump activation and WFs reaching full strength); and the development of responses that allowed the fish to reduce their exposure to high-intensity WFs (i.e., alternative behaviour). The need for investigating the effects of adjusting the WF procedures to the ecology and biology of *Betta splendens* is also discussed, particularly in regard to their territoriality and predominant defensive response (immobility) in relation to the experimental apparatuses and the target response (changing compartments).

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To

Leonor, Gina, Hernan, and Lina

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ABBREVIATIONS

ABA	Applied Behaviour Analysis
ABs	Air Bubbles
BL	Baseline
CABs	Curtains of Air Bubbles
cm	centimeter
dB	decibels
EA	Early Avoidance
Esc	Escape
F-F	Flow-Flow interval
FFA	Flow-Flow avoidance
FI	Fixed Interval schedule
FR	Fixed Ratio schedule
FT	Fixed Time schedule
IRT	Inter-Response time
kHz	kilohertz
LA	Late Avoidance
min	minutes
PR	Preference Ratio
PRP	Post-Reinforcement Pause
R-F	Response-Flow interval
R_o	Operant
R-S	Response-Shock interval
s	seconds
SA	Signalled Avoidance
SA-Pesc	Signalled Avoidance and Punishment-and-extinction for escape
S^D	Discriminative stimulus
S^R	Reinforcement
S-R	Stimulus-Response
S-S	Shock-Shock interval
UA	Unsignalled Avoidance
UA-Pesc	Unsignalled Avoidance and Punishment-and-extinction for escape
VI	Variable Interval schedule
VTS	Video Tracking System
WD	Water Disturbance
WF	Water Flow

Chapter I. Introduction

The present dissertation adheres to the behaviour analytic approach to learning and behaviour; i.e., operant theory and methodology. It specifically aims at contributing to (a) the research area within behaviour analysis known as *aversive control,* which focuses on the study of behaviour in relation to events that are commonly considered "noxious", "painful" or "annoying" – i.e., aversive stimuli (Hineline & Rosales-Ruiz, 2013); and (b) a research program that, over the past three decades, has intended to show that spatiotemporal analyses provide a more comprehensive and coherent understanding of learning and behavioural processes when compared to the traditional operant approach, which focuses only on rates of discrete responses (Pear, 2004). The inclusion of aversive control in the spatiotemporal research program is something that, to date, has had no precedents.

Behaviour analysis and learning

The study of learning has been a prominent area within experimental psychology for more than 100 years. Contemporary theory and research in this field is the result of a complex transition from global approaches, developed during the first half of the past century (e.g., the work of C. L. Hull, E. C. Tolman, B. F. Skinner, and E. R. Guthrie – Estes et al., 1954), to more specialized models that focus on diverse aspects of the processes involved in learning. The catalysts for this transition were the establishment of the cognitive paradigm during the 1960s, a renewed attention to classical conditioning phenomena, and the increasing interest in biological constraints on learning (Mowrer & Klein, 2000). As a result, modern research on learning and behavioural processes inherited diverse experimental procedures, phenomena, and concepts from the global theories, while incorporating important cognitive and biological perspectives (Bouton, 2007). It is in this contemporary panorama that behaviour analysis represents a unique and comprehensive approach to learning and behaviour.

Given its origins in B. F. Skinner's conceptual and empirical work, it is no surprise that behaviour analysis diverged fundamentally from coexisting mainstream learning theories and evolved to what is currently a productive field in basic and applied research areas (experimental analysis of behaviour and applied behaviour analysis, respectively – Fantino, 2008; Poling, 2010; Schlinger, 2010). Today, behaviour analysis is considered

> …a field dedicated to the development and application of behavioral principles to the understanding and modification of the psychological actions of organisms. As such, behavior analysis [is] committed to a comprehensive account of behavior, stretching from animal learning to complex human behavior (Vilardaga, Hayes, Levin, & Muto, 2009, p. 105).

The conceptual framework of behaviour analysis resulted from a diverse group of traditions dubbed "behaviorisms" (O'Donohue & Kitchener, 1999; Zuriff, 1985). Chief among them, B. F. Skinner's *radical behaviorism*, also known as *operant behaviorism*, provided the systematic context for the development of a wide variety of research programs (Catania & Harnad, 1988), which, despite specific differences, share general philosophical tenets in a "loose family resemblance" (Zuriff, 1985). These tenets include: (a) the behaviour of the individual organism is the subject matter of the discipline in its own right and not an index to causes in other dimensions (e.g., Moore, 2008; Morris, 1992); (b) research questions about the functional relationships between independent and dependent variables are preferred over hypothetically deduced theoretical questions, which, whenever considered, are also translated into questions about functional relationships between variables; and (c) single-organism research methods are favoured (Iversen, 2013; Lattal, 2013; Perone & Hursh, 2013; Johnston & Pennypacker, 2009; Zuriff, 1985).

As robust as the operant approach to learning and behaviour may be, it has never been mainstream in psychology; in fact, its contributions are frequently misunderstood and/or undervalued (Morris, 1992). One of the reasons for this is that operant theory entails assumptions,

concepts, and methodologies that not only continue to be mistaken for traditional learning models (O'Donohue, Ferguson, & Vargas, 2001; O'Donohue, & Kitchener, 1999; Zuriff, 1985), but also conflict with other perspectives in psychology (De Mey, 2003; Morris, 1992; Hineline, 1980, 2013; O'Donohue, Callaghan, & Ruckstuhl, 1998). The unique stance of the operant approach is particularly evident in its (a) very definition of behaviour (e.g., as a type of interaction between organism and environment, or as a functional response class - Catania, 2009; Johnston & Pennypacker, 1993a,b, 2009; Pierce & Cheney, 2013; Ribes-Iñesta, 2004); (b) the preference for single-organism designs and free-operant procedures (Johnston & Pennypacker, 1993a,b, 2009; Pierce & Cheney, 2013; Sidman, 1960); (c) importance assigned to the organism's history and environment in the understanding of learning and behavioural phenomena (e.g., the concepts of history of reinforcement and function of stimulus are central to the explanation of current behaviour – De Mey, 2003; Catania, 2013; Skinner, 1981); (d) non-mediational conceptualization of internal processes and mental concepts (e.g., De Mey, 2003; Hayes & Brownstein, 1986; Moore, 2001; Morris, 1992; O'Donohue et al., 1998; Rachlin, 1994; Skinner, 1945); and (e) use of *systematic replication* (attempts to "replicate a functional relationship under circumstances that differ from those of the original experiment" – Perone & Hursh, 2013, p. 123) for refining knowledge about the variables that control behaviour, and for testing the reliability and generality of experimental findings; i.e., the extent to which experimental findings are consistent and generalize over species, time, place, dependent measures, and similar experimental manipulations or independent variables (Johnston, 1979; Perone & Hursh, 2013; Sidman, 1960).

The following implications regarding how learning is conceptualized result from assuming the operant approach outlined above. First, learning refers to functional and historical aspects of behaviour, and not a process that occurs in another dimensional system or on a non-behavioural level. In this regard, Skinner (1988) stated that learning is "a process in the behavior of the

individual… we may define it as a change in probability of response, but we must also specify the conditions under which it comes about" (p. 94). Subsequent definitions of learning have closely followed Skinner's ideas. For instance, Johnston and Pennypacker (2009) and Pierce and Cheney (2013) assert that learning entails the acquisition, maintenance, and enduring changes in behaviour that occur as a consequence of conditioning processes involved in lifetime events. Further, Pear (2001) defines learning as "a dependency of current behaviour on the environment as a function of a prior interaction between sensory-motor activity and the environment" (p. 12). Second, explanations and predictions within operant theory typically follow the laws of conditioning, which can be understood as descriptions of how relationships between the organism's responses and environmental events affect future behaviour (Johnston & Pennypacker, 2009).

Finally, when an organism develops new ways of behaving in reaction to changes in its environment (i.e., some form of learning occurs), operant researchers recognize the necessity of providing both remote and immediate causal explanations for these learning phenomena. The predominant view within behaviour analysis is one that assumes "a general form of causation that goes beyond the push-pull mechanistic Newtonian model of physics" (Pierce & Cheney, 2013, p. 439) known as *selection by consequences* (Catania, 2013; Skinner, 1981). From this perspective, behaviour may be selected through phylogenic, ontogenic, or cultural processes that may complement or oppose each other. For instance, behaviour selected through an individual's lifetime (e.g., as a result of a particular history of reinforcement or punishment), may or may not reduce its reproductive fitness (e.g., substance abuse). Similarly, "patterns of behavior maintained through cultural selection among members of a group may oppose rather than support phylogenic or ontogenic contingencies [e.g., celibacy, suicide or modern war]" (Catania, 2013, p. 135). By following this perspective, behaviour analysis has integrated the study of learning and behavioural

processes with biology on the one hand, and the social sciences on the other (Pierce & Cheney, 2013).

Outline of the dissertation

The present dissertation is congruent with the tenets of the behaviour analytic perspective and is structured as follows. Chapter II introduces the relevant behaviour analytic foundations, both conceptual and methodological, of the research that is the subject of this thesis. The precepts detailed therein are critical to the following chapters, and aim to establish a "common ground" with the reader. This is particularly relevant for those who are not familiar with the behaviour analytic approach to aversive control phenomena. Moreover, such familiarity with the operant approach is crucial for the understanding of two shortcomings – which the research presented herein further aims to address – that are intrinsic to the behaviour analytic field itself: (a) the fact that studies on basic principles of aversive control today are almost nonexistent, primarily due to the lack of alternatives to electric shock procedures; and (b) the limited focus of the traditional operant methodology on rates of discrete responses.

Accordingly, Chapter III will discuss the relevance of aversive control research, and how its dramatic decline over the last decades is mostly related to an over-reliance on a single form of aversive stimulus, electric shock, which has been criticized on ethical, practical, and ecological validity grounds. Evidently, there is a need for the development of alternatives that allow for the continued study of aversive phenomena. This research, as a whole, is a contribution in that direction.

Chapter IV discusses (a) the rationale, efficacy, and limitations of the mainstream operant methodology, which focuses on rates of discrete responses (e.g., key pecks or lever presses), and (b) the alternative approach that has informed a research program on spatiotemporal analysis of behaviour at the University of Manitoba. The aim and methodology of such a program is

exemplified by foundational research with pigeons that confirmed the potential of spatiotemporal

analyses for providing a more comprehensive understanding of behavioural processes that

includes, but is not limited to, the study of response rates.

Unlike this first generation of studies of the spatiotemporal analyses program, the most

recent research has incorporated a fish species, *Betta splendens* (Siamese fighting fish – the term

"betta" will be used throughout this dissertation to refer to members of the species *Betta*

splendens). Therefore, Chapter V will review (a) the biological and behavioural characteristics of

bettas; (b) the rationale for utilizing fish in the study of learning and other behavioural

phenomena, including the basis for their use in behaviour analytic research and the benefits to the

spatiotemporal research program; and (c) the previous research conducted with bettas at the

University of Manitoba's Behaviour Analysis Fish Laboratory, which has extended the scope and

potential of the program on spatiotemporal analyses of behaviour.

The topics of the subsequent chapters originate from one aspect that has characterized

previous studies of the spatiotemporal research program, both with pigeons and bettas: an

exclusive focus on appetitive contingencies. Accordingly, Chapters VI and VII (a) review the

relevant antecedents of aversive control research with fish, namely studies on punishment and

avoidance with goldfish and bettas; and (b) sequentially reconstruct the development of a novel

methodology that has allowed, for the first time, the study of the spatial and temporal organization

of betta behaviour under aversive conditions. Specifically, these chapters include a review of six

preliminary studies, conducted on site, that were triggered by chance observations of betta's

reactions in their experimental and home tanks. These serendipitous observations led, for the first

time, to testing whether water disturbances have an aversive function on the behaviour of bettas.

The development of these experiments generated a versatile experimental paradigm that offers a

promising alternative to the use of electric shock in aversive control studies, as it does not involve painful stimulation and carries a higher level of inherent ecological validity.

Considering (a) that free-operant avoidance (Sidman, 1953a), one of the most important behaviour analytic paradigms for the study of aversive control, has not been demonstrated with bettas; and (b) the fact that the only study designed to show another form of avoidance in this species provided inconclusive results (Otis & Cerf, 1963), the decision was made to carry out an initial experiment that addressed these two issues. Chapter VIII describes this initial experiment, which was designed to test the effects of unsignalled and signalled free-operant avoidance contingencies that entailed water flows as the aversive stimulus and curtains of air bubbles as the warning stimulus. This experiment revealed that no consistent avoidance behaviour was developed by any of the subjects under both signalled and unsignalled conditions. Instead, a striking predominance of escape responses was developed.

In view of these unexpected findings, and based on what has been consistently replicated with other species, Chapter IX presents a second experiment in which the same avoidance contingencies remained in place, but where escape responses were arranged to produce the aversive stimulus (water flow) instead of interrupting it – i.e., a combined punishment and extinction contingency was scheduled. The widely replicated effects of punishment across different species, including bettas, led to the prediction that this change in the contingencies would favour the suppression of the target behaviour (escape) and the increment of an alternative response; in this case, avoidance. Nevertheless, avoidance behaviour did not increase during Experiment 2. Escape responses, however, were indeed affected by the contingencies of Experiment 2, in terms of a dramatic initial increase that was followed by a progressive decline.

Possible explanations for the unexpected findings of Experiments 1 and 2 are provided through the analysis of a series of responses that fish displayed towards different features of the

experimental tank, the potential effects of stimulation produced by the activation/operation of the

water pumps, apart from the water flows, and the patterns that emerged in the temporal and spatial

distribution of the subjects throughout the experiments (e.g., swimming trajectories and time

allocations to the compartments of the experimental tank). This discussion further demonstrates

the potential for spatiotemporal analyses to provide a distinct type of information, not obtainable

through the traditional operant approach, which should lead to a more comprehensive

understanding of behavioural processes.

Chapter II. Aversive control: the study of behaviour in relation to aversive events

The study of aversive control has played an essential role within Behaviour Analysis,

given that a complete understanding of behavioural processes must consider not only the effect of

appetitive forms of stimulation, but also the control from sources that abound in nature commonly

considered "noxious", "painful" or "annoying" (Baron, 1991; Crosbie, 1998; Hineline & Rosales-

Ruiz, 2013). In this regard, Crosbie (1998) has pointed out that

> an experimental analysis of [aversive control] is crucial if we are to achieve our goal of
> predicting, controlling, and interpreting the behavior of organisms. Studying only positive
> consequences of behavior will reveal only half of the picture. Furthermore, negative
> reinforcement and punishment are particularly powerful, and constantly present in the
> physical environment. (p.163)

Additional to the disciplinary relevance identified by Crosbie (i.e., predicting, controlling,

and interpreting behaviour), the study of aversive control has been deemed critical from an

applied perspective. Research in this field allows for the understanding and development of

treatments for dysfunctional behaviours that result from ways that organisms (human and non-

human) cope with aversive situations, and for testing the clinical value of drugs and other

physiological interventions (Baron, 1991). Moreover, basic knowledge about aversive control has

been used to address social issues, as exemplified by Sidman's (2001) examination of coercion as

a widespread system in our society that entails "the use of punishment and the threat of

punishment to get others to act as we would like, and … our practice of rewarding people just by

letting them escape from our punishments and threats" (p. 1). From Sidman's perspective,

coercion is a prevalent form of aversive regulation in society that leads to detrimental outcomes,

such as individuals "dropping out" of (i.e., escaping from) education, family, personal and

community responsibilities, civil duties, society, and even life itself (Pierce & Cheney, 2013).

The study of aversive control entails a variety of approaches and conceptual frameworks

that include strategies to asses interactions between respondent (classical) and operant processes

(e.g., conditioned suppression – Estes & Skinner, 1941; taste aversion – Garcia and Koelling, 1966), as well as numerous approaches directed to the study of emotional and social phenomena in relation to aversive events (e.g., learned helplessness – Seligman & Maier, 1967; reflexive aggression – Ulrich & Azrin, 1962; Ulrich, Wolff, & Azrin, 1964). Notably, the paradigms that have attracted the most attention within behaviour analysis – particularly through the use of the *free-operant* strategy, which is a distinctive feature of the behaviour analytic approach – are escape, avoidance, and punishment (Baron, 1991; Crosbie, 1998; Sidman, 1953a). The free-operant is but one of the many facets of operant methodology, described in detail in the following section.

The operant approach in the study of aversive control

Critical to operant theory are the notions of functional classes of responses and stimuli (Johnston & Pennypacker, 2009) and the concept of contingency (De Mey, 2003). These concepts are unique to the behaviour analytic perspective, and are consistent with its emphasis on functional relationships between the behaviour of an individual organism and its environment.

Response classes and stimulus classes

From an operant perspective, a *response class* is "a collection of individual responses that have common sources of influence in the environment" (Johnston & Pennypacker, 2009, p. 71), that falls under three general types (respondent, operant, and discriminated operant), and constitutes the basic "unit of analysis for the science of behavior" (Johnston & Pennypacker, 2009, p. 71). For example, in an operant experiment, all the possible ways in which a rat may operate a lever to produce a pellet of food constitute a single response class.

Analogous to response class, *stimulus class* is a term that refers to a collection of "stimuli that vary across physical dimensions but have a common effect on behavior" (Pierce & Cheney, 2013, p. 444). That is, classes of stimuli are defined by their common effect on a response class,

irrespective of the similarity or differences on physical dimensions (i.e., like classes of responses, classes of stimuli are defined functionally). For instance, if the deliveries of shock or intense noises have the same suppressive effect on a rat's rate of lever-pressing (the lever-press response class) in an operant experiment, both stimuli are said to belong to the same stimulus class (punishers). Classes of stimuli are named "reinforcers" or "punishers" depending on whether the effect observed after their presentation contingent on a response is an increase or a decrease of some aspect of the response class over baseline levels (e.g., when the contingent presentation of certain stimuli increases the response rate it is assumed that this class of stimuli has a reinforcing function). Similarly, the terms "positive" or "negative" – often carrying different connotations in non-behavioural literature – are assigned to a class of stimuli based on whether the response class that it controls produces or terminates any occurrence of stimuli in that class.

Depending on different combinations of these dimensions, *positive/negative* and *reinforcing/punishing*, classes of stimuli are dubbed *positive reinforcers*, *negative reinforcers*, *positive punishers*, and *negative punishers*. For instance, positive punishers, are stimuli that *occur* immediately following a response, and produce a *decrease* in some aspect of the response class that is influenced by those stimuli − e.g., a rat is trained to press reliably a lever to produce food; then, a shock is presented immediately after each response. This procedure results in a decrease in lever-pressing responses in similar circumstances (i.e., shocks had a positive punishing function for lever-pressing).

Aversive functions of stimulation

In accordance with the functional emphasis of the operant approach, the *aversive* quality of classes of stimuli is only determined by the effects that those stimuli have on a response class − i.e., their function − and not by other inherent characteristic of the stimulation, such as their

potential for inflicting pain, discomfort, or dislike. Accordingly, for Mallott and Trojan (2008) a

stimulus is aversive if it increases the future frequency of a response class when the latter is

followed by the removal or termination of the former (e.g., the rat that presses a lever for

interrupting a loud noise). More generally, Pierce and Cheney (2013) define aversive stimuli as

"those events that organisms evade, avoid, or escape from" (p. 153). Among the types of stimulus

classes reviewed in the previous section, punishers and negative reinforcers, both unconditioned

(their behavioural functions have a phylogenetic origin) and conditioned (their behavioural

functions have an ontogenetic origin) are considered *aversive*.

Aversive contingencies: punishment and negative reinforcement

From an operant perspective, *contingency* is a concept with descriptive and explanatory

functions that refers to specific types of relationships between classes of responses and classes of

stimuli (De Mey, 2003). Accordingly, the model to understand behavioural phenomena within an

operant framework is the three-term contingency ($S^D - R_o - S^R$, where S^D = discriminative

stimulus; R_o = operant; and S^R = reinforcement – Morris, 1992), which is defined as a "set of

functional relationships among distinct classes of antecedent stimuli, responses, and consequent

stimuli…" (Johnston & Pennypacker, 2009, p. 6). As Morris (1992) has pointed out, previous to

B. F. Skinner's development of his conceptual system, a two-term stimulus-response (S-R)

psychology was predominant. According to Morris,

> Skinner's genius was to see the inadequacy of such a unit of analysis and to propose a
> three-term psychology in which the Ss and Rs functioned differently. First, his consequent
> S is a reinforcing or punishing stimulus for responding. It is a cause of behaviour but not a
> mechanical push or a pull; rather it selects behaviour… Second, the antecedent S is a
> discriminative stimulus; it sets the occasion for responding but does not mechanically
> impel it…. Third, the response is an instance of an operant class of behaviour, not a reflex.
> It is functionally defined with respect to its consequences and antecedents, just as those
> consequences and antecedents are functionally defined to it." (p. 12).

Nevertheless, the three-term model and its functional emphasis have been "…often neglected by those who think of behaviour change only in terms of the instrumental and classical procedures of earlier conditioning theories" (Catania & Harnad, 1988, p. 6). As a result, behaviour analysis has been frequently misrepresented as a "stimulus-response" psychology that does not differ from the traditional learning models (e.g., Thorndike's, Hull's, Tolman's, or Guthrie's).

The three-term contingency model provides a framework for representing operant and discriminated operant behaviour, and distinguishing these from respondent behaviour (i.e., Pavlovian or classical). Four basic types of contingencies can be defined on the basis of this model – *positive reinforcement*, *positive punishment*, *negative reinforcement*, and *negative punishment*. This framework has been used to develop research programs, basic and applied, on the behaviour of human and non-human organisms. In the case of aversive control, operant research focuses on behaviour that is regulated by punishment and negative reinforcement contingencies of different levels of complexity (Baron, 1991; Crosbie, 1998; Hineline & Rosales-Ruiz, 2013). From this perspective, when aversive events are contingent on the occurrence of a response class and the resulting effect is a decrease in the probability of responding, the contingency is called *positive punishment*. Alternatively, when an ongoing stimulus is removed from a situation contingent on a specific response class, and such interaction results in a decrease in the probability of the response class, the contingency is called *negative punishment*. Finally, when specific events are terminated or prevented by a response class, and the resulting effect is the increase of probability of responding, the contingency is called *negative reinforcement* (Baron, 1991; Crosbie, 1998; Hineline & Rosales-Ruiz, 2013; Pierce & Cheney, 2013).

Behaviour maintained by negative reinforcement contingencies: avoidance and escape

The aforementioned definitions of response classes and aversive stimuli (punishers and negative reinforcers) lead to the definition of two types of behaviour of high relevance in the

study of aversive control: *escape* and *avoidance*. The following quote by Crosbie (1998)

exemplifies how the operant theory approaches these concepts:

> …negative reinforcement is defined as the operation by which response rate increases or is
> maintained when the response reduces, terminates (escapes), or postpones (avoids) an
> aversive stimulus. Aversive stimuli are those that affect behavior in this way. If an
> organism responds to escape or avoid a stimulus, then, in that situation, the stimulus is a
> negative reinforcer (i.e., aversive); a stimulus that does not increase responding in such a
> way is not. Punishment is defined as the operation whereby response rate is reduced
> following some response-dependent stimulus change (Azrin & Holtz, 1966). A punisher
> [i.e., aversive stimulus] is a response-dependent stimulus change that reduces responding.
> (p. 164).

Thus, escape and avoidance are response classes maintained by dissimilar negative

reinforcement contingencies. They differ from each other and from other forms of behaviour (e.g.,

punishment) on the basis of the specific relationships that are involved (e.g., reduction,

termination, or delay of aversive stimuli), and not on any intrinsic features. For instance, the same

lever-press response of a rat is functionally classified as escape if it terminates an ongoing

aversive event, such as a loud noise, or as avoidance if it postpones the occurrence of that same

event.

The free-operant approach

B. F. Skinner's seminal theoretical and methodological contributions resulted in a strategy

of proven efficacy, the free-operant approach. This method and the rationale for its use constituted

major aspects that differentiated, very early, behaviour analysis from other perspectives in the

study of learning (e.g., Hull, 1943a,b or Tolman, 1948). Fester (1953) summarized some of the

major characteristics and advantages of the free-operant method in the following quote:

> Contrary to the [general view], the "Skinner box" is not a specific technique, but rather a
> method of research employing the free operant. The use of the free operant is a method of
> wide generality; it refers to any apparatus that generates a response which takes a short
> time to occur, leaves the animal in the same place ready to respond again… [and is used]
> in experiments when the dependent variable is the frequency of occurrence of behaviour.
> Nearly all the problems of the science of behaviour fit this paradigm when the questions
> are of the form: what is the likelihood of a piece of behaviour occurring on this particular

occasion; how strong is the tendency to behave on this occasion relative to another occasion? The free operant has advantages in this respect [as compared to other approaches] because it removes restrictions on the frequency with which a response can occur and permits the observation of moment-to moment changes in frequency… (p. 21 - 22)

Thus, the free-operant approach differs from techniques that have been more characteristic of other psychological traditions (e.g., discrete-trials procedures in T-mazes), because it allows the experimental subject to respond repeatedly over long periods of time without the interference of the experimenter. Under these circumstances, the subject is "free" to respond any number of times – many, few, or none at all – during an experimental session. In this regard, Ribes-Iñesta, Torres, Correa, and Montes (2006) pointed out that

> …the free-operant method (Skinner, 1938; Ferster, 1953) eliminated the temporal and spatial restrictions on behavior in comparison to the traditional discrete-trial methodology of classical conditioning and the so-called 'instrumental learning' (Pavlov, 1927; Hull, 1943). In the free-operant situation, the organism is free to move within the experimental chamber and to respond across time to different properties of the environment without interruption. In colloquial terms, the free operant can be described as a situation in which the organism is free to do what it wants to do and can do. (p. 41)

The use of operant methods has resulted in the formulation of several principles of behaviour, such as reinforcement (unconditioned, conditioned, and differential), punishment, stimulus control, extinction, and avoidance. Furthermore, these methods have been implemented in a wide variety of experimental preparations and have been adopted in diverse basic and applied areas within and outside behaviour analysis (e.g., pharmaceutical and neurobiological research, drug addiction studies). Today, several behaviour analytic procedures share the following paradigmatic aspects of the free-operant method: (a) experimental devices (e.g., operant chambers) allow the subject to continuously interact with a single or several operanda (e.g., levers, illuminated discs, keys, running wheels); (b) several sources of stimulation are available for manipulation by the experimenter (e.g., lights, sounds, electric shocks); (c) positive or negative reinforcers (e.g., food, water, electric shocks) are delivered depending on different

schedules of reinforcement and punishment; (d) the rate of responding on a single or several

operanda is typically recorded for later analysis (e.g., cumulative records); and (e) the focus is on

a few subjects that are studied intensively, their data is not aggregated, and each typically serves

as its own experimental control.

The free-operant approach to avoidance

The distinction between free-operant methods and other procedures is particularly relevant

to the present research because it entails the study of aversive phenomena, which have been

studied differently in behaviour analysis than in other approaches to learning. As Steiner (1971)

pointed out, aversive control research has followed either a classical tradition associated with two-

factor theory (Mowrer, 1951, 1960) or a behaviour analytic tradition (e.g., Skinner's and

Sidman's free-operant procedures − Estes & Skinner, 1941; Sidman, 1953a). Steiner indicated

that the former uses procedures in which "…only responses that do or do not occur within

avoidance trials are considered… [and] responses occurring between the arbitrary presentations of

paired neutral and noxious stimuli are prevented, ignored, or considered problematic…" (p. 52).

For example, in a maze experiment the subject typically is removed from the maze after each trial

and its behaviour between trials is not considered. In contrast, Steiner noted that research that

implements free-operant methods does not have this limitation; for instance, avoidance learning is

seen "as a continuous sequence of emitted responses coming gradually under the control of an

avoidance contingency…" (Steiner, 1971, p. 52).

An additional argument in favour of free-operant methods relates to issues of measurement

associated with discrete-trials procedures. In non-operant procedures, an avoidance trial begins

with the presentation of a warning stimulus, and ends either with the occurrence of the aversive

stimulus (typically a brief electric shock), or with the response of the subject that stops or delays

the noxious stimulus (Sidman, 1953a). Sidman pointed out that a limitation of these procedures is

that the measure of interest consists of the count of an occurrence or non-occurrence of the avoidance response, which is "necessarily a gross one since there are no intermediate states between the extremes" (p. 157). Sidman considered that this difficulty has been traditionally solved in one of two ways: (a) by averaging the number of responses emitted by a group of subjects on each trial, or (b) by grouping the trials of a single individual into blocks of arbitrary length, and then summarizing them in some manner. Sidman argued against these two statistical approaches considering that in both cases a loss of a considerable amount of information is inevitable.

Sidman (1953a) devised an alternative procedure intended to solve these issues. In doing so, he became the first to report behaviour maintained by negative reinforcing contingencies in a manner that did not require discrete trials. This procedure, known as *Sidman avoidance* or *free-operant avoidance*, became one of the trademarks of behaviour-analytic research on aversive control. In this paradigm, periodic electric shocks are given to subjects unless they emit an operant response (press a lever or change compartments in a shuttle box) that delays the onset of the next shock. In addition, *shock-shock* (S-S) and *response-shock* (R-S) intervals are specified; these refer to the time between shocks if a response does not occur and the period between the emission of a response and the onset of the next shock if a response does not occur, respectively (Hineline, 1977; Hineline & Rosales-Ruiz, 2013).

The successful establishment and maintenance of lever-pressing in rats exposed to the unsignalled procedure devised by Sidman (1953a) was later replicated with a variety of species, which ranged from goldfish (Behrend & Bitterman, 1963) to humans (Ader & Tatum, 1961). Sidman's free-operant preparation became paradigmatic in aversive control research, and his findings were foundational for the non-mediational and molar conception of avoidance in behaviour analysis (Hineline & Rosales-Ruiz, 2013).

The behaviour analytic perspective to the "avoidance problem"

The operant approach to avoidance differs from other theoretical approaches such as two-factor or two-process theory (Mowrer, 1951) in that it does not assume hypothetical motivational functions for a warning stimulus to explain what has been called "the avoidance problem" – i.e., how can non-occurrence of an event be a reinforcing consequence (Hineline & Rosales-Ruiz, 2013)? According to the mainstream non-behaviour analytic interpretation, a warning stimulus occurs in all avoidance paradigms, even when one is not explicitly programmed. Initially the warning stimulus is a neutral event, but as a result of being paired with the primary aversive stimulus, it becomes a conditioned aversive or fear-eliciting stimulus (respondent or classical conditioning process). Consequently, the behavioural changes observed (e.g., in response times or frequency) are not explained by an avoidance contingency, but by an escape contingency (operant or instrumental conditioning process) because the organism's response *terminates* a warning stimulus instead of *preventing* or *delaying* a non-occurring aversive event – e.g., rats do not avoid a shock directly; instead, they escape from a warning stimulus such as a tone, which in their past experience has accompanied shocks (Hineline & Rosales-Ruiz, 2013).

Starting with the foundational studies by Sidman (1953a,b, 1955), increasing behaviour analytic research has shown that, contrary to two-process theory, avoidance can occur without the aid of warning stimuli; therefore, escape and avoidance indeed must be functionally differentiated (Hineline, 1977, 1981; Hineline & Rosales-Ruiz, 2013). Moreover, this evidence indicates that warning stimuli in avoidance procedures often have a discriminative function, instead of a fear-eliciting conditioned function. For instance, adding a warning stimulus (e.g., a tone) to the free-operant avoidance procedure during the last period of the R-S interval (just before the shock is due) typically results in subjects waiting for the onset of the warning stimulus before responding (Behrend & Bitterman, 1963; Hyman, 1971; Sidman, 1955; Simon & Kreuz, 1985; Steiner, 1971).

Finally, research has shown that avoidance behaviour can be maintained not only by postponement of the aversive stimulus, but also by changes in overall frequency of aversive events. For instance, Herrnstein and Hineline (1966) devised an unsignalled avoidance procedure for rats where, in the absence of responding, shocks were delivered based on a given baseline probability sampled every 2 s. A single lever press lowered that probability, without affecting the rate of sampling, where it remained until a shock occurred and the probability was reset to its baseline value. Herrnstein and Hineline found that lever-press responding was established and maintained by this procedure, notwithstanding the fact that some shocks were still inevitable (i.e., rat behaviour was maintained by the changes in the frequency of probabilistic aversive events) even though no explicit or implicit warning stimulus occurred. This finding show that two-process theory cannot account for all avoidance phenomena; as Hineline and Rosales-Ruiz (2013) put it, "warning stimuli are irrelevant [in these studies], making two-factor theory silent concerning the results" (p. 496). More generally, the outcomes of these and other related studies have supported the notion that consequences of behaviour that are extended in time and in complex patterns can still reinforce or punish behaviour (i.e., consequences need not be contiguous to behaviour if they are to affect it), an aspect that characterizes the molar approach of behaviour analysis to learning and behaviour (Hineline & Rosales-Ruiz, 2013; Malone, 2004; Pierce & Cheney, 2013).

Chapter III. Present state of research on aversive control

Despite the high relevance of the studies on basic principles of aversive control, several

authors have noted that there has been a dramatic decline of research in this area since the 1970s

(e.g., Baron, 1991; Catania, 2008; Crosbie, 1998; Pierce and Cheney, 2013). Baron (1991), in his

review of the paradigms of negative reinforcement and punishment, commented that

> [d]different interpretations can be placed on the sparsity of current studies of avoidance
> and punishment... An optimistic view – but one I find it difficult to subscribe to – is that
> the important questions are largely settled, in which case the procedures here are best
> regarded as tools for studying questions in other areas (e.g., behavioral pharmacology,
> sensory processes, behavioral economics, etc.). More pessimistic is the view that the
> promise of operant research for an understanding of aversive control remains to be
> fulfilled (cf. Perone et al., 1988). This must be my conclusion, insofar as too many of the
> essential questions remain unanswered. (pp. 211 - 212)

More than sixteen years later, Catania (2008) reviewed the research trends evidenced in

the flagship behaviour analytic publication (*Journal of the Experimental Analysis of Behavior* –

JEAB) and reached similar "pessimistic" conclusions:

> The analysis of aversive control has almost vanished from JEAB. Has the conduct and
> publication of research on punishment and escape and avoidance and conditioned
> suppression and related phenomena been punished? At the least, it has not been much
> reinforced. It is probably relevant that the links in the initiating chains for such research
> (Gollub, 1977) have become extended with the interposition of Institutional Review
> Boards and the corollary requirement that experimental protocols be specified in
> advance… Have we learned enough about aversive control phenomena in the past half-
> century that we do not need to study them anymore? …In a world so filled with aversive
> events that enter into various contingencies with behavior (Perone, 2003), can we entertain
> any extensions of our applications without continuing or expanding our experimental
> analyses of these phenomena? (p. 114)

Evidently, studies on basic principles of aversive control have declined since the 1970s, to

the point that today they are almost nonexistent. Moreover, this situation does not seem to be the

result of all the questions in this area having been answered, hence no further research, basic or

applied, being needed. On the contrary, it seems that research on aversive control has been

restricted and/or has not been encouraged, notwithstanding the fact that we have not yet learned

enough about the aversive regulation of behaviour, its side effects, and how to reduce its

prevalence (Pierce & Cheney, 2013).

As Catania (2008) indicated, the downward trend in experimental analyses of aversive

control is mainly related to ethical and welfare considerations. Overall, changes in cultural values

over the past decades, together with the influence of animal activist movements, have made it

almost impossible to conduct experiments on aversive control (Davis, 1981; Pierce & Cheney,

2013). One aspect that has clearly accentuated this issue is the fact that research on aversive

control has almost exclusively utilized a particular form of aversive stimulation, namely, electric

shock. This has been especially controversial because of the common view that it implies pain,

and therefore cruelty (Baron, 1991; Davis, 1981; Crosbie 1998; Hineline & Rosales-Ruiz, 2013;

Russell, 1997).

Electric shock in aversive control research: rationale and implications

The prominence of electric shock in aversive control research could be explained by its

convenience in terms of its reliability, broad range of intensities and durations, the feasibility of

titrating shock levels for individual subjects, and the fact that it is aversive at levels that do not

cause tissue damage (Barker, Sanabria, Lasswell, Thrailkill, Pawlak, & Killeen, 2010; Baron,

1991; Crosbie, 1998). Although these pragmatic aspects must have played an important role in the

success of preeminent research programs that systematically and exclusively employed shock-

based procedures (e.g., N. H. Azrin's et al. program on punishment – Azring & Holz, 1966), the

decision to extensively use electric shock in aversive control research has been based on a

conceptual rationale, summarized by Baron (1991) in the following remark:

> [One] characteristic of the aversive control literature is that the stimulus, with rare
> exception, has been electric shock − in rats administered through the grid floor of the
> experimental chamber, and in pigeons through electrodes… The conviction is that general
> principles will emerge. Investigations of lesser-studied aversive events usually have
> supported this expectation… (Baron, 1991, p. 176)

Although Baron thought that the behaviour analytic preference for electric shock is

justified by a conviction about generality, at the time of his review he recognized that this

conviction was only supported by a few studies with rodents and monkeys, in which stimuli such

as loud noise, bright light, and pinching of the tail were tested as having aversive functions

(Barnes & Kish, 1957; Brodie & Boren, 1959; Clark, Lange, & Belleville, 1973; Kaplan, Jackson,

& Sparer, 1965; Rohles, 1965; Spealman, 1978; Weiss & Laties, 1966). Moreover, this situation

did not change considerably for almost a decade following Baron's review. Crosbie (1998)

reported that an absence of studies comparing the effects of different aversive stimuli was still

evident in the field of experimental analysis of behaviour. He pointed out the need for

comparative studies by stating that, from a theoretical perspective,

> it is possible that organisms respond differently to biologically relevant physical stimuli
> than to biologically irrelevant [physical or] nonphysical aversive stimuli. Both are
> effective, but it is uncertain whether they have similar patterns of adaptation and recovery,
> and produce similar by-products such as emotion or overgeneralization. (p. 175)

Accordingly, it seems that the continued reliance on shock-based procedures for studying

aversive control is strategic, but not final; it is taken as an initial means of approaching the

complexity of certain phenomena, but there is an awareness about the need for replicating well-

studied facts about aversive control using other, perhaps more ecologically valid, forms of

stimulation, and in doing so, attempting to broaden the generality of the laws of behaviour and

learning.

<p align="center">*Alternatives to electric shock in aversive control research*</p>

Recent efforts specifically directed towards testing the aversive functions of forms of

stimulation alternative to electric shock have originated from two fronts. First, from the

acknowledgement of the dramatic decline of aversive control research due to ethical

considerations. This includes efforts to comply with one of the three principles endorsed

worldwide by agencies that regulate humane animal experimentation; namely, the refining of

procedures to enhance animal well-being and to minimize or eliminate pain and distress (National

Research Council, 2011).

A second factor that has encouraged research on alternative forms of aversive stimulation

is the recognition that shock-based procedures have practical limitations. Specifically, electric

shocks have secondary effects such as sleep disruption, altered social behaviour, interference with

electronic measurements, and reduction of certain activities that may be of interest for researchers

(e.g., rearing, grooming, and locomotion). Furthermore, experimental models of anxiety and

depression that involve electric shock have received criticism due to the fact that they entail a pain

component that does not seem to have a counterpart in such emotional phenomena in humans

(Brodie & Boren, 1959; Barker et al., 2010; McQuade, Creton, & Stanford, 1999).

Different forms of stimulation have been incorporated into aversive control paradigms

since the 1940's (e.g., loud noise – Barnes & Kish, 1957; bright light – Keller, 1941; cold –

Weiss & Laties, 1966; wind – Rohles, 1965; artificial gravity – Clark et al., 1973; pinching of the

tail – Brodie & Boren, 1959; and pressurized air – Spealman, 1978); however, the majority of

these have received little attention, with the notable exception of light and sound. Recently, these

two forms of stimulation have been studied more systematically under the explicit assumption that

they are feasible alternatives to electric shock in aversive control research.

Sound as an aversive stimulus

From the 1950s to the 1970s, a few studies conducted with rodents incorporated auditory

stimulation into aversive control paradigms (Barnes & Kish, 1957; Halpern & Lyon, 1966;

Harrison & Ableson, 1959; Harrison & Tracy, 1955; Hughes & Brett, 1976; Knutson & Bailey,

1974). This research obtained mixed findings regarding (a) the potential of auditory stimulation,

such as intense noise, to control behaviour under different aversive contingencies (e.g., escape, discrete-trials signalled avoidance, and free-operant avoidance), and (b) the possibility that certain auditory stimulation could produce the same patterns of behaviour observed extensively and reliably with electric shock. For instance, Harrison and Tracy (1955) reported successful control of bar-pressing behaviour by an escape contingency that entailed noise as the aversive stimulus, but acknowledged that behaviour developed more slowly and attained less stability as compared to other studies that implemented shock. Similarly, Knutson and Bailey (1974) could only reproduce free-operant avoidance and escape with high-intensity noise when it was previously paired with shock, while noise alone was ineffective.

More recently, Reed and colleagues (Reed, 2011; Reed, Collinsin & Nokes, 1995; Reed & Yoshino, 2001, 2005, 2008; Yoshino & Reed, 2008) have attempted to demonstrate that auditory stimuli could be a feasible alternative to electric shock. These studies have aimed at assessing the effects of presenting response-dependent auditory stimuli on the behaviour of rats that are exposed to operant paradigms; mainly, concurrent schedules of reinforcement. The roles of three variables have been specifically explored: (a) type of auditory stimulus (e.g., noise or clicks), (b) types of contingencies (e.g., extinction, continuous reinforcement, or schedules with different reinforcement rates), and (c) duration and intensity of the stimulus. Overall, these researchers have demonstrated that brief loud tones (e.g., a 1.5 s tone of 105 dB) have positive punishing functions for the operant behaviour of rats under choice paradigms. However, the suppressive effects have been less pronounced than those observed in analogous studies with electric shock, and have only been observed under schedules with low reinforcement rates (Reed & Yoshino, 2008). Reed and Yoshino (2008) acknowledged that more work is needed to improve their paradigm in order to offer a more ethically acceptable alternative that would allow for the continued study of punishment and other aversive control phenomena, including translational

research (e.g., a model for stressful disruption of eating patterns using response-contingent auditory stimuli – Reed, 2011).

Preliminary findings on the aversive functions of other forms of auditory stimuli for rat behaviour were reported by Norlund (2011). Unlike Reed et al.'s punishment paradigm, in this study a discrete-trials escape paradigm was implemented in which rats interrupted the presentation of ultrasound tones by poking their noses into a receptacle. The study showed a consistent control of behaviour by the ultrasound tones during negative reinforcement and extinction conditions.

Finally, MacKenzie, Foster, and Temple (1993) and McAdie, Foster, Temple, and Matthews (1993) tested the aversive function of various sounds for domestic hens. Unlike the previously described studies with rats, the specific purpose of this research was determining which sounds were aversive for hens, mainly with an interest towards animal welfare (machinery in animal production facilities produce intense noises for extended periods of time). MacKenzie et al. used an escape and avoidance procedure in which the termination of a constant or intermittent high intensity tone occurred when the animal moved to one end (the target area) of a tilting floor chamber; in addition, the sound remained off while the hen remained in the target side of the cage (i.e., the hen avoided further presentations of the sound). Alternatively, McAdie et al. (1993) used a choice paradigm in which concurrent variable interval food reinforcement schedules operated on two illuminated keys, and presentations of high intensity noise were made contingent on responses to one of these keys.

MacKenzie et al. (1993) reported that domestic hens spent high percentages of time in the target end of the chamber (i.e., with the sound off), and McAdie et al. (1993) found a consistent bias in responding and time allocation to the key that did not produce noise. These results allowed the authors of both studies to conclude that intense sounds indeed had aversive functions for

domestic hens. However, a paradoxical finding of this research is that changeover responses were

not affected by the contingencies. In MacKenzie et al.'s study, animals continued crossing to the

side of the chamber that triggered the onset of noise, and thus rarely allocated more than 80% of

the time to the "quiet" area of the cage; and in McAdie et al.'s study, animals continued pecking

regularly on the key that produced the noise – i.e., changes between schedules were not punished

as they remained similar across conditions.

MacKenzie et al. (1993) and McAdie et al. (1993) proposed that their unexpected findings,

in this case, dissociations between time allocation and changeover responses, could be explained

by the inherent characteristics of the auditory stimulation implemented, including a different

"degree of aversiveness" (McAdie et al., 1993, p. 224) when compared to other forms of

stimulation (e.g., electric shock). For instance, McAdie et al. concluded that "it is possible that

these sounds, although in some sense 'aversive' in that they biased behaviour, might not be

aversive enough to operate as punishers" (p. 236).

Light as an aversive stimulus

Similar to auditory stimulation, some research begun halfway through the last century

successfully developed experimental procedures that involved high-intensity light as the aversive

stimulus (e.g., Campbell & Messing, 1969; Kaplan, 1952; Kaplan, Jackson, & Sparer, 1965;

Keller, 1941, 1966; Lockard, 1961; O'Neil, Cooper, & Pedersen, 1968; Stern & Laties, 1989;

Zeaman & House, 1950). These early studies, however, were not concerned with attempting to

demonstrate that light could be an effective alternative to electric shock in view of the latter's

ethical and practical limitations. It was only recently that Barker et al. (2010) addressed this issue

in a series of experiments with rats, in which the efficacy of high-intensity light was

systematically tested across three aversive control paradigms: punishment, escape, and signalled

and unsignalled free-operant avoidance. Barker et al. reported that (a) high-intensity light used as

punisher of lever pressing maintained by concurrent schedules of food reinforcement suppressed overall response rates and produced bias to an alternative non-punished lever; (b) non-contingent presentations of the light produced reliable escape responding, consisting of the subject poking its head into an artificial burrow; and (c) avoidance contingencies increased and maintained responding, but only when escape was not functional and when a brief dim light, not a tone, signalled the imminent occurrence of intense light.

However, Barker et al. could not reproduce unsignalled free-operant avoidance using intense light as the aversive stimulus. Although they did not provide an explanation as to why this form of avoidance could not be replicated, they considered that the overall findings comprised enough evidence to support the use of light as an effective alternative to electric shock in aversive control research. This conclusion is strengthened by the consideration of particular advantages; for instance, the lack of any signs of habituation (reliable results were obtained throughout more than 100 daily exposures), the paradigm's cost effectiveness, and its potential for simple incorporation in other aversive control procedures (Barker et al., 2010).

Chapter IV. First generation of studies on spatiotemporal patterns of behaviour

Behaviour analysis has favoured the implementation of the free-operant approach, a unique research methodology that involves direct and continuous behavioural measures of single organisms. In addition to the advantages discussed in Chapter II, the free-operant approach has been considered central to behaviour analysis because of its typical use of *response rate*, which is a measure that is free from arbitrary constriction into trials, permits the continuous observation of responding, and provides a sensitive indicator of the effects of relevant variables (Sidman, 1953a).

Although response rate is widely accepted today as the fundamental datum in the study of operant behaviour, it is not without its conceptual and methodological limitations (e.g., Baum & Rachlin, 1969; Gilbert, 1958; Pear & Rector, 1979; Ribes-Inesta, 2006). In a foundational analysis regarding these shortcomings, Baum and Rachlin (1969) concluded the following:

> Skinner (1938) thought of the rat's lever press as a type of reflex. Accordingly, his basic measure of behavior was a count of the number of lever presses made during an experimental session… and [he treated] each lever press as an instantaneous event, as a point in time, having no duration... Since Skinner's work, experiments on operant behavior have usually treated responses as instantaneous. Indeed, response keys and levers… are so commonly used today that available apparatus tends to enforce response counting as the means of measuring behavior. [Conversely,] if we admit that behavior has duration, an alternative scheme of measurement becomes available. Behavior that can be counted can also be timed. Response duration, or time spent responding, can be just as basic a measure of behavior as response frequency. (p. 861)

Although major conceptual and empirical efforts have resulted from these considerations, such as analyses of time allocation and choice (e.g., Baum & Rachlin, 1969; Baum, 2012) and the development of a molar approach to behavioural processes (e.g., Baum, 1989; 1990; 2002), none have employed a systematic focus on both the spatial and temporal dimensions of behaviour as that of the spatiotemporal research program at the University of Manitoba (Eldridge & Pear, 1987; Eldridge, Pear, Torgrud, & Evers, 1988; Martin, 2010; Pear & Rector, 1979; Pear, 1985, 1988; Pear & Eldridge, 1984; Pear & Legris, 1987; Pear, Silva, & Kincaid, 1989; Silva & Pear, 1995;

Silva, Pear, Tait, & Forest, 1996; Silva, Silva, & Pear, 1992; Sales, 2014). The research presented

in this dissertation represents recent advances in this program, and as such, a review of the

conceptual foundations, methodology, and representative research of the spatiotemporal program

become integral to the development of the present studies.

Rate of responding: rationale and limitations

Established measures such as latency, duration, and frequency are utilized to quantify

some of the fundamental qualities of behaviour (e.g., temporal locus, temporal extent, and

repeatability, respectively). Response rate, however, is the most utilized measure in behaviour

analytic research, especially when compared to other fields that use measurement strategies more

suitable for discrete trials (e.g., latency, running speeds, distances and trajectories, and visits to

specific areas of mazes – Church, 2003).

The relevance of response rate in behaviour analytic research can be traced back to B. F.

Skinner's seminal ideas, which advocated methods that allowed for the direct and precise study of

behavioural processes, thus helping to achieve the major goals of behaviour analysis – i.e., the

prediction and control of behaviour.

> The resolving power of the microscope has been increased manifold, and we can see
> fundamental processes of behavior in sharper and sharper detail. In choosing rate of
> responding as a basic datum… we make important temporal aspects of behavior visible.
> (Skinner, 1956, p. 229)

Focusing on rate of responding has been well justified on conceptual and pragmatic

grounds. First, it is assumed that this measure is an index of the probability of behaviour, which is

the crucial datum of interest for the science of behaviour (Pear, 2004). Second, it is a useful

feature of the basic and applied behaviour analytic methods (Johnston & Pennypacker, 2009;

Pierce & Cheney, 2013). For instance, the use of response rate favoured the development of a

basic research program begun in the 1950s (Fester & Skinner, 1957), which, to date, continues to

be mainstream in behaviour analytic research; namely, the study of schedules of reinforcement

(Catania, 2008; Morse & Dews, 2002; Schlinger, Derenne, & Baron, 2008). Research in applied

behaviour analysis (ABA) has also benefited from using rate of responding as a measurement

tactic. For example, research on modifying target behaviours such as throwing objects, eating

disorders, hitting, or problem-solving typically implements a measurement approach based on

response rate (Pear, 2004; Martin & Pear, 2010).

The experiments that incorporate rate of responding as the measure of interest focus on

operant responses that are very short in duration and that tend to occur very frequently (e.g.,

pressing a lever or pecking a response key); thus, the calculation of response rate can be obtained

by dividing the number of responses over a given period of observation (e.g., a session time

measured in minutes). Overall, it is assumed that the rate of a response is an indication of its

probability, in that a response that occurs at a high rate in one situation has a high probability of

being displayed in a similar situation in the future. Furthermore, different independent variables

can be manipulated in order to test their effect on the rate of response, and the observation of

changes in response rates can be understood as changes in the probability, or "strength", of a

given response class.

Unfortunately, response rate seems to be a less appropriate measure under certain

circumstances, such as when: (a) the target behaviour has long and/or varied durations; (b) the

target behaviour does not occur very often; (c) the target behaviour's relevance or applied

significance does not reside in its repeatability but in other properties, such as temporal extent; or

(d) it is problematic to delimit specific instances of the target behaviour. Johnston and

Pennypacker (2009) present an example that illustrates some of these limitations. They propose a

situation in which 10 responses are recorded on each of two 20-min experimental sessions (A and

B). In this example, a basic response rate calculation will provide a frequency of 0.5 responses per

minute for both sessions A and B (10 responses/20 min = 0.5). However, if the total duration of

the responses is calculated to be 10 minutes in session A, and 15 min in session B, it is evident

that the response rate by itself may be misleading. One alternative is to calculate the rate of

response for each session adjusting for the amount of time in which there was an actual

opportunity for the response to be emitted. Therefore, since the total duration of the responses in

session A was 10 minutes (i.e., behaviour could occur only during the remaining 10 min of the

session), the frequency can be adjusted to 1 response per minute (10 responses/10 min = 1).

Alternatively, given that the period available to respond in session B was 5 min, the rate can be

adjusted to 2 responses per minute (10 responses/5 min = 2). From this example, it is evident that

using response rate while ignoring the duration of the responses, or the opportunity for responses

to occur, may result in misleading conclusions or loss of information.

        Pear (2004) described other situations in which rate of responding may not be an

appropriate measure. For example, researchers or practitioners are sometimes specifically

interested in the duration of certain behaviours (e.g., exercising, waiting for an event to occur,

sustaining a conversation, or maintaining eye contact). In other circumstances, the target

behaviour involves continuous movement, for which isolating specific non-arbitrary instances is

not an easy task (e.g., tantrums or verbal interactions). A common strategy to solve this problem

consists of defining and sampling intervals in order to detect if at least one instance of the target

response occurred (i.e. interval-sampling measures). However, Pear argued that this is not an

actual solution to the limitations of response rate, since it seems more like an alternative that is

focused on a temporal dimension of behaviour. He affirmed that these interval-sampling

calculations are concerned with the total number of intervals in which at least one response was

observed, and not with the frequency of the target response itself.

The limitations described above have not been so salient under the traditional operant

methodology, because in most of the experimental arrangements the responses are typically

defined by the instantaneous closing of an electronic switch that has little to no spatial or temporal

extent (e.g., discrete responses, such as a pigeon's peck on a response key). Pear (2004)

commented in this regard that

> …although the spatial aspect of operant behaviour is minimized or ignored in Skinner's
> approach, it is not absent or negligible. On the contrary, operant behaviour generally does
> have spatial extent, even though it may not be directly recorded… Skinner did not ignore
> the spatial problem. On the contrary, he recognized it and proposed an ingenious solution:
> namely, the concept of the operant (Skinner, 1953, p. 65). Essentially, an operant is a set
> of topographically distinct responses that have the same effect. According to this definition
> it is not necessary to be concerned with the ways in which, for example, a rat presses the
> lever. The lever-pressing operant encompasses all the ways in which a rat can conceivably
> press a lever. This definition enabled Skinner to ignore the various topographies of lever
> pressing, key pecking, etc., and focus on switch closures as the primary datum of interest.
> The definition worked because the operant turned out to be an orderly unit of analysis. The
> reason the operant is such an orderly unit appears to be because after many reinforced
> repetitions, an operant typically comes to be composed of only one, or at most a few,
> topographically distinct responses (see Pear, 2001, p. 38). Reflecting this fact, the terms
> operant, response, and operant response tend to be used interchangeably in Skinner's
> terminology. (p. 131 - 132)

In Pear's view, using brief and discrete responses has been a very successful strategy in

operant research. It has allowed discovering orderly behaviour-environment relationships, such as

the function of different schedules of reinforcement, while disregarding spatial properties of

behaviour. Furthermore, it is precisely the effect that is observed when this type of response is

repeatedly reinforced – the actions involved becoming stereotyped – that has supported the

decision of focusing only on their rate, disregarding other properties.

As effective as the response-rate strategy may be in operant research, it is limited in terms

of the types of responses to which it can be applied, and the circumstances in which it can be

relevant. This has a major implication for the science of behaviour: its over-reliance on response

rates may have been hampering a more complete understanding of learning and behavioural

phenomena. Accordingly, Pear (2004) has proposed that, in addition to rate of responding, other

spatial and temporal aspects need to be integrated into a more comprehensive approach (e.g., the

amount of time that an organism spends engaging in different behaviours and their structure and

patterning). One of his arguments is that Skinner's ideas regarding rate of responding as a

fundamental measure were based on the assumption that frequency is related in some way to the

probability of a behaviour to occur, which in turn is the real datum of interest for the science of

behaviour. However, Skinner did not specify a method to translate rate of responding into

probability (Pear, 2004). He stated that:

> What is required is an analysis of the conditions that govern the probability that a given
> response will occur at a given time. Rate of responding is by no means to be equated with
> probability of responding, as frequency theories of probability and comparable problems
> in physics have shown. Many investigators prefer to treat rate of responding as a datum in
> its own right. Eventually, however, the prediction and control of behaviour call for an
> evaluation of the probability that a response will be emitted. The study of rate of
> responding is a step in that direction. (Skinner, 1963, p. 505)

Accordingly, Skinner made the "first step" by considering rate of responding as a major

measure of behaviour and showing that it could lead to very successful research programs; but

developing the strategy to translate rate into probability was a "task left open for others to carry

out later" (Pear, 2004, p. 134). Behavioural activity theory (Pear, 2004) addresses this issue and

establishes the underlying framework for a research program on spatiotemporal aspects of

behaviour. The objective of this program has been "determining whether a spatiotemporal

analysis provides additional information that is not evident from studies that have focused only on

response rate as the dependent variable" (Pear, 2004, p. 139), and in doing so, has developed

alternative directions in behaviour analytic research.

First generation of studies: rationale

Behavioural activity theory (Pear, 2004) incorporates rate of responding and

spatiotemporal aspects of behaviour into a single framework. From this perspective, an activity is

defined as a sequence of responses and its main measure is duration. Two assumptions are

fundamental to this approach: activities have a structure that occurs both in spatial and temporal

dimensions, and contrary to the mainstream approach, analyses based on rate of responding are

not the only option (Pear, 2004).

On the basis of this conceptualization, Pear has argued that activities have some

mathematical properties that provide a solution to the limitations of response rate. For example,

one of the problems of translating rate of responding into probability is that the rate of "non-

responding" is also needed in order to estimate the probability of these complementary events

(Pear, 2004). As Pear has noted, rate of non-responding is a measure that has not been explored in

the traditional operant methodology, and if it is to be incorporated in the analyses, a common

behavioural unit to responding and non-responding needs to be developed (i.e., some sort of

scaling is needed for when activities with different structure are taken into consideration).

Conversely, a solution to this limitation can be derived from behavioural activity theory. Given

that units of time are common across activities – i.e., time is the same for any activity and no

scaling is required – a proportion can be obtained for any activity simply "…by taking the ratio of

the total time spent engaging in the activity and the total time available for the activity to occur"

(Pear, 2004, p. 135). Accordingly, one of the major advantages of this approach is that it allows

for formulating and testing quantitative behavioural predictions without requiring the scaling of

arbitrary units, such as a key peck or a lever press, in order to make them equivalent across

activities. This strategy seems to be even more relevant in those earlier described cases, in which

response rate is not an important measure of the target behaviour, or when it is not possible to

obtain a meaningful measure of response rate (Pear, 2004).

With behavioural activity theory as a framework, Pear and colleagues initiated a research

program on spatiotemporal aspects of behaviour at the University of Manitoba which is still in

effect (Eldridge & Pear, 1987; Eldridge et al.,1988; Martin, 2010; Pear & Rector, 1979; Pear, 1985, 1988; Pear & Eldridge, 1984; Pear & Legris, 1987; Pear et al., 1989; Silva & Pear, 1995; Silva et al., 1996; Silva et al., 1992; Sales, 2014). This program follows some basic premises that constitute a corollary of the previous discussion on the limitations of the traditional behavioural measurement approach: (a) rate of responding has been useful for obtaining a fundamental understanding of basic behavioural processes, but there are conceptual, methodological, and empirical reasons that limit its usefulness – alternatives need therefore be explored; (b) behavioural activity theory provides a conceptualization that treats rate of responding as a special case, and incorporates spatial and temporal measures as aspects that enrich the understanding of behavioural phenomena; and (c) the structure of activities occurs in spatial and temporal dimensions, it is therefore relevant to study their patterns; e.g., beginnings and ends (Pear, 2004).

In accordance with these premises, the main purpose of the research program has been to determine if spatiotemporal analysis provides information that is not evident from the exclusive use of discrete response rates as the dependent variable (Pear, 2004, p. 139). The first generation of experiments strategically utilized operant paradigms with well-known effects of certain variables on the response rate. These experimental setups were variable-interval schedules of reinforcement (Pear, 1985), differential reinforcement of low and high rates (Pear, 2004), behavioural contrast (Pear & Eldridge, 1984), autoshaping (Eldridge & Pear, 1987), and superstitious behaviour (Eldridge et al., 1988). The following section describes some of these experiments in detail in order to demonstrate the methodology that has been developed in the spatiotemporal program, the type of data analyses that are typically conducted, and the major findings that have been consistently replicated throughout several studies.

First generation of studies: methodology and findings

A three-dimensional video tracking system (VTS) was developed for the purposes of recording and representing the continuous movement of animals exposed to different reinforcement contingencies (Pear et al., 1989). The subjects in the first generation of experiments were white pigeons whose heads were painted black in order for the VTS to track their movements. The VTS was capable of recording the position of a pigeon's head along the three spatial dimensions at a rate of 10 data points per second.

In order to explore the spatiotemporal patterns developed under variable-interval (VI) schedules of food reinforcement, Pear (1985) exposed two pigeons to VI 15-s, VI 5-min, and Extinction – where VI 15-s consisted of reinforcement (delivery of food) contingent on the first response (key peck) after an interval varying around a mean of 15 s following the previous reinforcement; VI 5-min consisted of reinforcement contingent on the first response after an interval varying around a mean of 5 min following the previous reinforcement; and Extinction consisted of no provided reinforcement. Different measurement strategies were implemented, such as the continuous tracking of the distances between a pigeon's head from a key, and plotting the paths described by a bird's movement inside the experimental chamber.

Pear (1985) found that the VI 15-s produced a close-to-key pattern between responses, whereas the VI 5-min resulted in more extensive and elaborate spatiotemporal patterns of behaviour (e.g., more intricate "looping" and "excursions"). Even though the spatiotemporal patterns of each pigeon were idiosyncratic and reached a high degree of regularity, they also preserved a certain degree of variability within and across sessions. Figure 1 (taken from Pear, 1985) exemplifies the type of data that can be obtained when conducting this type of spatiotemporal analyses of behaviour.
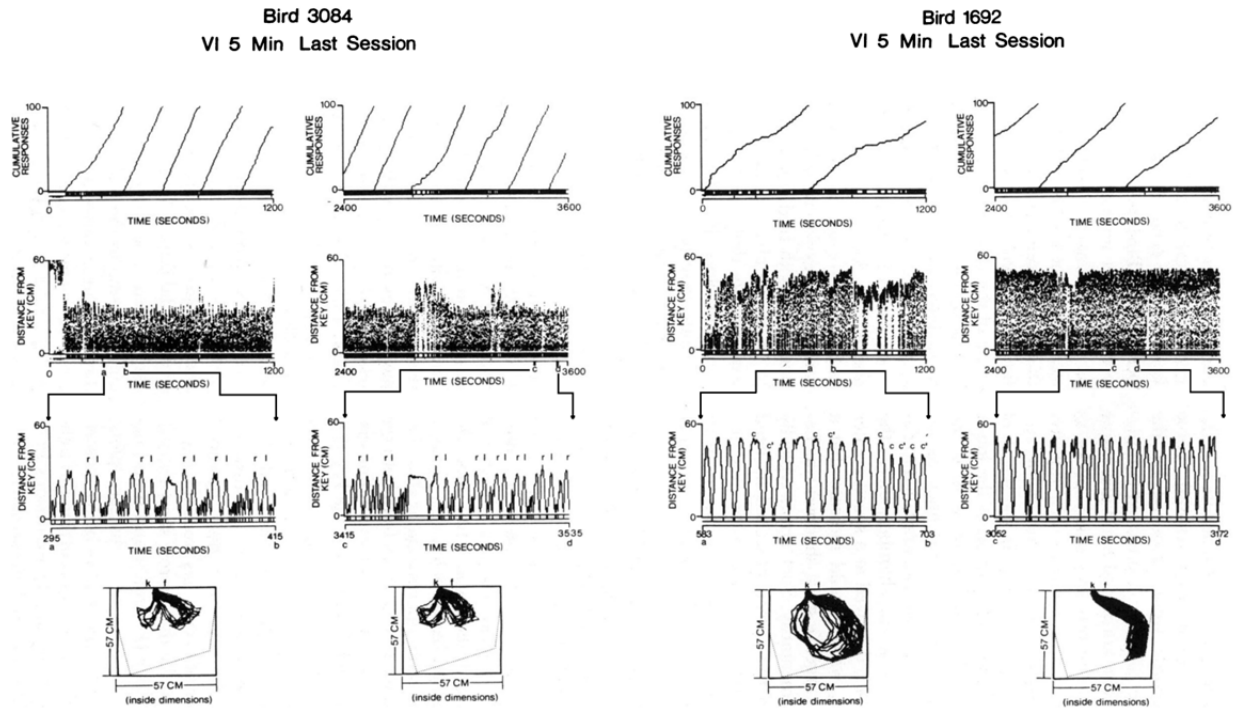
Figure 1. Last session of the VI 5-min schedule for each of the two pigeons utilized in Pear's (1985) study. The cumulative record of the first 1200 s of this session is shown in the top left graph for each subject. The top right graph shows the last 1200 s of the session. In the second row of graphs the distance of the bird's head from the key is shown for the first and last 1200 s of the session. The third row of graphs shows expansions of the regions between "a" and "b" and between "c" and "d" indicated in the second row of graphs. The labels "r" and "l" (Bird 3084) and "c" and "c'" (Bird 1692) correspond to excursions away from the key that the pigeons displayed in the form of circuits or elongated "figure-eight" patterns (clockwise or counter clockwise and to the right or to the left of the key). The bottom row of graphs corresponds to plots of these expanded data from an overhead view. Each graph traces the path described by the pigeon's head in producing the data that appears in the graph immediately above it. The letters "k" and "f" represent the location of the key and feeder in the experimental chamber.
Adapted from Figure 2 in Pear, J. J. (1985). Spatiotemporal patterns of behavior produced by variable-interval schedules of reinforcement. *Journal of the Experimental Analysis of Behavior, 44*(2), 217 - 231. © Used with permission by publisher.

Given that a standard behaviour analytic design (single-subject) was implemented, in

which two birds were exposed to all the experimental conditions (VI 15-s, VI 5-min, and

Extinction), Pear (1985) was able to detect that the patterns developed under each schedule

returned very quickly when the value of the VI schedules was reversed (e.g., from VI 5-min to VI

15-s). Additionally, when the extinction schedule was introduced "the patterns that had developed

under each of the two schedules appeared and gradually deteriorated as extinction progressed"
(Pear, 1985, p. 229).

The resurgence and dissipation of these patterns during extinction suggests that an often
reported phenomenon in the operant literature could be interpreted differently when considered
through a spatiotemporal approach. That is, changes in the probability of reinforcement – of
which extinction is one particular case – not only produced the well-known variability in response
rate (Pierce & Cheney, 2013), but also generated the re-emergence, variation, and fading of
previously reinforced spatiotemporal patterns.

Pear (1985) concluded that variations in the parameters of the schedules not only resulted
in the well-known changes in response rates, but also affected the behaviour that occurs between
the discrete responses that are typically utilized for calculating rates. It seemed that the activities
in which the pecking responses were embedded – such as alternating movement circuits or
elongated "figure-eight" patterns – were under the control of the reinforcing contingencies. This
was evident in the gradual development and dissipation of these activities during the different
conditions. In this regard, Pear (2004) commented that

> …to compare response rates in these activities without taking into account the differences
> in the topography or structure of the activities is misleading… [for example,] [t]he gradual
> decrease in key pecking tells only a part of what happens to an activity when it is no
> longer reinforced. The spatiotemporal data help to fill in the details by revealing a gradual
> disintegration in the structure of the activity as opposed to simply the gradual decrease
> suggested when the response rate is alone examined (p. 141).

The findings of this study are representative of the research program in spatiotemporal
analyses of behaviour, and have led to the general notion that the rate of the discrete responses
typically utilized for behaviour analyses (e.g., key pecks) tends to be dissociated from the
activities in which the response is embedded. Moreover, different contingencies may have
separate effects on response rate and the spatiotemporal patterns of behaviour. In view of this, it is

evident that it is important to examine response rates and activities separately, since different

information can be obtained from each, resulting in a better understanding of behavioural

phenomena.

Recently, the research program has followed the overall behaviour analytic strategy of

incorporating other species with the aim at testing the generality of learning. In particular, Pear et

al. have incorporated fish – specifically, bettas – with the purpose of replicating various

behavioural phenomena in another species while conducting further spatiotemporal analyses. The

rationale for incorporating fish models in learning research, including its benefits, are described in

the next chapter, together with the main findings of a series of studies on spatiotemporal aspects

of betta behaviour.

Chapter V. Second generation of studies of the spatiotemporal program:

research with fish

In line with one of the major aims of Behaviour Analysis – probing the generality of

behavioural processes (Johnston, 1979) – the second generation of studies of the spatiotemporal

research program has incorporated a fish species as its experimental model.

Fish models in the study of learning and behaviour

Fish behavioural processes have been studied using a wide range of experimental

paradigms. As a result of these studies, and especially since the early 1990s, researchers have been

continually reporting that fish are by no means "primitive" in terms of cognitive phenomena such as

perception, attention, memory, and social learning (Brown, Laland, & Krause, 2006). Several

conditioning phenomena observed in rats and pigeons have been systematically replicated with fish

(e.g., conditioned reinforcement – Salzinger, Freimark, Fairhurst, & Wolkoff, 1968; punishment –

Adler & Hogan, 1963; avoidance – Behrend & Bitterman, 1963; effects of reinforcement delay –

Elcoro, da Silva, & Lattal, 2008; temporal discrimination – Gee, Stephenson, & Wright, 1994).

Also, a growing body of research has demonstrated the flexibility of fish behaviour throughout a

wide range of ecological problems, such as finding food, spatial navigation, modulating aggression

towards conspecifics, and cooperating (Brown et al., 2006). Finally, the demonstration of

similarities in learning capabilities between fish and other vertebrates have stimulated prominent

research programs on the evolution of cognitive processes and the underlying neural mechanisms of

learning (Braithwaite, 2006; Overmier & Hollis, 1990; Sloman et al., 2006).

Appetitive and aversive conditioning procedures with fish have been valuable models in

behavioural neuroscience for a variety of reasons, including: (a) the neurodevelopmental processes

can be continuously visualized in certain species of fish that have a clear chorion (cellular coat

surrounding the mature egg); (b) the proliferation, differentiation, migration, and projection of

specific neural structures can be identified in fish by means of reporter systems; (c) the assessment

of molecular mechanisms can be assisted by the use of numerous mutating agents and techniques

now available; and (d) the cost of experimentation can be reduced and the resources maximized,

because fish are easily bred in great numbers, develop rapidly, and are relatively inexpensive to

maintain (Levin & Cerutti, 2009). As a result, two major areas have incorporated learning

paradigms that entail fish as animal models: research on the effects of toxic and pharmacological

chemicals (e.g., Eddins, Cerutti, Williams, Linney, & Levin, 2010; Eddins, Petro, Williams, Cerutti,

& Levin, 2009) and the study of the neurobiological aspects of development, aging, and associated

diseases (e.g., Blank, Guerim, Cordeiro, & Vianna, 2009; Cofiel, & Mattioli, 2009; Zhdanova, Yu,

Lopez-Patino, Shang, Kishi, & Guelin, 2008). In this context, the zebrafish (*Danio rerio*) has

become one of the most prominent experimental models for studying the molecular basis of

neurodevelopment and associated disorders (Blaser & Vira, 2014). Today, the genetics and

physiology of learning and memory are more widely studied in zebrafish than with other species,

including popular models such as goldfish and rats (Levin & Cerutti, 2009).

Operant research with fish

Operant research has incorporated fish species with the aim of testing the generality of

learning phenomena observed in other mainstream models, such as rats and pigeons. As a result,

diverse behavioural processes have been studied with different paradigms (e.g., classical,

instrumental, or free-operant), response systems (e.g., defensive or alimentary), and forms of

reinforcement (e.g., conditioned or unconditioned). Despite the large number of fish species

available (over 20,000 – Sloman et al., 2006), behaviour analytic research has almost exclusively

utilized *Carassius auratus* (Goldfish) and *Betta splendens* (Siamese fighting fish). Research on

spatiotemporal analyses of behaviour has not being an exception to this trend, as its most recent

studies have been conducted with *Betta splendens* (e.g., Martin, 2010; Sales, 2014). A major

advantage that has justified the use of bettas in this program is the avoidance of artefactual

behaviour, which is likely to be observed in other species kept in laboratory conditions with

greater restricted freedom of movement (e.g., birds and mammals). For this reason, betta mobility

and body size relative to the experimental tank has made this species an excellent model for

studying spatiotemporal phenomena associated with different contingencies.

<p align="center">*Betta splendens* (*Siamese fighting fish*)</p>

The Siamese fighting fish is a member of the anabantid family and is native to Southeast

Asia. Their typical habitats are quiet fresh water ponds with muddy bottoms and flooded rice

paddies, where they tend to hide beneath aquatic plants, presumably to avoid predators (e.g.,

egrets, herons, and kingfishers). Males build one bubble nest in the center of their territory, court

females, and care for a single batch of developing eggs and larval fish at a time (Jaroensutasinee

& Jaroensutasinee, 2001; Jaroensutasinee & Jaroensutasinee, 2003). Each male defends a territory

near the surface, the radius of which is typically less than half of the distance to the nearest bubble

nest of a conspecific. Male bettas are very territorial, especially during courtship and isolation.

Their aggressiveness is typically expressed by biting, changes in body color intensity, fin

expansion, and gill cover extensions (Karino & Someya, 2007; Simpson, 1968). Finally, wild and

domesticated bettas significantly differ in size (wild variants are smaller), color (wild variants are

dull brown or green, whereas domesticated bettas have been selected for more intense color

variations), and behaviour – e.g., domesticated variants are more aggressive, since they have been

selected for combat (Jaroensutasinee & Jaroensutasinee, 2001; Jaroensutasinee & Jaroensutasinee,

2003; Monvises, Nuangsaeng, Sriwattanarothai, & Panijpan, 2009; Goldstein, 2004).

Bettas began to be incorporated into behaviour analytic research when it was found that

the exposure to live conspecifics, models of conspecifics, and mirror images had positive

reinforcing effects on the males of this species (e.g., Baenninger & Mattleman, 1973; Thompson, 1963). Some of the earliest operant experiments with bettas consisted of manipulations of simple positive reinforcement schedules, respondent conditioning procedures, and the positive punishment of aggressive displays (Adler & Hogan, 1963; Fantino et al., 1972; Melvin & Anson, 1969; Melvin & Ervey, 1973; Thompson & Sturm, 1965a,b). Overall, this first generation of studies confirmed the generality of learning phenomena using a form of reinforcement – mirror images – distinct from reinforcers more frequently implemented in operant research, such as food or water.

Subsequent behaviour analytic experiments with bettas focused on the effects of different reinforcement schedules and variables that affect performance during those schedules. For example, Turnbough and Lloyd (1973) tested the effects of two classes of reinforcers (light from a projector and a film of another male fish) during fixed-ratio (FR) and differential-reinforcement-of-other-behaviour (DRO) schedules. As has been typically reported with other species and forms of reinforcement, Turnbough and Lloyd found that the rate of responding observed during FR 1 was substantially higher than the operant level, while remaining low during DRO and extinction. More recently, Lattal and Metzger (1994), Wirth, Lattal, and Hopko (2003), and Elcoro et al. (2008), have replicated other behavioural phenomena, such as discrimination training and the effects of reinforcement delay.

Early spatiotemporal analyses of betta behaviour

A series of studies conducted at the University of Manitoba's Behaviour Analysis Fish Laboratory have explored the effects of different variables on the spatial and temporal distribution of betta behaviour. More specifically, these studies investigated the effects of: (a) response-dependent schedules of mirror presentations (Chitty, 2003); (b) response-independent schedules of mirror presentations (Martin, 2010); (c) manipulating the response-location and the response-

feedback stimuli on the fixed-ratio performance of bettas (Sales, 2014); and (d) delivering water

disturbances in the form of air bubbles and water flows (Hurtado-Parrado, Pear, & Froese, 2009;

Hurtado-Parrado, Pear, & Khattiyakornjaroon, 2010). These studies are aligned with the research

program's main objective, the broadening of the scope of information available from the use of

spatiotemporal analysis, and represent developments in the methodology that was originally used

with pigeons. For example, the video tracking system (VTS – Pear et al., 1989) has been adapted

to (a) detect and record movements of dark objects in an aquatic medium; (b) measure distances

of the fish body to different locations in an experimental tank; (c) plot the trajectories of fish; (d)

deliver different forms of feedback (fish pellets, mirror reflections, lights, streams of air bubbles,

and water flows) based on contact with one or several virtual target areas (e.g., a computer-

defined virtual cylinders) as the target response; and (e) implement different behavioural learning

paradigms (e.g., respondent conditioning and schedules of reinforcement). A review of this

research is presented in order to show the sensitivity of bettas' behaviour to different conditioning

paradigms, and to exemplify the type of spatiotemporal analyses that have been developed in this

second generation of studies.

Chitty (2003) explored the effects of changing the FR requirements and the location of a

target on the swimming patterns of male bettas. In this study, the reinforcer consisted of a 20-s

presentation of a mirror image, and the operant response consisted of the fish entering the area of

a virtual sphere – i.e., a sphere defined by and identifiable only with the tracking software and not

by any explicit cues. This sphere had the same diameter of the circular rings utilized in other

experiments with bettas and goldfish (Wirth et al., 2003), and, depending on the experimental

condition, was placed near or far from the wall in which the reinforcer was delivered. Chitty's

(2003) findings were consistent with previous research on schedules of reinforcement that

reported longer post-reinforcement pauses (PRPs) and higher response rates as indicators of

sensitivity to the changes in the FR requirement. In other words, Chitty confirmed the generality

of these schedule-related phenomena in bettas when mirror reflections were utilized as positive

reinforcers. In addition, Chitty found that increments in the FR requirement only affected the

response rates and PRPs when the target sphere was placed far from the wall in which the mirror

images were presented. This suggests that schedule-controlled behaviour of bettas is sensitive to

the interaction between the schedule requirements and the location in which reinforcement is

delivered.

Martin (2010) explored the effects of non-contingent (i.e., response-independent)

reinforcement on the habituation of betta aggressive display using fixed-time (FT) and variable-

time (VT) schedules of mirror presentations. Specifically, he implemented an FT 2-min schedule,

in which reinforcement occurred every 2 min irrespective of the subject's behaviour, and a VT 2-

min schedule, in which the period of time that elapsed before the delivery of response-

independent reinforcers averaged at 2 min. Considering that behaviour analytic research on the

effects of non-contingent schedules of reinforcement (e.g., "superstitious behaviour"; Skinner,

1948; Timberlake & Lucas, 1985) has been conducted almost exclusively with pigeons and rats,

the purpose of Martin's study was to test the generality of these findings using fish and a response

system that was not feeding-related. Although the effects observed by Martin (2010) could not be

replicated consistently across subjects and all treatment conditions, this study provided enough

evidence to consider that VT schedules have a dishabituating function on the agonistic responses

of bettas previously exposed to FT schedules, which in this case consisted of highly stereotyped

"lap-swimming" in front of a mirror wall.

More recently, Sales (2014) tested the effects of manipulating a response-location stimulus

(a coin) and a response-feedback stimulus (a light) on the fixed-ratio performance of bettas. In her

study, bettas received food reinforcement for contacting a virtual target area (cylinder) located in

the center of the tank. Sales analyzed the rate of responding, spatiotemporal patterns, and

accuracy of responses (i.e., ratio target responses per approximation of target response area) under

different schedule requirements. She found that accuracy of responding was most strongly

controlled by the combination of the response-location stimulus and the response-feedback

stimulus, less strongly by the response-location stimulus alone, still less strongly by the response-

feedback stimulus, and least strongly by the absence of both stimuli. She did not find the well-

known distribution of responding observed in FR schedules – i.e., a consistent PRP break-and-run

response pattern – found with birds and mammals.

Overall, the findings of the research summarized above indicate that the spatiotemporal

organization of betta behaviour is sensitive to (a) variations of time, periodicity, and location of

positive reinforcers, such as food or mirror images, (b) the presence of response-feedback and

response-location stimuli, and (c) the response-requirements of certain schedules of

reinforcement. More generally, they support the notion that *Betta splendens* is an excellent model

for studying spatiotemporal phenomena associated with different contingencies.

Nevertheless, it is also evident that all research conducted over the past decades on

spatiotemporal patterns of behaviour, both with pigeons and bettas, was entirely focused on

appetitive contingencies (positive reinforcement), with a notable absence of aversive control

studies. This trend changed recently, when informal observations of male betta behaviour during

the course of a follow-up of Chitty's (2003) study suggested, for the first time, the possibility of

testing whether water disturbances (WDs) had an aversive function for the behaviour of bettas

(e.g., positive punishment or negative reinforcement functions). A series of preliminary studies

aimed at refining a methodology that would allow for the investigation of the effects of WDs on

the behaviour of bettas are described in detail in the next chapter. The findings of these

preliminary experiments support the notion that WDs have aversive functions for the behaviour of

bettas, and more generally, they confirm the feasibility of a WD model for the study of aversive

control phenomena with bettas.

Chapter VI. Aversive control of fish behaviour

The formal study of aversive control phenomena in fish can be traced back to E. L. Thorndike's early experiment with "[t]he common Fundulus" (Thorndike, 1899, p. 923). In this study, an aquarium was divided in two compartments by a partition, each with a different behavioural function: a sunlit compartment with an unconditioned aversive function, and a shaded compartment with an unconditioned reinforcing function. On each trial, the fish had the opportunity to escape the sunlit compartment through an opening in the partition. After several daily trials, Thorndike found a marked decrease in the time the fish took to escape. Moreover, Thorndike added partitions with openings in different locations and found that fish readily learned to go through the new openings in succession to escape the sunlight. This study is seminal for two reasons: it exemplifies the early interest in testing the generality of learning phenomena (in this case, aversive learning phenomena) across species, a tendency that continues to date; and it entails an unconditioned aversive stimulus (sunlight) different from electric shock, which would become mainstream in the field of aversive control and has been questioned from ethical, practical, and ecological validity perspectives (Baron, 1991; Brodie & Boren, 1959; Barker et al., 2010; Davis, 1981; Crosbie 1998; McQuade et al., 1999 – see Chapter III for the implications of the over-reliance of behaviour analytic research on this form of stimulation).

Historically, the fundamental rationale behind aversive control research with fish has been comparative (e.g., an exploration of the generality of conditioning phenomena). Similar to studies conducted with other species (pigeons, rats, monkeys), electric shock has predominantly been the only form of aversive stimulation implemented in these studies. Their overall findings have confirmed the generality of several learning phenomena observed in other species (e.g., response suppression under punishment contingencies, conditioned suppression, and free-operant avoidance). Nevertheless, an important feature of this body of research is that negative

reinforcement has been practically ignored with bettas, with the only study to make such an attempt (Otis & Cerf, 1963) showing inconclusive results.

Negative reinforcement: signalled and unsignalled free-operant avoidance in goldfish

Behrend and Bitterman (1963) and Scobie (1970) demonstrated, several decades ago, that the behaviour of goldfish is sensitive to free-operant avoidance contingences in a fashion similar to what was previously found with other species. To date, these same phenomena have not been tested in other fish.

In Behrend and Bitterman's experiment, every time that a goldfish changed compartments in a shuttle tank the delivery of a shock was interrupted and/or postponed by 20 s (R-S = 20 s). Given that the main goal of Behrend and Bitterman was to systematically replicate the effects of signalled and unsignalled free-operant avoidance observed in rats (Sidman, 1953a,1953b; 1955), the authors assessed the effects of (a) delivering response-independent (i.e., unavoidable) and response-dependent (i.e., avoidable) shocks; (b) scheduling two values for the S-S interval (20 s vs. 2.5 s); and (c) delivering shocks in the absence or presence of a warning stimulus, consisting of a light presented during the last 5 s of the R-S interval - i.e., unsignalled vs. signalled free-operant avoidance, respectively. Regarding *a*, the authors found that the rate of crossings increased only when the avoidance contingency was in place. Also, fish that were exposed first to unavoidable shocks showed a subsequent impairment in the acquisition of the avoidance learning when the contingency was established. Concerning *b*, there were no noticeable differences between scheduling a 2.5 and a 20 s S-S interval. And in relation to *c*, the introduction of a warning stimulus (light) decreased the rate of crossing in the first 15 s of the R-S interval, while at the same time increasing the rate of crossing during the last 5 s (i.e., when the light was present). Behrend and Bitterman concluded that the negative reinforcement contingencies scheduled in their experiments produced the same effects in goldfish that were previously observed in rats.

In Scobie's (1970) experiment, goldfish were exposed to a free-operant avoidance procedure in which shocks were delivered in the absence of a warning stimulus. Scobie was interested in comparing the effect of different lengths of the S-S and R-S intervals (15, 30 and 60 s). One of the major findings of this study is that the average inter-response times (IRTs) were very similar across conditions regardless of the lengths of the S-S and R-S intervals (IRTs remained between 15 - 25 s regardless of the avoidance schedule). Scobie concluded that goldfish indeed learned to avoid the shocks by changing compartments, but the subjects were responding at a rate that was higher than necessary to avoid all the shocks during the conditions in which S-S and R-S intervals were 30 and 60 s.

Goldfish and bettas: studies on punishment

Conditioned suppression and punishment of operant responding using brief electric shocks have been replicated successfully with goldfish (Geller, 1963, 1964) and bettas (Adler & Hogan, 1963; Fantino, Weigele, & Lancy, 1972; Melvin & Ervey, 1973). In Geller's (1963, 1964) studies with goldfish, lever pressing was acquired and maintained on VI schedules of food reinforcement. Conditioned suppression was demonstrated by lower rates of lever-pressing during presentations of a flashing light that was previously paired with electric shocks. Moreover, response suppression occurred when shocks were made contingent on lever-pressing (i.e., suppression produced by a positive punishment contingency).

Adler and Hogan (1963) demonstrated the suppressive effects of punishing the extension of gill-covers in bettas, which is an unconditioned response that is part of a display of aggressiveness in this species (Simpson, 1968). Brief electric shocks were contingent on extensions of the gill membrane, which occurred during the presentation of a mirror image or a live conspecific; this produced long-lasting response suppression in all fish that were exposed to these contingencies. Later, Melvin and Ervey (1973), Fantino et al., 1972, and Melvin, Prentice-

Dunn, Adams, and Herring (1986) replicated the findings of Adler and Hogan and demonstrated the effects of implementing different intensities of punishment. These studies, together with that of Melvin and Anson (1969), showed that mild intensity shocks, instead of producing suppression, resulted in (a) facilitative effects – i.e., gill-extensions became more intense (e.g., lasted longer), and (b) high self-punitive behaviour – i.e., notwithstanding the punishment contingency, bettas continued displaying operant responding, which led them to receive over 250 shocks per 2 hr session.

Goldfish and bettas: aversive control differences

Not only has free-operant avoidance never been tested in bettas to date, but Otis and Cerf's (1963) study is the only study that has attempted to demonstrate other types of avoidance using this fish species (discrete-trials avoidance). Nevertheless, the results of this research, together with the findings of other studies conducted with a mix of theoretical and methodological traditions (i.e., combining operant and traditional learning theory approaches), provide some evidence for the behavioural differences between goldfish and bettas in aversive control procedures.

Otis and Cerf (1963) compared the performance of goldfish and bettas during a discrete-trials avoidance task using shock as the unconditioned aversive stimulus and light as the signalling, or "warning" stimulus. The task consisted of crossing from a dark compartment to one that was illuminated across several trials. They concluded that goldfish were "superior in learning ability" (Otis & Cerf, 1963, p. 679) when compared to bettas. Only four out of twelve bettas met the criterion of displaying five consecutive avoidance responses, whereas ten out of twelve goldfish reached the same criterion. Otis and Cerf's informal observations of betta freezing episodes in the dark compartment led them to suggest that the differences in avoidance acquisition between goldfish and bettas were related to phylogenetic differences in the natural reactions to aversive stimulation. According to these authors,

[g]oldfish evolved from bottom-feeding scavenger fish and flight appears to be high in the innate repertoire of responses as a reaction to sudden stimulus. Bettas, on the other hand, are primarily surface swimmers and capture live food, their initial reaction to a novel stimulus appears to be immobility and, under appropriate conditions, attack (Otis & Cerf, 1963, p. 681).

This interpretation of Otis and Cerf's relates to the concept of "competing responses", which later appeared in the aversive control literature and describes how certain characteristics of aversive stimuli evoke specific responses that may or may not "compete" with the responses that are critical for learning the contingencies (Anderson, Crowell, Cunningham, & Lupo, 1979). Moreover, the fact that the majority of the bettas did not learn to avoid the delivery of electric shocks by crossing from a dark to a lighted compartment – despite several trials – suggests that bettas display behavioural sensitivity to the natural aversive quality of brightly illuminated environments (i.e., *scototaxis* – Maximino et al., 2007; Maximino et al., 2010a; Maximino, et al., 2010b; Maximino, da Silva, Gouveia, & Herculano, 2011). Although there is no specific report of scototaxis-related behaviour in bettas, this phenomenon has been confirmed in several other fish species, such as zebrafish, guppies, and nile tilapia (Maximino et al., 2007).

Alternatively, it is possible that bettas (as opposed to goldfish) do not easily discriminate changes of illumination in a shuttle tank. Additional evidence regarding the poor effect of light as a discriminative stimulus for bettas appears in the context of non-aversive research that utilized free-operant procedures (Wirth et al., 2003) and discrete trials in submerged T-mazes (Craft, Szalda-Petree, Brinegar, & Haddad, 2007). Wirth et al. (2003) tested the effects of varying the duration of mirror image presentations on the behaviour of bettas exposed to a task in which curtains of air bubbles were used as a discriminative stimulus, and where operant responses consisted of swimming through a ring. They demonstrated successful stimulus control of betta behaviour under different schedules of reinforcement signalled by air bubbles. More recently, Craft et al. (2007) tested three different forms of signalling stimuli (green and red colors, curtains

of air bubbles, and the location of food placement) on the preference of male bettas for target

areas in a T-maze task. Craft et al. (2007) found that fish only discriminated the location of larger

portions of food (the target area) when air bubbles signalled them, and not when color or position

was used.

Overall, the studies of Otis and Cerf (1963), Wirth et al. (2003), and Craft et al. (2007)

suggest species-specific differences between bettas and goldfish that have not yet been

systematically explored. First, bettas and goldfish may differ in their capacity to adjust to negative

reinforcing contingencies (e.g., escape or signalled avoidance), which would explain the poor

performance of bettas in Otis and Cerf's (1963) experiment. Second, bettas and goldfish may

differ in their behavioural sensitivity and reactions to aversive stimuli, including illuminated

environments and electric shock. For instance, it is possible that aversive stimulation produces

more immobility in bettas than in goldfish, which may interfere with the establishment of active

responses involved in certain experimental paradigms, such as changing compartments to avoid a

shock (e.g. Otis and Cerf's, 1963 task). Third, bettas and goldfish may differ in their capacity to

discriminate changes in illumination that signal aversive or appetitive stimulus. This is apparent

from the fact that stimulus control in bettas could only be obtained when curtains of air bubbles

were incorporated in the procedure as a discriminative stimulus that signalled food and mirror

images.

Evidently, more research is needed to confirm these differences, especially because

research on aversive control with bettas is almost nonexistent. Thus, it seems that one critical test

would involve confirming if betta behaviour could be regulated by free-operant avoidance

contingencies (signalled and unsignalled), as has been successfully demonstrated in goldfish

(Behrend & Bitterman, 1963; Scobie, 1970). The studies presented here are precisely aimed at

testing, for the first time, the effects of implementing negative reinforcement contingencies on

betta behaviour using a form of aversive stimulation different than the traditional electric shock.

Chapter VII. A methodology for the study of aversive control of betta behaviour using water

disturbances: Preliminary Experiments 1 - 6

Informal observations of male betta behaviour during the course of a follow-up of Chitty's (2003) study suggested the possibility of testing, for the first time, whether water disturbances (WDs) had an aversive function for the behaviour of bettas (e.g., positive punishment or negative reinforcement functions). In this follow-up study, bursts of air bubbles (ABs) were tested as a response feedback stimulus for contacting a virtual target area (every time the fish entered a target region a brief stream of air bubbles was introduced to the tank using an external air pump). Two observations were made during this experiment: first, fish displayed signs of agitation towards the ABs, and hesitation to approach the targeted area (see sample video, Hurtado-Parrado, 2012a). Second, fish in their housing tanks showed a "freezing" response when the water filters were running, with the animal remaining stationary on the opposite side of the tank from which the filters created a water flow. Apparently, such immobility was evoked by the filters and/or the water disturbances which they produced, because it was not observed at any other time (filters were only run twice a week for few hours).

These informal observations, together with some additional considerations, led us to initiate a series of interrelated preliminary studies (Experiments 1 - 6) aimed at developing a methodology that would test whether WDs had an aversive function for the behaviour of bettas (Hurtado-Parrado, 2013; Hurtado-Parrado et al., 2009, 2010). First, as described in Chapter V, the natural habitats of wild male betta are quiet fresh water ponds with muddy bottoms or flooded rice paddies, where they establish a territory, build bubble nests, and breed (Jaroensutasinee & Jaroensutasinee, 2001; Monvises et al., 2009; Goldstein, 2004). Accordingly, it seemed plausible that WDs could be unconditioned aversive events for bettas since WDs disrupt their territorial, bubble-nesting, and breeding activities and/or could be associated with the intrusion of other

males or predators into their territory. Second, as previously discussed (Chapter III and VI), all studies on aversive control of fish behaviour have been conducted with electric shock, which is a form of stimulation that has been questioned for its lack of generality and ecological validity (e.g., electric shock is not a stimulus that animals would face regularly in their habitats, and has a pain component that may not be common to all naturally occurring aversive interactions). We concluded that identifying and studying the functions of other, perhaps more naturally occurring, classes of stimuli (such as WDs) was important.

The behaviour analytic rationale for testing unknown aversive functions of classes of stimuli

The approach of the preliminary studies conducted in the Behaviour Analysis Fish Laboratory in order to test the functions of WDs was consistent with certain operant notions pointed out in Chapter II. First, behaviour analytic theory emphasizes rigorous operational definitions of basic technical terms. Second, operant concepts have a strong functional emphasis. For these reasons, the definition of a new class of stimuli requires the explicit demonstration of its effects on some properties of a response class. In the particular case of negative reinforcing and positive punishing functions (i.e., aversive functions) – which were the functions suspected for WDs – the behavioural literature includes specific effects on a response class that define a class of stimuli as aversive. The clear implication is that (within the behavioural approach) it is not sufficient to assume that a given stimulus class, such as WDs, is aversive, and that it will function in all respects exactly in the same manner as other aversive stimuli, such as electric shock. Instead, an operant approach requires that this be demonstrated empirically before it can be accepted within the established body of behavioural scientific knowledge.

In line with this approach, we considered that demonstrating the aversive functions of WDs required confirmation that betta behaviour changed in accordance with the predictions of operant theory for behaviour under aversive control. Moreover, we took into consideration the

behavioural activity theory regarding the relevance of spatiotemporal analyses of behaviour. For example, in order to confirm the positive punishment function of WDs, a decrease in some temporal aspect of the target response class, such as its duration or latency, should have been evident when the deliveries of WDs were contingent on instances of that same response class. With this rationale, we conducted a series of interrelated experiments using different paradigms and methodological refinements; together, they confirmed the unconditioned aversive function of WDs and their positive punishing function. In addition, these studies provided, for the first time, some evidence that spatiotemporal analyses of betta activity regulated by aversive events have the potential to enrich our understanding of basic behavioural processes, in a similar manner as has been demonstrated with appetitive stimulation.

Preliminary Experiments 1 - 6: effects of non-contingent deliveries of WDs in the form of air bubbles and water flows, and the introduction of a punishment contingency

In view of the lack of previous research utilizing WDs as a form of aversive stimulation for fish behaviour, a process of development, refinement, and testing of appropriate devices and procedures was carried out. A first study − consisting of four pilot experiments − tested the function of two forms of WDs: air bubbles (ABs) and water flows (WFs) (Hurtado-Parrado, 2013; Hurtado-Parrado et al., 2009). A subsequent study − divided in two experiments − was conducted to test the effect of response-dependent deliveries of WFs (positive punishment contingency) on the frequency of responding and the spatiotemporal distribution of betta behaviour (Hurtado-Parrado, 2013; Hurtado-Parrado et al., 2010). The VTS described in previous chapters, adapted for conducting spatiotemporal analysis of betta behaviour in our lab (e.g., Chitty, 2003; Martin, 2010, Sales, 2011), was further modified at different stages to suit the varying requirements of these six preliminary experiments.

Preliminary Experiment 1: non-contingent presentations of ABs.

Experiment 1 used ABs as a form of WD and the subject was a single male betta. In every

20-min daily session, simultaneous and continuous presentations of ABs were introduced in three

corners of an experimental tank with dimensions of 40 x 20 x 20 cm and a water depth of 10 cm

(see Figure 2). The remaining corner, in which no disturbances were introduced, was labeled the

"target area" (i.e., undisturbed area of the experimental tank). Across 12 daily sessions, the

position of the target area was changed randomly once the pattern of location-preference of the

fish became stable (usually within 2 - 3 consecutive sessions).
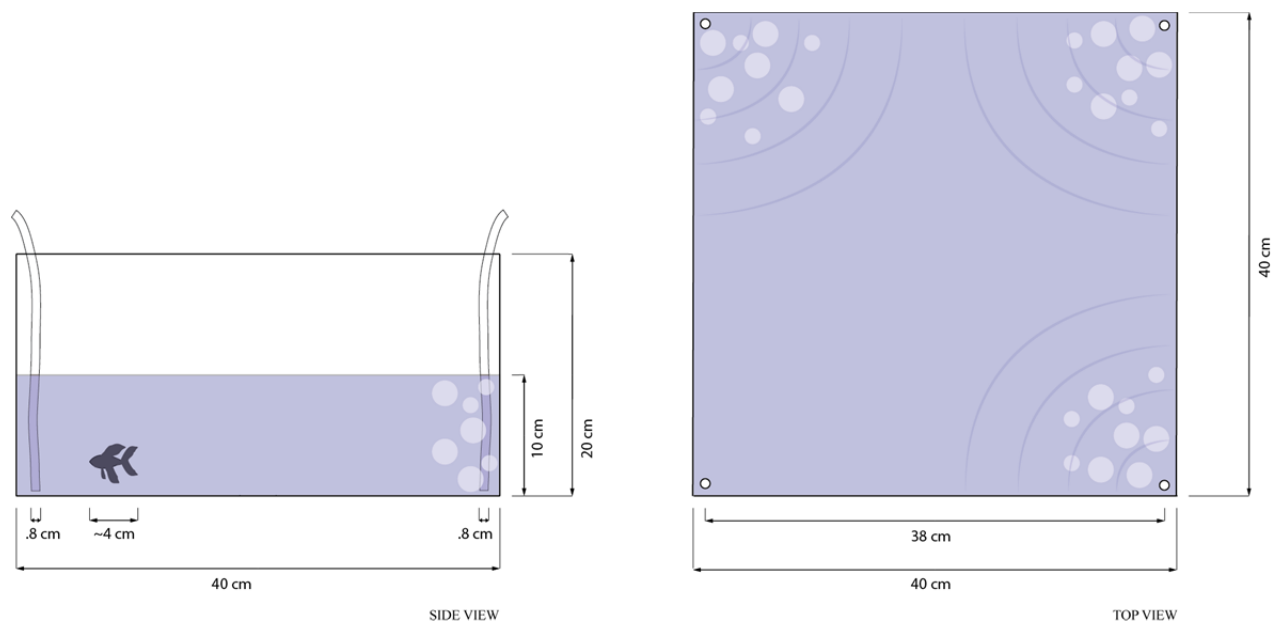
Figure 2. Configuration of the fish tank during Preliminary Experiment 1. Three plastic tubes located on each corner created WDs by introducing non-contingent, continuous, and simultaneous streams of air bubbles (ABs). The remaining corner, in which no disturbances were introduced, was labeled the "target area" (i.e., undisturbed area). Across 20-min daily sessions, the position of the target area was changed randomly once the pattern of location-preference of the fish became stable.

The main finding of this first test was that throughout all the scheduled sessions and

changes of the location of the target area, the fish preferred the undisturbed corner (see Figure 3).

This approximated the effect that was informally observed in the home tanks when the water

filters were running.
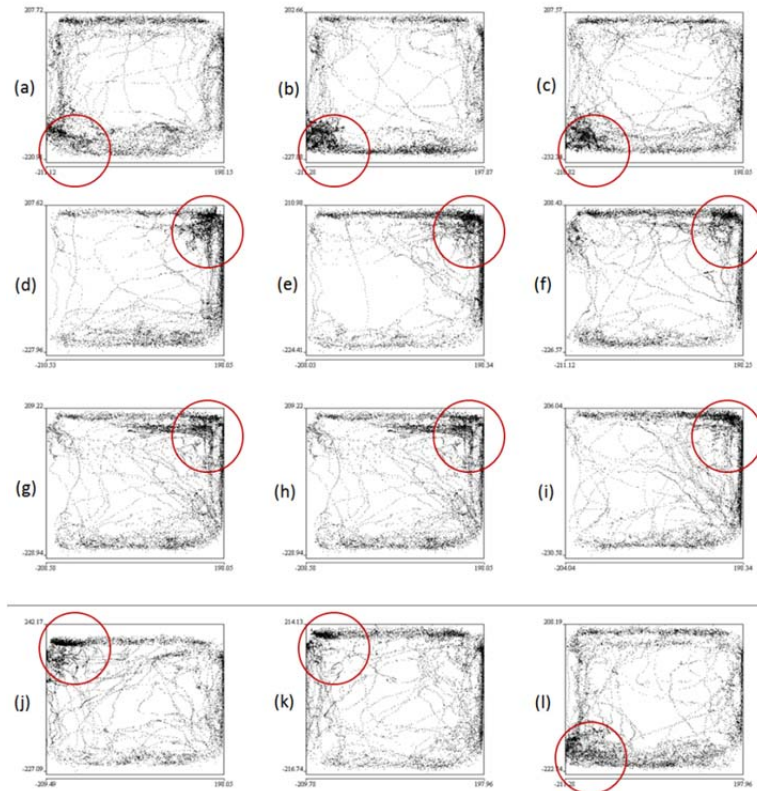
Figure 3. Spatiotemporal data of Preliminary Experiment 1. Each square (a, b, c, d... l) constitutes an overhead view of the spatiotemporal distribution of the fish inside the experimental tank during a 20-min session of continuous response-independent presentations of air bubbles (ABs). Each dot represents the position of the fish at every tenth of a second. Darker areas therefore indicate that the fish spent more time in that location of the tank. The circle on each plot indicates the location of the undisturbed corner, or "target area" (i.e., no ABs were introduced in that corner).

Experiment 1 provided, for the first time, important information regarding the preferences

of the fish for undisturbed areas of the tank; however, some limitations were evident: first, the

tubes placed in the corners of the tank were not segregated or shielded, which resulted in WDs

affecting, to some extent, the entirety of the experimental environment; second, software

limitations and the unavailability of a more optimal strategy for measurement limited the analyses

of the subject's preferences (e.g., researchers could only use visual inspection of plots

representing the spatiotemporal distribution of the fish behaviour during each session – see Figure

3). This situation demanded the implementation of a different technique.

Preliminary Experiment 2: non-contingent deliveries of ABs in a shuttle tank

Considering that previous research on aversive control of fish behaviour via shocks was

conducted in shuttle tanks (Behrend & Bitterman, 1963; Scobie, 1970), an analogous preparation

was implemented in Experiments 2, 3, 4, and 5: the tank was divided into two equal-sized

compartments by an opaque plastic partition; the two areas were connected by a central opening;

and WDs were introduced to only one of those compartments (see Figure 4).

SIDE VIEW   TOP VIEW
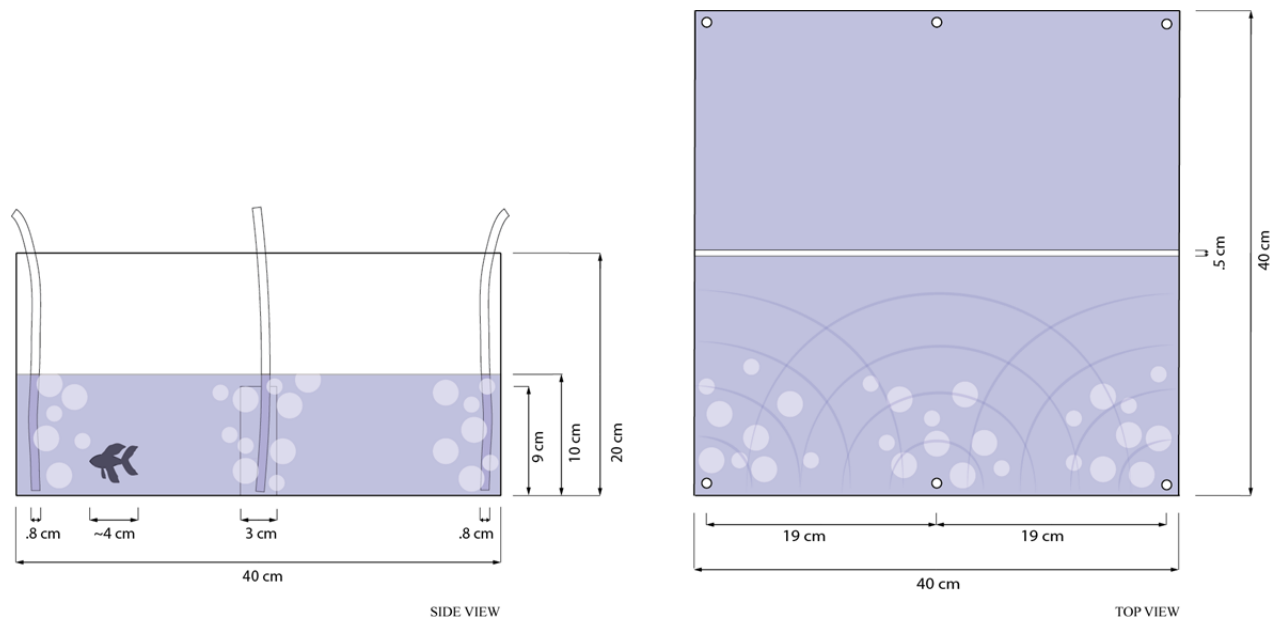
Figure 4. Arrangement of the shuttle tank during Preliminary Experiment 2. Two equally-sized compartments were created by means of an opaque plastic partition. The two areas were connected by an opening in the center of this partition. Three plastic tubes located on each corner and sidewalls of the tank created water disturbances (WDs) by introducing non-contingent, continuous, and simultaneous streams of air bubbles (ABs).

Since the subject could go freely from one compartment to the other at any point in the

session – as is characteristic of the free-operant methodology – alternative quantifications of the

amount of time that the fish spent in each compartment and the number of changes of

compartment (i.e., crossings) were now available. This strategy provided a more precise measure

of the subject's preference with regard to the presence or absence of WDs.

Experiment 2 used the same fish as was used in Experiment 1. The experiment began with

a *baseline condition* – no WDs introduced in either of the two compartments of the shuttle tank. A

treatment condition was then introduced, characterized by the presence of WDs. One

compartment was disturbed until the subject's time allocation to each compartment stabilized

across several sessions (i.e., the experimental condition changed when the fish displayed a stable

pattern of temporal distribution across sessions), at which point WDs were introduced in the other

compartment. These two treatment conditions (WDs on left compartment and WDs on right

compartment) followed the sequence: left, right, left. Finally, the baseline condition was

reintroduced after the last alternation, for a total of five experimental conditions.

Unlike Experiment 1, the time the fish allocated to each compartment of the tank was

recorded and utilized for drawing conclusions on the subject's preferences for the disturbed or

non-disturbed areas. This allowed a new measurement of preference to be introduced into the

analysis: the preference ratio (PR). This measure consisted of the ratio of the difference between

the time that the fish spent in either compartment of the tank over the total session time. That is,

$$PR = \frac{Time\ in\ the\ right\ compartment - Time\ in\ the\ left\ compartment}{Session\ time}$$

As the difference between the amount of time that the fish spent in the right and left

compartments of the tank increased, PR approached 1.0 or -1.0 respectively. Accordingly, a

positive value of PR indicated a preference of the fish for the right compartment of the tank, a

negative PR indicated a preference for the left side of the tank, and a PR of zero indicated no

preference for either side of the tank. We expected that the PR would provide a convenient index

of the subject's time allocation to the two sides of the tank and its preference for non-disturbed

areas.
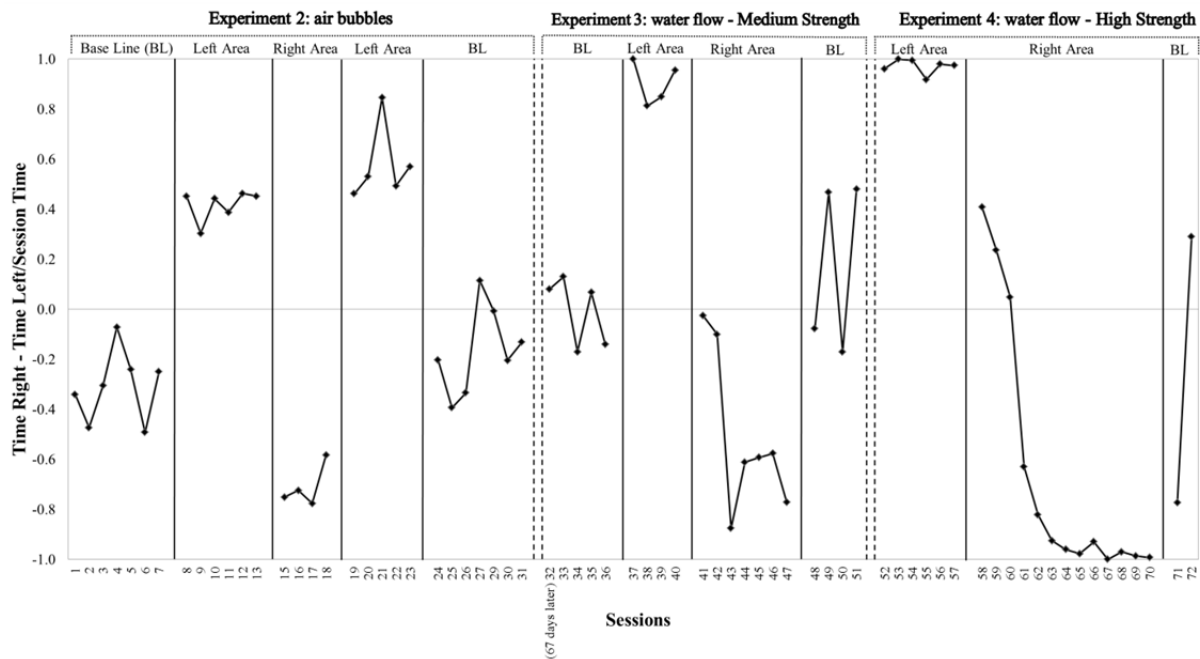
Figure 5. Preference of a male betta for the right or left compartment of the shuttle tank during
preliminary Experiment 2, 3, and 4, indicated by the Preference Ratio (PR; calculated for every
daily session). This consisted of the difference between the amount of time the fish spent on the
right compartment of the tank and the time that the fish spent on the left compartment of the tank,
divided by the total session time (i.e., PR = [Time Right – Time Left] / Session Time). Preference
for the right compartment is indicated by PR > 0.0, whereas a preference for the left side is
indicated by PR < 0.0. Experiment 2 (left panel) used ABs as a form of WD; Experiment 3 (centre
panel) used submersible water pumps to create water flows (WFs) of medium strength;
Experiment 4 (right panel) used submersible water pumps to create WFs of high strength. In each
of the 20-min sessions, the fish could change compartments any time through the opening located
in the tank's partition. WDs were absent during baseline sessions. "Left Area" and "Right Area"
labels specify the compartment of the tank in which the non-contingent WDs occurred
continuously for each session. No data were collected between sessions 31 and 32 (67 days of
inter-semester recess). The final baseline of Experiment 4 was cut short due to unforeseen
circumstances.

As predicted, the results of Experiment 2 (see Figure 5 – *Experiment 2: air bubbles*)

showed that throughout treatment conditions (sequence of vertical panels "Left Area" – "Right

Area" – "Left Area"), the fish spent substantially more time in the non-disturbed compartment, as

indicated by the corresponding PRs above 0.4 during the WD exposure on the left compartment

(first and third treatment condition; "Left Area") and below -0.7 during WD exposure on the right

compartment (second treatment condition; "Right Area"). Moreover, the changes in time

allocation were immediate, as indicated by the fact that PRs changed (from positive to negative

values) with the very first session of each treatment condition. Finally, the effect of the ABs in the

time distribution is also evident in the return to initial baseline levels immediately after the final

treatment condition.

Preliminary Experiments 3 and 4: non-contingent presentations of WFs

Experiment 3 and 4 were variations of the procedure and apparatus of Experiment 2, and

aimed at systematically replicating the effects of delivering continuous response-independent

WDs in a single compartment of the tank. The reason for these modifications was that ABs only

created WDs around the tank walls and tubes, while other areas remained less disturbed.

Additionally, Experiments 1 and 2 indicated that, while WDs had an effect in terms of causing a

clear change in the spatiotemporal distribution of the fish behaviour (i.e., preference for the non-

disturbed compartment), the subject continued crossing to the compartment in which the WDs

were presented. It appeared that the disturbances created by ABs drastically decreased the time

allocated by the fish to the target compartment, but its crossings to this area were not completely

suppressed. This observation was unexpected, because the literature reports that aversive

stimulation in the form of electric shock typically produces complete suppression of responding

(Geller, 1964; Melvin & Ervey, 1973); we therefore suspected that this pattern may have been

caused by the uneven disturbances produced by the ABs.

The unexpected crossings to the disturbed compartment and the "water tunnels" that some

biological laboratories utilize for studying fish behaviour (e.g., Graham, Dewar, Lai, Lowell &

Arce, 1990; Priede & Holliday, 1980) inspired a new set of modifications to the apparatus, which

were implemented in Experiments 3, 4 and 5. Instead of introducing ABs to the tank, submergible

water pumps, encased in white plastic covers to avoid interference with the tracking, were utilized

to create three levels of water flow (WF): no flow, medium-strength flow, and high-strength flow

(see Figure 6).
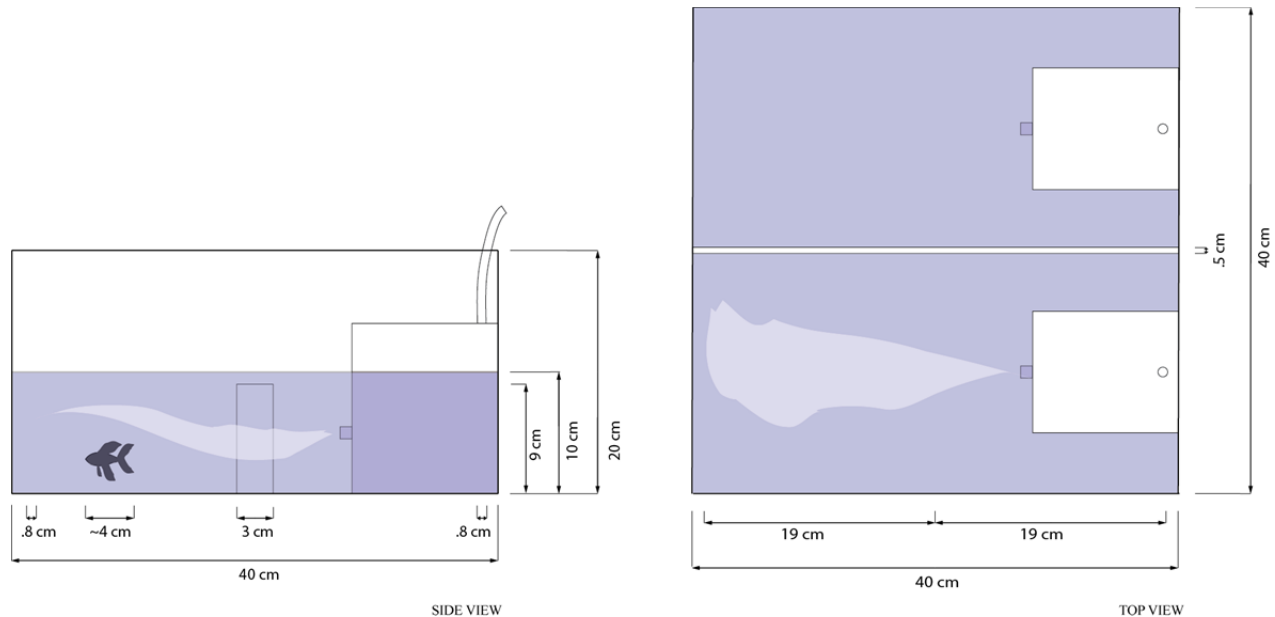
Figure 6. Arrangement of the shuttle tank during Preliminary Experiment 3 and 4. A water pump
located in each compartment of the tank (covered in white plastic to avoid interference with
tracking) produced WFs of two different magnitudes (medium and high strength).

Experiment 3 followed the same procedure as Experiment 2; the only difference consisted

in the use of water pumps, which produced continuous medium-strength WFs in only one

compartment of the tank (see Figure 6). Experiment 4 was a replication of Experiment 3, except

with high strength WFs. The same fish utilized in Experiment 1 and 2 was used in Experiment 3

and 4. The decision to test two different strengths of flows was based on the assumption that the

magnitude of preference for the target compartment was dependent on the intensity of the WFs;

specifically, a more pronounced preference for the non-disturbed area of the tank was expected

when a higher intensity of WDs was introduced.

The results of Experiments 3 and 4 replicated the general finding of Experiment 2. The

subject consistently showed a marked preference for the compartment of the tank in which the

water was not disturbed (i.e. extreme positive and negative PR values coincided with the introduction of WF to the left and right compartment of the tank, respectively – see Figure 5, centre and right panel).

Each plot in Figure 7 shows the spatial distribution of the fish during the last three sessions of the final treatment condition of Experiment 4 (WFs in the right compartment), once stable time allocations across the compartments were reached. It is evident that an extreme preference for the undisturbed compartment of the shuttle tank (in this case, the right side; PRs close to 1.0) was accompanied by regular swimming patterns inside that area: mainly along the long wall of the right compartment, with long periods of time spent between the water pump container and outer right wall of the tank (Figure 7 – region "A"). As previously discussed (Chapter VI), brightly illuminated environments may affect betta behaviour in the same way as in other species of fish (zebrafish, guppies, tilapia – Maximino et al., 2007). It is possible that the subject's preference for spending considerably more time in certain areas of the tank could be explained by the fact that the angle of the experimental chamber's light created areas of lower illumination, such as the corner between the water pump container and the wall of the tank. This is somewhat consistent with the observation that in the wild, bettas hide under vegetation, presumably to avoid predators. What is more significant about this interpretation is that it exemplifies how the spatiotemporal analysis approach has the potential to provide additional information, which leads to a more complete understanding of behaviour.
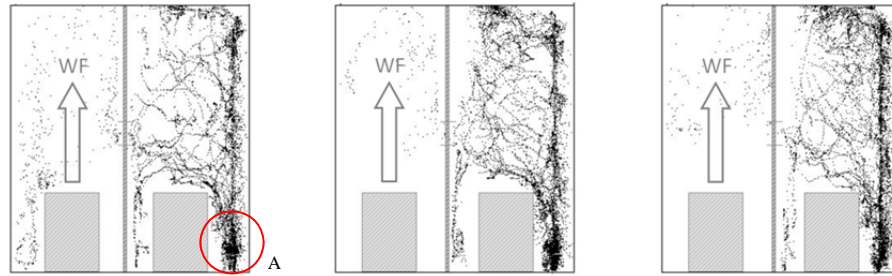
Figure 7. Each plot is an overview representation of spatial distribution of the fish inside the shuttle tank during the last three sessions (20 min each) of the final treatment condition of Preliminary Experiment 4 (continuous WFs delivered only in the left compartment). The central dashed line represents the partition dividing the shuttle tank. The shaded grey boxes represent the water pumps that produced the WFs (one pump in each compartment). Each dot represents the location of the fish every tenth of a second; accordingly, darker areas indicate that the fish spent more time in that location. "A" indicates the area between the water pump container and the outer right wall of the tank, where the fish consistently remained for longer periods. WF = "water flows". Arrows indicate the direction of the flows.

Finally, while two different strengths of WFs were tested in Experiment 3 and 4, PR values across the treatment conditions did not differ considerably. However, when the PRs of Experiments 3 and 4 were compared to those of Experiment 2, it was evident that the WFs produced more extreme PRs than the ABs procedure. As a result, high-strength WFs were chosen for subsequent experiments.

Preliminary Experiment 5: Effects of non-contingent WF deliveries and a punishment contingency on time allocation and crossings.

Experiment 5 was a systematic replication of Experiment 4 (i.e., it was an "attempt to replicate a functional relationship under circumstances that differ from those of the original experiment – Perone & Hursh, 2013, p. 123). Specifically, a new subject was utilized, the experimental design was modified, a new measure was introduced (crossings between compartments), a different aversive contingency was scheduled (positive punishment), and additional adjustments were made to the tank.

The additional measure in this experiment consisted of the number of times the fish crossed the partition, going from one compartment to the other. Consistent with the general approach of the spatiotemporal analyses research program, measuring the crossing response of the fish allowed examination of the relationship between the subject's time allocation to the compartments and the frequency of crossing between them. In other words, this experiment focused on examining the relation between relative preference (i.e., spatiotemporal patterns) and frequency of crossing (i.e., a discrete response measure).

Informal observations during Experiments 3 and 4 indicated that the fish occasionally reduced its exposure to WDs by hiding in gaps between the surfaces of the tank and the water pump containers. As a result, the apparatus was modified in Experiment 5 to remove these "safe areas" that could be favouring the fish's continued presence in the disturbed compartment. As for the experimental design, additional baseline conditions were interspaced between every treatment condition. These baselines were meant to allow for a replication of the rapid changes in the PRs observed in previous experiments when the WDs were introduced and removed.

Finally, response-dependent deliveries of WFs were scheduled during the last treatment condition of Experiment 5. The purpose of introducing this condition was to gather direct evidence of the positive-punishing function of WDs for the behaviour of bettas. It was expected that response-dependent deliveries of WFs would produce a decrease in some aspect of the subject's behaviour; either frequency and/or duration. During this treatment condition, WFs were delivered only in the left compartment of the tank, and only when the fish crossed from right to left. The left side of the tank was designated as the target area because the fish systematically displayed a high time allocation to that compartment throughout the four previous conditions of Experiment 5. Another procedural aspect of this experimental condition is that the pump continued producing WFs in the target (left) compartment during the entirety of the subject's time

there, and it was turned off only when the fish completely left that compartment (i.e., returned to

the right side of the tank). Given this contingency, the water pump remained off when the fish was

located in the right compartment of the tank (see sample video – Hurtado-Parrado, 2012b).

  Overall findings. The effect of presenting non-contingent and continuous WDs, observed

in the previous experiments, was systematically replicated throughout the first conditions of

Experiment 5. That is, extreme PR values indicated that the subject's relative distribution of time

was clearly affected by the constant presence of WFs in a particular compartment (see Figure 8,

graph "a"). The eighth (fourth treatment) condition was an exception to this generalization, in that

the subject showed a preference for the same compartment (left) that the WFs occurred (see eight

panel of graph "a"; *WF – Left side*). Furthermore, except for the fourth treatment condition, the

rapid changes in the subject's preference that were observed throughout the previous experiments

were also replicated (i.e., the fish changed its relative time allocation to the compartments almost

immediately after a change in conditions). Regarding the new measure incorporated to the

procedure of this experiment (number of crossings per session), it was found that fish changed

compartments more frequently throughout all the baseline condition in comparison to the

experimental conditions (see Figure 8, graph "b").
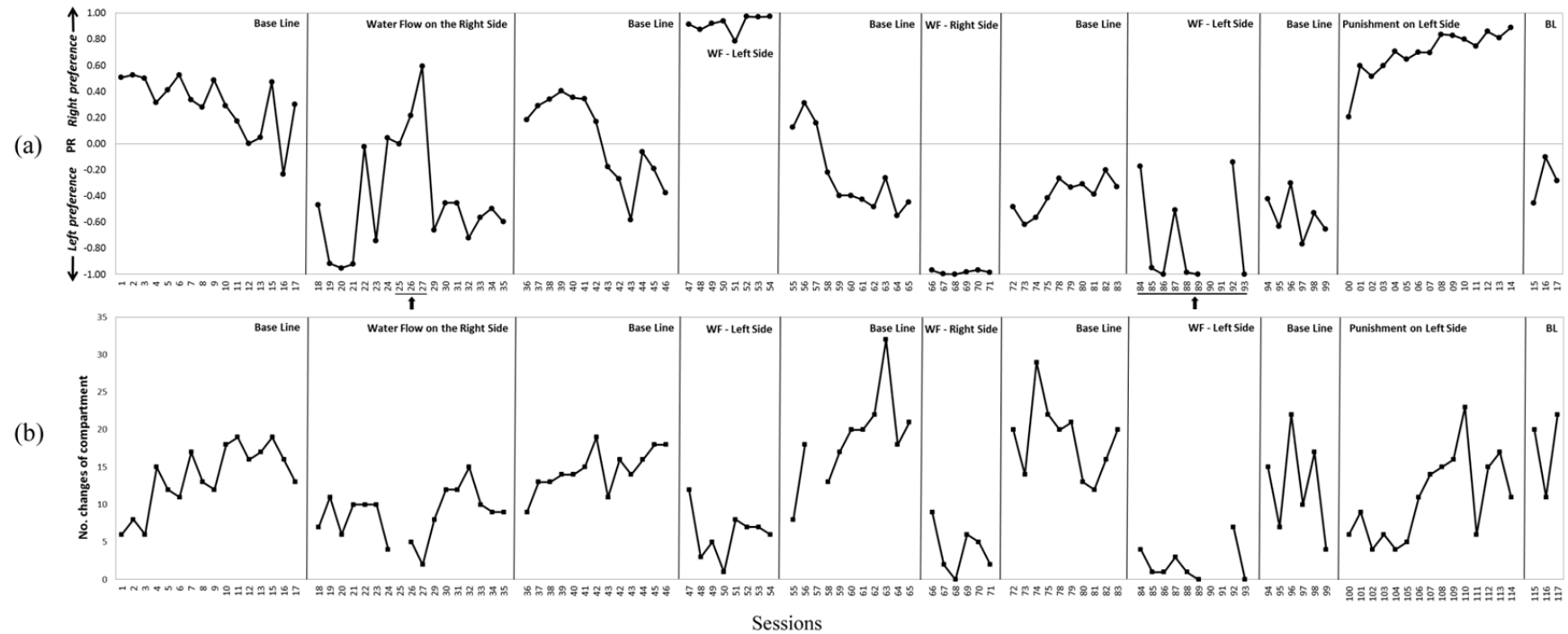
Figure 8. Preference Ratio (top graph – *a*) and the number of crossing responses (bottom graph – *b*) of a male betta throughout control and treatment conditions of Preliminary Experiment 5. Three different treatments conditions were introduced: WFs occurring on the right, and left compartment (non-contingent deliveries of WFs), followed by punishment on the left compartment of the tank (contingent deliveries of WFs). Baseline (BL) conditions were scheduled before and after every change of treatment condition. Each treatment and baseline condition is represented in a vertical panel. Underlined sessions on graph *a* indicate observations of "freezing" responses. Data points missing for sessions 90 and 91 of graph *a*, and sessions 25, 57, 90, 91 of graph *b* correspond to loss of information.

Freezing responses. Similar to Experiment 3 and 4, the subject continued crossing to the disturbed compartment and seemed to be reducing its exposure to the WFs by remaining immobile in a particular area. Specifically, session videos showed that the fish was occasionally being dragged, or allowed itself to be dragged, by the returning WF current to a crevasse between the tank wall and the water pump covers. The fish remained motionless in this location for considerable periods of time, even though it could remove itself at any moment (i.e., "freezing" responses – see Figure 9).
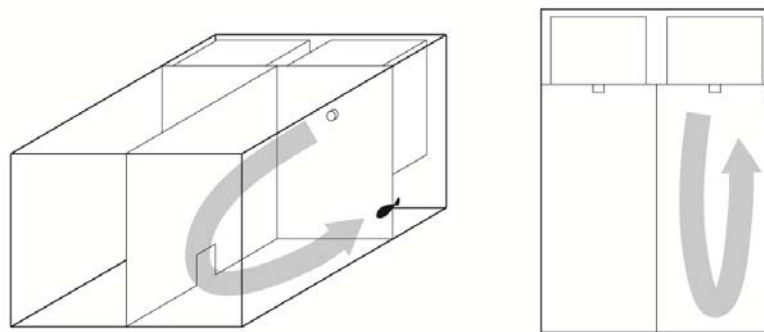
Figure 9. Representation of subject being dragged by the returning WF current between the tank wall and the water pump covers during Preliminary Experiment 5, where it remained motionless for considerable periods of time (i.e., freezing response).

Moreover, these freezing responses occurred across several sessions and seemed to explain many dramatic fluctuations in the subject's time allocation between disturbed and undisturbed compartments. For example, the unexpected high allocation of time to the disturbed compartment that occurred during the first and fourth treatment conditions of Experiment 5 coincided with this "freezing" response (see Figure 8, graph "a", sessions 25- 27 and 84 – 93).

Effects of scheduling a punishment contingency. In view of the recurrent and extreme periods of freezing observed throughout the second condition, in which WFs were presented in the left compartment (sessions 84 - 93), this condition was terminated and a subsequent baseline was introduced (sessions 94 - 99). Considering the subject's high preference for the left compartment throughout these two conditions, it was decided to introduce a punishment

contingency, consisting of deliveries of WFs contingent to the fish visiting the left compartment

(target area).

Throughout this condition the subject (a) did not display freezing episodes, (b) showed a

consistent increase in time allocation to the non-disturbed compartment of the tank (i.e. right

compartment, reaching levels above 90%), and (c) displayed a fluctuating number of crossings

which, overall, remained close to previous and subsequent baseline levels (see Figure 8, graph

"a" and "b", sessions 94 - 117). Findings "b" and "c" were unanticipated, given the assumption

that WDs should have produced an analogous effect on the two behavioural measures under

consideration – i.e., a reduction in time allocation to the target compartment and suppression of

crossings to that area. A sixth experiment was conducted with naïve subjects and additional

adjustments to the experimental procedure in order to replicate this dissociation between time

allocation and number of crossings and to confirm the consistency of these results.

Preliminary Experiment 6: replication of punishment effects

A systematic replication of the effects produced by the punishment condition of

Experiment 5 was attempted with two new subjects (Fish 13 and 14). Fish were exposed to

response-dependent deliveries of WFs in a target compartment chosen on the basis of

preferences observed during an initial baseline. The only procedural difference between the last

condition of Experiments 5 and Experiment 6 was that in the latter WFs were delivered

intermittently instead of continuously: WFs of 10 s were delivered every 10 s and only during the

time that the fish stayed in the target compartment. This change in the procedure was based on

the findings of previous research that indicate that the temporal characteristics of aversive

stimuli, at least in the case of electric shocks, evoke specific responses in the subjects that may or

may not compete with the responses that are critical for the learning of the negative reinforcing

contingency (e.g., freezing – Anderson et al., 1979; Lawry, Lupo, Overmier, Kochevar, Hollis, & Anderson, 1978). In this context, in order to incorporate WFs into aversive control paradigms that have traditionally used electric shocks, it was necessary to differentiate and adapt certain standard parameters, such as the interval between aversive stimuli (Flow-Flow interval) and the duration of the aversive stimulus (the length of WF periods).
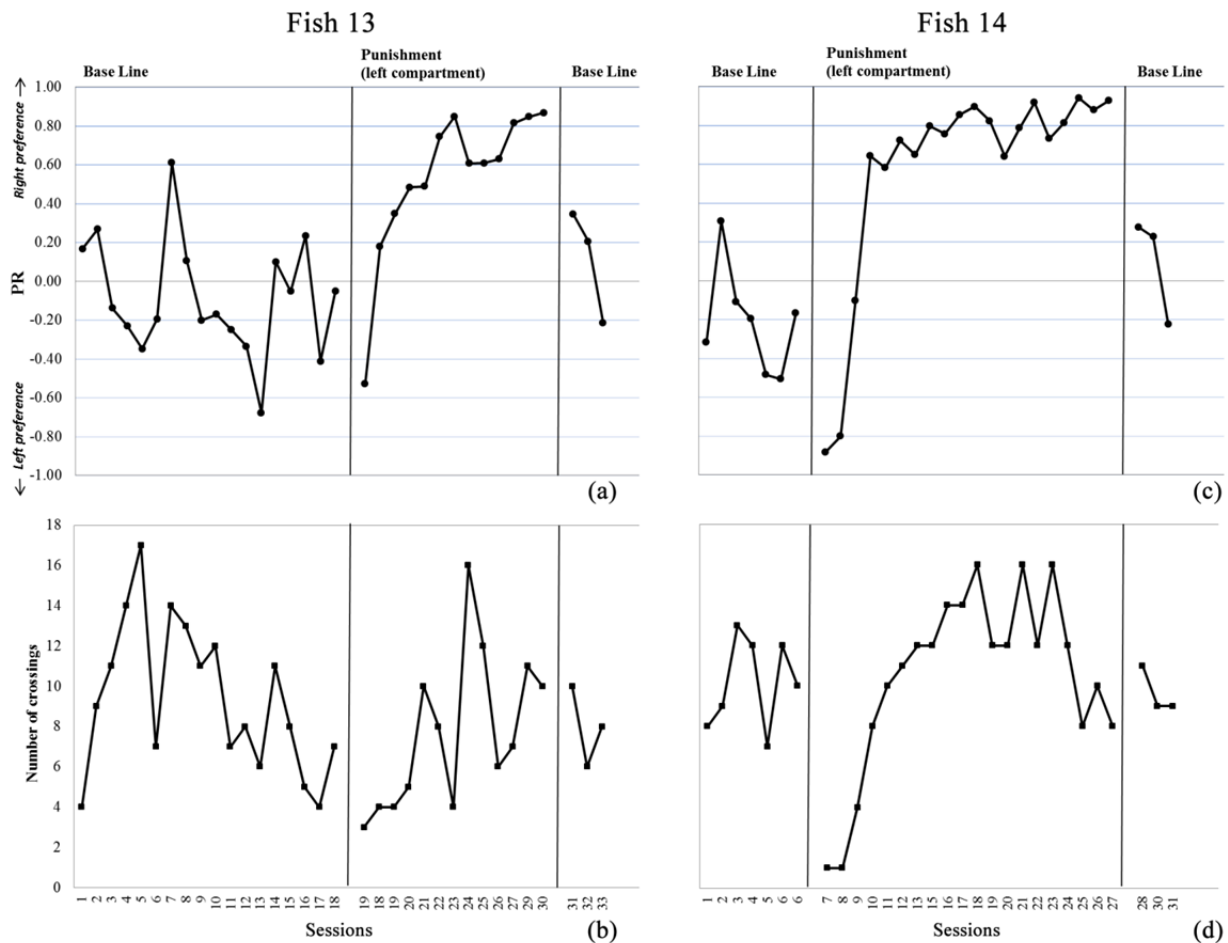
Figure 10. Preliminary Experiment 6: systematic replication of the punishment condition of preliminary experiment 5 – i.e., response-dependent and intermittent deliveries of WFs in the left compartment. The top row of graphs (*a & b*) corresponds to the PRs across baseline and treatment conditions for Fish 13 and 14. The bottom graphs (*b & d*) correspond to the number of crossings observed throughout those conditions.

As Figure 10 shows, Experiment 6 systematically replicated the findings of Experiment 5's last treatment condition (punishment on the left side) and baseline (see Figure 8). These include (a) the overall preference of both subjects for the left compartment in the absence of WFs

(i.e., during baselines), and (b) the dissociation between a reduction in time allocation to the target compartment and a close-to-baseline frequency of crossing. More specifically, contingent and intermittent deliveries of WFs produced the same ascending trend in the PR values, which surpassed .85 for both subjects (see Figure 10, graph "a" and "c"). Moreover, during the first sessions of the last baseline condition (i.e., immediately after the contingency was removed) the rapid return to more evenly distributed time allocations to both compartments was observed in both fish.

Regarding the number of crossings, Fish 13 maintained a very similar number of crossings throughout both baseline and treatment conditions (see Figure 10, graph "b"). Fish 14 stopped crossing during the first sessions of the treatment condition (see Figure 10, graph "d", sessions 7 - 9), but an increasing trend in the number of crossings was observed as the condition progressed. Furthermore, the frequency of crossing observed in Fish 14 during the last sessions of the treatment condition remained similar to that of the subsequent baseline.

Conclusions regarding the function of WDs for betta behaviour

Unconditioned aversive functions of WDs

Consistent with the traditional operant approach reviewed in Chapter IV, systematic changes in the frequency of responding are typically accepted as evidence of the reinforcing or punishing functions of a given class of stimuli (Hineline & Rosales-Ruiz, 2013). However, some behaviour analytic literature has proposed that changes in other aspects of the response class allow for the confirmation of the behavioural function of a given stimulus as well. For instance, Johnston and Pennypacker (2009) stated that, from a methodological perspective, if a class of stimuli is terminated immediately following responding and the result is an increase *in some aspect* of the response class, members of that stimulus class are called negative reinforcers.

Moreover, two of the studies reviewed in Chapter III – MacKenzie et al., (1993) and McAdie et al. (1993) – demonstrated that temporal measures, such as percentages of time that a given stimulus is off and time allocations to a response option, were informative about the aversive function of intense sounds that were tested with domestic hens. The approach of the preliminary studies presented above is not only aligned with these perspectives but also with the overall assumptions of the research program on spatiotemporal analyses (Pear, 2004). That is, orderly changes in the spatiotemporal distribution of behaviour allow for the confirmation of the function of a given class of stimuli. Following this line of thought, it is argued that the significant changes in time allocation to the disturbed and non-disturbed compartments of the shuttle tank indicate that WDs certainly have a positive punishing function, and perhaps other unconditioned aversive effects for bettas.

The specific psychobiological mechanisms by which WDs affect the behaviour of bettas cannot be identified from the studies conducted in our lab thus far, mainly because this has not been the purpose of the studies. However, it is possible to provide a tentative notion of the processes involved based on an ecological perspective. The unconditioned aversive function of WDs could be related to the disruption of territorial, bubble-nesting, and/or breeding activities, or high energetic costs of locomotion. Alternatively, WDs could be associated with predatory risks, and/or the presence of intruders. This last account regarding predatory risks and/or the presence of intruders is somewhat consistent with the dissociation between time allocations to the tank's compartments (fish spent the majority of the session time in the non-disturbed area) and the close-to-baseline frequency of crossing observed in the experiments. Specifically, the short visits to the disturbed side of the tank could be interpreted as activities related to assessing for intruders or predatory risks (Pitcher, Green, Marrugan, 1986).

From a different perspective, the sensitivity of betta behaviour to WFs may be associated with the effects of intense and/or discordant stimulation affecting the lateral line and/or ear systems (Popper & Carlson, 1998). It is also possible that WFs utilized in our experiments require bettas to swim at speeds that are above a certain critical (or "comfort") level (Popper & Carlson, 1998; Anderson, Berzins, Fogarty, Hamlin, & Guillette, 2011), especially considering that this species is native to quiet bodies of water (Jaroensutasinee & Jaroensutasinee, 2001). In any case, it appears that more research is needed to explain the unconditioned aversive function of WFs for the behaviour of bettas. As Popper and Carlson (1998) concluded,

> that fish respond to the movement of the water they live in is obvious. Less obvious are the details of this response… Although some guidelines describe aspects of fish response to flow, such as the critical swimming speeds of various species and sizes of fish (Clay 1961; Bell 1991), the response of [some fish species] to flow remains poorly understood. (pp. 674-675)

Dissociation between time allocation and number of crossings

An example of one such "poorly understood response" of bettas to flow, as Popper and Carlson (1998) would put it, is evident in the dissociation between time allocation and number of crossings; in particular, the fact that fish never ceased to enter the disturbed compartment. During treatment conditions of preliminary Experiments 5 and 6, fish showed a consistent increase in time allocation to the non-disturbed compartment of the tank, while displaying a fluctuating number of crossings which, overall, remained close to previous and subsequent baseline levels (see Figure 8 and 10). Although the data of these experiments do not provide the necessary information for a satisfactory explanation for this unanticipated dissociation, some tentative accounts can be formulated.

It is plausible that the negative reinforcing effect of WFs was stronger than its punishing effect, which was weakened somewhat by the opportunity to escape the WFs (fish could enter

and leave the target compartment without long exposures to the punishing stimulus). This interpretation requires that some form of uncontrolled reinforcement must have been available in the left compartment throughout the experiment (i.e., crossings must have been maintained by a source of reinforcement). For instance, unintended mirror images could have been appearing on one of the tank's glass walls; the activation of the water pump could have produced other forms of stimulation with appetitive functions (e.g., noises or vibrations); oxygenation levels could have varied throughout the tank due to the WFs (Magnus, 2010); or the compartments could have contained different illumination levels. In addition, unfamiliarity with a new environment or some form of confinement-aversion due to the size of the compartments could have also been unintended negative reinforcing factors, as suggested by research on the stress-responsiveness of wild and domesticated bettas (Verbeek, Iwamoto, & Murakami, 2008). Such negative reinforcers could have competed with the aversive function of the WFs.

In any case, uncontrolled sources of reinforcement could explain the preference of the fish for the left compartment throughout baseline conditions of Experiment 5 and 6, in which no WDs were present, and the close-to-baseline level of crossings throughout the punishment conditions. Specifically, since the presumed form of reinforcement must have remained available in the target compartment during the punishment condition, these competing contingencies (reinforcing and punishing) could have resulted in the emergence of a behavioural pattern in which the fish spent the majority of the time in the non-punished area – reducing to a minimum level its exposure to the WFs – but still briefly accessed the target compartment. Previous research on the effectiveness of punishment and response alternatives supports this interpretation. Such studies have demonstrated that response suppression by punishment depends on the parameters of the positive reinforcement contingency and the availability of a competing

response to produce the same reinforcement (Azrin, Holz, & Hake, 1963; Azrin & Holz, 1966;

Hineline & Rosales-Ruiz, 2013; Pierce & Cheney, 2013). Observations of the session videos

seem to support this notion. The fish appeared to have learned how to enter and leave the target

compartment during WFs without much effort (i.e., using the direction of the flow instead of

swimming against the current) and on several occasions seemed to interrupt the crossing

response (e.g., the fish swam around the opening, slowly poked its head through, and returned

without completing the change of compartments – see sample video Hurtado-Parrado, 2012c).

Finally, an alternative interpretation of the dissociation between crossing frequency and

time allocation data results from considering that the configuration by which the WDs are

delivered in the shuttle tank may elicit anti-predator responses in bettas. Accordingly, both the

patterns of time allocation to the compartments and the frequency of crossing could be associated

with defensive behaviour caused by the experimental setup. In other words, an ecological

analysis suggests that the short visits of the bettas to the disturbed side of the tank could be

interpreted as activities of recognition and assessment of predatory risk (e.g., inspection of an

intruder – Pitcher, Green, Marrugan, 1986).

Potential of the WDs paradigm for research on spatiotemporal patterns of behaviour

As demonstrated by previous research conducted in our lab with pigeons and bettas

exposed to positive reinforcement preparations, the spatiotemporal analysis of betta activity

controlled by aversive events has the potential to enrich our understanding of basic behavioural

processes. Experiments 5 and 6 revealed a dissociation between time allocation and frequency of

crossing under the effects of WFs, and provided evidence regarding the possible influence of

other stress-related variables on betta behaviour (e.g., bright illumination or confinement). More

generally, preliminary Experiments 1-6 indicated that the presence or absence of a contingency

between responses and the deliveries of WDs (ABs or WFs) affects the spatiotemporal

organization of behaviour, including the incidence of freezing responses. Compared to the

conditions in which the deliveries of WDs were response-independent (i.e., non-contingent

deliveries of WDs – Experiment 2, 3, and 4), response-dependent deliveries of WFs (Experiment

5 and 6) produced very few episodes of freezing (evidenced only in one subject), and solely

during early stages of the conditioning procedure (Fish 14, first two sessions of the treatment

condition). Furthermore, the frequency of crossing observed during the response-dependent

deliveries of WFs were higher than those of the non-contingent conditions, and, in fact, reached

baseline levels at the end of the condition. Overall, it seems that response-dependent deliveries of

WDs produce high time allocations to non-disturbed compartments, and affect, to a certain

degree, the incidence of immobility responses. In contrast, the frequency of crossings does not

seem to be affected by these stimuli.

WDs as an alternative to electric shock

        The findings obtained with WDs confirmed the feasibility of using them in an alternative

method for studying phenomena that belong to the area of aversive control; an area that "seems

to be in jeopardy" (Catania, 2008, p. 114) due to its over-reliance on electric shock procedures

(see Chapter III). Moreover, the WD model is a particularly relevant alternative in that it entails

stimulation that bettas are likely to encounter in their natural environment, and is therefore more

ecologically valid when compared to shocks. Overall, the successful development of the WD

approach with bettas supports Baron's (1991) conviction on "the feasibility of deviations from

the rat-pigeon-shock methodology" (p. 176) in aversive control research.

        Finally, the findings of these preliminary experiments suggested possible differences

between the effects of WDs and electric shocks. For instance, contingent deliveries of WFs did

not produce suppression of crossings, which differs from the findings of previous research on

punishment of betta behaviour with electric shocks in which complete suppression was indeed

achieved (Adler & Hogan, 1963; Fantino et al. 1972; Melvin & Ervey, 1973). In view of these

findings, further development and testing of the WDs model gain particular relevance due to the

need for expanding upon potential differences between the effects of shocks and WDs on betta

behaviour. This is especially pertinent considering the practical and ethical issues associated with

shocks (see Chapter III) and their implications for the continued viability of the field of aversive

control. Moreover, such research would respond to Crosbie's (1998) call for comparative studies

to answer whether organisms respond differently to biologically relevant stimuli as opposed to

biologically irrelevant aversive stimuli (e.g., electric shocks); this includes whether these stimuli

are equally effective and produce similar patterns of adaptation, recovery, and by-products (see

Chapter III).

Further implications, applications, and prospective developments of the WFs paradigm

The notion that fish are capable of pain perception has received substantial empirical

support from the use of different paradigms, including negative reinforcement (Braithwaite &

Huntingford, 2004; Chandroo, Duncan, & Moccia, 2004; Reilly, Quinn, Cossins, & Sneddon,

2008). Aversive conditioning impairments and reductions in behavioural and physiological

responses to noxious stimulation have both been observed when morphine was previously injected

in fish (Braithwaite & Boulcott, 2007). These findings, together with evidence that fish possess

nociceptive systems comparable to those of other vertebrates (Sneddon, 2004; Sneddon,

Braithwaite, & Gentle, 2003), have resulted in an increasing concern for fish welfare (Huntingford

et al., 2006; Posner, 2009; Volpato, 2009). Accordingly, the development of research methods that

do not involve pain are clearly a welcome addition to the tools for studying not only aversive

control of behaviour in fish (as demonstrated thus far), but also for conducting research on fish

welfare. The WDs paradigm shows promise for the latter in terms of being consistent with the

approach defended by Volpato (2009), who extensively reviewed the strategies and challenges

on the study of fish welfare and concluded that operant techniques play an important role in this

field. In this regard, Volpato commented that

> …although [behavioural, physiological, or feeling-based] approaches [to study welfare of fish] are among the best choices, they are still incomplete… Attempts to determine the welfare state of nonhuman animals are far from a solution… In hopes of enhancing such efforts, I change the focus from trying to identify an animal's internal state of well-being or trying to demonstrate that organisms suffer, to searching for conditions in which an animal is in a "good state," which I define as one that the animal would choose. Although animal wants are easily detectable from choice tests, they are also identifiable through operant conditioning techniques, in which the animal performs an activity either to obtain something necessary or pleasant or to avoid something harmful or aversive. These techniques require more complex and more time-consuming experiments, but they are useful not only for studies of fish cognition but also for studies to determine how much an animal wants a condition — the more desirable the stimulus, the faster the learning acquisition. (Volpato, 2009, p. 332)

It should also be kept in mind that, as with humans, what an animal "wants" is not also in

its best interest. For example, a fish, like a human, may prefer not to engage in strenuous health-

promoting exercise. However, it may be that using WFs to require fish to engage in such exercise

may, within reasonable limits, be beneficial to the animals.

Recently, the WFs paradigm has had further developments. In particular, creating an

underflow system that prevents fish from finding "safe" areas in the tank (which seemed to

favour freezing responses during the preliminary studies), and automatizing the experimental

setups in different aspects; these include the timing of, the duration of, and criteria for WF

delivery, and the recording of different measures, such as response types (e.g., crossings during a

water flow or during a warning stimulus) and durations and frequencies of WFs. These

improvements allowed for conducting the studies that are presented in the following chapters of

this dissertation, which aimed at testing the WFs experimental paradigm with three complex

aversive contingencies that previously have only been studied using shock-based procedures:

unsignalled free-operant avoidance, signalled free-operant avoidance, and punishment.

Statement of the problem

Over the past decades, several authors have drawn attention to the dramatic decline in aversive control research (Baron, 1991; Catania, 2008; Crosbie, 1998; Pierce & Cheney, 2013). This is not the result of having settled all of the area's major questions; instead, it is primarily a consequence of an over-reliance on shock-based procedures which, notwithstanding their role in the success of preeminent behaviour analytic research programs during the past century, have been increasingly criticized on ethical, practical, and ecological validity grounds.

Considerable research efforts have been made to develop alternatives that allow for the continued study of basic principles of behaviour regulated by aversive stimuli (e.g., intense light and noises have been tested across several operant paradigms with rats). The experimental setup with bettas exposed to WDs, developed at the University of Manitoba's Behaviour Analysis Fish Laboratory, has shown promise as an ecologically valid and non-painful alternative, as demonstrated by a set of six preliminary experiments (Chapter VII). Moreover, throughout the development of this paradigm, the scope and potential of the spatiotemporal program was broadened by the addressing of aversive control phenomena (e.g., testing the effects of contingent and non-contingent deliveries of WDs). The findings of these preliminary studies (Chapter VII) support the overall premise of the program; namely, that spatiotemporal analyses of behaviour provide information that is not evidenced by the traditional operant approach, which is based on rates of discrete responses.

Free-operant avoidance (Sidman, 1953a) is a standard paradigm of behaviour-analytic research on aversive control. Considering that the only studies that have attempted to test free-operant avoidance in fish have been with goldfish, and the fact that the only research that intended to show a form of avoidance in bettas produced inconclusive results (Otis & Cerf,

1963), a first experiment was designed to test signalled and unsignalled free-operant avoidance in bettas. The most direct antecedents of this experiment were the studies of Behrend and Bitterman (1963) and Scobie (1970), which successfully demonstrated free-operant signalled and unsignalled avoidance with goldfish. Specifically, Experiment 1 aimed to: (a) assess different aversive functions of WFs for the behaviour of bettas, specifically, negative reinforcement; (b) investigate the generality of aversive control phenomena studied almost exclusively with electric shock, in this case, signalled and unsignalled free-operant avoidance and escape behaviour; and (c) test the versatility of the new aversive control paradigm that would serve as an alternative to preparations with electric shock.

The second experiment was a follow-up to the principal and unexpected findings of Experiment 1: that no consistent avoidance behaviour was developed by any of the subjects under both signalled and unsignalled conditions, and that a dramatic increase and predominance of escape responses was established across all fish. These observations, coupled with the fact that (a) previous research with bettas using shock-based procedures effectively replicated the suppressive effect of punishment observed across different species (Adler & Hogan, 1963; Fantino et al., 1972; Melvin & Ervey, 1973), and (b) preliminary experiments verified that contingent presentations of WFs do indeed have an effect on the spatial and temporal organization of betta behaviour (e.g., a punishment contingency dramatically reduced the time allocation of the subjects to a target compartment and partially suppressed the occurrence of crossings), a second experiment was conducted in which the same avoidance contingencies of Experiment 1 remained in place, but where escape responses, instead of interrupting the WFs, triggered their activation; i.e., a two-component contingency was scheduled for escape, namely, positive punishment and extinction (punishment-and-extinction).

The widely replicated effects of punishment observed across different species, namely target-response suppression and an increase of frequency of alternative responses (Azrin & Holz, 1966; Hineline & Rosales-Ruiz, 2013), led to the prediction that the punishment procedure implemented in Experiment 2 would generate a suppression of escape responses (target behaviour) and an increase in frequency of alternative responses – in this case, avoidance. Related to this problem is the issue of whether the contingent WFs for escape are best conceptualized as punishment, extinction, or a combination of those processes.

Chapter VIII. Experiment 1: betta behaviour under unsignalled and signalled avoidance contingencies composed of WFs

The WFs model described in Chapter VII was adapted to schedule unsignalled and signalled avoidance contingencies that entailed WFs as the aversive stimulus and curtains of air bubbles (CABs) as the discriminative or "warning" stimulus. The decision for implementing CABs resulted from a series of considerations based on the few relevant studies available (reviewed in Chapter VI). First, stimulus control phenomena have only been demonstrated in bettas when CABs were incorporated as a discriminative stimulus (signaling food and mirror images − Craft et al., 2007; Wirth et al., 2003); conversely, light and spatial cues have failed to produce operant discrimination. In addition, stimulus control of betta behaviour using CABs was accomplished in the absence of stress reactions, which indicates that CABs do not have unconditioned aversive functions for the behaviour of this species. This last consideration was critical because, as Braithwaite and Boulcott (2007) have indicated, confounding effects may result from the fact that the discriminative stimulus used in conditioning experiments also has unconditioned aversive functions. Light is a good example, because considerable research has shown that different species of fish show behavioural sensitivity to bright illumination (i.e., *scototaxis* − Maximino et al., 2007; Maximino et al., 2010a, 2010b; Maximino et al., 2011), and some evidence suggests that bettas may react similarly. The poor performance of bettas in Otis and Cerf's (1963) task involving bright illumination supports this notion, together with the preference for less illuminated areas observed in the fish of our preliminary experiments.

Conversely, CABs were not expected to have aversive functions related to WDs, because in the studies of Craft et al. (2007) and Wirth et al. (2003) this stimulus was introduced with air stones, which would have produced (if any) only small turbulences in the environment. Finally,

an additional reason for choosing CABs over light as the warning stimulus resulted from the fact

that some preliminary tests showed that changes of illumination, produced by a flashing a light,

negatively affected the tracking accuracy of the VTS. Overall, the decision of implementing

CABs in the signalled condition of Experiment 1 provided the opportunity to test if this form of

stimulation could function as a discriminative stimulus for operant behaviour controlled by

consequences different to food and mirror images (i.e., positive reinforcers).

Antecedents and predictions

The procedure implemented in Experiment 1 was adapted from the research of Behrend

and Bitterman (1963) and Scobie (1970) with goldfish exposed to electric shocks. Specifically,

shock-shock (S-S) and response-shock (R-S) intervals were translated to flow-flow (F-F) and

response-flow (R-F) intervals, and signalled conditions consisted of CABs being presented

during the last seconds of the R-F interval. The anticipated outcomes of Experiment 1, mostly

based on the findings of these two studies, are presented below.

First, frequency of crossings between compartments was expected to increase above

baseline levels as a result of the introduction of the negative reinforcing contingencies;

correspondingly, a systematic reduction in WFs was predicted to accompany such increments in

number of crossings. These two fundamental outcomes were expected to confirm the negative

reinforcing function of WFs and the generality of free-operant avoidance phenomena.

Second, Scobie (1970) reported that subjects of his experiment were crossing "too early"

during the R-S intervals, which led to a higher than necessary frequency of crossings to avoid all

of the shocks. Alternatively, Behrend and Bitterman (1963) showed that goldfish changed

compartments more frequently during the last part of the R-S interval – i.e., when the warning

stimulus was present. Although Behrend and Bitterman did not report if the minimum rate of

crossings necessary to avoid all the shocks was exceeded during the signalled or unsignalled avoidance conditions, it seems plausible that the rates during the signalled conditions, when compared to the unsignalled conditions, were closer to the minimum rate required to avoid all the shocks. This is due to the fact that consistent early crossings during the R-S intervals require the fish to display higher response rates in order to avoid all the scheduled shocks. In view of these antecedents, it was expected to find higher frequencies of crossings between compartments during signalled, as opposed to unsignalled, conditions. Hyman's (1971) study with monkeys supported this prediction, since he found that removing the warning stimulus from the avoidance schedule increased both response and shock rates, and produced shorter inter-response times.

Third, in the preliminary studies each visit of the subject to a single target compartment represented a programmed exposure to the WFs (contingent or non-contingent to the crossing response – *Preliminary Experiments 3 - 6, Chapter VII*). Conversely, implementing a free-operant avoidance paradigm provided a different setting, in which the subject received WFs regardless of the compartment in which it was located unless the rate of crossings met certain negative reinforcing contingencies. Accordingly, the free-operant procedure allowed the fish to explore and spend time in both compartments – a previously observed natural tendency of bettas – without being necessarily exposed to the WFs. This situation was possible because the deliveries of the WFs were interrupted (escaped) or postponed (avoided) depending on the pace of crossings between compartments. In view of these conditions, and the expectation that bettas would more closely match the minimum rate of crossings necessary to avoid all flows, it was predicted that more uniform patterns of time allocation to the compartments would emerge during signalled, as opposed to unsignalled, conditions. Moreover, it seemed plausible that bias for one of the compartments, evident throughout preliminary experiments (e.g., fish in

preliminary experiment 6 showed a clear preference for the left compartment previous to any experience with the WFs – Figure 10), would not be affected by the avoidance contingencies, since WFs would be delivered only when the subject reached a maximum time of permanence – i.e., subjects could maintain an overall bias for a specific compartment by crossing regularly without having to stay "too long" in the non-preferred side of the tank. This would occur notwithstanding the equal distribution of the negative reinforcers (WFs) across both compartments.

Fourth, traditional aversive control procedures deliver very brief instances of shocks (less than 1 s); therefore, analyzing behaviour that occurs during those intervals, or calculating the amount of time that the subject is exposed to them, has not been of interest. Conversely, the process of adapting the WFs paradigm for avoidance resulted in raising the duration of the aversive stimulus to more than a few seconds; this, due to the fact that preliminary tests to the system indicated that activation of the water pumps for shorter periods was unreliable in producing the intended intensity of water currents inside the experimental tank. This previously unintended modification resulted in important analytic possibilities, not typically available in traditional avoidance research: (a) examining the effects of incorporating an escape contingency in a free-operant avoidance procedure, in which the subject could terminate an ongoing WF by changing compartments during its presentation (escape responses), and (b) determining the frequency and duration of the aversive stimulus (WFs) for each experimental session (i.e., *how often* and for *how long* the subject was exposed to the WFs). Dissociations between these variables (frequency and duration of the WFs) were expected; for instance, one possibility was that the frequency and duration of WFs varied according to how escape and avoidance behaviour developed throughout the sessions. If avoidance behaviour required more sessions to develop

and surpass escape in frequency, only reductions in WF duration would occur during the early

prevalence of escape responses, while reductions in the frequency of WFs would not be observed

until avoidance behaviour became predominant. The possibility to study these potential

dissociations was a notable advantage of the WF paradigm developed for this experiment, since

the traditional preparations utilized in the aversive control field focus only on the effects of brief

presentations of aversive events (primarily shocks), thus the interactions between escape,

avoidance, and the consequences that affect and maintain them are rarely studied.

Finally, considering that previous studies of the spatiotemporal analyses program have

repeatedly shown that different behavioural patterns are developed as a result of implementing

different contingencies (e.g., intricate movement patterns in pigeons exposed to short variable-

interval food schedules – Pear, 1985; highly stereotyped lap-swimming in bettas exposed to

fixed-time schedules of mirror presentations – Martin, 2010), the establishment of different

swimming patterns (e.g., regular trajectories in the tank) under baseline, signalled, and

unsignalled avoidance conditions was expected. The idea that such patterns would emerge was

supported by preliminary analyses and anecdotal observations of session videos of the

preliminary experiments described in Chapter VII. For example, in the punishment conditions of

preliminary Experiments 5 and 6, the fish displayed a swimming pattern inside the non-disturbed

compartment that can be described as a combination of quick approaches to the opening of the

partition, with sudden stops or changes of trajectory just before crossing to the target

compartment (see sample video Hurtado-Parrado, 2012c). The fish showed this pattern several

times, often accompanied by immobility near the entrance, slowly poking its head into the target

compartment, and quick withdrawals. Conversely, visits to the disturbed partition were very

short, and characterized by the fish swimming in the same direction of the WF currents. This

pattern apparently allowed the fish to continue visiting, very briefly, the target compartment, while the amount of time – and possibly effort – spent on each entry was considerably reduced.

Method

Subjects

Six male bettas were obtained from a local pet store. They were selected on the basis of being dark enough to be detected by a video tracking system (VTS), described in the *Apparatus* section of this chapter. These fish were labeled C01, C02, C03, C04, C05, and C06. Each subject was housed in an individual tank (40 cm x 20 cm x 30 cm) and was transported to the experimental tank before each daily session. A 12/12 hr. light/dark cycle (lights on at 8:00 AM) was in effect, and all the experimental sessions occurred between 10:00 AM and 3:00 PM. The experiment was conducted between May and August 2012. Fish were fed in their housing tanks at least 1 hr before the daily session was conducted. The feeding procedure consisted of the experimenter providing unlimited access to food pellets during a 3-min period. A record of the number of pellets ingested daily was maintained.

The only reason for removing fish from the study was for illness. Previous experience indicated that common behavioural symptoms of betta illness were (a) large reduction in amount of food ingested, (b) severe reduction in mobility (indicated by sudden and unexpected changes in the data); and (c) fin clamping. Accordingly, animal caretakers and the experimenter monitored the health and food intake of the fish daily. Fish C01, C02, and C04 developed health issues (infection) at different times of the study and received pharmacological treatment under supervision of the animal caretakers of the Psychology Department at the University of Manitoba. Illness was detected in Fish C01 on day 65 of the study, but it continued to be subjected to tests until day 80 when, in consultation with animal caretakers, it was determined

that its eating and movement patterns were compromised enough to be euthanized. Fish C02 and

C04 showed clear signs of health issues on day 60 and 65 of the study, respectively. These

subjects were euthanized on day 76 and 82 under the same considerations of Fish C01.

All experimental procedures and animal maintenance were approved by the Institutional

Animal Care and Use Committee (IACUC) of the University of Manitoba (Protocol No. F12-

024).

Apparatus

The shuttle tank utilized in the preliminary experiments (Chapter VII) was adapted for

this study. The clear glass test tank (40 cm × 40 cm × 20 cm) was filled to a depth of 12 cm with

dechlorinated water of a temperature between 26°- 27° C, and was emptied, cleaned, and refilled

before each session. The outer surfaces of the test tank were covered with translucent

polystyrene panels to permit diffuse light to enter while reducing any visual effects of objects

outside the aquarium. The tank was located in a room separate from the experimental control

equipment and illuminated by overhead fluorescent lights.

As shown in Figure 11, the test tank was divided by white opaque plastic dividers into

three compartments. Compartment 1 contained four AquaClear 50® water pumps and was

inaccessible to the subject, which was confined to compartments 2 and 3. For brevity's sake, the

term "compartment" will hereafter be a reference to compartment 2 or 3, unless otherwise

indicated. A small opening (3-cm wide) connected these equal-sized compartments. The water

pumps introduced WFs to the compartments and were calibrated to produce a constant flow of

approximately 3600 ml per minute. Water projected by the water pumps was recycled through a

channel consisting of a plastic divider and a wire mesh. Due to a mechanical failure of one of the

four water pumps, only pumps 1 and 4 were active during the initial sessions of the first

experimental condition. Therefore, WFs for those days were delivered using only one pump for each compartment. Immediately after the technical issues were resolved, all four pumps were utilized for the remainder of the first treatment condition. Inspection of the data collected during this condition led to the deliberate use of just pumps 1 and 4 throughout the rest of the study. Although pumps 2 and 3 were not active, they were not removed from the tank in order to block access of the fish to compartment 1.
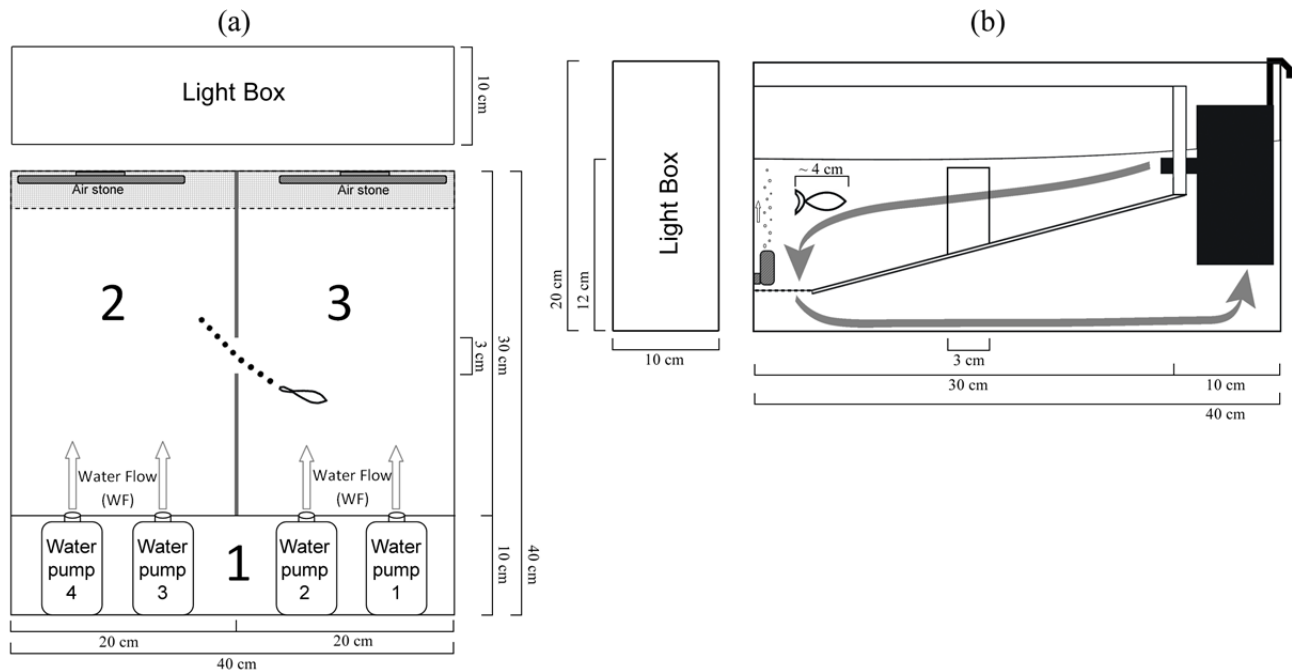
Figure 11. Overhead view (a) and side view (b) of the experimental shuttle tank (40 cm × 40 cm × 20 cm). The tank was divided into 3 different areas by opaque white plastic dividers (a). In compartment 1 (40 cm × 10 cm × 20 cm), four water pumps provided WFs to the equally-sized compartments 2 and 3 (20 cm × 30 cm × 20 cm each). The fish was allowed to swim freely and cross between areas 2 and 3 through a 3 cm wide opening in the divider connecting these compartments. Compartment 1 was inaccessible to the fish. One air stone was attached with suction cups near the lower back walls of compartments 2 and 3. The air stones were connected to a pump located outside the tank in order to produce curtains of air bubbles (CABs – warning stimulus). Water was recycled by a channel created by a plastic divider and a wire mesh through which pumps forced the water (b). A light box with two 15-watt bulbs and located 5 cm away from the tank was constantly on and, in combination with overhead fluorescent lights, evenly illuminated the inside of the tank.

Two "air stones", which could be electronically controlled to deliver CABs, were attached by suction cups to the wall opposite to compartment 1. Air was pushed, by means of tubing, from an air pump outside the tank to these air stones, from where it produced CABs. These CABs served as the warning stimulus (WS – i.e., discriminative stimulus).

A custom-made 3D video tracking system (VTS) based on that used in a number of studies by J. J. Pear and colleagues (e.g., Pear, 1985; Pear, 2004; Pear & Eldridge, 1984; Pear, et al., 1989) was utilized for recording behavioural measures. Like the earlier VTS, the system operated on the basis of brightness contrast, with the difference that rather than being oriented perpendicularly to each other, cameras were positioned side-by-side, 80 cm apart, and attached to a scaffold over the shuttle tank. Both cameras scanned 10 times per second from the outer wall of compartment 3 to the outer wall of compartment 2, and recorded the position of the central pixel as soon as a dark region (3 pixels in length) was encountered. Since the fish was the darkest object visible to the cameras, all other regions being white, this video output was processed to plot a single point location in 3 spatial coordinates every $1/10^{th}$ of a second. This output was then run through another computer containing additional custom-made software ("Operant Behaviour Laboratory", or OBL; programmed by Wayne S. Chan) that recorded the following behavioural measures: (a) number of crossings between compartments; (b) time allocation to each compartment of the tank, (c) amount of time that the subject was exposed to the WFs in each compartment, (d) duration of each visit to a compartment; and (e) trajectories of the fish. The OBL software also controlled the timing of stimulus events – specifically, WFs and CABs – through relays connected to the water pumps and the air pump.

A light box with two 15-watt bulbs was constantly on, and located 5 cm away from the experimental tank, nearest the wall opposite to Compartment 1. This box illuminated the tank's

interior evenly, with the aim of improving the tracking by reducing the occurrence of spurious

dark areas (see Figure 11).

Parameters. The free-operant avoidance procedures reported by Behrend and Bitterman

(1963) and Scobie (1970) were adapted for the present study. Due to the fact that the deliveries

of shocks were replaced with deliveries of WFs, the standard terminology of *response-shock* (*R-S*) and *shock-shock* (*S-S*) intervals was replaced by *response-flow* (*R-F*) and *flow-flow* (*F-F*)

intervals. Additional to these parameters, the duration of the aversive stimulus was also adapted

for the present study. While the traditional free-operant avoidance procedures that utilize electric

shock tend to use deliveries that last less than 1 s, the characteristics of the WFs in this study

required scheduling flow presentations of longer duration. This was due to the intrinsic delay (of

few seconds) between the activation of the water pumps and the moment when the WFs reached

their maximum strength, as well as a similar delay for WFs to dissipate when the water pumps

were turned off. In addition, unlike the case of electric shock, it was felt that WFs require a non-

zero amount of time to be aversive. Since intermittent and contingent 10 s deliveries of WFs

were effective in producing sizeable changes in betta behaviour in the preliminary experiments

(see Chapter VII), the same duration of the WFs was utilized in the present study.

Finally, since the procedure of the present study was never used with bettas before, the

value of the R-F was derived from research with goldfish in which different configurations of the

R-S and S-S intervals produced consistent signalled and unsignalled free-operant avoidance

(Pinckney, 1968; Scobie, 1970). Scobie reported that equal values of S-S and R-S between 30

and 60 s produced consistent unsignalled avoidance in goldfish. Alternatively, Pinckney (1968)

found the highest level of non-discriminated shuttle responding with S-S = 5 s and R-S = 15 – 20

s, and the greatest reduction of shocks with S-S = 5 s and R-S = 40. However, Behrend and

Bitterman (1963) found high rates of shuttle responding under signalled and unsignalled avoidance when S-S and R-S = 20 s. By extrapolating these heterogeneous data from the experiments with goldfish, and considering the particularities of WF build-up and dissipation (e.g., delay for WFs to dissipate after the water pumps were turned off), the parameters that seemed most appropriate for the production of stable free-operant avoidance in bettas were R-F = 30 s and F-F = 30 s.

Overview of the experimental conditions. Unlike the design of Behrend and Bitterman's (1963) and Scobie's (1970) studies, a baseline condition was scheduled at the beginning of Experiment 1 (see *Procedure* below). In doing so, Steiner's (1971) contention regarding the relevance of information about the operant level of crossings in shuttle-based avoidance procedures was taken into account (e.g., operant levels of crossings are relevant if the species that are utilized are known to be sensitive to any of the general aversive properties of the experimental setting).

Apart from baseline conditions, two treatment conditions were scheduled: (a) unsignalled free-operant avoidance (i.e., response-dependent deliveries of WFs without a warning signal), and (b) signalled free-operant avoidance (response-dependent deliveries of WFs with a warning signal). The six subjects utilized in this experiment were exposed to both treatment conditions, differentiated only by the sequence of these conditions (see *Design* below).

Procedure

Daily baseline and treatment sessions were 20-min long (including WF periods), and tested only one subject at a time. Before each daily session, the subject was removed from its housing tank using a water container and was introduced to one of the compartments of the shuttle tank (assigned randomly). A piece of white plastic was used to temporary obstruct the

opening between Compartments 2 and 3 of the shuttle tank, in order to keep the fish in the

assigned compartment until the start of the session. The session began three minutes after the

subject was put in the shuttle tank, under the assumption that the transportation may have

produced some amount of stress (although fish were never out of water). At the end of the 3-min

rest period, the barrier obstructing the opening was removed and the VTS system started tracking

the fish. Room lights were controlled automatically by the VTS system – i.e., they were turned

on at the start of the session when tracking began and turned off immediately when the session

time ended. As a result, room lights remained off during the entire extra session time.

Immediately after the session ended, the fish was removed from the experimental tank

using the same water container and returned to its housing tank. In addition to the data saved

automatically by the OBL at the end of the session, general observations were recorded on a log,

and basic data were entered into an EXCEL spreadsheet (e.g., number of crossings, time

allocation to compartments, number of WFs delivered and frequency of different crossing types),

which allowed for the plotting of daily performance for each subject, and informed decisions for

the next session (e.g., change of conditions when stability criteria were reached – see *Stability*

criteria below). Subsequently, the experimental tank and VTS system were prepared for the

session of the next subject (i.e., test tank was emptied, cleaned, refilled, and the OBL software

was programmed with new parameters).

Specific procedural details of each condition of the experiment were:

Baseline. WFs were not delivered during either of the 20-min baseline sessions. The

number of crossings between compartments, the duration of each visit to each compartment, and

the preference ratio (PR - the difference between the amount of time the fish spent on the right

compartment of the tank and the time that the fish spent on the left compartment over the total session time) were recorded for each baseline session.

Unsignalled avoidance (UA). WFs were delivered every 30 s (F-F = 30 s) in the compartment where the fish was located, unless the fish crossed to the opposite compartment of the tank (i.e., response-dependent deliveries of WFs). With each crossing the timer was reset to 30 s (i.e., R-F interval = 30 s); if WFs were running at that moment they were immediately stopped.

Additional to the measures described for baseline conditions, three more were recorded on each UA session: (a) number and percentage of WFs delivered (percentages were calculated assuming that a total absence of crossings would have resulted in the delivery of 30 WFs – i.e., 100%); (b) duration and percentage of exposure to WFs (percentages were calculated assuming that a total absence of crossings would have resulted in a total exposure to WFs of 300 s – i.e., 100%); and (c) frequencies of different types of crossings, as described below.

Crossing during a WF, categorized as escape (Esc), interrupted the WF and initiated an R-F interval. Crossings that occurred during R-F or F-F intervals were classified as avoidance, and likewise initiated an R-F interval. F-F intervals occurred only following a full WF event without any incidence of response.

Crossings in the present study were further differentiated in order to obtain more detailed information about the temporal distribution of the avoidance responses, especially considering the distinction that Behrend and Bitterman (1963) used regarding "early" and "late avoidance". Specifically, a crossing was recorded as: early avoidance (EA) when it occurred during the first 25 s of the R-F interval (i.e., fish changed compartment within 25 s of the last response); late avoidance (LA) when it occurred during the last 5 s of the R-F interval (i.e., fish changed

compartment 25 - 30 s after the last response); and Flow-Flow avoidance (FFA) when it occurred

anytime during the F-F interval (i.e., fish changed compartment within 30 s of the last full

incidence of a WF without response). Due to software limitations, it was not possible to

distinguish between early and late Flow-Flow avoidance.

    Signalled avoidance (SA). The parameters and measures scheduled for the UA condition

were the same as those for the signalled avoidance (SA) condition, with the addition of a

warning stimulus presented during the last 5 s of each R-F interval. This stimulus consisted of

presentations of curtains of air bubbles (CABs) that lasted for the remaining 5 s of the R-F

interval, or until the fish changed compartments.

Design

    A single-case design was implemented (Johnston & Pennypacker, 1993, 2009; Perone &

Hursh, 2013). Table 1 shows the sequence in which each subject was exposed to the different

conditions of the experiment, which changed whenever a stable pattern of crossings was

observed (see stability criteria in *Data analysis*). During the first treatment condition of subjects

C02, C03, C04, and C05 (marked with a "*"), WFs were produced by two water pumps per

compartment (two-pump configuration), as originally planned. However, after considering the

poor performance of these fish during this initial condition, it was decided to use a single water

pump per compartment (one-pump configuration) for the rest of the study. Fish C01, C02, and

C04 were removed from the study, due to infection, before completing all the programmed

conditions.

Table 1.

Sequence of conditions for each subject during Experiment 1

Fish	Conditions									
C01	BL-1	UA-1	BL-2	UA-2	BL-3	SA-1	BL-4	SA-2		
C02	BL-1	UA-1*	BL-2	UA-2	BL-3	UA-3	BL-4	SA-1	BL-5	
C03	BL-1	UA-1*	BL-2	UA-2	BL-3	UA-3	BL-4	SA-1	BL-5	SA-2
C04	BL-1	SA-1*	BL-2	SA-2	BL-3	SA-1	BL-4	UA-1	BL-5	
C05	BL-1	SA-1*	BL-2	SA-2	BL-3	SA-1	BL-4	UA-1	BL-5	UA-2
C06	BL-1	SA-1	BL-2	SA-2	BL-3	SA-3	BL-4	UA-1	BL-5	UA-2

Note. BL = Baseline; UA = Unsignalled Avoidance; SA = Signalled Avoidance; * = condition with two functional water pumps per compartment (two-pump configuration).

Video recording and manual scoring of responses

Although the VTS used for this study provided a low-resolution digital video of every session (the output of the right camera of the VTS was captured and saved by the software *VirtualDub 1.9.11*), informal observations throughout the study made it apparent that better quality videos were needed. Specifically, casual observations during the first half of Experiment 1 indicated that fish were systematically displaying certain responses (e.g., air gulping, approaching the air stones, and swimming by the door) during the sessions, but it was impossible to score and analyze such behaviours using the VTS video feed due to its low resolution and angle of the cameras. In view of these observations, together with the unexpected low incidence of LA crossings (i.e., the rates of changing compartments during the CABs presentations was low, as predicted from previous research), it was decided to use manual scoring to explore the behaviour of the fish during CABs and non-CABs periods.

A high-definition digital camera, placed on a tripod behind compartment 1 (see Figure

12) was used to record every session starting on day 45 of the study. The sessions chosen for

scoring were those corresponding to the steady-state of the last SA condition and its preceding

baseline; that is, the last three sessions of the last SA condition and the last three sessions of the

previous baseline for fish C03, C05, and C06. The data of these subjects and the specific sessions

were selected on an availability basis − high-definition videos were not available before day 45,

and the remaining fish did not complete all the programmed conditions.

Figure 12. Arrangement and position of the high-definition digital camera (behind compartment
1 of the experimental tank).

The high definition videos allowed the experimenter and a research assistant to identify

those responses which consistently occurred within and across sessions (see Table 2). Each

selected video was scanned in 20-s blocks, noting the frequency of target responses on a

spreadsheet designed specifically for the purposes of this analysis (see Appendix). The following

responses were scored during CABs intervals (abbreviations and a detailed definition of each

response appear on Table 2): (a) fish displayed an incomplete crossing; (b) fish turned around

when it was in front, under, or above the air stones; (c) fish swam or stayed under/above the air

stones; (d) fish swam towards the door; (e) fish approached CABs; (f) fish swam away from

CABs (but not towards the door); (g) air gulps; and (h) fish aligned itself or swam towards the

nozzles of the water pumps.

Alternatively, the following responses were recorded during the non-CABs periods of the

signalled and baseline sessions: (a) air gulps during WFs; (b) air gulps when WFs were not

occurring; (c) fish swam or stayed under/above the air stones; (d) fish approached the door

without crossing; (e) fish approached or poked the nozzles of the water pumps; (f) fish remained

near the door for more than 5 sec; (g) fish crossed part-way (i.e., incomplete crossing in which at

least a third of the subject's body was in the opposite/destination compartment); (h) lap-

swimming by the walls of the experimental tank.

Table 2.

Abbreviations and definitions of each response scored manually through observation of high-

definition videos.

Responses during warning stimulus – CABs	Responses during non-CABs periods
IC = incomplete crossing (fish did not cross entirely to the opposite compartment by the time CABs stopped and a WF began). **ASA** = fish swam in the air stones area – i.e., under, besides or above them. **AG** = air gulps during CABs. **STD** = fish swam towards the door. **TA** = fish turned around while in front, under, or above the air stones (clockwise or counter clockwise). **AppCABs** = fish approached CABs (i.e., fish began several cm away from the air stones and swam towards them when CABs were presented). **SAW** = fish began near the air stones and swam away when CABs started (but not	**IC** = incomplete or part-way crossing (fish did not entirely cross to the opposite compartment. At least a third of its body was in the destination compartment). **ASA** = fish swam in the air stones area – i.e., under, besides or above them. **AG** = air gulps when WFs were not occurring. **SBTD** = Fish remained beside the door for 5 sec or more (fish usually looked like it was "waiting" to cross). **AppD** = fish approached the door without crossing. **AppAS =** fish approached air stones. **AGDWF** = air gulps during WF. **AppN**: fish approached and/or poked nozzles. **Lap-Swimming (LS)** = fish moved between two

towards the door). **OWF** = fish oriented towards WFs (fish rotated so it faced the nozzles of water pumps and/or swam towards water pumps).	of the tank's outer corners, within the same compartment, while swimming along one of the tank's walls.

Data analysis

   Steady-state approach, stability criteria, and visual inspection of data. The present study

used the steady-state approach that has evolved from the work of B.F. Skinner (Skinner, 1956),

was described by Sidman (1960), and has been detailed and extended by Johnston and

Pennypacker (1993a, 2009). An important aspect of this analytic approach is the use of *stability*

criteria. Among the numerous such criteria that have been developed and implemented in

experiments with different species (e.g., rats, fish, and humans) and procedures (e.g., positive,

punishing, and negative reinforcement contingencies – Martin, 2010; Sales, 2014; Critchfield,

Paletz, MacAleese, & Newland, 2003; Green & Estle, 2003; Magoon & Critchfield, 2008), the

stability criteria utilized by Sales (2014) was considered to be the most suitable for the present

study. Two reasons justified this decision: first, the guidelines developed by Sales have been

successfully implemented in our lab with the same species (bettas), and using similar techniques

(VTS for fish – adapted from Pear et al., 1989); and second, these criteria closely resembled the

guidelines that have been used in the other representative operant studies with positive,

punishing, and negative reinforcing contingencies (Critchfield et al., 2003; Green & Estle, 2003;

Magoon & Critchfield, 2008). Accordingly, the specific criteria for the present study were as

follows.

   Any condition (baseline or treatment) was terminated when no consistent trend was

noticeable (no increase or decrease in responding for three consecutive data points, confirmed

through visual inspection of the graphed data) and either: (a) the total number of crossings

between compartments for a session fell within the average of the last 5 sessions ±5 responses, or

(b) 20 consecutive sessions were run in a condition and there was no consistent trend when this 20 session cut off was achieved.

Visual inspection strategy. Historically, one of the distinctive features of the behaviour analytic field is that researchers have been very successful in detecting functional relationships between dependent and independent variables by visually attending to the data of single individuals (e.g., analyzing level differences, upward and downward trends, and magnitude of the variability under both baseline and treatment conditions – Johnston & Pennypacker, 1993a, 2009). An example of a fruitful implementation of this strategy is the analysis of the characteristic effects produced by different schedules of reinforcement, which has been traditionally done by visual inspection of response patterns in cumulative records (e.g., the "scalloping" effect produced by fixed-interval schedules is identified in the cumulative records as a gradual increase in the rate of responding up to the moment of reinforcement – Fester & Skinner, 1957; Lattal, 2012; Pierce & Cheney, 2013).

As part of the steady-state approach, the results of the present study were analyzed by visual inspection of patterns in graphed data. For instance, graphical representations of response frequencies were created and interpreted using the stability criteria previously described. Moreover, additional analyses resembled the developments of the research program on spatiotemporal patterns of behaviour (e.g., Pear, 1985). For example, XY ("overview") plots that represented the movements of fish throughout the entire experimental session, or during specific periods, were created and analyzed.

Results

Overall number of crossings per session for each fish

Figure 13a and 13b show the total number of crossings made by each fish during each session of baseline (BL), SA, and UA conditions. Specifically, each horizontally arranged graph shows a given subject's crossings throughout daily sessions of BL and treatment conditions (UA and SA), with each condition being represented in a separate vertically arranged panel. As explained in the section in this chapter titled *Method − Design*, C01, C02, and C03 differed from C04, C05, and C06 regarding the sequence in which they were exposed to SA and UA conditions: the former were exposed to UA first, while the latter were exposed to SA first.
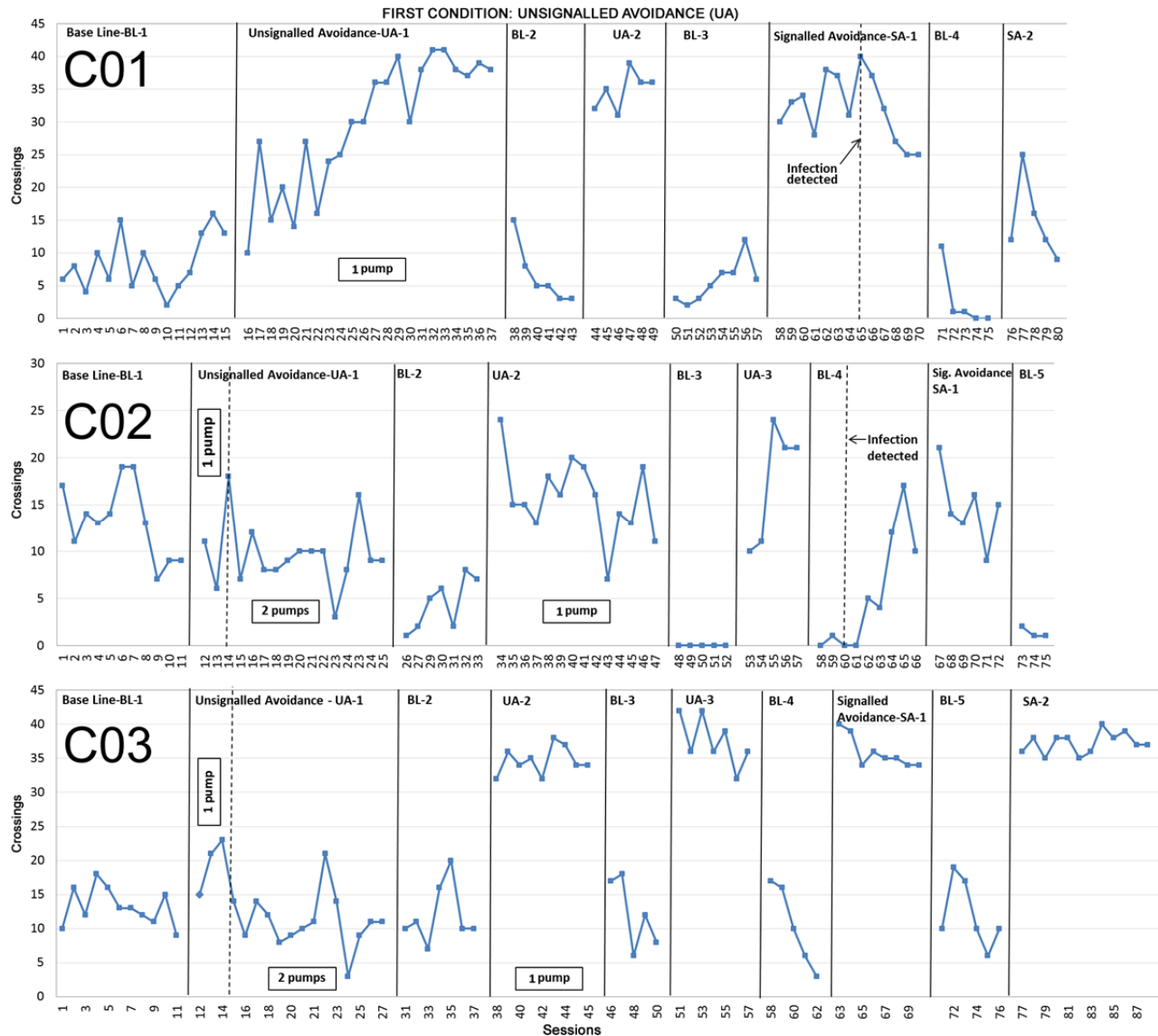
Figure 13a. Number of crossings of C01, C02, and C03 during baseline and treatment conditions of Experiment 1 (UA and SA). Each horizontally arranged graph shows the indicated subject's crossings throughout daily sessions, each condition being represented in a separate vertically arranged panel (e.g., the top graph shows all of C01's data, with the second panel of this graph representing its first treatment condition; UA-1). Technical difficulties caused only two water pumps to be active during the first sessions of the initial treatment condition (one pump for each compartment i.e., compartments 2 and 3, see Method section, Apparatus, of this chapter for details). The dashed line in the first treatment condition of C02 and C03 indicates the day on which all four water pumps were first functional (day 15). As of the second treatment condition and throughout the rest of the experiment, only one water pump remained active for each compartment. C01 was always exposed to a single functional water pump per compartment. Early signs of infection were first observed in fish C02 on day 35. These were clearly detected in C01 and C02 on days 59 and 60, respectively, as indicated by corresponding dashed lines. BL = Baseline; SA = signalled avoidance; UA = unsignalled avoidance.
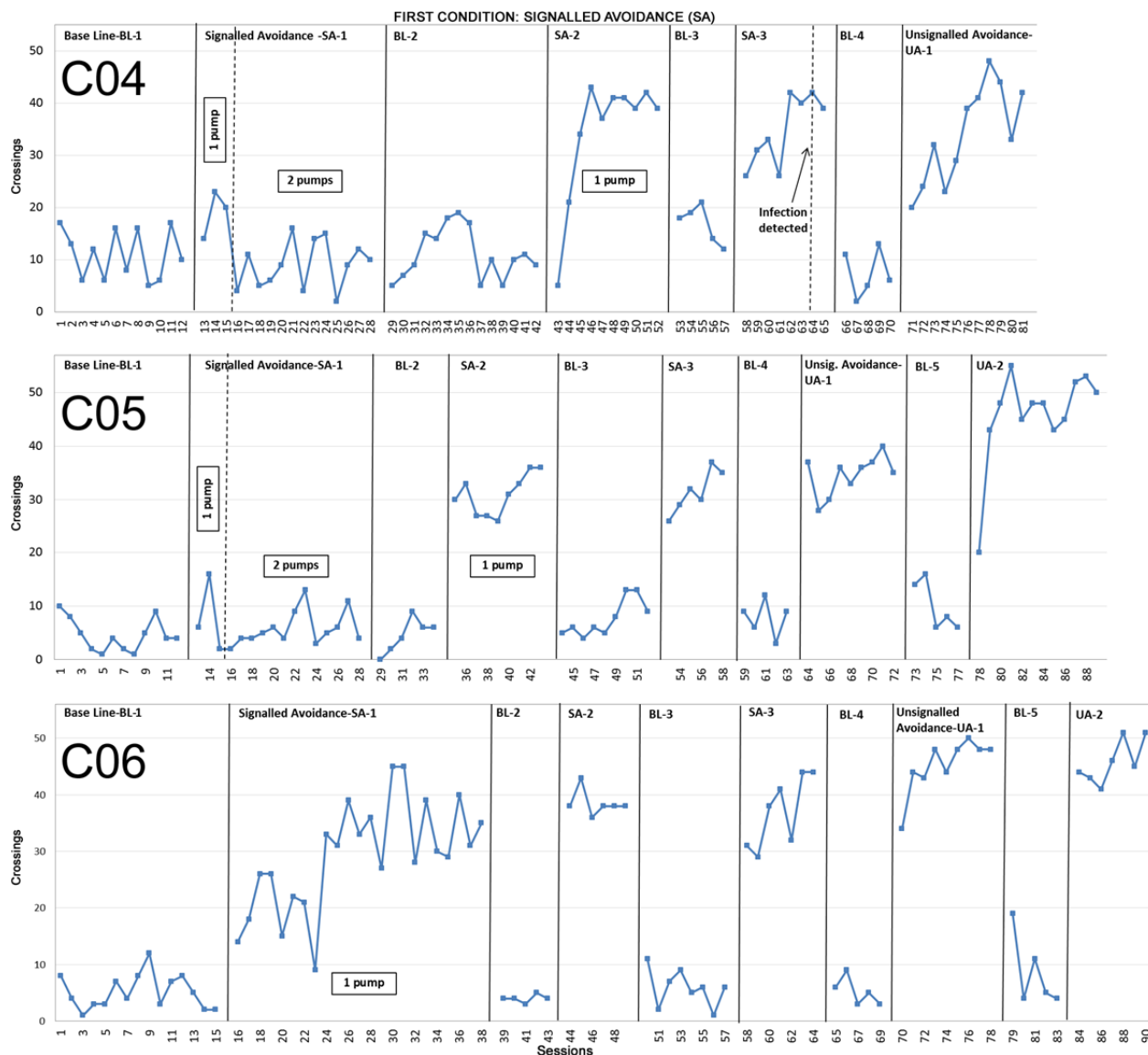
Figure 13b. Number of crossings of C04, C05, and C06 during baseline and treatment conditions of Experiment 1 (SA and UA). Each horizontally arranged graph shows the indicated subject's crossings throughout daily sessions, each condition being represented in a separate vertically arranged panel (e.g., the bottom graph shows all of C06's data, with the second panel of this graph representing its first treatment condition; SA-1). Technical difficulties caused only two water pumps to be active during the first sessions of the initial treatment condition (one pump for each compartment i.e., compartments 2 and 3, see Method section, Apparatus, of this chapter for details). The dashed line in the first treatment condition of C04 and C05 indicates the day on in which all four water pumps were first functional (day 15). As of the second treatment condition and throughout the rest of the experiment, only one water pump remained active for each compartment. C06 was always exposed to a single functional water pump per compartment. Infection was detected in C04 on day 63, as indicated by the corresponding dashed line. BL = Baseline; SA = signalled avoidance; UA = unsignalled avoidance.

As can be seen from Figure 13a and 13b, all fish showed a low but consistent level of

crossings prior to any exposure to WFs and CABs (i.e., during BL-1), to which they returned

throughout subsequent interspersed baseline conditions. Although the intention was for WFs to

be delivered into each compartment using two water pumps, a malfunction made it necessary to

begin the first treatment condition using a single water pump for each compartment (see *Method*

−Apparatus, in this chapter). Accordingly, fish C02, C03, C04, and C05 were initially exposed

to three sessions in which only one pump was running for each compartment (one-pump

configuration) before both pumps began to be functional (two-pump configuration). Fish C03,

C04, and C05 showed an increment in the number of crossings above baseline levels during

these first three sessions of the condition in which the one-pump configuration was active. C02's

crossings also showed an increase, although not as clearly, during this same period.

Subsequently, with the introduction of the two-pump configuration (first dashed line in

graphs C02, C03, C04, and C05 of Figure 13a and 13b), C02, C03, C04, and C05 showed a

decrease in crossings − in some cases, reaching frequencies below the previous baseline − which

did not increase again throughout the rest of the condition. The fact that the number of crossings

of fish C02, C03, C04, and C05 fell as soon the two-pump configuration became functional

suggested that having a single active pump for each compartment produced more crossings.

Moreover, session videos indicated that these fish struggled at escaping the WFs produced by the

two-pump configuration, as opposed to escaping the flow produced by only one pump. In view

of these data, and since C01 and C06 had not yet begun the first treatment, it was decided to test

if the one-pump configuration produced more frequent and consistent levels of responding.

Following this initially unplanned manipulation, C01 and C06 showed a consistent ascending

trend in the number of crossings, reaching from two to three times the original BL-1 frequencies.

This was consistent with the performance of fish C02, C03, C04, and C05, which showed an

increase during the one-pump configuration of their first condition.

With the introduction of BL-2 (Figure 13a and 13b - third panel for all fish), the number

of crossings of C01 and C06 rapidly returned to baseline levels, whereas the crossings for the

rest of the subjects (C02, C03, C04, and C05) remained near the low levels observed throughout

their first treatment condition. It was thus decided to test if the increasing trend observed in the

crossings of C01 and C06 with the one-pump configuration could be reinstated in all fish during

a second treatment condition. The fourth panel of each fish in Figure 13a and 13b corresponds to

this condition, in which the high levels of crossings observed in C01 and C06 were not only

replicated with these same fish, but also across the rest of the subjects (C02, C03, C04, and C05).

Moreover, the crossing frequencies of fish C03, C04, and C05 were clearly higher during this

second treatment when compared to all previous conditions (BL-1, BL-2, and the first treatment).

Although C02 replicated the trend previously described for the other fish in that it showed a

higher number of crossings during the second treatment condition (UA-2) than BL-2 (Figure

13a), this subject was anomalous in that its crossing frequency was only slightly higher than that

observed during the first treatment condition (when the two-pump configuration was in place),

and very similar to the one observed during BL-1 (previous to any exposure to WFs). Notably, it

was precisely in the early sessions of UA-2 that C02 first showed some signs of stress and/or

infection such as fin clamping (second session – day 35).

Due to the suppressive effect on crossings produced by the use of two pumps (as opposed

to one for each compartment), only one pump in each compartment remained active following

condition BL-3. During the rest of the experiment, every treatment condition thus produced

crossings that remained well above baseline levels, reaching frequencies as high as three or four times those of the interspersed baselines.

When comparing UA and SA conditions, it is important to note that the frequency of crossing during UA sometimes reached higher levels than during SA conditions. Although this was observed at some point across the data of all fish, it was more marked and consistent for fish C05 (UA-2) and C06 (UA-1 and UA-2). However, as explained in the *Method* section of this chapter, the data of C01, C02, and C04 are limited in this regard, since these subjects did not complete all the programmed conditions of the experiment due to illness.

Total WF frequency and duration

Figure 14a and 14b show the frequency and duration of WFs expressed in percentages during baseline and treatment conditions (UA and SA), which allows for quantifying both measures as proportional and easily comparable ratios. Similar to Figure 13a and 13b, each horizontally arranged graph in Figure 14a and 14b shows a given subject's data, with conditions being represented in separate vertically arranged panels. Percentages were calculated given that a total absence of crossings during an entire 20-min session would have resulted in the fish being exposed to 30 instances of WFs and a total of 300 s of flow time. For instance, if a fish in a given session received 15 instances of WFs and was exposed to a total of 200 s of flow time, these values would correspond to 50% of WFs (15/30 x 100 = 50%) and 66.7% of total flow time (200/300 x 100 = 66.7%). The horizontal dashed line indicates 100% of both WF frequency (30 instances) and duration (300 s), and allows for the unified comparison of both measures. Note that frequency and duration of WFs may rise above 100% depending on the subject's temporal distribution of crossings.
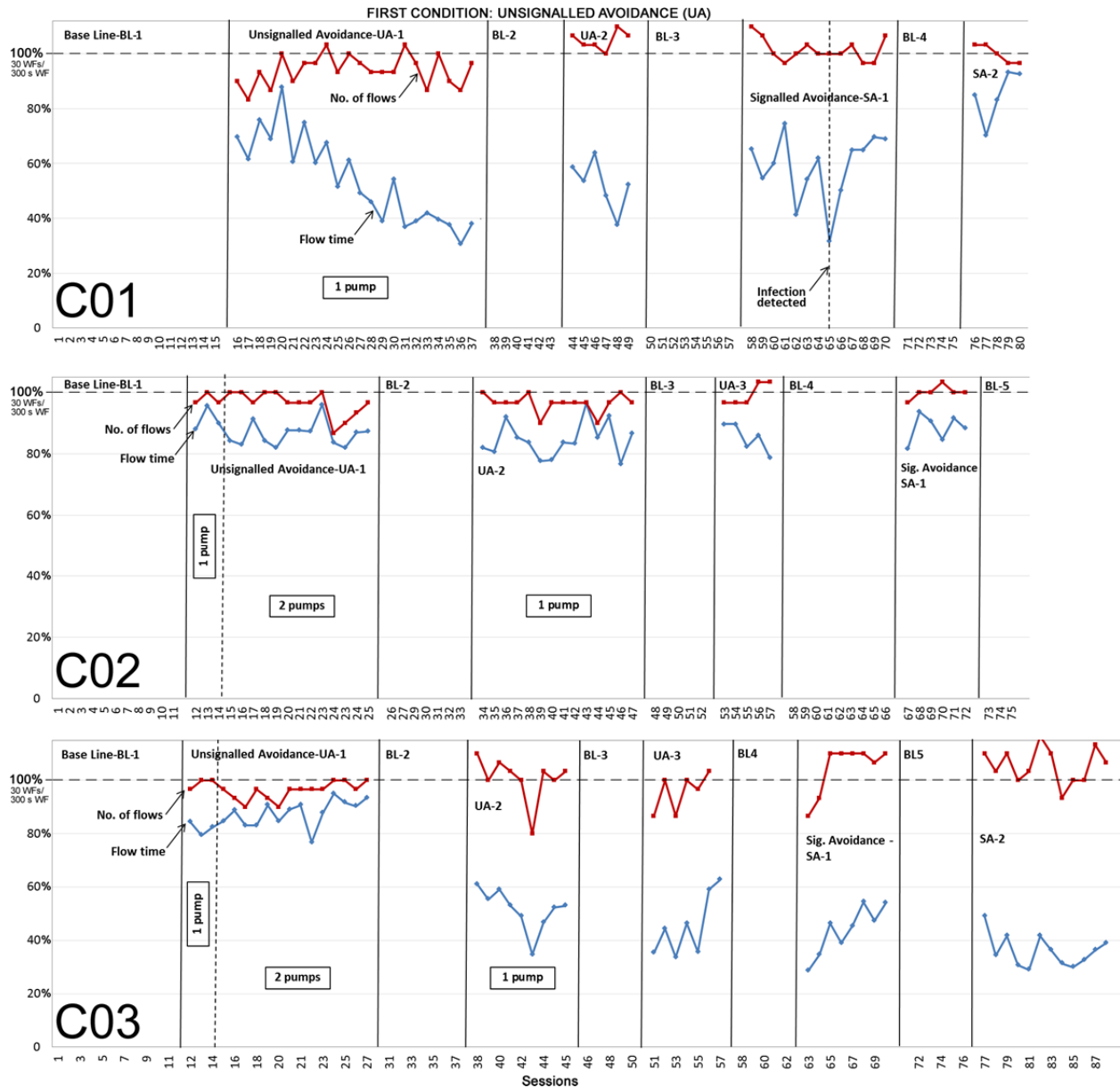
Figure 14a. Frequency and duration of WFs, expressed in percentages, during UA and SA conditions of Experiment 1 for fish C01, C02, and C03. There are no data points during baselines because WFs were not delivered during these conditions. Each horizontally arranged graph shows the indicated subject's data throughout daily sessions, and each one of the conditions is represented in a separate vertically arranged panel. For instance, the top graph shows C01's data, and the second panel of this graph represents its first treatment condition (UA-1). Percentages apply to both WFs and flow time, where 100% refers to the total number of WFs and flow time given a total absence of crossings (30 WFs and 300 s of flow time), indicated by a horizontal dashed line. It was thus possible for this percentage to exceed 100%, based on the subject's crossing patterns. For instance, fish C03 was exposed to 32 instances of WFs (out of 30 originally planned) and 117 s of flow time (out of 300 s initially scheduled) during the last session (day 88), representing 107% and 39% of total number of flows and flow time scheduled, respectively. SA = signalled avoidance; UA = unsignalled avoidance. Details about the number of water pumps active during the first treatment condition and the infection of fish C01 and C02 are the same as for Figure 13a.
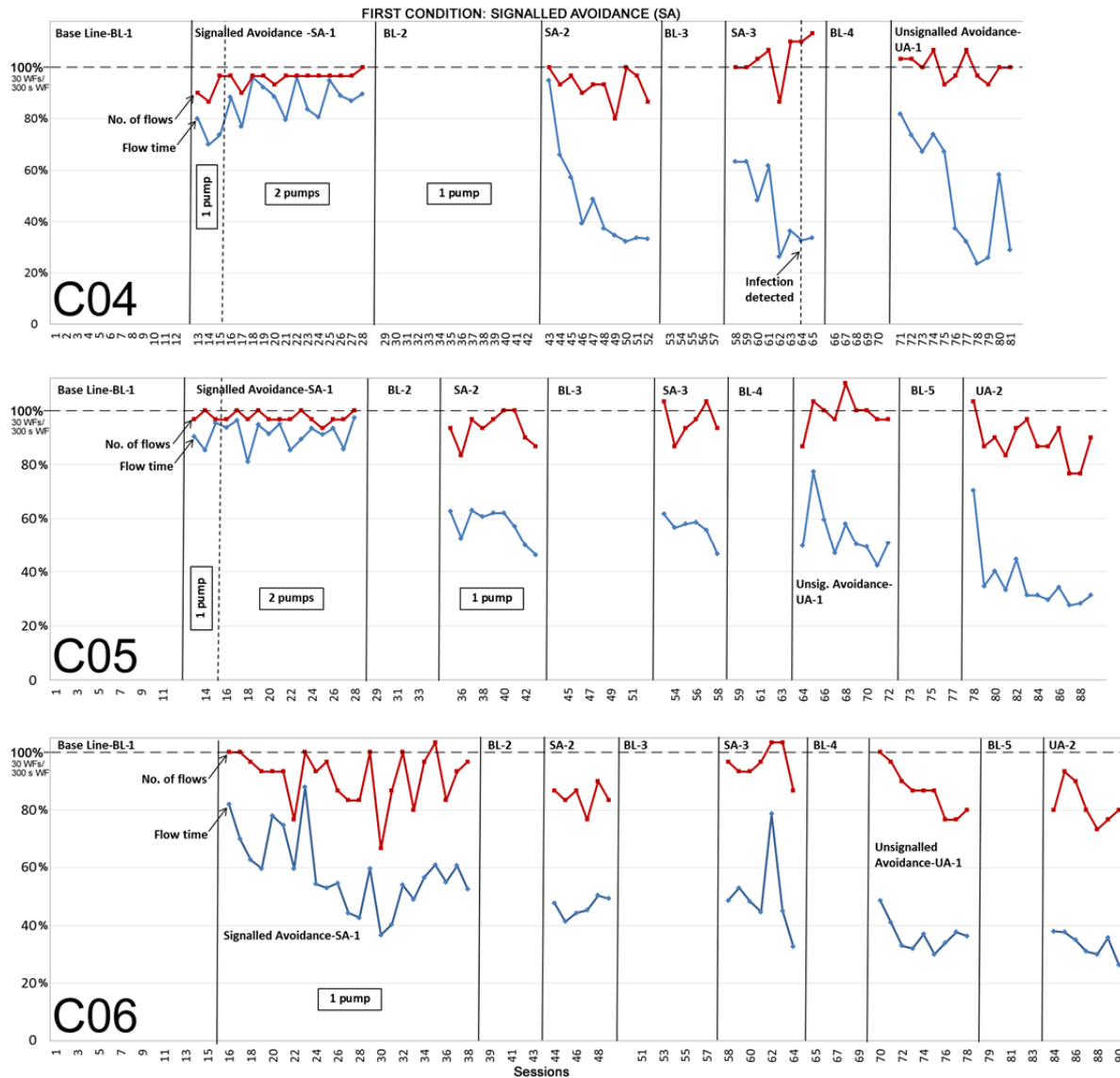
Figure 14b. Frequency and duration of WFs, expressed in percentages, during UA and SA conditions of Experiment 1 for fish C04, C05, and C06. There are no data points during baselines because WFs were not delivered during these conditions. Each horizontally arranged graph shows the indicated subject's data throughout daily sessions, and each one of the conditions is represented in a separate vertically arranged panel. For instance, the bottom graph shows C06's data, and the second panel of this graph represents its first treatment condition (SA-1). Percentages apply to both WFs and flow time, where 100% refers to the total number of WFs and flow time given a total absence of crossings (30 WFs and 300 s of flow time), indicated by a horizontal dashed line. It was thus possible for this percentage to exceed 100%, based on the subject's crossing patterns. For instance, fish C06 was exposed to 24 instances of WFs (out of 30 originally planned) and 79 s of flow time (out of 300 s initially scheduled) during the last session (day 90), representing 80% and 26% of total number of flows and flow time scheduled, respectively. SA = signalled avoidance; UA = unsignalled avoidance. Details about the number of water pumps active during the first treatment condition and the infection of fish C04 are the same as for Figure 13b.

As can be seen from Figure 14a and 14b, percentages of WFs per session rarely approximated or went below 80% throughout the experiment; this was specifically the case of C03 C05, and C06, which only seldom reduced the number of WFs per session to 24 instances out of the originally scheduled 30 (80%). In contrast, WFs percentages reached values that were equal or above 100% (i.e., fish received more than the 30 WFs originally scheduled) in several cases.

Unlike the small or nonexistent reduction in the frequency of WFs observed across all fish, the total duration of flows per session saw large reductions below 100% in five of the six subjects, with the exception being C02. More specifically, overall duration of WF exposure was consistently reduced to levels below 35% (~ 100 s out of the originally scheduled 300 s), and sometimes fell below 25%.

A systematic decline in WF time was observed in fish C01 and C06 during their first treatment condition, coinciding with the one-pump configuration (Figure 14a and 14b, respectively). In contrast, such a pattern was not observed in the analogous condition of fish C02, C03, C04 and C05, during which the two-pump configuration was active. It was only with the introduction of the second treatment condition for these fish, during which the one-pump configuration became active, that WF frequencies and durations replicated the pattern observed in the first treatment condition of C01 and C06 – i.e., slight changes in the incidence of WFs, mostly decrements, were accompanied by a substantial progressive reduction in the percentages of total flow duration.

With the exception of fish C04, overall frequencies of WFs reached lower values during UA conditions than during SA conditions. However, this tendency was only consistent throughout a subject's two UA conditions in the case of C06, both containing frequencies lower

than those observed in SA conditions. Flow time percentages similarly reached lower values

during UA conditions as opposed to SA conditions, although the contrast was not always as

substantial and the effect was not replicated in fish C02 and C03

Frequency of different types of crossings: escape, early avoidance, late avoidance, and flow-flow

avoidance

Figure 15a and 15b show the frequency of Escape (Esc), early avoidance (EA), late

avoidance (LA), and flow-flow avoidance (FFA) displayed by all the subjects during treatment

conditions (UA and SA). Each horizontally arranged graph of Figure 15a and 15b shows a given

subject's data, with conditions being represented by separate vertically arranged panels. For

instance, the top graph in Figure 15a shows C01's data, and the second panel of this graph, from

left to right, represents its first treatment condition (UA-1).
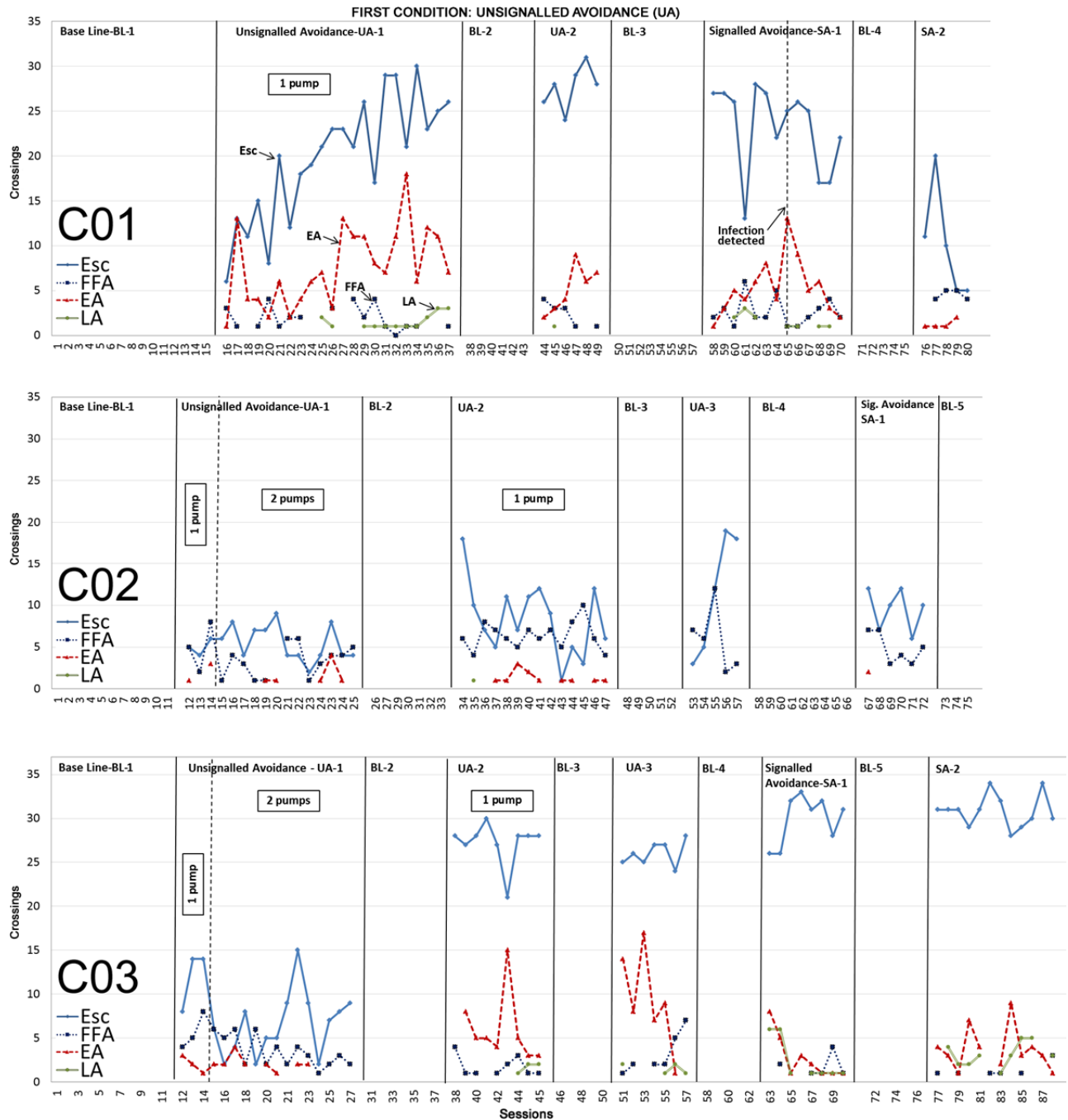
Figure 15a. Frequency of different types of crossings during UA and SA conditions of Experiment 1 for fish C01, C02, and C03. Each horizontally arranged graph shows the indicated subject's data throughout daily sessions, with each condition is represented by a separate vertically arranged panel. For instance, the top graph shows C01's data, and the second panel of this graph, from left to right, represents its first treatment condition (UA-1). Esc = escape; EA = early avoidance; LA = late avoidance; FFA = F-F avoidance (crossing during flow-flow interval); SA = signalled avoidance; UA = unsignalled avoidance. Details about the number of water pumps active during the first treatment condition and the infections of fish C01 and C02 are the same as for Figure 13a.
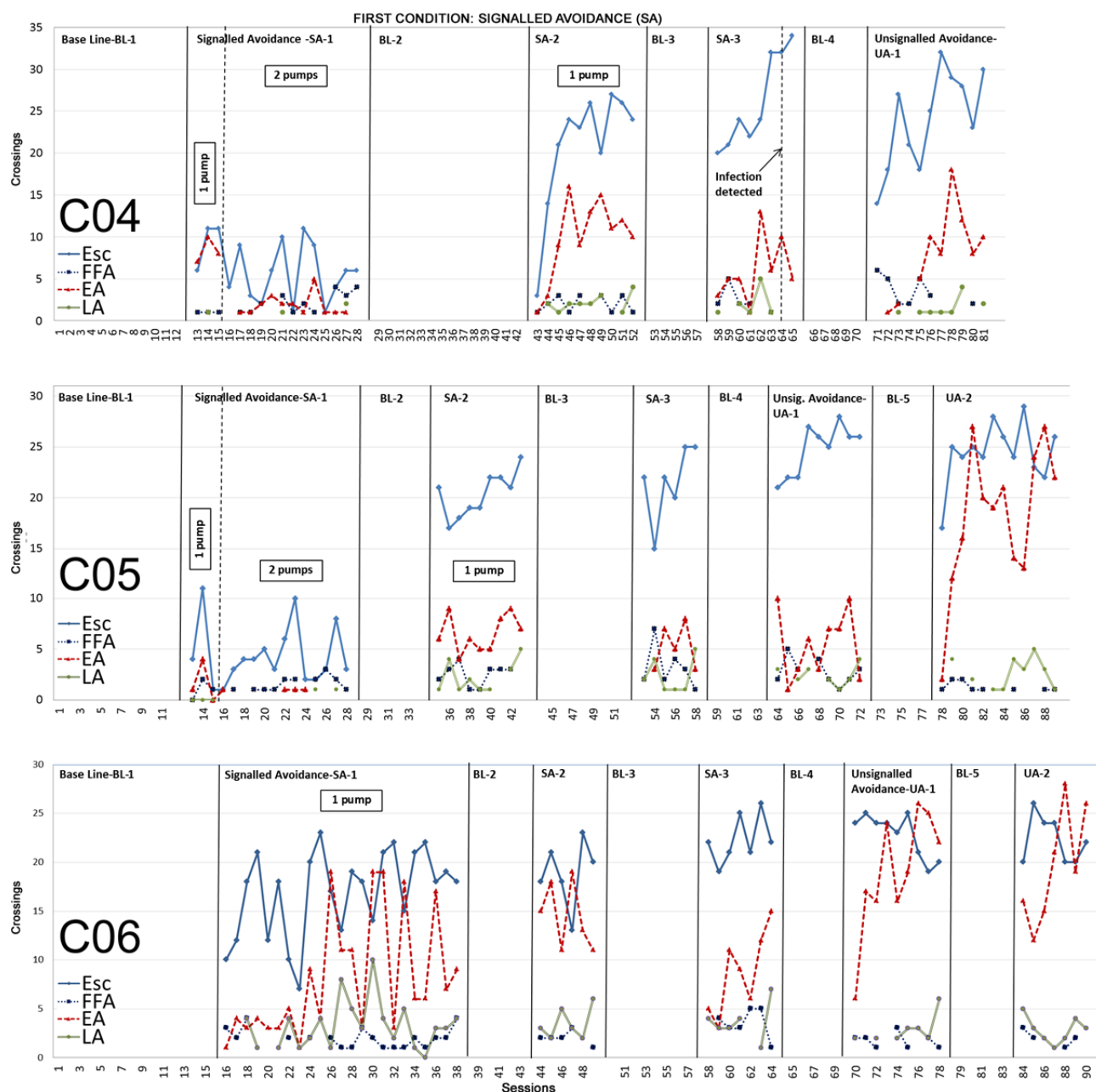
Figure 15b. Frequency of different types of crossings during UA and SA conditions of Experiment 1 for fish C04, C05, and C06. Each horizontally arranged graph shows the indicated subject's data throughout daily sessions, with each condition represented by a separate vertically arranged panel. For instance, the top bottom graph shows C06's data, and the second panel of this graph, from left to right, represents its first treatment condition (SA-1). Esc = escape; EA = early avoidance; LA = late avoidance; FFA = F-F avoidance (crossing during flow-flow interval); SA = signalled avoidance; UA = unsignalled avoidance. Details about the number of water pumps active during the first treatment condition and the infection of fish C04 are the same as for Figure 13b.

Figure 15a and 15b demonstrate a rising trend in Esc responses for fish C01 and C06 across their first treatment condition; as a result, Esc became the dominant type of crossing for these fish at a very early stage. This trend in Esc was accompanied by a somewhat irregular increment in the number of EA responses. This tendency in Esc and EA of C01 and C06 contrasts with that observed in fish C02, C03, C04, and C05 during their first treatment condition (in which the two-pump configuration was in place). Overall, these latter fish showed an unstable frequency of Esc that rarely surpassed 11 instances and was only slightly higher than that of EA. Furthermore, EA responses were both sporadic and irregular for this fish across the condition.

Once the one-pump configuration was established for all fish in the second treatment condition (Figure 15a and 15b, panel 3), the trend of Esc and EA displayed by fish C03, C04, and C05 changed substantially becoming similar to what was observed in C01 and C06. Subject C02 initially showed higher frequencies of Esc compared to the previous UA-1 condition, which resembled the trend observed in C03, C04, and C05 at the beginning of their second condition. However, unlike these latter subjects, frequency of Esc dropped during subsequent sessions, became unstable, and remained at levels that did not differ substantially from those observed in UA-1. Moreover, C02's EA did not increase throughout the condition, occurring sporadically and with a very low rate, and virtually did not occur again during the rest of the experiment.

Throughout the treatment conditions that followed (Figure 15a and 15b, panel 5 onward), Esc remained as the predominant response displayed by all fish. A somewhat increasing trend in this response type was further observed across treatment conditions of all subjects, with the exception of C01 and C02. EA was the second most frequent type of crossing displayed by all fish, except for C02. Although EA in fish C05 and C06 closely matched or slightly exceeded the rate of Esc at some points of the experiment, especially during the last treatment conditions

(Figure 15b, UA-2 for C05 and UA-1 and UA-2 for C06), this did not coincide with a significant

reduction in the overall level of escape. LA and FFA were the least consistent and most

infrequent response types across all fish; in fact, these responses were not displayed at all in

several sessions. Although C02's frequency of FFA sometimes closely matched and exceeded

Esc (see Figure 15a), it is worth noting that this subject's overall frequency of crossings during

treatment conditions was only slightly above baseline levels.

When comparing the data of SA and UA conditions in terms of frequency of different

response types, it is important to first note that only fish C03, C05 and C06 completed all the

programmed treatment conditions (see *Method* section in this chapter). In this comparison, the

frequency per session of Esc reached higher levels during UA conditions for all fish excepting

C03, in which it consistently occurred with more frequency during the SA conditions. Although

EA crossings reached higher frequencies during the UA conditions of all fish, this was

particularly evident and consistent in fish C05 and C06, and was only consistent throughout both

UA conditions of C06 (see Figure 15b). Notwithstanding its low frequency, rarely exceeding five

instances per session, the highest and most consistent frequencies of LA were observed under SA

for all fish except C02, which displayed a single instance of LA during the entire experiment.

Finally, no clear distinction between the frequencies of FFA crossing was observed when

comparing SA and UA conditions.

Time allocation (preference ratio – PR)

Figure 16a and 16b shows each subject's relative time allocation to the right or left

compartments of the experimental tank during baseline and treatment conditions. Each

horizontally arranged graph shows a given subject's data throughout daily sessions of control

and treatment conditions, with each one being represented by a separate vertically arranged

panel. For instance, the top graph of Figure 16a shows C01's data, and the second panel of this graph represents its first treatment condition (UA-1).

Time allocation is expressed in preference ratio (PR), which was calculated for every session and consisted of the difference between time spent on the right compartment and time spent on the left compartment of the tank, divided by the total session time (i.e., PR = [Time Right – Time Left] / Session Time). As a result, preference for the right compartment is evidenced by PR > 0.0, whereas a preference for the left compartment is indicated as PR < 0.0.
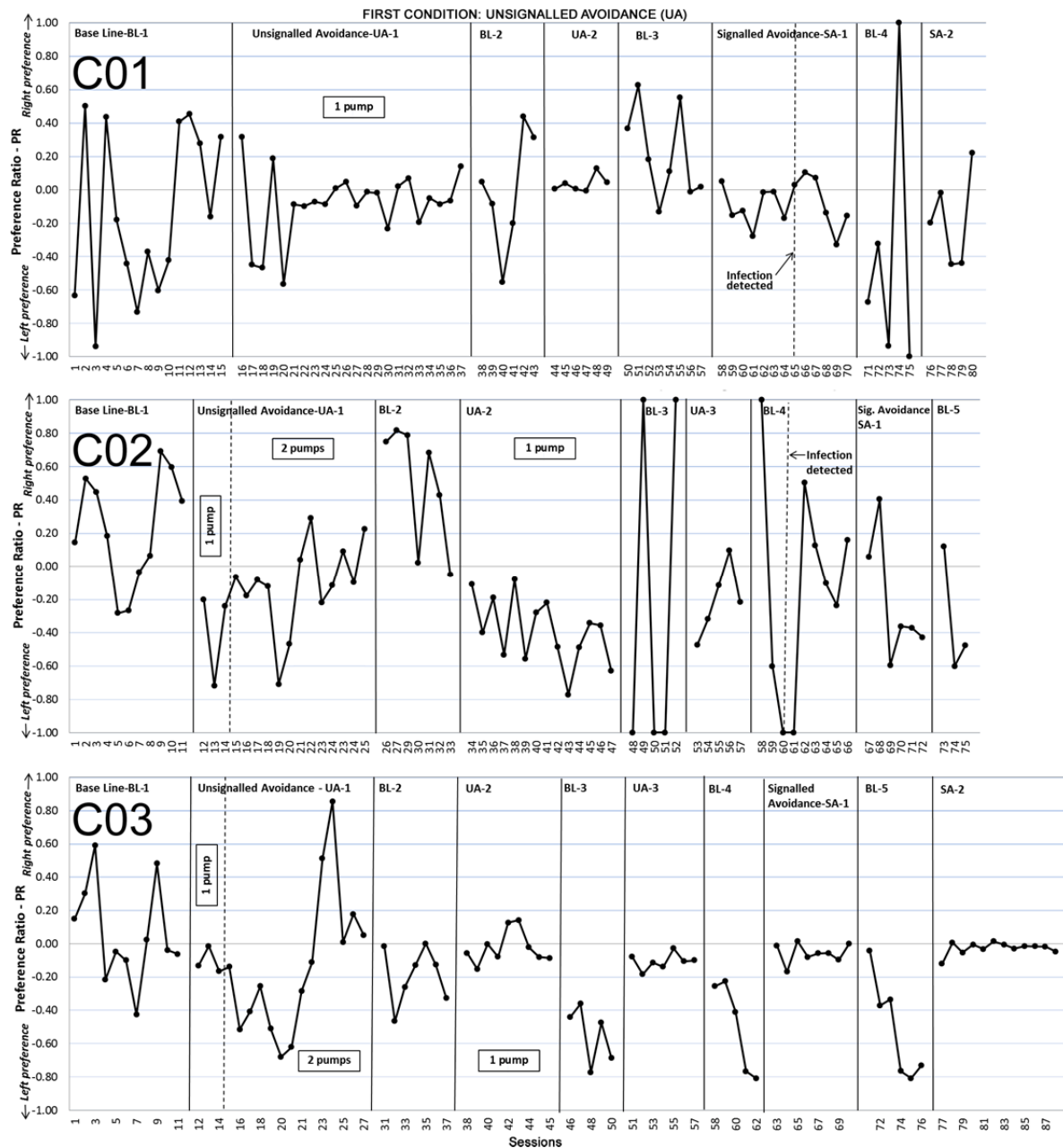
Figure 16a. Fish C01, C02, and C03 time allocation to right and left compartment of the experimental tank, expressed in preference ratio (PR), during baseline and treatment conditions of Experiment 1. Each horizontally arranged graph shows the indicated subject's PRs throughout daily sessions, and each one of the conditions is represented in a separate vertically arranged panel. For instance, the top graph shows C01's data, and the second panel of this graph represents the first treatment condition (UA-1). PR was calculated for every session and was constituted of the difference between the amount of time the fish spent on the right compartment of the tank and the time that the fish spent on the left compartment of the tank over the total session time (i.e., PR = [Time Right – Time Left] / Session Time). Preference for the right compartment is indicated by PR > 0.0, whereas preference for the left compartment is indicated by PR < 0.0. SA = signalled avoidance; UA = unsignalled avoidance; BL = baseline. Details about the number of water pumps active during the first treatment condition and the illness of fish C01, C02, and C04 are the same as for Figure 13a.
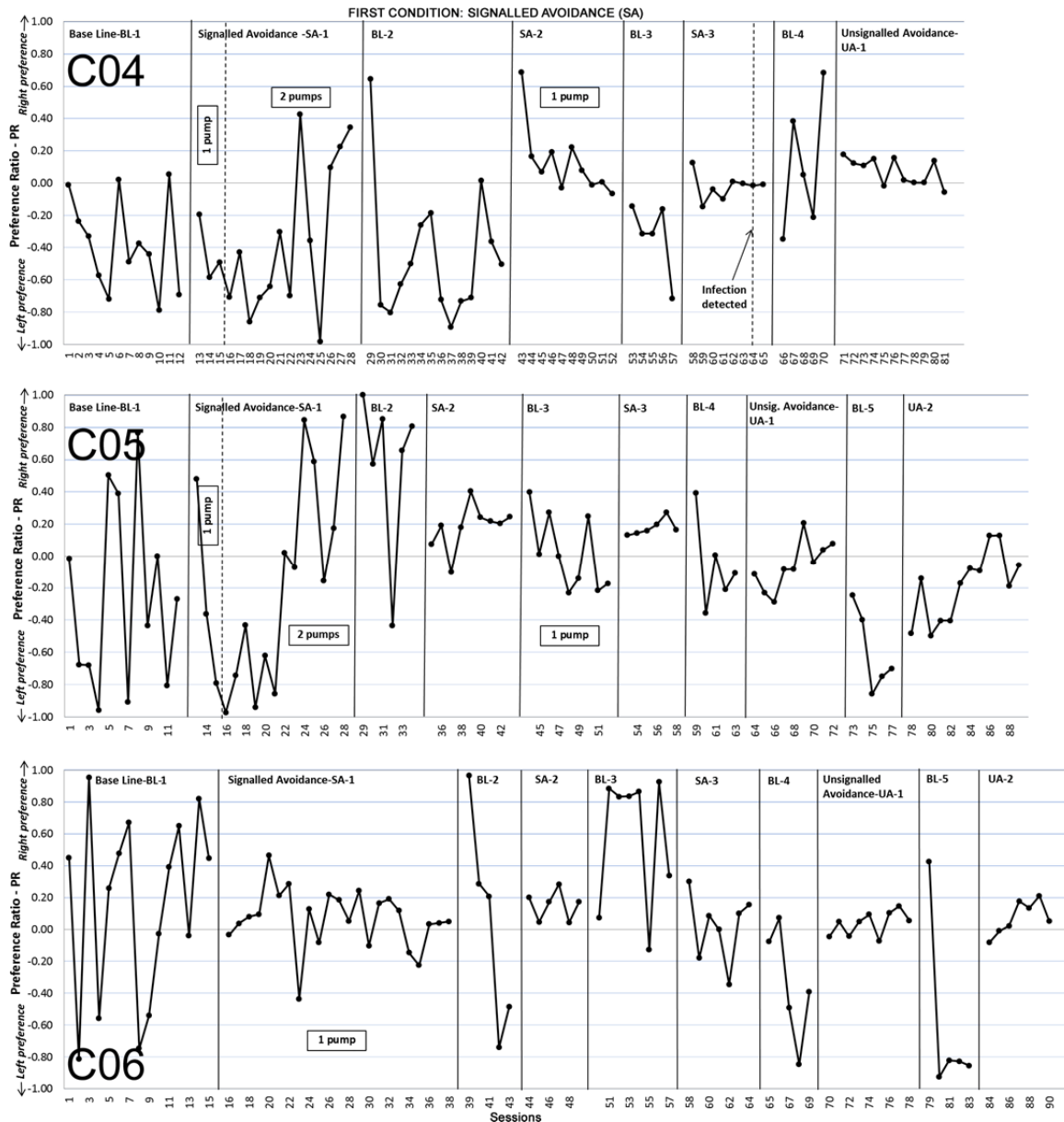
Figure 16b. Fish C04, C05, and C06 time allocation to right and left compartment of the experimental tank, expressed in preference ratio (PR), during baseline and treatment conditions of Experiment 1. Each horizontally arranged graph shows the indicated subject's PRs throughout daily sessions, and each one of the conditions is represented in a separate vertically arranged panel. For instance, the bottom graph shows C06's data, and the second panel of this graph represents the first treatment condition (SA-1). PR was calculated for every session and was constituted of the difference between the amount of time the fish spent on the right compartment of the tank and the time that the fish spent on the left compartment of the tank over the total session time (i.e., PR = [Time Right – Time Left] / Session Time). Preference for the right compartment is indicated by PR > 0.0, whereas preference for the left compartment is indicated by PR < 0.0. SA = signalled avoidance; UA = unsignalled avoidance; BL = baseline. Details about the number of water pumps active during the first treatment condition and the illness of fish C04 are the same as for Figure 13b.

Initial baseline conditions in Figure 16a and 16b (BL-1) show that time allocations to each compartment were very irregular for all subjects, fluctuating between extreme positive and negative PR values across sessions, previous to any experience with WFs and CABs. That is, all fish remained for long periods in either the right or left compartment during a given session, and a given preference would sometimes persist across several sessions (e.g., C04's consistent predilection for the left compartment – Figure 16b), and other times would change from one day to the other (e.g., C06's dramatic fluctuations throughout BL-1). Similarly, in subsequent baseline conditions time distributions across compartments were typically extreme (PR values closer to -1.0 or 1.0) and were consistently biased to one compartment across consecutive sessions and, sometimes, conditions (e.g., C03's systematic preference for the left compartment throughout BL-2, BL-3, BL-4, and BL-5 – see Figure 16a). Though less frequently, time distributions across the tank compartments during baselines also fluctuated across sessions with substantial variations in PRs across consecutive sessions and baseline conditions (e.g., C01's BL-4, and C02's BL-3 and BL-4 – Figure 16a).

Whereas C01's and C06's time allocations across compartments of the tank stabilized and approached even distributions during their first treatment condition (Figure 16a and 16b, UA-1 and SA-1 respectively), C02, C03, C04 and C05's time distributions remained unstable and uneven during the first treatment condition. Only with the second treatment condition of these fish, which differed from the first due to the use of the one-pump configuration, did time allocations gain stability and approach even distributions across compartments (PR values ~ 0.0) in a fashion similar to that of C01 and C06. Although C02's pattern of time allocations was initially similar to that of the rest of the fish, throughout this condition it progressively shifted towards a bias for the left compartment (Figure 16a).

Starting with BL-2 (Figure 16a and 16b, panel 4 onwards), five of the six subjects, the exception being C02, showed a pattern exemplified by (a) steady and even time allocations across compartments during treatment conditions (PRs close to 0); (b) steady and extremely uneven time distributions across compartments during baseline conditions; and (c) no clear differences in time allocations between UA and SA conditions. The data of fish C03 exemplifies this pattern most clearly: starting on BL-3 (Figure 16a, panel 5), and during the rest of the experiment, this subject showed a very steady trend that consisted of modest to extreme time allocations to the left compartment during baselines (PR values ranged between -.23 and -.81) that alternated with very even time allocations (PRs ~ 0) during unsignalled and signalled conditions (UA-3, SA-1, and SA-2). By the end of the experiment, fish C03 was allocating almost exactly the same amount of time to each compartment (PRs ranging from -.05 to .01), as shown in the last sessions of SA-2.

Temporal distribution of crossings and swimming patterns

　　　　Figures 17 – 23 show the temporal distribution of crossings and swimming patterns during the last session of each baseline and treatment condition for each fish. The top graph of each figure is the cumulative record of crossings that occurred during the 20-min session. Vertical bars or lines on the horizontal bands located below the cumulative records of treatment conditions show CABs and WF periods and the occurrence of crossings, indicating when they were escape (ESC) or avoidance (AV), the latter encompassing EA, LA, and FFA. The bottom graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during the non-WF periods of the session (i.e., both R-F and F-F intervals). Dotted lines on each plot indicate the tank's divider and door (opening), and solid bars represent the air stones.
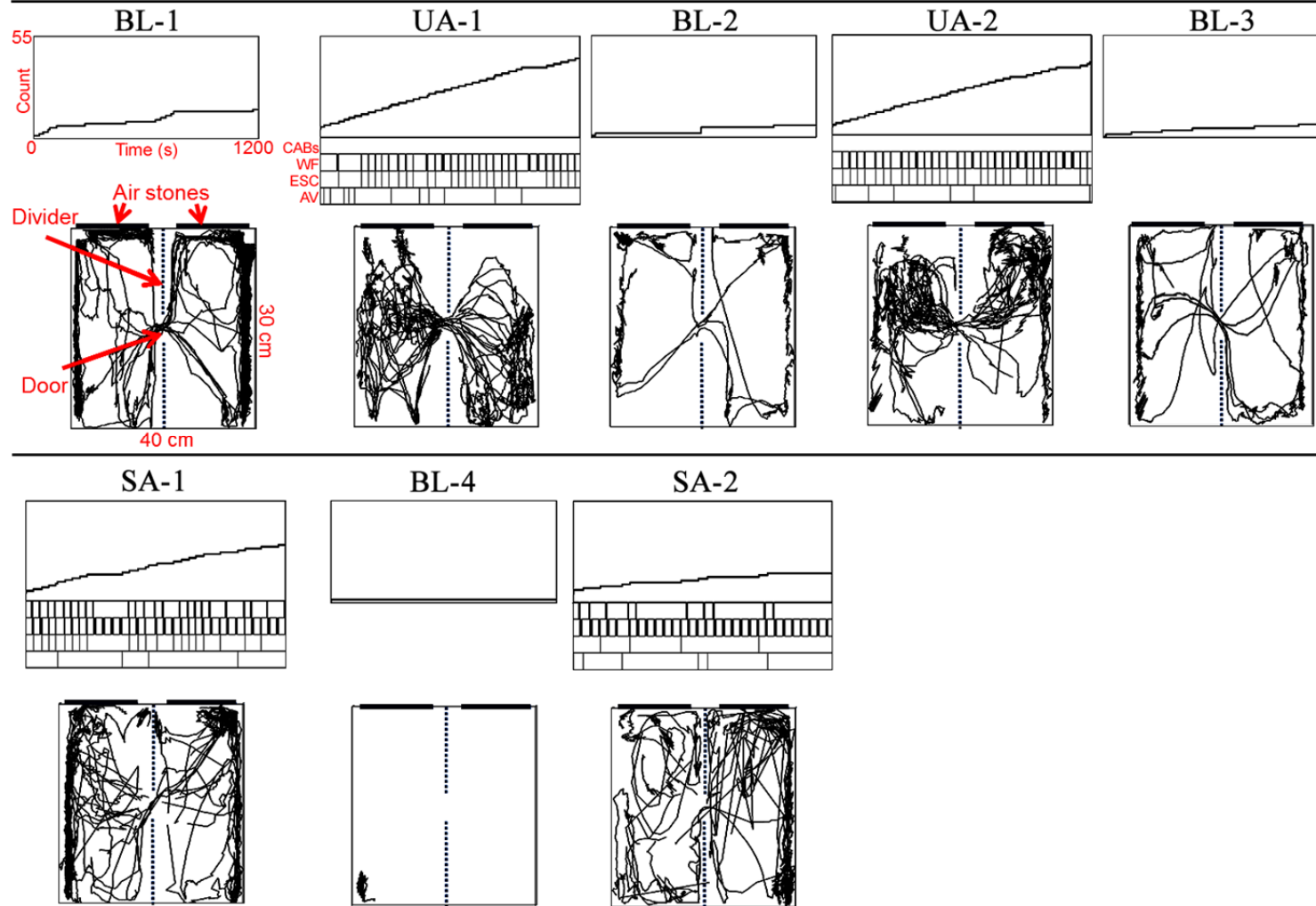
Figure 17. Top row graphs show the cumulative records of fish C01's crossings during the last session (1200 s) of each baseline (BL) and treatment condition (UA and SA). Horizontal bands below the cumulative records of UA and SA sessions show the occurrence of CABs, WF periods (WF), escape (ESC), and avoidance responses (AV). Bottom row graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during each corresponding session. Dotted lines indicate the location of the divider and the door, and solid bars describe the location of the air stones.
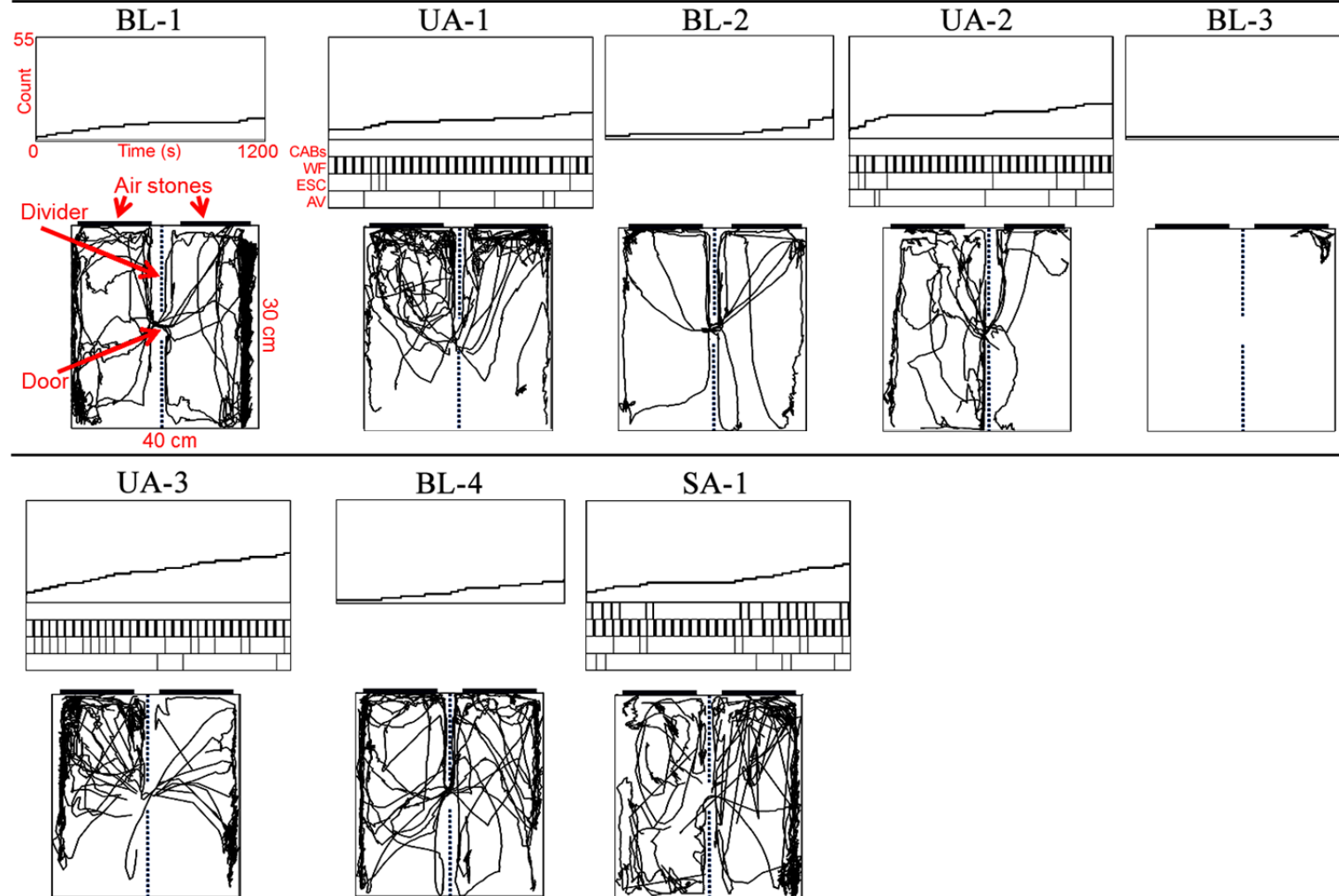
Figure 18. Top row graphs show the cumulative records of fish C02's crossings during the last session (1200 s) of each baseline (BL) and treatment condition (UA and SA). Horizontal bands below the cumulative records of UA and SA sessions show the occurrence of CABs, WF periods (WF), escape (ESC), and avoidance responses (AV). Bottom row graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during each corresponding session. Dotted lines indicate the location of the divider and the door, and solid bars describe the location of the air stones.
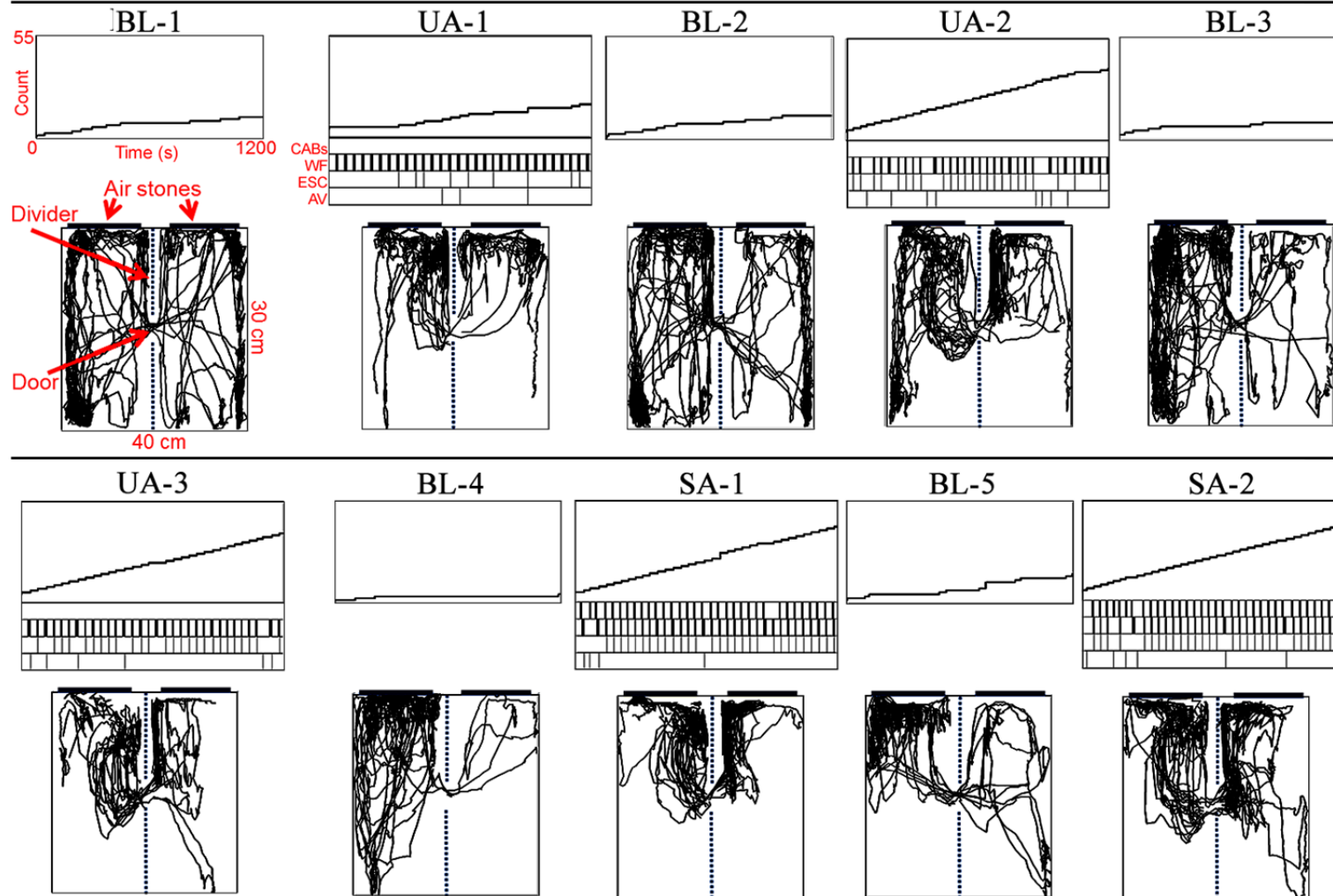
Figure 19. Top row graphs show the cumulative records of fish C03's crossings during the last session (1200 s) of each baseline (BL) and treatment condition (UA and SA). Horizontal bands below the cumulative records of UA and SA sessions show the occurrence of CABs, WF periods (WF), escape (ESC), and avoidance responses (AV). Bottom row graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during each corresponding session. Dotted lines indicate the location of the divider and the door, and solid bars describe the location of the air stones.
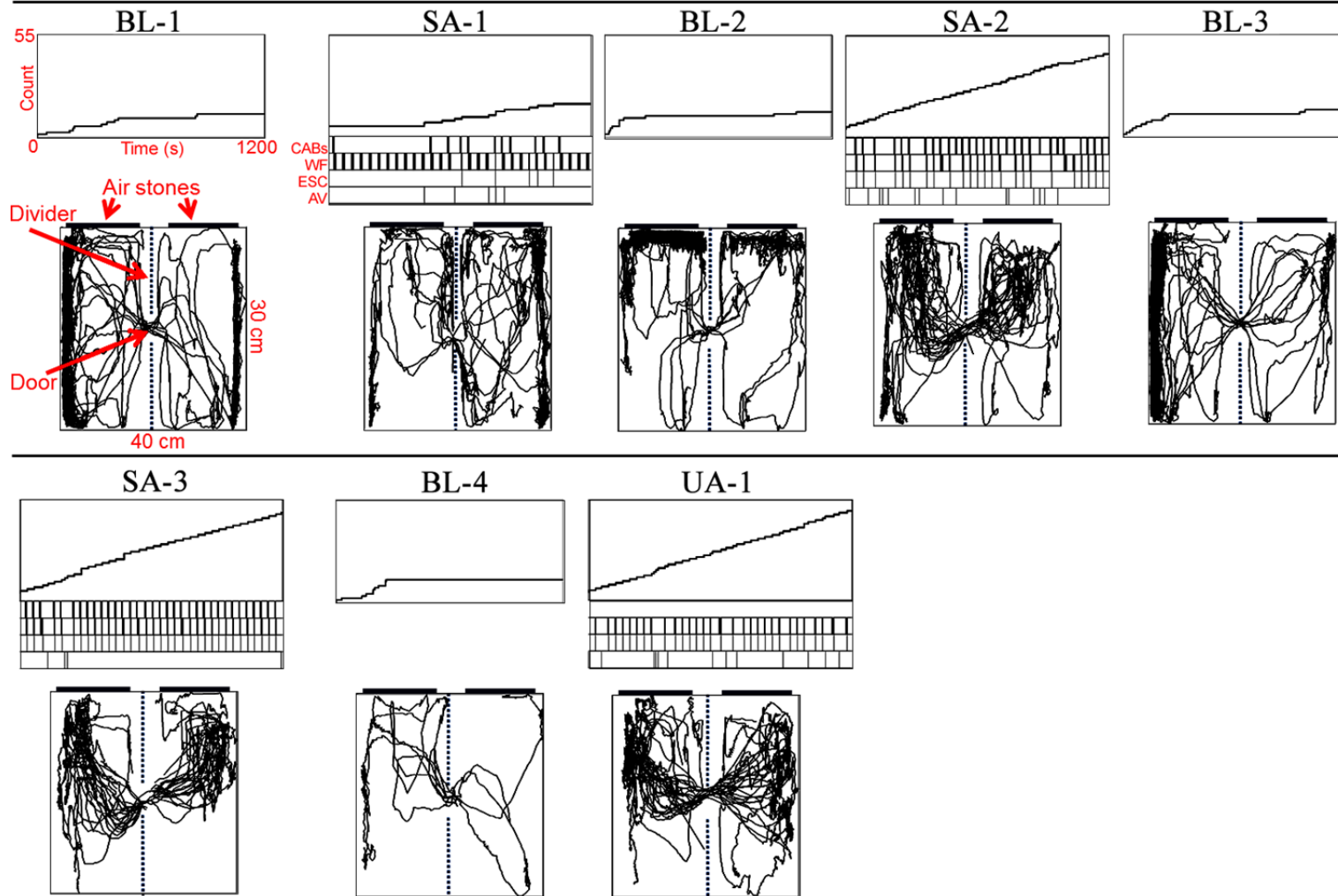
Figure 20. Top row graphs show the cumulative records of fish C04's crossings during the last session (1200 s) of each baseline (BL) and treatment condition (UA and SA). Horizontal bands below the cumulative records of UA and SA sessions show the occurrence of CABs, WF periods (WF), escape (ESC), and avoidance responses (AV). Bottom row graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during each corresponding session. Dotted lines indicate the location of the divider and the door, and solid bars describe the location of the air stones.
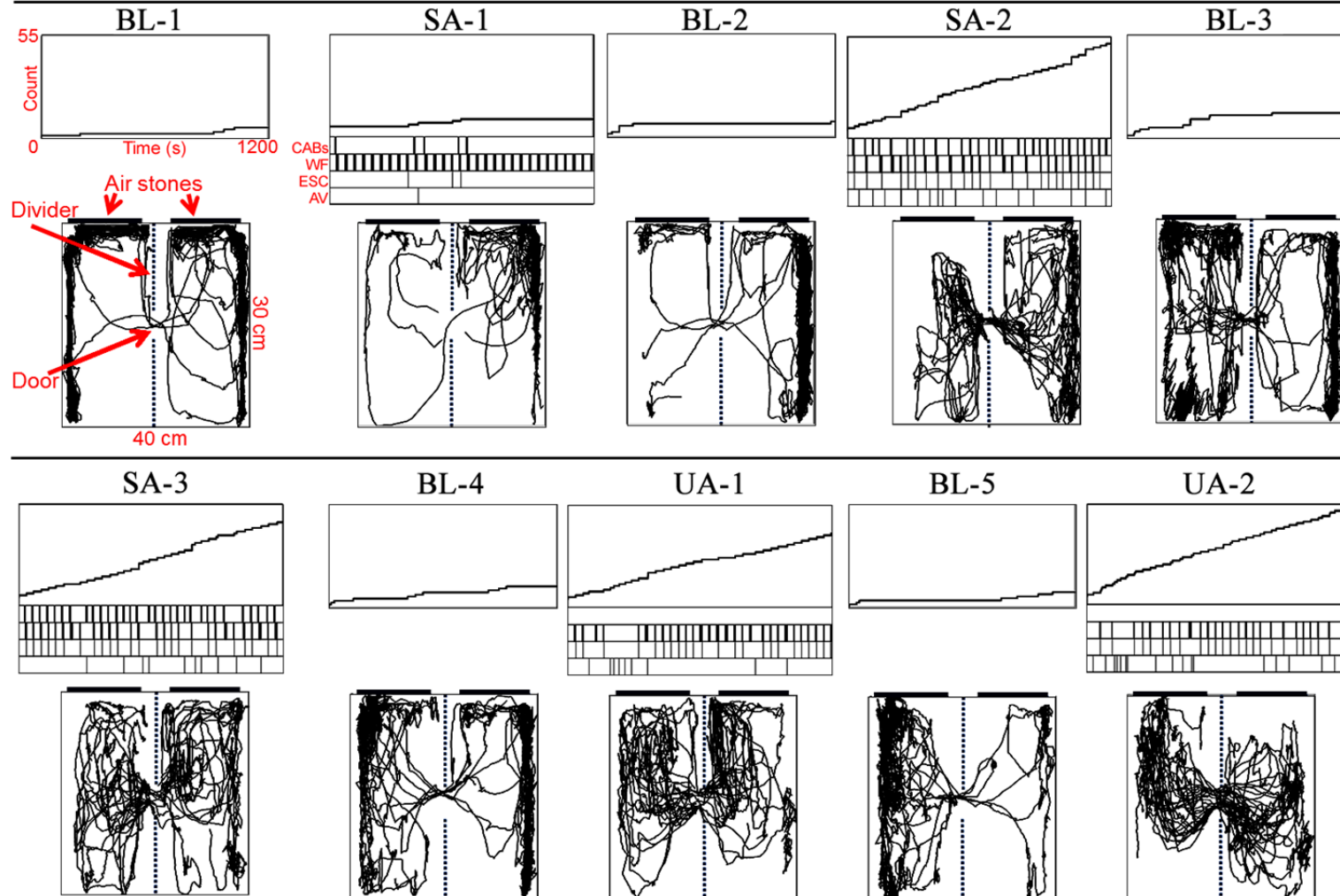
Figure 21. Top row graphs show the cumulative records of fish C05's crossings during the last session (1200 s) of each baseline (BL) and treatment condition (UA and SA). Horizontal bands below the cumulative records of UA and SA sessions show the occurrence of CABs, WF periods (WF), escape (ESC), and avoidance responses (AV). Bottom row graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during each corresponding session. Dotted lines indicate the location of the divider and the door, and solid bars describe the location of the air stones.
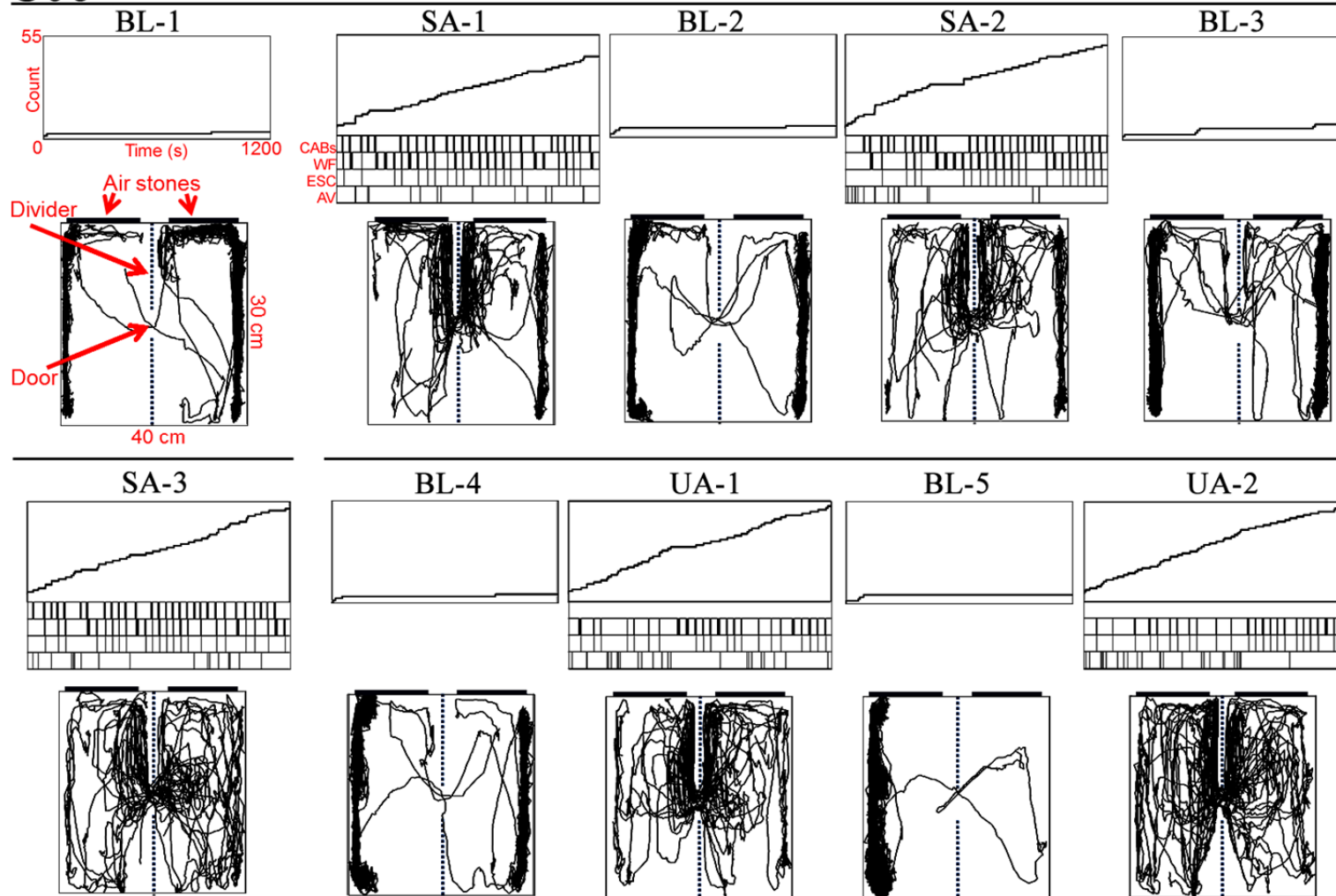
Figure 22. Top row graphs show the cumulative records of fish C06's crossings during the last session (1200 s) of each baseline (BL) and treatment condition (UA and SA). Horizontal bands below the cumulative records of UA and SA sessions show the occurrence of CABs, WF periods (WF), escape (ESC), and avoidance responses (AV). Bottom row graphs show XY (or "overhead") plots of the swimming trajectories described by the fish during each corresponding session. Dotted lines indicate the location of the divider and the door, and solid bars describe the location of the air stones.

Temporal distribution of crossings. As shown in the cumulative records of Figures 17 – 22 (top rows), crossings during the last sessions of baseline conditions, when stability was reached, were low in number and were generally spread out throughout the 20 min session; only in few cases were short bursts of crossings observed (BL-2 of C02 and BL-4 of C04 – see Figures 18 and 20, respectively) or fish remained without crossing during entire sessions (e.g., BL-4 of C01 and BL-3 of C02 – see Figure 17 and 18, respectively).

In contrast, crossings during steady states of treatment conditions showed a continuously increasing "staggered" pattern that was replicated within and across all fish (excepting C02, where it was only observed in SA1), and did not differ across signalled and unsignalled conditions. This pattern consisted of fish changing compartments at a very regular pace just after the beginning of each WF delivery; thus, the thin (indicating single instances) and periodic vertical bars ("WF" and "ESC") show the WF periods and the occurrence of escape responses. In those fish that began the experiment with the two-pump configuration (C02, C03, C04, and C05), this staggered pattern only emerged during the second treatment condition, in which one pump was disabled in each compartment (during the first treatment condition crossings by these fish were erratic throughout the sessions, and in many cases similar in distribution and rate to baseline conditions – e.g., see Figure 21, SA1 of fish C05). Finally, avoidance responses of any kind (EA, LA, FFA), appearing as vertical bars "AV" were somewhat irregular and spread out throughout the sessions. Moreover, the pattern of these responses did not seem to vary from UA to SA conditions.

Swimming patterns during baseline and treatment conditions. Two notable observations regarding swimming patterns became apparent throughout the study: (a) swimming trajectories became more regular (followed recognizable patterns), though never entirely stable, as they continued to change, at least to some extent, within and across sessions; and swimming patterns

that emerged during BL conditions differed overall from those that developed during treatment conditions.

The patterns that emerged during the first BL condition (i.e., previous to any exposure to WFs) were very similar across all fish (see the first overhead plot under BL-1 in Figures 17 – 22). Such a pattern could be described as highly repetitive back-and-forth swimming alongside the glass walls of the experimental tank that run parallel to the divider (lap-swimming), and in many cases extended to the adjacent walls upon which the air stones were mounted. Lap-swimming was noticeable in subsequent BLs for all fish, though with a certain degree of variability, and in some cases it was also part of the swimming patterns during treatment conditions. For instance, Figure 22 shows clear lap-swimming for C06 during BL-2, BL-3, BL-4, and BL-5, despite any variations (BL-5 for example, shows a noticeable bias for the left compartment); such behaviour was also evident in the trajectories this fish developed during SA-1 and SA-2.

With regards to treatment conditions, two distinctive swimming patterns were identified across all fish. These tended to fade during the interspersed BLs and reappear when treatment conditions were reinstated. The first pattern, illustrated in Figure 23, was most salient in fish C01 (UA-1 and UA-2), C04 (SA-2, SA-3, and UA-1), and C05 (SA-2 and UA-2). The left overhead plot of this figure shows the swimming trajectories described by C04 during the last session of condition SA-3. Although the swimming paths for the entire session can be seen to be fairly regular, a representative 60-s segment (800 - 860 s) was extracted for greater detail. This pattern could be described as a repeated "twisting" trajectory in which at a pace determined by WF deliveries (every 30 s, as dictated by the R-F interval) the fish looped, crossed, and repeated a similar path in the other compartment.
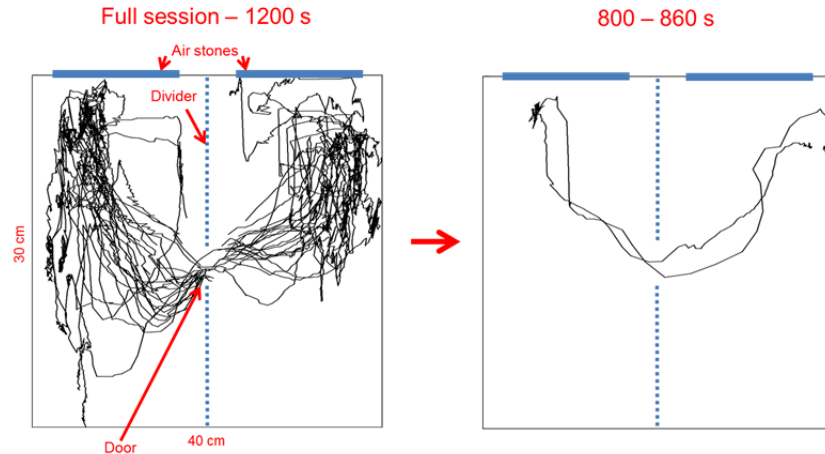
Figure 23. The left overhead plot shows the swimming trajectories of fish C04 during the last session of SA-3, excluding WF periods. A 100-s segment of the same session (800 – 860 s) provided a representative detail and is shown in the right plot.
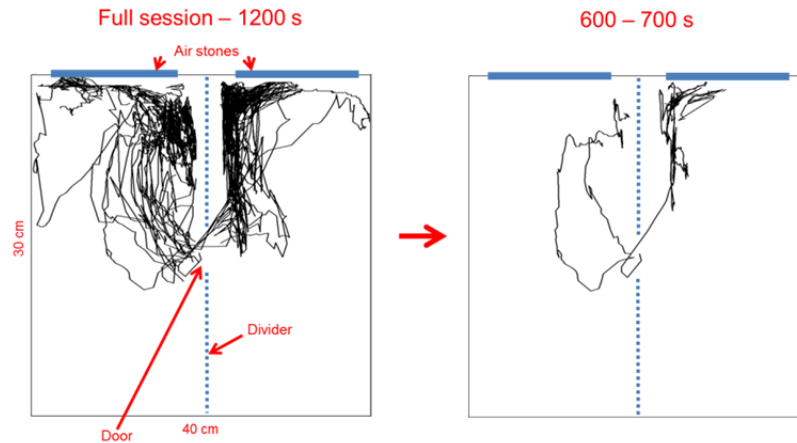
Figure 24. The left overhead plot shows the swimming trajectories of fish C03 during the last session of SA1, excluding WF periods. A 100-s segment of the same session (600 – 700 s) provides representative detail and is shown in the right plot.

The second swimming pattern that emerged during treatment conditions was detected in

C03 and C06 and is illustrated in Figure 24. The left overhead plot of this figure shows all the

swimming trajectories described by C03 during the final session of condition SA-1, and the right

plot describes the pathing for a 100 s segment of the same session (600 – 700 s). This pattern

could be described as repetitive swimming along the wall of the divider, from the connecting door

to the wall where the air stones were attached (in many cases extending underneath, in front, or

above the air stones). The side of the divider in which the fish described this form of lap-

swimming changed in accordance with WF deliveries (every 30 s, as determined by the R-F

interval).

Responses displayed during CABs and non-CABs periods

Informal observations during the first half of Experiment 1 indicated that fish were

systematically displaying certain responses (e.g., air gulping, approaching the air stones, and

swimming by the door) during the experimental sessions. In view of these observations, together

with the unexpected low incidence of LA crossings (i.e., fish were not changing compartments

during the CABs as predicted), the decision was made to produce high-definition videos of the

sessions and explore the behaviour of the fish during CABs and non-CABs periods using manual

scoring (see section *Method, Video recording and manual scoring of responses*, in this chapter).

Figure 25 shows the cumulative count of different responses displayed by fish C03, C05,

and C06 during CABs and non-CABs periods of the last three sessions of their final SA condition

together with the last three sessions of the preceding baseline. The data of these specific subjects

and sessions were selected on an availability basis (no relevant video data are available for

previous conditions – see *Method* in this chapter), and the fact that the remaining fish did not

complete all programmed conditions.

Each row of two graphs on Figure 25 shows the indicated subject's total instances of

different response types during the three indicated days of baseline or SA condition. Counts of

responses that were observed only during the delivery of CABs periods are shown on the left

graph, and cumulative counts of responses displayed only during the non-CABs periods (i.e.,

responses that occurred during WFs, R-F intervals and F-F intervals) appear on the right graph.

For instance, fish C03's data for non-CABs periods is shown on the rightmost graph of the first

row, and the first bar represents the total number of approaches to the air stones (AppAS)

observed during the last three days of BL-5.
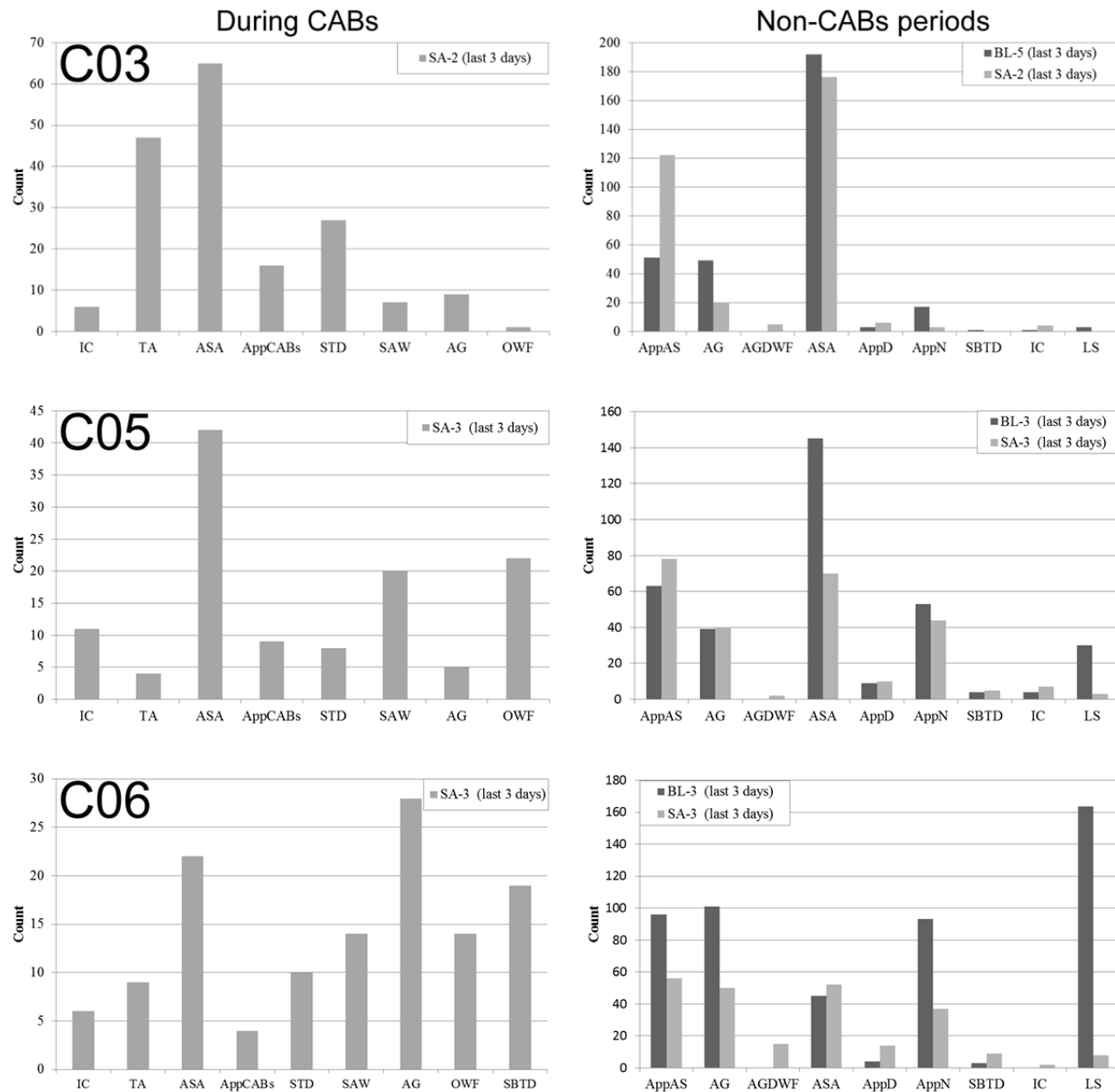
Figure 25. Frequency of different responses displayed by fish C03, C05, and C06 during deliveries of CABs (left panel) and non-CABs periods (right panel) of their last exposure to sequential baseline and SA conditions of Experiment 1 (SA-2 for C03 and SA-3 for C05 and C06). Only the total instances of different response types during the three indicated days of the conditions are shown; i.e., each bar shows the aggregated incidence of each response type during the three corresponding days of baseline or SA condition.

Note. IC = incomplete crossing; TA = turned around; ASA = swam in the air stones area (under, beside or above); AppCABs = approached CABs; STD = swam towards door; SAW = swam away from CABs; AG = air gulped; OWF = Oriented towards WF; SBTD = swam or "remained" beside the door; AppAS = approached air stones; AGDWF = air gulped during WF; AppD = approached door; AppN = approached nozzle; LS = lap swimming.

The data in Figure 25 indicate that fish displayed three overall types of responses related to different objects/sites of the tank during presentations of CABs, namely: (a) related to air stones/CABs (ASA, AppCABs, SAW); (b) related to the source of the WFs, in this case the water pump nozzles (forms of preparatory or anticipatory responses - AG, TA, OWF); and (c) related to the opening or door (STD, IC, and SBTD). Notably, each fish engaged more in some of these response types than others. More specifically, C03 primarily displayed responses related to air stones/CABs and the door, C05 responses related to air stones/CABs and the WF source, and C06 showed a mixed pattern of the three types.

Regarding responses during the non-CABs periods, three somewhat analogous response types were primarily noticeable; namely, (a) related to the air stones area (AppAS and ASA), (b) related to the door (IC, SBTD, AppD), and (c) related to the WF sources (AppN). All three fish showed a high incidence of responses related to the air stones during both BL and SA conditions (AppAS and ASA), and no clear pattern regarding differences between these conditions is noticeable. Responses related to the WF sources (AppN) were more frequent in C05 and C06, and responses related to the door had an overall low frequency with no clearly noticeable pattern across the three fish. Finally, C06 showed a unique frequency of LS and AG, the latter occurring frequently during both non-CABs and (more notably) CABs periods.

Discussion

The main goal of the present study was to test the negative reinforcement function of WFs for the behaviour of *Betta splendens*. Specifically, the study aimed at investigating the generality of two aversive control phenomena, previously studied almost exclusively with electric shock, and never with *Betta splendens*: free-operant escape and avoidance. A consistent and marked increase in crossing frequency during the conditions that had WFs scheduled, reaching three to four times the levels observed in interspersed baselines, was replicated within and across subjects without signs of habituation. Such a high number of crossings produced a steady reduction in total exposure time to WFs, in some cases reaching below 75% of the total WF duration scheduled per session.

These findings confirm the unconditioned negative reinforcing function of WFs for the behaviour of bettas, as their crossing behaviour was reliably and repeatedly brought and maintained under aversive control of the scheduled contingencies. Moreover, these results expand the generality of a negative reinforcement phenomenon – escape – using a form of stimulation different than the mainstream electric shock. In accordance with Johnston's (1979) terminology, the study extended the generality of a basic behavioural process across species and settings.

Nevertheless, free-operant avoidance phenomena observed in other species, including fish (Behrend & Bitterman, 1963; Scobie, 1970; Simon & Kreuz, 1985), was not reliably replicated in this study, notwithstanding the extensive number of treatment conditions sessions (in some cases surpassing 50 days of training). Specifically, whereas escape became the predominant response across all fish very early in the experiment, avoidance – primarily early avoidance – typically had a low frequency and only occasionally matched or slightly exceeded the frequency of escape (as observed in subjects C05 and C06, though this pattern was only replicated in C06). Even in those cases in which there were substantial avoidance responses, they did not result in a sizable

reduction in the overall frequency of WFs or of escape responses. Additionally, it is this predominance of escape behaviour that explains the dissociation between the overall frequency and duration of WFs that was observed in the study. The fact that WF time substantially decreased over sessions, whereas WF frequency did not, was the result of the fish developing a highly regular response pattern that consisted of changing compartments during the first few seconds of the WFs. As fish developed faster escape responses (evidenced in the progressive reduction in WF time across sessions), and incidence of avoidance remained low, the frequency of WFs delivered per session approached, or even surpassed, the number of WFs originally scheduled per session. When an escape response occurred, a WF was cut short before its full 10 seconds, and thus the next WF occurred sooner than it would have otherwise. Given the fixed duration of each session (20 min), this tended to increase the number of WFs in a session so that more than the originally scheduled number of WFs could occur. Only in cases where fish showed an increase in avoidance levels was WF frequency decreased to a certain degree (e.g., both UA conditions of C06 and UA-2 of C05; Figure 14b).

The finding that bettas engage in learned escape behaviour from a non-shock aversive stimulus systematically replicates previous research in which aversive control has been demonstrated using unconditioned aversive stimuli different from electric shock (e.g., escape and punishment using intense noise or bright light – Barker et al., 2010; Knutson & Bailey, 1974). However, the apparent impossibility to establish free-operant avoidance in this experiment adds to a series of previous efforts with non-human animals in which replication of this phenomenon has been attempted with non-shock stimuli, but has only been demonstrated a few times. For instance, whereas Rohles (1965) and Clark et al. (1973) successfully demonstrated free-operant avoidance in monkeys using wind and artificial gravity, Knutson and Bailey (1974) and MacKenzie et al. (1993) could not replicate such phenomena in rats and domestic hens using intense noise; in fact,

similar to the present study, subjects in MacKenzie et al.'s experiments learned to escape, but not to avoid the aversive stimulus. Finally, in Barker et al.'s (2010) study with rats, positive punishment of lever pressing and escape responding (head entries into a hole) were successfully replicated using intense light as the aversive stimulus. However, free-operant avoidance could only be established and maintained when escape was not functional, and when a brief dim light, not a tone, signalled the imminent occurrence of intense light. As will be detailed later, Barker et al.'s successful findings regarding the effect of making escape not functional was one of the considerations that led to the design of Experiment 2 in the present study.

Effectiveness of WFs in controlling betta behaviour: one-pump vs. two-pump configuration

One factor that seemed to reduce the effectiveness of WFs in controlling the behaviour of bettas was the use of two water pumps to produce the WFs in each compartment (two-pump configuration). When fish C02, C03, C04 and C05 were exposed to a first treatment condition in which the two-pump configuration was active, their crossing responses did not increase above baseline levels. Conversely, high and reliable crossing behaviour over baseline levels occurred consistently when the one-pump configuration became functional (a single pump produced the WFs in each compartment). The results of this unplanned manipulation suggest that similar to electric shocks (Boren, Sidman, & Herrnstein, 1959; Das Graças de Souza, Alves de Moraes, & Todorov, 1984; Glazer & Weiss, 1976; Lawry et al., 1978), the effects of WFs depend on their parameters, such as speed. For instance, Boren et al. (1959) and Das Graças de Souza et al. (1984) reported that intensities of shock much above the level necessary to establish and maintain negatively reinforced behaviour in rats have a "disruptive effect on behavior" (Das Graças De Souza et al. 1984, p. 73). It is possible that the force of the WFs produced by two water pumps competed with the swimming behaviour of the fish, resulting in the inability to develop consistent crossing behaviour. However, more research is required in this regard, since the parameters of the

WFs were not systematically manipulated in this experiment, and no within-subject replications were attempted (as this was not the purpose of the study).

    Of greater relevance is the fact that the crossing pattern that emerged in fish that went from a two-pump to a single-pump configuration did not seem to differ from that of other fish that started the experiment with only one pump active in each compartment. All fish gradually acquired high rates of crossings throughout the conditions in which only one pump was functional for each compartment. This suggests that bettas exposed to uncontrollable WFs may not show the deficits in escape-avoidance learning that have been observed in dogs and rodents that have previously experienced uncontrollable electric shocks, a phenomenon known as *interference effect* (Anderson et al., 1979; Anisman & Merali, 2009; Glazer & Weiss, 1976; Lawry et al., 1978) or *learned helplessness* (Maier & Seligman, 1976). The fact that the interference effect has only been demonstrated using electric shock calls for further research using the WF paradigm, especially considering that Behrend and Bitterman (1963) provided preliminary evidence of such a phenomenon with shock in goldfish. To date, this effect has not been explicitly tested in fish of any kind.

UA and SA conditions: Predictions and outcomes

    A second purpose of Experiment 1 was to compare the effects of signalled and unsignalled free-operant escape and avoidance contingencies on the behaviour of bettas. Considering that fish C01, C02, and C04 did not complete all the programmed UA and SA conditions of the experiment due to their infections (which may have affected their behaviour throughout the experiment), only the data of C03, C05, and C06 seem relevant to discuss for the purpose of this study.

    Previous research with goldfish and monkeys exposed to signalled and unsignalled shocks are the most direct precedents for discussing the present experiment with signalled and unsignalled WFs. Behrend and Bitterman's (1963) goldfish, when exposed to signalled

conditions, typically postponed crossing until near the end of the R-S interval when the warning

stimulus was present. Thus, one may infer that lower response rates were established during

signalled than during unsignalled conditions. This inference is supported by the results of a

subsequent study where goldfish were only exposed to unsignalled contingencies. Scobie (1970)

reported that the crossing frequency displayed by his subjects was higher than necessary to

prevent all the scheduled shocks per session, resulting from the fact that fish consistently crossed

during the first part of the R-S intervals. Finally, Hyman (1971) showed that monkeys responded

at a lower rate during signalled than during unsignalled avoidance, which was evidenced by the

fact that the removal of the warning stimulus from the avoidance schedule (i.e., changing from a

signalled to an unsignalled scheduled) increased response rates.

In view of these antecedents, it was expected that betta crossing frequency would be

higher during unsignalled than during signalled conditions. Such an effect was observed, to a

certain degree, in that fish C05 and C06 showed higher crossings during conditions in which the

warning stimulus was absent than when it was present. However, considering that this outcome

was only replicated during the last conditions of the experiment for C06 and C05, whereas it was

not observed for C03, suggests that it was caused by the extended exposure to the experimental

task rather than to the treatment conditions per se. Specifically, higher frequencies of crossings

may have resulted from extended training, and not from the presence or absence of the warning

stimulus. This interpretation is supported by the fact that the highest crossing frequency of C05

and C06 was evident during their last scheduled treatment conditions (Figure 13b), which

happened to be unsignalled, and that for C03 the frequency of crossings during its last condition

of the experiment (SA-2, Figure 13a) was overall higher than previous conditions (in particular

SA-1, Figure 13a).

Nonetheless, additional information regarding a possible difference in crossing frequency across signalled and unsignalled conditions results from analyzing the types of responses displayed by the fish (escape, early avoidance, late avoidance, and flow-flow crossings). The outcomes of such analyses are unique in that previous research in free-operant avoidance typically has not differentiated escape and avoidance behaviour. This is generally the case since the aversive stimuli in these studies, shocks, are too brief for escape to occur except when the S-S interval is so low as to, in effect, approach continuous shock. Even in the case of one of Behrend and Bitterman's (1963) experiments, in which a very brief S-S interval (2.5 s) was interpreted as an escape contingency, a detailed analysis of the behavioural effects of such a contingency was not pursued.

UA and SA conditions: Escape and avoidance

An examination of betta response types indicates that the apparent differences in crossing frequencies throughout the conditions of the experiment actually represented specific variations in two types of crossings, namely escape and early avoidance. Regarding escape, its frequency increased as the experiment progressed. Although noticeable in all three pertinent fish (C03, C05, and C06), this was clearest in C03 and C05 (Figure 15a and 15b). As for early avoidance, substantial increases in these responses were observed at some stages of the experiment, which matched and sometimes exceeded frequency of escape. Although this effect was substantial and steady in the case C05 and C06, it was still noticeable in C03, as the highest levels of early avoidance for this fish were observed during unsignalled conditions (Figure 15a). This result partially replicates the finding from Behrend and Bitterman's (1963) unsignalled conditions, in which goldfish showed more crossings during the first part of the R-S interval (analogous to early avoidance responses in the present study). However, the finding from Behrend and Bitterman's signalled conditions was not observed; that is, during signalled conditions of the present

experiment bettas did not consistently increase their frequency of crossing during the last part of

the R-F interval, moment at which the warning stimulus (CABs) was presented. A detailed

analysis of this outcome accompanies a forthcoming discussion on the role of the warning

stimulus.

It therefore appears that the difference in the overall frequency of crossings across

signalled and unsignalled conditions represents, in fact, two separate outcomes. First, fish tended

to engage more in escape as the experiment progressed, which adds to the previously mentioned

interpretation regarding a "training effect" – i.e., increments in crossings throughout the

experiment seem to be the result of more exposure to the task, regardless of the treatment

condition. And second, scheduling a warning stimulus (CABs) near the end of the R-F interval

seemed to have affected the incidence of avoidance to a certain degree; specifically, its presence

appeared to have some limited control – although more would have been expected based on the

findings of Behrend and Bitterman (1963) – while its absence appeared to have favoured

increments in crossings during the first part of the R-F interval (early avoidance).

The role of the warning stimulus

The fact that late avoidance did not reach higher frequencies during signalled conditions,

notwithstanding previous research with bettas in which successful stimulus control was

accomplished using this same form of stimulation (Craft et al., 2007; Wirth et al., 2003), merits a

separate analysis. This is especially the case considering that (a) in the present experiment, the

warning stimulus (CABs) seemed to have exerted some effect, as indicated by the fact that despite

the overall low rates of LA, the highest and most consistent frequencies of this response were

observed under signalled conditions (in particular C03 and C06 – Figure 15a and 15b); and

moreover (b), systematic observations of the session videos indicated that the fish indeed respond

in different ways to the presentation of CABs – e.g., on CABs onset, fish turned away from this stimulus, approached the air stones, swam towards the door, or air gulped (Figure 25). Although the data of the present experiment do not provide the information necessary to formulate a satisfactory explanation for this situation, some possible accounts that may be explored by further research are proposed as follows.

A first interpretation is that the duration of the CABs (5 s), though the same as in Behrend and Bitterman (1963) study, may have been too brief. This is supported by the fact that bettas showed fewer early avoidance responses during the signalled conditions (Figure 15a and 15b), which indicates that subjects tended to postpone their crossings until near the end of the R-F interval (similar to Behrend and Bitterman's fish). However, subjects may not have been able to complete the change of compartment before the advent of the WF, so their responses occurred during the first seconds of the WF and were recorded as escape (which is consistent with the high incidence of this response type). Adding to this notion is the possibility that the response requirement in the present experiment may have made the task more challenging, and therefore more time consuming. Specifically, subjects were required to cross through a somewhat narrow door instead of, as in Behrend and Bitterman's (1963) and Scobie's (1970) experiments, swimming over any point of a hurdle that divided the two compartments. Finally, the incidences of incomplete crossings and swimming toward the door provide additional support to this notion, as these responses were observed in all three pertinent fish (Figure 25), and suggested that subjects in several occasions were "in the process" of crossing when the CABs period ended.

Points against this explanation include the data on the swimming patterns (e.g., repetitive swimming along the wall of the divider, going from the connecting door to the air stones wall – Figure 24) and other responses displayed during the CABs (swimming by the air stones or beside the door – Figure 25). Specifically, these data indicate that the fish typically were very close to the

air stones and the door at the onset of the warning stimulus, which suggests that they would have

had enough time to experience the CABs and cross to the other compartment. Future tests in this

regard are required, including increasing the length of the CABs and adapting the apparatus to

allow for the use of a target response similar to Behrend and Bitterman's (1963) and Scobie's

(1970).

A second interpretation is that CABs had a weak discriminative stimulus function. This

may have been related to a specific aspect of the avoidance schedule utilized. CABs were

scheduled to be delivered only at the end of the R-F interval, which occurred after escape or

avoidance responses were displayed, but not during the F-F interval. As a result, whenever a fish

failed to escape a given WF, CABs did not precede the following delivery of WFs. Consequently,

the function of the CABs as a discriminative stimulus may have been weaker because they not

always preceded the presentation of the aversive stimulus. Nevertheless, this explanation seems

unlikely considering the very low incidence of situations in which fish failed to escape an instance

of WF; therefore, F-F intervals occurred on very few occasions. Instead, the high frequency of

escape displayed by all fish guaranteed that CABs were almost always presented before every

WF.

A third interpretation of the data regarding low frequency of crossings during the warning

stimulus is that the experimental procedure of the present study inadvertently favoured the

combined occurrence of sign- and goal-tracking effects. When a given event (conditioned

stimulus – CS) is paired with a biologically relevant stimulus (unconditioned stimulus – US) the

CS may evoke approach or contact with itself (or its location) or with the US (or its location).

These effects are termed sign-tracking (approaching the CS or its location – Hearst & Jenkins,

1974), and goal-tracking (approaching the US or its location – Boakes, 1977), respectively.

Although both effects typically have been studied with appetitive contingencies involving food,

and replicated in different species, including fish (Nilsson, Kristiansen, Fosseidengen, Fernö, &

van den Bos, 2008), there are reports that analogous effects could also be reproduced when the

US is an aversive stimulus, such as shock (Leclerc, 1985; Leclerc & Reberg, 1980) or, as in the

present experiment, WFs.

Among the variables that have been proposed as responsible for modulating goal- and

sign-tracking effects is the spatial distribution of the stimuli, especially the distance between the

CS and the US. In Silva et al.'s (1992) study, pigeons were exposed to different distances between

a light (CS) and the site in which food (US) was presented (food hopper). It was found that

subjects sign-tracked when the CS was closest to the food hopper, goal-tracked when the CS was

farthest from the site of the US, and "engaged in both sign- and goal-tracking (or something

intermediate) at intermediate conditioned-stimulus-to-unconditioned-stimulus distances" (Silva et

al., 1992, p. 17). Extrapolating from these findings, it seems plausible that fish in the present

study engaged in a mixed pattern of sign- and goal-tracking as a result of the contingencies

involved (CABs reliably predicted WFs) and the spatial configuration of relevant stimuli; namely,

the WFs and their source (water pump nozzles), CABs and their source (air stones), and the door

connecting the compartments of the shuttle tank. Whereas CABs could be interpreted as a CS and

WFs as a US, the source of the WFs (nozzles) and the site of the door are not easily translated into

traditional appetitive sign-/goal-tracking procedures. Nevertheless, data regarding betta behaviour

during presentations of CABs (Figure 25) indicate that subjects showed three types of responses

related to different elements and/or sites of the tank: (a) responses related to the air stones and

CABs, such as approaching or swimming away from them; (b) responses related to the WFs and

their source (nozzles), such as apparent preparatory or anticipatory responses (air gulps, turning

away from CABs, or facing the WF); and (c) responses related to the door, such as approaching,

swimming in its vicinity, and incomplete crossings.

Considering the spatial arrangement of the water pump nozzles (i.e., WFs source), the air stones/CABs (located in the wall opposite to the nozzles), and the opening in the tank's divider wall (somewhat halfway between the air stones and the nozzles), it seems plausible that fish showed mixed response patterns in relation to these three locations. That is, similar to what Silva et al. (1992) reported, fish may have engaged in both sign- and goal-tracking, or something intermediate, given the spatial configuration and distances between the door, nozzles and air stones/CABs (e.g., in the last SA sessions of C03, responses with higher frequency during CABs were a combination of actions aimed both at the air stones/CABS and the door – Figure 25). This suggests that due to these combinations of sign- and goal-tracking responses, fish did not display the expected target response more frequently or reliably (crossing the door to prevent the next WF), especially since the CABs interval was of limited duration (5 s).

This goal-/sign-tracking explanation is supported by previous research on what has been referred to as *omission responding* (Peden, Browne, & Hearst, 1977). It seems plausible that the observed paradoxical effect – fish missing the opportunity to avoid the WF due to engaging in responses during the CABs – is analogous to an omission responding phenomenon, reported in previous studies with other species, including fish (Bottjer, Scobie, & Wallace, 1977); namely, subjects miss the opportunity to access reinforcers that are available for limited periods of time as a result of responding towards a CS that has been paired with those same reinforcers (Peden et al., 1977). Future research should systematically explore this interpretation by manipulating spatial characteristics of the experimental procedure in order to see if, as in Silva et al.'s study, changing the location of the air stones (e.g., placing them closer to or farther from the door) affects the incidence of the different types of responses observed during the present study (related to the door, the air stones/CABs, or the source of the WFs). Moreover, considering that Craft et al.

(2007) showed high and reliable betta discrimination using a procedure in which "when entering

the goal box, the subject swam through bubbles" (Craft et al., 2007, p. 577), suggests that placing

the air stones beneath door is a configuration that could favor higher stimulus control in the WF

experimental paradigm – i.e., bettas may show better performance during signalled conditions if

they also swim through CABs to change compartments.

WF frequency and duration across UA and SA

Another prediction based on the findings of Behrend and Bitterman (1963) and Scobie

(1970) was that bettas would more closely match the frequency of crossings necessary to prevent

all the WFs during signalled, as opposed to unsignalled, conditions. Clearly, this outcome was not

observed as all the subjects of the present study received the majority of the scheduled WFs per

session, and occasionally slightly more, regardless of the treatment condition. Nevertheless, it is

worth taking a closer look at the data regarding frequency and duration of WFs, since some

differences across UA and SA conditions were noticeable – notwithstanding the fact that they

were not always substantial or consistently replicated across and within subjects. This effort is

especially relevant because previous research has not explored the possible dissociations between

these variables, due to the fact that the duration of the aversive stimulus (electric shock) has been

practically negligible.

Fewer instances of WFs were delivered during unsignalled as opposed to signalled

conditions (i.e., the fish were more "successful", to a certain degree, in reducing the number of

WFs when no warning stimulus was presented – Figure 14a and 14b). This was observed across

the three subjects that completed all programmed conditions (C03, C05, and C06), though it was

not replicated across both UA conditions of subject C05 (the effect was only clearly observed in

its second UA condition). This outcome is counter to that reported by Bitterman and Behrend

(1963) and Scobie (1970), whose findings indicate that goldfish were more efficient in reducing

the number of shocks during signalled instead of unsignalled conditions (i.e., fish displayed less

responses to avoid all the shocks during signalled conditions).

Similar to WF frequency, percentages of flow time reached lower levels during

unsignalled conditions (i.e., the fish achieved greater reductions in WF duration when no warning

stimulus was presented – Figure 14a and 14b). Nevertheless, the fact that this effect was not

replicated in C03, which (unlike C05 and C06) experienced the UA conditions before the SA

conditions, suggests that the previously discussed "training effect" could have been responsible

for the apparent differences. That is, it is suggested that fish became more successful at escaping

the WFs as the experimental sessions progressed, resulting in a reduction in WF duration. This

account is consistent with the fact that greater reductions of WF time occurred during the last

condition for the three fish, regardless of it being signalled (C03) or unsignalled (C05 and C06);

more generally, this is also consistent with the previously discussed observation that fish tended to

engage more in escape as the experiment progressed.

The contingencies in effect for this experiment determined that frequency and duration of

WFs depended on the patterns of crossings (high and reliable avoidance responses reduced the

frequency of WFs, whereas escape reduced the duration of WFs, but served to ultimately increase

their frequency). Accordingly, it seems that the differences observed throughout the study

regarding WF frequency and duration were the result of specific patterns of escape and avoidance

that emerged across the experiment, and not directly resulting from whether the condition was

signalled or unsignalled. Consequently, an explanation of these patterns is inevitably linked to the

understanding of what caused the variations on incidence of escape and avoidance. As will be

discussed in the last section of this chapter, it was suspected that such variations could be

explained by procedural aspects.

Swimming patterns

Previous studies in the spatiotemporal analysis research program have repeatedly shown that distinct behavioural patterns are developed as a result of implementing different contingencies (e.g., intricate movement patterns in pigeons exposed to short variable-interval food schedules – Pear, 1985; highly stereotyped lap-swimming in bettas exposed to fixed-time schedules of mirror presentations – Martin, 2010). Accordingly, it was expected that bettas would develop distinctive swimming patterns (e.g., regular trajectories in the tank) throughout the different conditions of the experiment.

As predicted, swimming trajectories became regular, although never entirely stable, indicated by the fact that they changed to some extent within and across sessions, as well as across fish. The swimming patterns that emerged during baselines differed overall from those that developed during treatment conditions, and no differences were detected between signalled and unsignalled conditions (Figures 17 - 22). Specifically, whereas baseline patterns primarily consisted of stereotyped lap-swimming, two behavioural patterns were identified during treatment conditions (twisting trajectories and repetitive swimming along the dividing wall; Figure 23 and 23), which tended to fade during the interspersed baselines and reappear when treatment conditions were reinstated. Interestingly, lap-swimming observed during baselines was occasionally noticeable in the swimming patterns displayed during treatment conditions.

These outcomes, together with the previously discussed results regarding frequency of crossings, extend the generality of some of the key findings of the spatiotemporal research program. Specifically, they demonstrate that similar to what has been repeatedly shown across a variety of appetitive experimental paradigms (see Pear, 2004), aversive contingencies regulate not only the incidence of discrete responses (crossings through an opening), but also the behaviour that occurs between such discrete responses. Analogous to what previous studies in this program

have reported in pigeons (e.g., Pear, 1985), distinct swimming patterns developed and disappeared

during the different conditions (see Figures 17 - 22). Furthermore, the fact that such contingencies

did not explicitly target those swimming patterns, which still emerged, disappeared, and resurged

in accordance with the scheduled treatment conditions (notwithstanding some degree of

variability across and within subjects), supports the notion that behaviour occurring between the

reinforced responses (e.g., a pigeon's movements between reinforced key pecks or a fish's

swimming trajectories between crossings) could be generated and maintained by adventitious

reinforcement (Skinner, 1948; Pear, 1985).

Two aspects of the specific swimming patterns that emerged throughout the experiment

merit discussion. The first relates to a previous study by Martin (2010) that explored the effects of

delivering non-contingent reinforcement (mirror-images on FT or VT schedules) on the

habituation of bettas' aggressive displays, where bettas typically developed highly stereotyped

lap-swimming in front of a glass wall in which mirror images were presented. The fact that all

bettas displayed similar swimming patterns throughout the present study (starting with the first

baseline – i.e., previous to any exposure to WFs; see Figures 17 - 22), despite the fact that no

mirror images were presented, suggests that unintended reflections may have been occurring in

the glass walls. This would explain why fish consistently returned to lap-swimming behaviour

whenever baseline conditions were established. Moreover, this supports the interpretation of the

data of preliminary studies suggesting that fish reduced their time allocations to a target

compartment in which WFs were delivered contingently (i.e., punishment of crossings to a target

compartment – Chapter VII, *Preliminary experiments 5 and 6*), but continued visiting it as a result

of an unintended availability of reinforcement in that target compartment, perhaps reflections on

the glass wall. As will be discussed, the data regarding swimming patterns coupled with time

allocation to the compartments of the shuttle tank suggests that the escape and avoidance

contingencies of the experiment could have had inadvertent punishing effects.

Second, one particular swimming pattern, which emerged during treatment conditions,

involved regular trajectories between the door and air stones areas (repetitive swimming along the

wall of the divider, going from the connecting door to the air stones wall – see Figure 24). The

fact that an adjustment to the scheduled escape and avoidance contingencies did not explicitly

require that fish C03 and C06 display these specific swimming trajectories suggests that other

factors could have influenced each fish to respond very similarly to their environment. Two

previously discussed variables could account for these homogeneous swimming patterns, namely

sign-/goal tracking effects (Silva et al., 1992) and/or adventitious reinforcement (Skinner, 1948;

Pear, 1985).

Time allocation to compartments

In preliminary studies with the WF experimental paradigm (Chapter VII), each visit of a

subject to a target compartment represented an exposure to the WFs – i.e., WFs were contingent

on visits to a pre-defined compartment. A major finding across those studies was that bettas

dramatically reduced their time allocation to a target compartment in which WFs were delivered,

but continued visiting it for very short periods of time (Chapter VII – Figure 8 and 10); that is, the

aversive contingency affected betta time allocation to the tank compartments. By implementing

free-operant avoidance contingencies using the same WF experimental paradigm it was expected

that time allocations would be affected as well, albeit with a different pattern, since the

contingencies now allowed the fish to explore and spend time in both compartments. Specifically,

and in view of previous research in which goldfish tended to postpone their crossings until near

the end of the R-S interval or the moment at which a warning stimulus was presented, it was

predicted that betta time allocation would be more uniform during signalled, as opposed to

unsignalled, conditions. This is because systematically "waiting" until the end of each R-F

interval to cross would have resulted in fish spending very similar amounts of time in each

compartment on every visit. In addition, it was expected that biases for one of the compartments

during baseline conditions (i.e., when no WFs were scheduled), evident throughout the

aforementioned preliminary experiments, would not be affected by the avoidance contingencies,

since subjects could maintain an overall preference for a specific compartment by crossing

regularly without having to stay too long in the non-preferred side of the tank.

As predicted, betta time allocations to the shuttle tank compartments were systematically

affected by the introduction of free-operant escape and avoidance contingencies that involved

WFs as the aversive stimulus. Moreover, the pattern of such time distributions differed from that

observed in the preliminary studies in which other contingencies were scheduled (e.g., positive

punishment produced extreme time allocations to the compartment in which no WFs were

delivered – Chapter VII, *Preliminary experiments 5 and 6*).

In the present experiment, betta baseline time allocations tended to be extremely biased

(Figure 16a and 16b), favoring one compartment over the other, and either remained consistent

(i.e., similar extreme PRs across consecutive sessions and, sometimes, conditions) or fluctuated

unreliably (i.e., extreme PR changes across consecutive sessions and baseline conditions). These

notably biased time allocations changed to more even and steadier distributions every time the

aversive contingencies were introduced (Figure 16a and 16b). However, this outcome differs from

the prediction derived from the studies of Behrend and Bitterman (1963) and Scobie (1970) in that

betta time allocation did not differ across signalled and unsignalled conditions. In addition, the

prediction that bettas would maintain baseline preferences for one compartment throughout

treatment conditions was not confirmed. Nevertheless, an aspect that limits any conclusion

regarding these predictions is that both were based on the assumption that bettas would show

patterns and levels of avoidance behaviour similar to those demonstrated in research with

goldfish. This was not the case however, as avoidance only occasionally reached levels

approximating or slightly above those of escape, which as previously discussed, was the

predominant response in all fish.

Tentative explanations of the study's main outcomes

One possible explanation for the low incidence of avoidance, the major unexpected

outcome of the study, is that a *warm-up effect* (an initial low rate of response – the *warm-up*

period – followed by its increase and stabilization; Sidman, 1955) could have been involved.

Specifically, it is possible that the length of the experimental sessions (20-min) was not long

enough for the initial warm-up period (if it occurred) to dissipate. Nevertheless, this

interpretation seems unlikely because the within-session temporal distribution of crossings

indicates that avoidance responses did not increase by the end of the session; in fact, these

responses very often appear clustered at the beginning of the session (e.g., last session of SA-2 for

C06 – Figure 22). Moreover, Behrend and Bitterman (1963), though using a different fish species

(goldfish), successfully reproduced signalled and unsignalled free-operant avoidance

implementing daily sessions of same length to those used in this study. Alternatively, a joint

analysis of the data on patterns of time allocation during treatment conditions, within-session

temporal distributions of crossings, and swimming patterns leads to a series of considerations that

ultimately serves to provide a comprehensive interpretation of the outcomes of the study.

First, the even distributions of time across the compartments observed during treatment

conditions appear to be, in fact, the result of an established crossing pattern – changing

compartments at a very regular pace just after the beginning of each WF delivery (indicated by

the staggering pattern in the cumulative records). That is, fish spent nearly the same amount of

time in each compartment due to escaping back and forth in time with the WFs deliveries, which

occurred at regular spacings determined by the R-F interval of 30 s.

Second, time allocations to the compartments during treatment conditions clearly differ

from the pattern that subjects typically displayed during baseline conditions, both in this

experiment and the preliminary studies (biased distributions of time across compartments – e.g.,

Figure 8 and 10 of Chapter VII and Figure 16a and 16b of this chapter). This suggests that the

avoidance and escape contingencies produced a deviation in the fish's otherwise regular activity,

which is evident in the fact that extremely uneven distributions of time resurged every time such

contingencies were removed during baselines (see Figure 16a and 16b). Taking this into

consideration, together with the fact that swimming patterns during baseline conditions tended to

be similar across fish and reappeared whenever treatment conditions ended (highly repetitive lap-

swimming alongside the longer outer walls, whether in one or both compartments; Figures 17 -

24), it seems that the contingencies of the experiment were having inadvertent punishing effects.

Specifically, behavioural patterns typically triggered in these fish by the circumstances provided

by the experimental setup (e.g., certain dimensions of the tank, patterns of illumination, or

unintended reflections on the glass walls) could have been punished during the treatment

conditions. This account seems to be consistent with the fact that lap-swimming, a behavioural

pattern apparently caused by unintended mirror images in the glass walls (as previously

discussed), typically disappeared during treatment conditions and resurged during baselines (e.g.,

swimming patterns of C06 during baseline and treatment conditions – Figure 17).

Third, data regarding time allocation (Figure 16a and 16b), predominant response type

(escape – Figure 15a and 15b), and within-sessions temporal distributions of crossings (Figures

17 - 22) indicate that the fish typically postponed crossing until the onset of each WF, regardless

of the treatment condition, which mirrored the effect that Behrend and Bitterman (1963) observed

in goldfish exposed to signalled conditions (their fish systematically postponed crossing until near the end of the R-S interval when the warning stimulus was present). This suggests that some form of stimulation, distinct from CABs, may have been functioning as a warning stimulus throughout both signalled and unsignalled experimental conditions. In this line of thought, it is plausible that the purported stimulus, perhaps sound or vibrations, may have been produced at some point during water pump activation (at start-up and/or while running). Considering that WFs take some time to gain full strength once they are turned on, subjects were able to avoid the full-strength disturbances by crossing during the first seconds of the WF period. Specifically, this interpretation accounts for the predominance and regularity of crossings during the first seconds of each WF delivery, and classifies them not as escape, but as discriminated avoidance – i.e., an unintentional signalled avoidance contingency was scheduled throughout the experiment. Furthermore, CABs exerted poor stimulus control because other stimuli (related to the water pump activation and/or operation) occurred more contiguous to the aversive stimulus; thus, fish typically postponed their crossing past the CABs periods (first seconds of the WF interval). This interpretation is supported by the observation that the introduction of a warning stimulus during signalled conditions did not substantially change the incidence of late crossings (LA) – i.e., betta behaviour was already under stimulus control.

However, it is unclear how, if at all, the higher levels of early avoidance during unsignalled conditions could relate to this proposed account. One possibility results from considering the biological relevance of CABs; specifically, their potential for eliciting unconditioned responses related to different behavioural functions, such as territorial, parental, or foraging. Craft et al. (2007) proposed that air bubbles could be a "more salient stimulus compared to color or location" (p. 576) for bettas due to the fact that males of this species construct bubble nests on the surface of the water to attract mates, which further serves as territorial marker. It is

therefore plausible that, during signalled conditions, the fish showed fewer crossings during the early portions of the R-F interval as a result of being engaged in CABs-air-stones-related behaviour. For instance, data of the session videos showed that CABs repeatedly elicited different responses, and that fish spent a considerable amount of time in the air stones areas, even during non-CABs periods, very often displaying repetitive swimming behaviour (see Figure 25).

The data of the present experiment raise a number of questions, such as why some of the findings of relevant precedents were not replicated. However, one aspect common to the discussions of the present study's results is that differences in methodology could be responsible for the differential outcomes, especially the lack of demonstration of free-operant avoidance. Among such methodological variations, what seems to be of particular relevance is the fact that in the present study both escape and avoidance responses were functional, whereas in previous studies only avoidance responses were effective in controlling the aversive stimulus. Consequently, it is possible that replicating the avoidance effects observed in the studies of Behrend and Bitterman (1963) and Scobie (1970) requires arranging contingencies that explicitly favour high rates of avoidance, such as changing the consequences for escape responses. The fact that Barker et al. (2010) could only reproduce free-operant avoidance using intense light when escape became nonfunctional supports this notion. Consequently, a second experiment, to be described in the next chapter, was conducted aiming at producing higher avoidance levels by changing the contingencies operating on escape responses. In so doing, it was expected to replicate the effects observed in signalled and unsignalled conditions reported in previous research.

Chapter IX. Experiment 2: effects of scheduling extinction and punishment for escape in free-operant avoidance

Experiment 2 was a follow-up to the primary findings of Experiment 1, which found that: consistent avoidance behaviour was not developed by any of the subjects under either signalled or unsignalled conditions. Instead, a dramatic development and predominance of escape responses occurred across all fish. Experiment 2 was based on these observations and the knowledge that: (a) previous research with bettas using shock-based procedures has demonstrated suppressive effects of punishment (Adler & Hogan, 1963; Fantino et al., 1972; Melvin & Ervey, 1973), (b) preliminary experiments conducted by the author verified that the spatial and temporal organization of betta behaviour is sensitive to contingent presentations of WFs (e.g., a punishment contingency dramatically reduced the time allocation of the subjects to a target compartment and partially suppressed the crossings – see Chapter VII, *Preliminary experiments 5 and 6*), and (c) Barker et al. (2010) could only obtain free-operant avoidance of intense light in rats when this stimulus was made inescapable. Experiment 2 maintained the same avoidance contingencies of Experiment 1, but escape responses, instead of interrupting the WFs, triggered their activation. That is, the contingency programmed in the study had two components – extinction for escape and positive punishment for escape (punishment-and-extinction).

It was expected that extinguishing and punishing escape responses in this experiment would permit the replication of the effects observed in signalled and unsignalled conditions in previous research with other species, including a different species of fish (Behrend & Bitterman, 1963; Scobie, 1970). The effects of punishment across different species, namely target-response suppression and increase of frequency of alternative responses (Azrin & Holz, 1966; Hineline & Rosales-Ruiz, 2013), led to the prediction that this punishment procedure would generate (a) a

suppression of the target behaviour (escape) and (b) an increase in frequency of an alternative

response, in this case, avoidance.

Method

Subjects and apparatus

Fish C03, C05, and C06 from Experiment 1 served in Experiment 2. All aspects of the

apparatus and measures were the same as for Experiment 1. Sessions were conducted between

August and September 2012.

Parameters

Duration of the WFs (10 s), F-F intervals (30 s), CABs presentations (i.e., last 5 s of the R-

F interval during signalled conditions), and R-F intervals for EA, LA, and FFA crossings

remained the same as for Experiment 1. The only difference was that the R-F interval for Esc

responses was set to 0 s (i.e., extinction and punishment for Esc). For instance, if a fish crossed

from the right to left compartment during the delivery of a WF, the WF would stop in the right

compartment and immediately start in the left compartment. During the signalled conditions of

Experiment 2, CABs were not delivered after an Esc response occurred because the R-F interval

for Esc was set to 0. Instead, CABs only appeared during the last 5 s of the R-F interval initiated

by EA, LA and FFA responses.

Procedure and design

Daily sessions for Experiment 2 started the day following the final session of Experiment

1 for each fish. Procedural aspects were the same as for Experiment 1 (length of the sessions,

system set up, transportation of the fish, signalled and unsignalled contingencies, stability criteria,

etc.); the only difference, as already mentioned, was that the duration of the R-F assigned to Esc

responses was 0 s. The punishment WF lasted 10 s, which was the same as for the

avoidance/escape WF. As with the avoidance/Escape WF, the fish could escape the punishment

WF by swimming to the other compartment. But then a punishment WF would occur in that compartment; and so on. Thus, Experiment 2 consisted of UA and SA conditions (as in Experiment 1) in which Esc responses (crossing during a WF) produced the immediate delivery of a WF in the compartment to which the fish crossed. Hereafter, the condition in which signalled avoidance and punishment-and-extinction contingencies for escape were scheduled is referred to as *SA-Pesc*, and the condition in which unsignalled avoidance and punishment-and-extinction for escape contingencies were scheduled is referred to as *UA-Pesc*.

As Table 3 shows, fish C03 was first exposed to SA-Pesc because its last condition during Experiment 1 was SA. Conversely, fish C05 and C06 initiated Experiment 2 with UA-Pesc because their last condition during Experiment 1 was UA.

Table 3.

Scheduled sequence of conditions for each subject during Experiment 2

	Treatment conditions
Fish C03	SA-Pesc UA-Pesc
Fish C05 & C06	UA-Pesc SA-Pesc

Note. UA-Pesc = unsignalled avoidance with punishment-and-extinction for Esc responses; SA-Pesc = signalled avoidance with punishment-and-extinction for Esc responses.

Results

The data of each subject across treatment conditions of Experiment 2 (UA-Pesc and SA-Pesc) regarding (a) overall number of crossings, (b) frequency and duration of WFs, (c) frequency of different types of crossings (Esc, EA, LA, and FFA), and (d) time allocation to the compartments of the shuttle tank are analyzed first. Subsequently, (e) the temporal distribution of crossings and swimming patterns that emerged during baseline and treatment conditions is presented.

Figure 26 shows the number of crossings of each fish during the last baseline and treatment condition of Experiment 1, and the two treatment conditions of Experiment 2 (SA-Pesc and UA-Pesc). The last two conditions of Experiment 1 are included for comparison purposes. Each horizontally arranged graph of Figure 26 shows a subject's crossings across daily sessions, and each condition is represented in the separate vertically arranged panels.
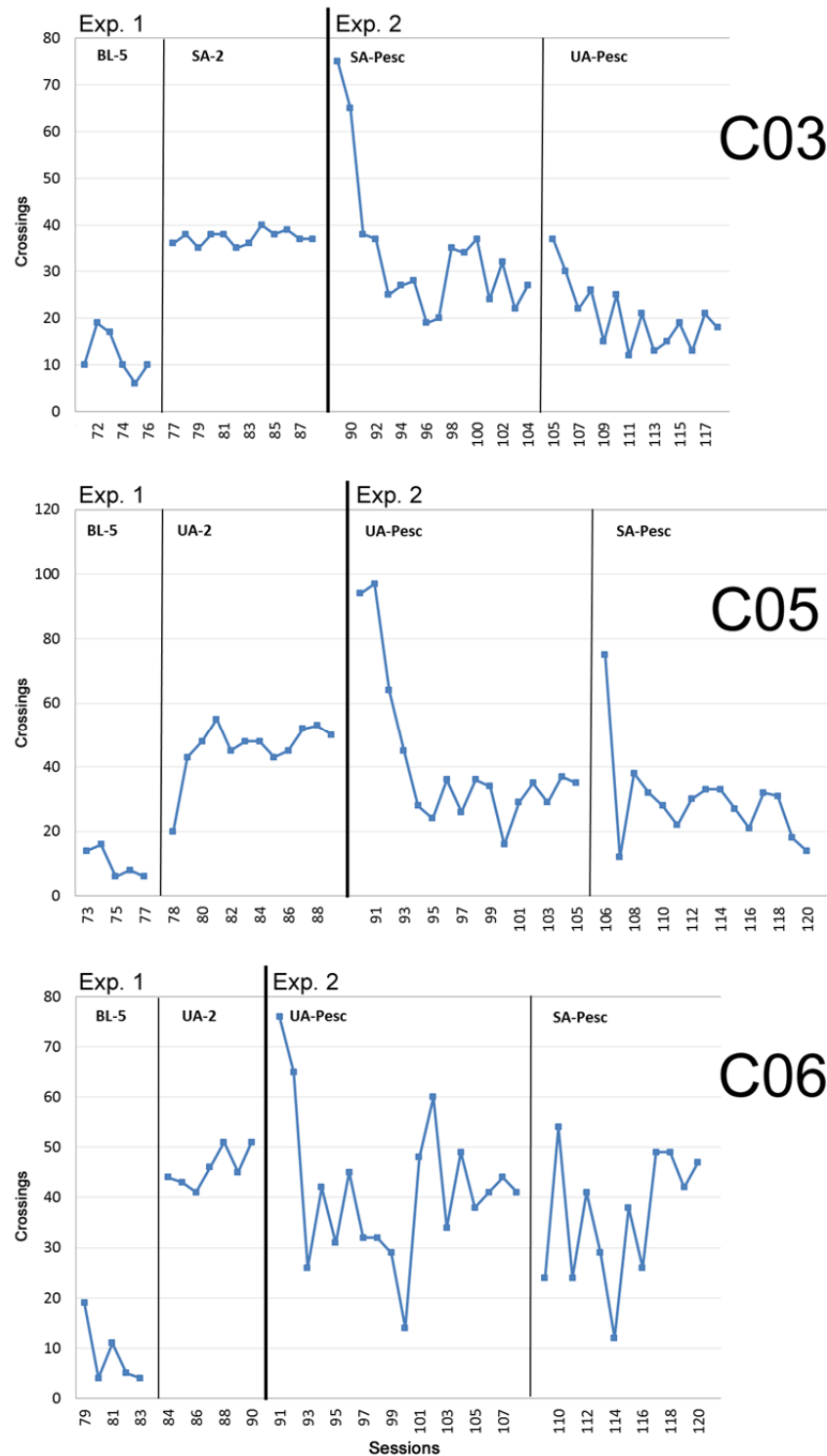
Figure 26. Overall number of crossings of fish C03, C05, and C06 during the last two conditions of Experiment 1 and during treatment conditions of Experiment 2. Every horizontally arranged graph shows a subject's crossings throughout daily sessions, and each condition is represented in a separate vertically arranged panel. For instance, the top graph shows C03's data and the third panel of this graph represents the first treatment condition of Experiment 2 (SA-Pesc).
Note. BL-5 = Baseline five (Experiment 1); UA-2 = Unsignalled avoidance 2 (Experiment 1); UA-Pesc = unsignalled avoidance with punishment-and-extinction for Esc (Experiment 2); SA-Pesc = signalled avoidance with punishment-and-extinction for Esc (Experiment 2).

Note that all three fish began the first condition of Experiment 2 with a dramatic increase in the number of crossings above the level observed during the last condition of Experiment 1. Throughout the condition, this frequency of crossings proceeded to decrease and stabilize at or below levels of the last condition of Experiment 1. The change from UA-Pesc to SA-Pesc or from SA-Pesc to UA-Pesc in the last condition of Experiment 2 had no clear or consistent effect across fish. In general, as in Experiment 1, there was no clear difference between signalled and unsignalled conditions.

Figure 27 shows the frequency and duration of WFs, expressed in percentages, for each fish during the last condition of Experiment 1 and both conditions of Experiment 2 (UA-Pesc and SA-Pesc). Percentages were calculated assuming that a total absence of crossings during an entire 20-min session would have resulted in the fish being exposed to 30 instances of WFs and a total of 300 s of flow time (the horizontal dashed line indicates the 100% level for both variables). For instance, if a fish in a given session received 30 instances of WFs and was exposed to a total of 200 s of flow time, these values correspond to 100% of WFs (30/30 x 100) and 66.7% of total flow time (200/300 x 100). It should be noted that, as a result of implementing a punishment contingency in Experiment 2, as in Experiment 1, the fish could receive considerably more than 100% WFs flows and flow time.
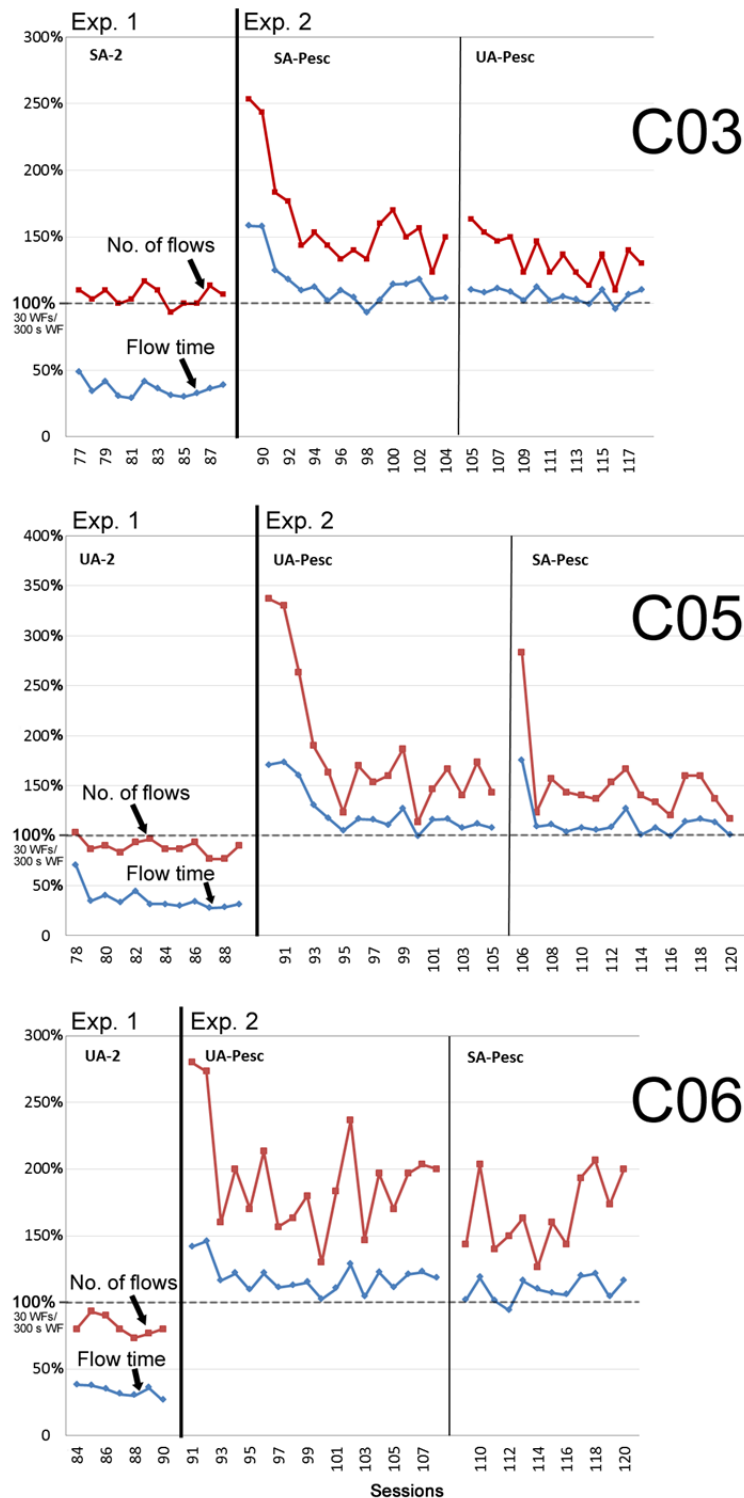
Figure 27. Frequency and duration of WFs during the last condition of Experiment 1 (UA-2 for C05 and C06 and SA-2 for C03) and both conditions of Experiment 2 (UA-Pesc and SA-Pesc). Every horizontally arranged graph shows each subject's data throughout daily sessions, and each condition is represented in a separate vertically arranged panel. Frequency and duration of WFs are expressed in percentages, and refer to the total number of WFs and total flow time for each session and for each fish. Percentages were calculated assuming that a total absence of crossings would have resulted in the fish being exposed to 30 instances of WFs and 300 s of flow time (the horizontal dashed line indicates 100 % level for both measures).

As Figure 27 shows, both number of WFs and WF duration per session across conditions closely mirrored the number of crossings over the conditions shown in Figure 26, coinciding with the addition of the punishment-and-extinction contingency. In addition, the level of these two variables decreased throughout both conditions of Experiment 2, and stabilized well above their levels during the last condition of Experiment 1. Again, there were no clear differences between UA-Pesc and SA-Pesc.

Figure 28 shows the frequency of each type of crossing (Esc, EA, LA, FFA) displayed by each fish during the last treatment condition of Experiment 1 and both treatment conditions of Experiment 2. Similar to Figure 26, every horizontally arranged graph of Figure 28 shows a subject's data throughout daily sessions, and each condition is represented in separate vertically arranged panels. In addition, the last condition of Experiment 1 is included for comparison purposes. Changes of compartment that occurred during the last five sec of the R-F interval were counted as late avoidance (LA), regardless if the condition was unsignalled avoidance (no warning stimulus) or signalled avoidance (CABs presented during the last 5 s of the interval).
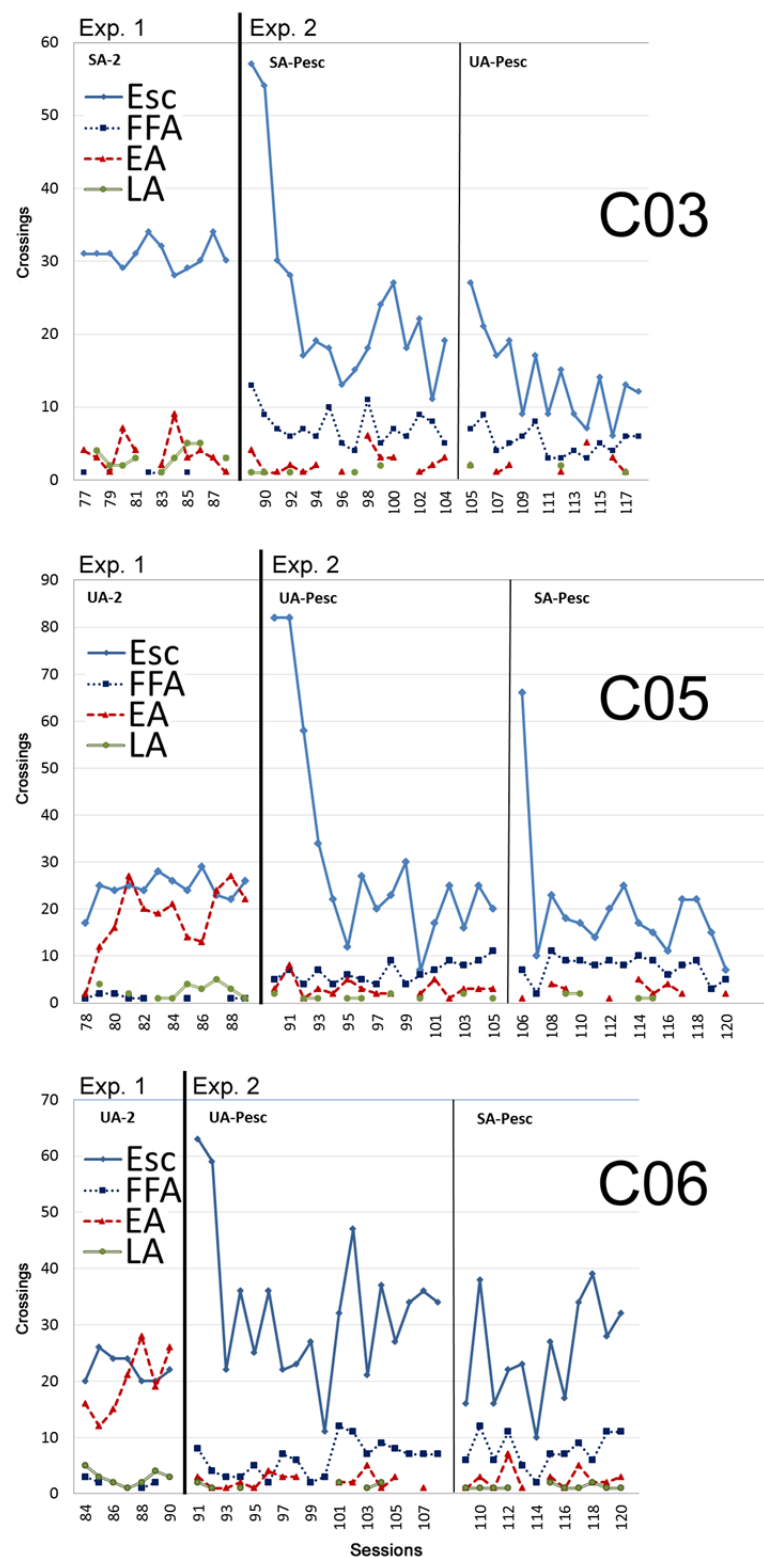
Figure 28. Frequency of different types of crossings during last condition of Experiment 1 and treatment conditions of Experiment 2 (UA-Pesc and SA-Pesc). Every horizontally arranged graph shows each subject's data throughout daily sessions, and each one of the conditions is represented in a separate vertically arranged panel.
Esc = escape; EA = early avoidance; LA = late avoidance; FFA = flow-flow avoidance.

As Figure 28 shows, all three fish initiated Experiment 2 with a dramatic increase in the

number of Esc responses, that doubled (C03) or tripled (C05 and C06) the levels observed during

the last condition of Experiment 1. The frequency of Esc crossings then decreased until they were

at or below the levels of the last condition of Experiment 1. FFA was the second most frequent

response type displayed by each fish throughout Experiment 2. The incidence of this type of

crossing stabilized in both treatment conditions at levels that were considerably higher during

both conditions of Experiment 2, than in the last treatment condition of Experiment 1. Compared

to the other types of responding, the frequency of EA and LA responses was considerably lower

during both conditions of Experiment 2. Finally, there appears to be little or no difference

between any of the different types of responding as a function of the signalled or unsignalled

conditions.

Figure 29 shows each subject's time allocation to the right and left compartments of the

experimental tank during last conditions of Experiment 1 and treatment conditions of Experiment

2. Similar to Figure 26, the last two conditions of Experiment 1 are included, as they provide

additional comparison points for the analysis. Time allocation is expressed as the preference ratio

(PR), which, as already described, was calculated as the difference between the time the fish spent

on the right compartment and the time the fish spent on the left compartment over the total session

time (i.e., PR = [Time Right – Time Left] / Session Time). As a result, preference for staying in

the right compartment is indicated by PR > 0.0, whereas a preference for the left compartment is

indicated by PR < 0.0.
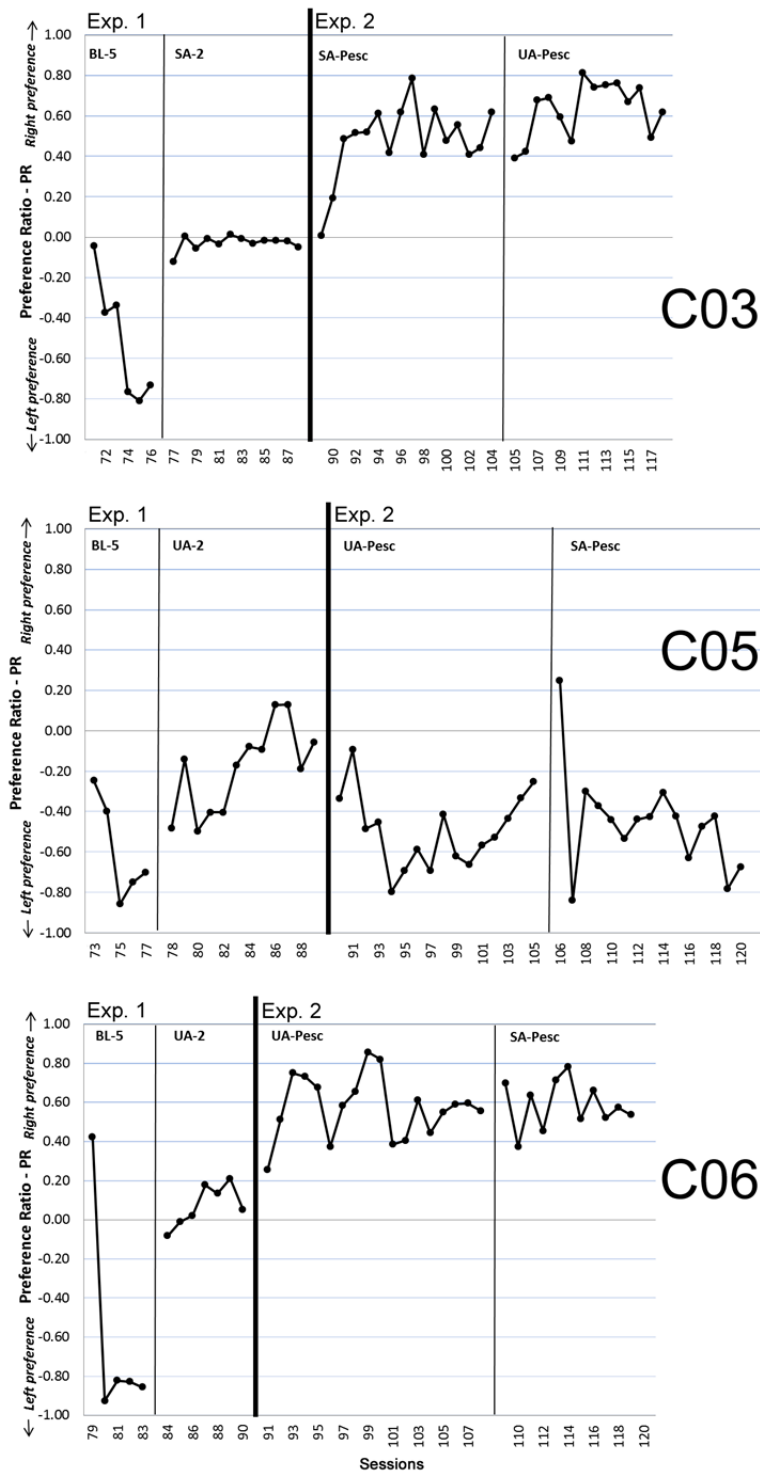
Figure 29. Fish C03, C05, and C06's time allocation to right and left compartment of the experimental tank during last conditions of Experiment 1 (BL-5, SA-2, and UA-2) and treatment conditions of Experiment 2 (UA-Pesc and SA-Pesc). Every horizontally arranged graph shows a subject's data throughout daily sessions, and each condition is represented in a vertically arranged panel. Time allocations are expressed in preference ratios – PR, which consisted of the difference between the amount of time the fish spent on the right compartment of the tank and the time that the fish spent on the left compartment over the total session time (i.e., PR = [Time Right – Time Left] / Session Time). Preference for staying in the right compartment is indicated by PR > 0.0, whereas a preference for the left compartment is indicated by PR < 0.0.

As shown in Figure 29, throughout both conditions of Experiment 2 each fish showed a clear preference for either compartment that started to develop immediately after the punishment-and-extinction contingency was introduced (as this was not present during Experiment 1's treatment conditions). Moreover, it is important to note that a similar consistent preference for one compartment was also evident during the baseline conditions of Experiment 1.

Figures 30, 31, and 32 show C03, C05, and C06's (respectively) temporal distributions of crossings and swimming patterns during the last session of each condition of Experiment 2. The last two conditions of Experiment 1 are also included (left column) for comparison purposes. More specifically, the top row of graphs in each figure, coupled with the adjoining horizontal bands, correspond to cumulative records of crossings that occurred during the corresponding 20-min session.

CABs and WF periods and escape or avoidance crossings are shown as vertical bars or lines on each corresponding band ("CABs", "WF", "ESC", and "AV", respectively). Thicker response bars indicate a higher density of crossings. The middle and bottom rows of graphs show XY ("overhead") plots of the swimming trajectories described by the fish during both non-WF and WF periods of the corresponding session. WF periods were plotted separately because the duration of WFs increased substantially during Experiment 2. Dotted lines on each plot indicate the location of the tank's divider and door, while horizontal solid bars represent the air stones.
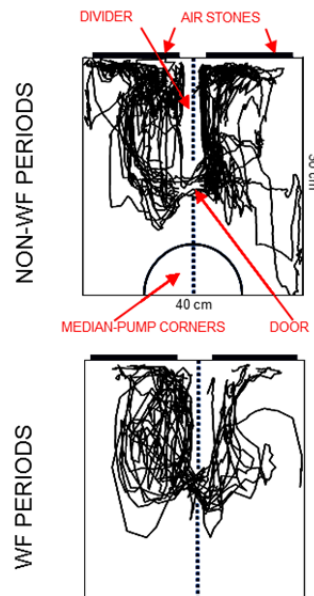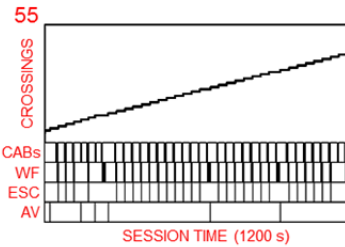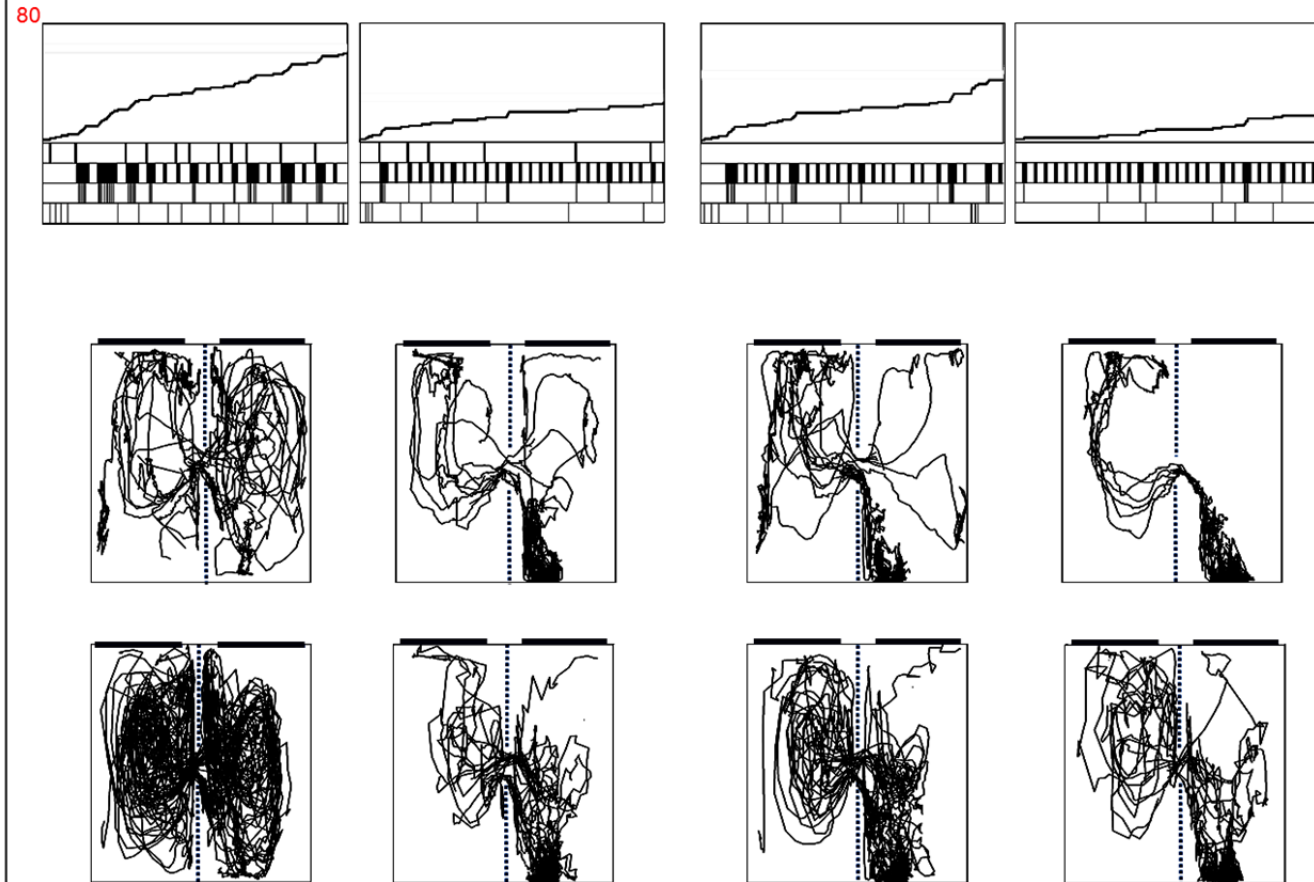
Figure 30. Graphs on the top row show the cumulative records of fish C03's crossings during the last session of Experiment 1, and the first and last session of each condition of Experiment 2 (SA-Pesc and UA-Pesc). Bars on the horizontal bands show the occurrence of CABs periods ("CABs"), WF periods ("WF"), escape crossings ("ESC") and avoidance crossings ("AV"). When clustered, indicating repeated occurrences, such bars form a solid strip. The lower two rows show XY (overhead) plots of the swimming trajectories during non-WF and WF periods of each corresponding session. Dotted lines indicate dividing wall and door, solid bars indicate air stones, and the semi-circle indicates two corners in both compartments formed by the intersection of divider and water pump wall, referred to as the "median-pump corners".
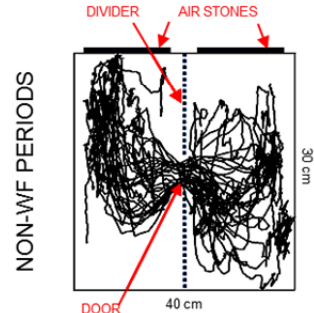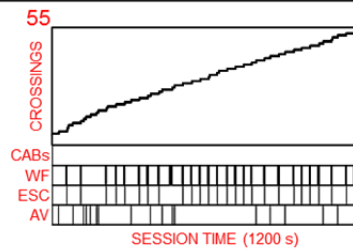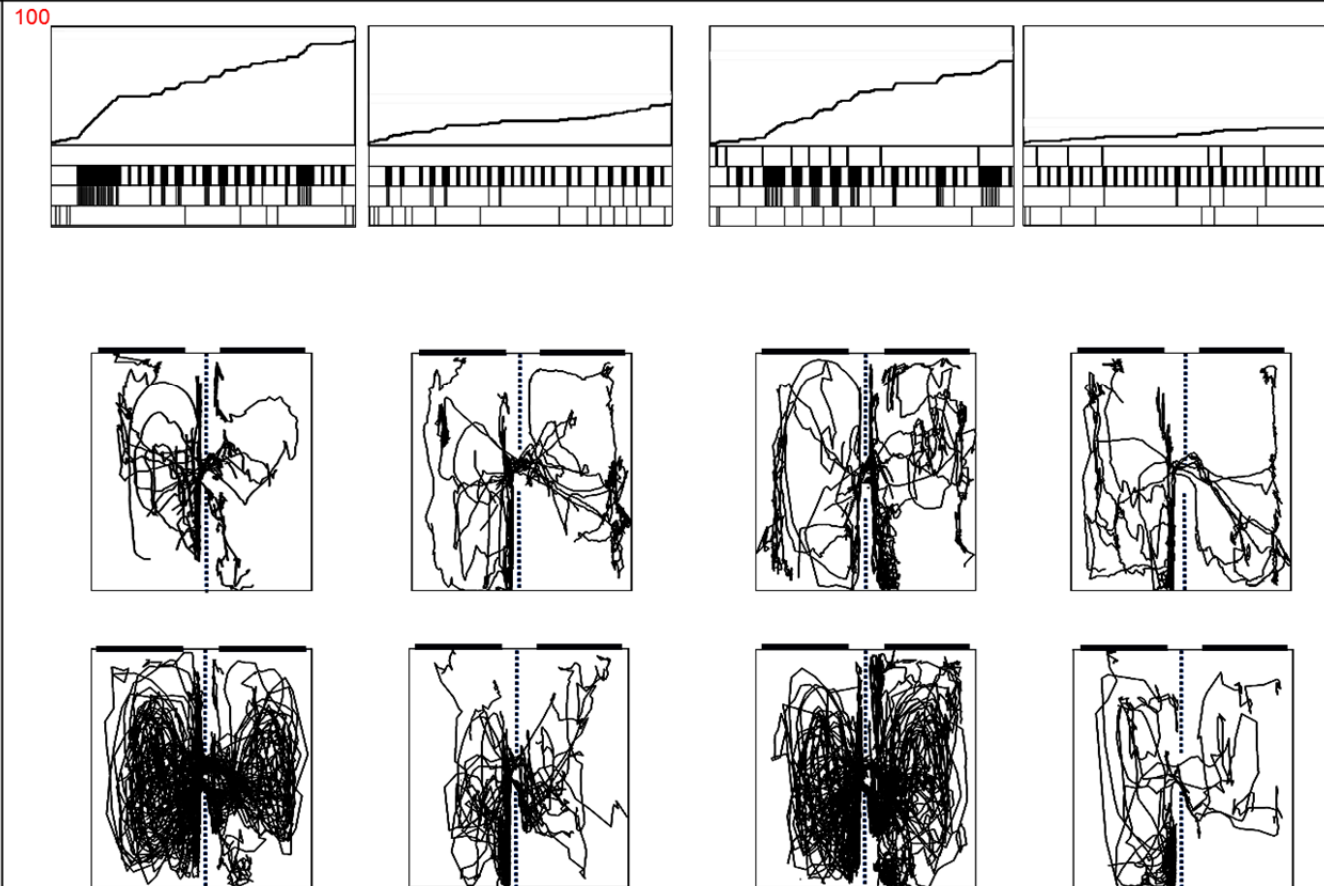
Figure 31. Graphs on the top row show the cumulative records of fish C05's crossings during the last session of Experiment 1, and the first and last session of each condition of Experiment 2 (SA-Pesc and UA-Pesc). Bars on the horizontal bands show the occurrence of CABs periods ("CABs"), WF periods ("WF"), escape crossings ("ESC") and avoidance crossings ("AV"). When clustered, indicating repeated occurrences, such bars form a solid strip. The lower two rows show XY (overhead) plots of the swimming trajectories during non-WF and WF periods of each corresponding session. Dotted lines indicate dividing wall and door and solid bars indicate air stones.
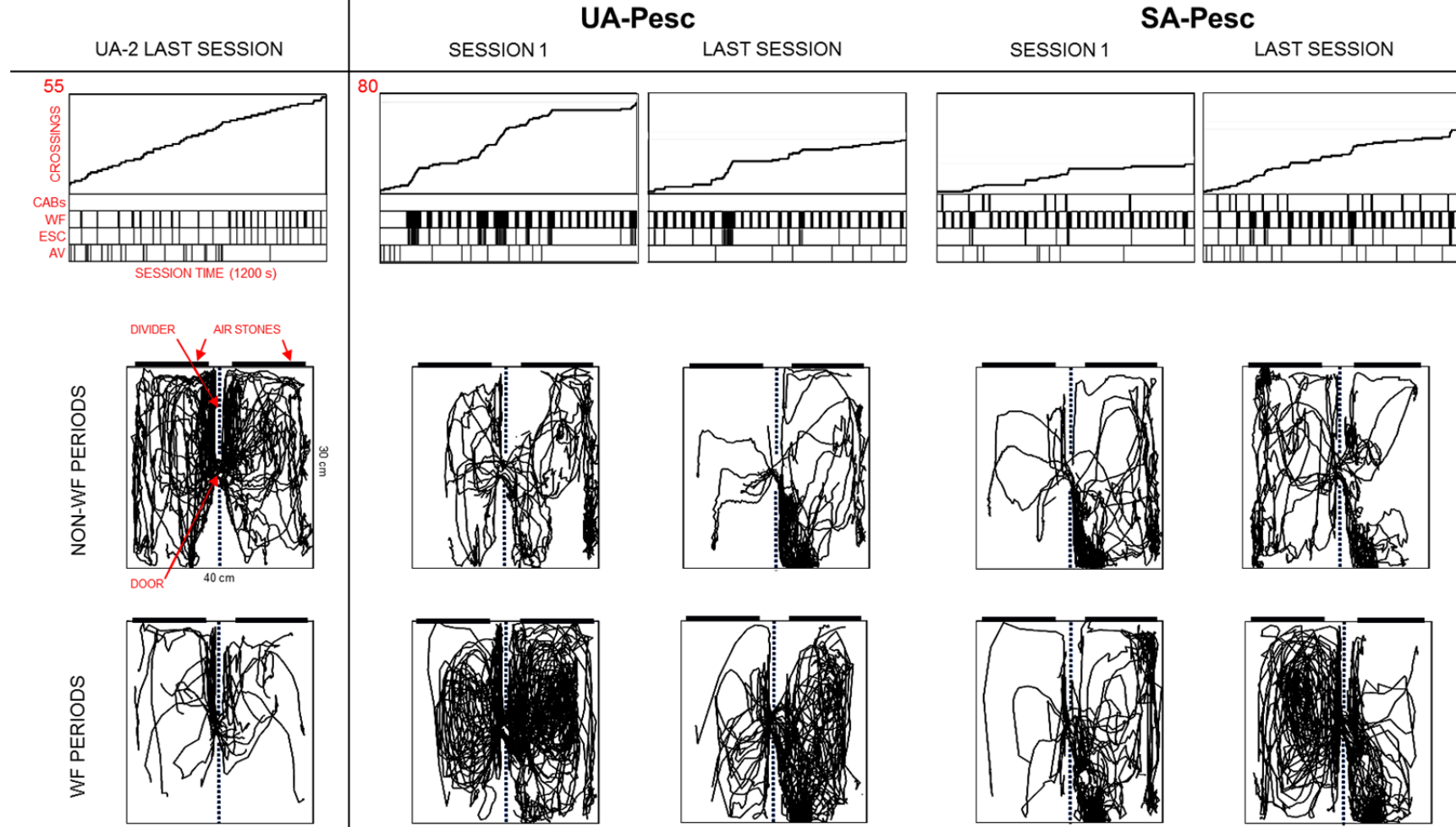
Figure 32. Graphs on the top row show the cumulative records of fish C06's crossings during the last session of Experiment 1, and the first and last session of each condition of Experiment 2 (SA-Pesc and UA-Pesc). Bars on the horizontal bands show the occurrence of CABs periods ("CABs"), WF periods ("WF"), escape crossings ("ESC") and avoidance crossings ("AV"). When clustered, indicating repeated occurrences, such bars form a solid strip. The lower two rows show XY (overhead) plots of the swimming trajectories during non-WF and WF periods of each corresponding session. Dotted lines indicate dividing wall and door and solid bars indicate air stones.

As shown in the cumulative records on the top rows of Figures 30 - 32, all three fish showed that the consistent crossing pattern characteristic of the treatment conditions of Experiment 1 (first cumulative record of each figure) was immediately affected by the introduction of the punishment-and-extinction contingency of Experiment 2 (i.e., Session 1 of the first treatment condition). Specifically, the very regular crossing pace that coincided with the onset of each WF delivery (hence the interspersed and consistent replication of bars in bands "WF" and "ESC", and the steady increase in the curve of the cumulative record) changed immediately to an irregular distribution of responses with the introduction of the punishment-and-extinction contingencies of Experiment 2 (however, note the difference in the upper limit of crossings for the cumulative records of each subject during Experiment 2). This uneven crossing pattern, evident in all three fish, was characterized by (a) numerous sequential escapes ("escape bursts") followed by periods of no crossings, and (b) a few scattered instances of avoidance. That is, the fish engaged in quick back-and-forth crossings initiated by the onset of a given WF, sometimes reaching more than 15 consecutive compartment changes (indicated by the steep increases in the cumulative record and dense bars on band "ESC"), which produced the delivery of several consecutive WFs (indicated by dense bars on band "WF"). Following an escape burst, a fish remained in one compartment for the duration of one to several F-F intervals, then typically engaged in a subsequent burst, or, though much less frequently, changed compartments during an F-F interval (evidenced by scattered bars on band "AV").

By the end of the first treatment condition, all fish substantially reduced the incidence and duration of the escape bursts, as observed in the cumulative record for the last session of each subject's first condition (Figure 30, 31 and 32). This is evident in the more flattened curves, together with shorter and consecutive WF periods (bars on the "WF" band") without

accompanying escape crossings (bars on the "ESC" band). However, this change in distribution of escape occurred without any noticeable variation in avoidance (band "AV"), which continued to be scattered without any noticeable pattern.

The cumulative records corresponding to the first session of the second treatment condition of fish C03 (UA-Pesc) and C05 (SA-Pesc) showed a return to the pattern observed during the first session of Experiment 2; that is, an increment in the incidence of escape bursts was noticeable in these fish during the first session of the second condition. However, by the end of the experiment, the cumulative records of these fish showed again flat curves and practically no escape bursts (see last cumulative record of Figure 30 and 31). Conversely, this trend in escape bursts was not observed in C06.

The swimming trajectories identified during Experiment 1 ("twisting" trajectories in fish C05, and swimming along the dividing wall in fish C03 and C06) disappeared during the first session of Experiment 2 (corresponding plots of Figures 30 - 32). Specifically, all three fish showed unsystematic trajectories during their first session (see Figures 30 - 32), which in the case of WF periods appeared to primarily follow the elliptical current of flows in the shuttle tank.

As seen in the bottom two rows of Figures 30 - 32, a distinct swimming pattern was noticeable for all fish (though never entirely stable, as indicated by the fact that they changed, to some extent, within and across sessions). This pattern could be described as lap-swimming between the door and the intersection of water pump walls and divider (the "median-pump corner"; see Figure 30) all while remaining close to the dividing wall, and interspersed periods of immobility in that same corner. This pattern, hereafter referred to as "door-corner swimming", occurred more consistently in the right compartment for fish C03 and C06, and in the left compartment for fish C05. Moreover, this pattern was clearly discernible in the non-WF periods

of all fish, as well as during the WF deliveries of C03 and C06 (though less apparent in C06). A

final matter of note, evident in the comparison of overhead plots for WF and non-WF periods, is

the fact that the presence of WFs generated a much greater number of crossings between

compartments.

Discussion

The purpose of Experiment 2 was to follow-up on the primary finding of Experiment 1; namely, that free-operant avoidance was not reliably established, notwithstanding the extensive training to which all fish were exposed. This outcome was particularly unexpected considering that previous research with other species, including fish, has consistently replicated such aversive control phenomena. Conversely, the results of Experiment 1 showed a dramatic increase and predominance of escape responses across all fish. Whereas subjects developed a highly regular response pattern that consisted of changing compartments during the first seconds of each WF, avoidance remained at low levels, and only occasionally matched or slightly exceeded levels of escape. As a result, fish were successful in reducing the time exposed to WFs but not the number of actual WFs presentations.

One aspect that was common throughout the discussion of the findings of Experiment 1 was that major procedural differences with respect to the relevant research antecedents could have been responsible for the absence of free-operant avoidance using WFs as the aversive stimulus. One procedural difference that seemed to be of particular relevance was the fact that both types of responses – escape and avoidance – were functional, whereas in previous research, only avoidance was effective in controlling the aversive stimulus (due to its short duration). Accordingly, it was thought that replicating the avoidance effects observed in the studies of Behrend and Bitterman (1963) and Scobie (1970) with goldfish required arranging contingencies that explicitly favoured high rates of avoidance, such as changing the consequences for escape. The fact that Barker et al. (2010) could only produce free-operant avoidance in rats using intense light when it became inescapable strongly supported this notion. Experiment 2 thus aimed at producing higher avoidance levels by changing the contingencies operating on escape responses;

namely, stipulating that crossing during a WF would trigger a new WF in the destination

compartment – i.e., a two component positive-punishment and extinction contingency.

Considering that two major behavioural effects of scheduling extinction and punishment are the

decline in the frequency of the target response and the increase of alternative responses (Azrin &

Holz, 1966; Hineline & Rosales-Ruiz, 2013), it was predicted that the procedure would produce

substantial decrements in escape behaviour in exchange for increased in frequency of alternative

responses – in this case, avoidance.

All fish in this experiment saw an immediate and dramatic increase in frequency of

escape (see Figure 28), which began to decline in the second session. In the case of C03 and

C05, escape continued to drop throughout the conditions, with the exception of the first session

of the second condition, which saw a burst of escape responses similar to that of the first session

of the first condition. By the end of the experiment, the incidence of escape in fish C03 and C05

was clearly below levels observed throughout the treatment conditions of Experiment 1.

Alternatively, C06's incidence of escape remained very unstable throughout the study,

fluctuating at levels below and above those occurring during the last condition of Experiment 1.

Regarding the other types of behaviour, despite a low incidence of early and late avoidance (not

occurring at all in several sessions) F-F avoidance was more consistent and increased in

frequency above levels observed throughout Experiment 1. However, such increments were not

substantial and never surpassed those of escape.

These patterns of crossings caused all subjects to start the experiment with nearly a three-

fold increase in the frequency and duration of WFs as compared to Experiment 1, which declined

as sessions progressed (see Figure 27). Nevertheless, by the end of the experiment subjects were

typically receiving a number of WFs above the originally scheduled amount, and were exposed

to WFs in a way that would be expected for a subject that did not cross at all during the experiment (i.e., ~100% of the programmed WF time per session). Finally, introducing or removing the warning stimulus (i.e., changing from a signaled to an unsignalled condition and vice versa) only had a noticeable effect in the first session of fish C03 and C05, which showed a substantial increase in escape, similar to that observed at the start of the experiment.

These findings provide additional evidence that crossing responses in Experiment 1 were indeed maintained by a negative reinforcing contingency between the interruption of WFs and the changing of compartments, as indicated by the fact that all fish showed instances of *extinction burst,* a well-established phenomenon that is characteristic of the extinction of an operant response (Cooper, Heron, Heward, 1987; Pierce & Cheney, 2013). Specifically, a within-session analysis of the temporal distribution of crossings (cumulative records data; Figure 30 - 32) showed that the substantial increase in compartment changes at the beginning of Experiment 2 represented the occurrence of escape bursts, where fish engaged in several back-and-forth crossings triggered by an initial WF. As the experiment progressed, such escape bursts progressively declined in incidence and duration, which further supports the notion that the punishment-and-extinction contingency had indeed an effect on the escape-maintained behaviour.

However, the fact that escape gradually decreased and did not reach complete suppression suggests that, out of the two components of the contingency programmed in the study – extinction and punishment – the latter had a weak, or perhaps negligible effect on the subjects' behaviour. This interpretation is based on previous research (Azrin & Holz, 1966) that compared the efficacy of different procedures to reduce response rates, and reported that effective punishment differed from extinction in that it typically produces complete suppression

and has immediate effects. As Hineline and Rosales-Ruiz (2013) have put it, "punishment applications should work fairly quickly. If they do not, one or more of the [variables known for modifying the effectiveness of punishment after Azrin and Holz's (1966) review] need adjustment" (p. 487). The classification of such variables in terms of the (a) characteristics of the punishing stimulus, including its intensity and schedule; (b) control over the maintaining variables of the punished behaviour; (c) availability of alternative behaviour; and (d) availability of alternative sources of reinforcement provides a useful framework for interpreting the data of the present study. It should be noted, however, that practically all of the studies reviewed by the above authors used shock as the aversive stimulus.

Regarding the characteristics of the punishing stimulus ("a" above), this factor does not seem to be responsible for the weak suppressive effect of the punishment contingency, as the procedure of Experiment 2 entailed that every instance of the target response (escape) triggered the same WFs that were effective in the previous experiment in maintaining this target response under a negative reinforcing contingency. This suggests that the aspects of the punishing stimulus (WF), by remaining unchanged from one experiment to the other, should have favored a suppression of the target behaviour by the punishment contingency. Similarly, lack of control over the maintaining variables of escape ("b" above) is unlikely to explain the absence of response suppression because Experiment 2 precisely entailed the withholding of the reinforcement that maintained escape during Experiment 1 (by establishing that crossings during a WF no longer interrupted this stimulus).

Regarding the possibility that the absence of an alternative behaviour could explain why the punishment contingency was not effective ("c" above), this factor also appears to not be responsible since the experimental settings were arranged so that avoidance (a behaviour that

was already in the repertoire of the subjects and occurred, albeit infrequently, during

Experiment 1) was functional in producing the same reinforcement originally generated by

escape – i.e., it was an available alternative response, with the same consequence in terms of

producing the identical amount of reprieve from WFs. In fact, the prediction was that avoidance

responses would have increased, given their alternative function.

Finally, considering that the findings of the preliminary experiments (Chapter VII) and

Experiment 1 have been, to a certain degree, consistent with the notion that unintended

reinforcement could have influenced the adjustment of fish to the experimental contingencies

(e.g., mirror reflections could have induced lap-swimming in Experiment 1), it is worth

exploring the possibility that alternative sources of reinforcement could have inadvertently

competed with the punishment-and-extinction contingencies of Experiment 2 ("d" above),

resulting in the observed lack of suppression of escape responses.

Potential alternative sources of reinforcement for escape

In discussing the data of Experiment 1, it was proposed that stimuli possibly produced by

the operation of the water pumps (e.g., acoustic, mechanical, and/or electromagnetic) could have

acquired discriminative functions as a result of being paired with the onset of the WFs and/or as

a result of predicting the full strength of the WFs (i.e., pump-related stimuli may have been

functioning as warning stimuli), hence fish showed reliable crossings during the first seconds of

the WF periods. In view of the fact that the acquisition of discriminative functions also entails

the establishing of conditioned reinforcing functions for the same stimuli (Pierce & Cheney,

2013; Pear, 2001), it is possible that pump-related stimuli not only continued exerting stimulus

control during Experiment 2, but also functioned as conditioned reinforcers, especially in view of

their long history of predicting both the onset and termination of WFs. In other words, it is

plausible that pump-related stimuli acquired discriminative and conditioned reinforcement functions during Experiment 1 that were carried over to Experiment 2. This is consistent with the view that the behavioural effects observed throughout Experiment 2 were extinction-related, instead of outcomes of the punishment component of the contingency, as the progressive declining trend observed in escape (Figure 28) resulted from those functions becoming extinguished. Specifically, whereas the occurrence of escape-bursts at the beginning of the experiment is not consistent with the view that the punishment component was effective, their reappearance in C03 and C05 during the second treatment condition (Figure 28) is congruent with the notion that a discriminated operant was being extinguished. For instance, it is possible that the change of conditions (going from SA-Pesc to UA-Pesc, and vice versa) involved a variation in the experimental settings (CABs were reintroduced in the case of C05, and removed in the case of C03) that could have triggered a well-established phenomenon of extinction known as *spontaneous recovery* (Pierce & Cheney, 2013).

Also consistent with this account is the fact that, during Experiment 2, the delay between water pump activations and WFs reaching their full strength remained unchanged from Experiment 1, which led to the notion that such a delay allowed the subjects to avoid full-strength disturbances by crossing during the first few seconds of the WF period. This appears to be linked to the ineffectiveness of the punishment component, in terms of a slow and irregular decline of escape throughout the experiment. Specifically, it appears that escaping (a) kept producing a somewhat reinforcing consequence (briefly interrupted the WFs), (b) allowed fish to escape from punishment (full-intensity WFs), and (c) permitted fish to continue experiencing pairings of the pump-related stimuli and WF interruptions, hence the presumed functions of such stimuli being extinguished at a slow pace. However, it is plausible that these reinforcing

variables may not have been enough to maintain escape throughout Experiment 2, especially

considering that it entailed fish constantly and successively changing compartments during the

first seconds of the WFs (as observed in escape bursts), which may have been a markedly

strenuous situation for the subjects. A result of such circumstances seems to be that fish

developed other response patterns throughout the experiment that allowed them to more

efficiently reduce their exposure to the WFs.

Swimming patterns and alternative behaviour

A supplementary interpretation of the results of the study, contributing to the

understanding of the trend of escape and, more importantly, of the fact that avoidance behaviour

did not increase to the expected levels, is related to how certain swimming patterns, interpreted

as alternative behaviour, could have produced unintended reinforcement. This notion results

from the spatiotemporal data collected in the study, namely, patterns of time allocation to the

compartments and the emergence of systematic swimming trajectories.

During Experiment 2, all fish progressively developed a pattern of time allocation that

consisted of a marked and steady preference for one of the compartments of the shuttle tank (C03

and C06 favoured the right compartment, and C05 favoured the left compartment – Figure 29).

When looking at the swimming trajectories associated with this preference, it is evident that

distinctive patterns, different from those identified during Experiment 1, emerged and persisted

with a certain degree of consistency throughout the study (Figure 30, 31, and 32). All three fish

changed from displaying very unsystematic trajectories during the first session of Experiment 2

to show a pattern, during both WF and non-WF periods, that consisted of door-corner swimming

on either side of the divider. Careful inspection of the experimental setup indicated that WFs

have a reduced strength in the areas associated with these swimming patterns, due to being at the

farthest extension of a current that first bounces against the wall opposite to the water pump (see

Figure 33 below). Consequently, it is plausible that particular behavioural patterns related to

such areas developed in all fish because they allowed them to reduce their exposure to high-

intensity WFs.
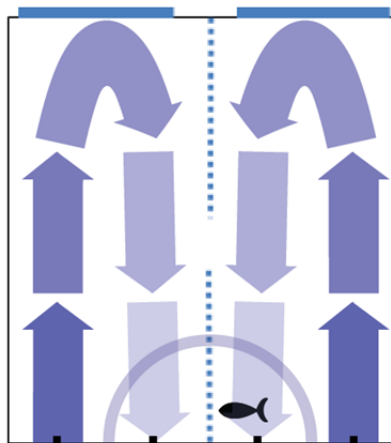
Figure 33. Schematic representation of the direction of WFs inside the experimental tank, and
areas where their strength was reduced due to being at the farthest extension of a current that first
bounces against the wall opposite to the water pump. Dotted lines indicate dividing wall and
door, solid bars indicate air stones, small black squares indicate the pump nozzles, and the semi-
circle indicates corners in both compartments formed by the intersection of divider and water
pump wall, referred to as the "median-pump corner" (area associated with door-corner
swimming – see Figure 30).

In addition, it is possible that swimming patterns related to areas with reduced WF

intensity could have been reinforced by the fact that by enduring a complete WF delivery (10 s),

fish gained access to 30 s of no WFs, corresponding to the F-F interval. From this perspective,

door-corner swimming could be seen as alternative response that was established and maintained

by a reduction in WF intensity and access to the same amount of WF-free time that had earlier

been produced with escape responses. A series of aspects seem to support this interpretation.

First (and as mentioned earlier), fish were withstanding several consecutive WFs without

crossing by the end of the experiment; in other words, fish were going through several successive

cycles of WF (10 s) and non-WF (30 s) periods (as shown in cumulative records of Figure 30,

31, and 32). Second, Experiment 2 showed higher and steadier frequencies of FFA crossings

than those observed in Experiment 1 (Figure 28). From a response-deprivation perspective,

which establishes that restricted access to a behaviour below its free-choice level makes access to

that behaviour a positive reinforcer (Timberlake & Allison, 1974), the postponement of crossings

to the F-F interval (by enduring complete WFs) allowed fish to reach baseline levels of crossings

(e.g., Experiment 1 showed that fish displayed similar and steady minimum levels of crossings

across baseline sessions, to which they rapidly returned whenever the contingencies were

removed – see Figure 13b, BL-4, SA-1, and BL-5 of fish C06). Third, responses analogous to

door-corner swimming were identified throughout preliminary experiments (Chapter VII), in

which bettas developed preferences for areas of the tank where they remained immobile and

which seemed to reduce their exposure to WFs (e.g., a fish developed a pattern that consisted of

remaining motionless in a gap between the tank wall and the water pump covers for considerable

periods of time – see Chapter VII, *Preliminary experiment 3 - 5*). Additional support for this

interpretation comes from the study by Verbeek et al. (2008), which showed that the behavioural

strategy to which bettas tend to resort to when reacting to challenging situations (e.g., unfamiliar

environments and confinement) involves immobility. Extending Verbeek et al.'s (2008) notion

that "remaining immobile while being faced with inescapable confinement is likely to be an

adaptive strategy linked to metabolic rate" (p. 86), it is plausible that the particular behavioural

patterns developed by subjects of the present and preliminary experiments could also be related

to adaptive strategies intended to regulate energy expenditure in the face of stressors.

Finally, the account discussed thus far is consistent overall with what has been observed

in research with rats exposed to shock-based procedures, where subjects develop unspecified

responses that allow them to reduce exposure to, or fully avoid an aversive stimulus. A

prominent example was provided by Azrin and Holz (1966), in which "a rat learned to press a

response lever with its foot while lying on its back, thereby eliminating the possibility of shock

while still producing the food pellets" (p. 384). Other examples are the "awkward, but shock-

reducing, posture [consisting of] putting only one foot on the grid" (Azrin & Holz, 1966, p. 384),

and the report of Imada, Shimai, and Imada (1981), in which one of their rats exposed to brief

shocks (0.3 s) "could jump up effectively to reduce the shock duration to… which it would have

received otherwise" (p. 52). Considering that it is unlikely that the WFs used in this study were

comparable in quality or intensity to the shocks used in the above-mentioned studies, it is notable

that analogous effects could be produced.

Chapter X. General discussion and conclusions

Research on spatiotemporal analysis of behaviour has demonstrated over the past decades

that a comprehensive understanding of behaviour and learning phenomena requires an approach

that includes, but is not limited to, the study of rates of discrete responses. By developing and

implementing the WF experimental paradigm for *Betta splendens* throughout a series of studies,

the scope of the spatiotemporal program was extended to aversive control phenomena,

something that had no precedent. Throughout this research, the comprehensive analysis of

frequency and temporal distribution of behaviour, incidence and duration of the aversive

stimulus (WF), patterns of swimming trajectories, and occurrence of various responses related to

different features of the experimental tank provided a more enriched interpretation of the

outcomes of the experiments than if only the traditional focus on the rate of the target response

(crossings) would have been applied.

The major findings of present experiments could be summarized as follows: (a) *Betta*

splendens passively avoided water disturbances in the form of ABs and WFs, confirming the

aversive function of these stimuli for this species; (b) when exposed to a free-operant avoidance

procedure, *Betta splendens* reliably escaped periodically delivered WFs; (c) although escape

behaviour improved with practice, *Betta splendens* did not appear to learn to avoid periodic

presentations of WFs, despite the fact that, based on escape behaviour, WFs were clearly

aversive to this species by definition; (d) adding a warning stimulus to the free-operant procedure

did not result in more or more efficient avoidance behaviour, as has been demonstrated in other

species, including goldfish; (e) when *Betta splendens'* escape from WFs was punished with more

instances of WFs, the result was not a substantial decrease of escape – instead, punishment

caused an initial increase of escape followed by a decrease to a level that was approximately the

same that occurred when escape responses were not punished; and (f) punishment of escape

responses in *Betta splendens* did not appear to result in increased avoidance responding as an

alternative response, unlike the effect of punishment on rats, which can result in the

establishment of an alternative response.

Although the spatial and temporal analyses conducted were limited in terms of providing

satisfactory answers to the unexpected findings, there is a major heuristic value to this approach

in terms of suggesting a series of interpretations that are easily translatable to further research.

As Sidman (1960) observed, "failure of replication will serve as a spur to further research rather

than lead to a simple rejection of the original data" (p. 74).

In Experiment 1, analysis of the different types of data led to different explanations for

the lack of replication of negative reinforcement phenomena, including the complete avoidance

of WFs and control by the warning stimulus (CABs). These interpretations were related to (a) the

short duration of the warning stimulus, (b) the spatial configuration of the sources of stimuli

(e.g., the location of the air stones/CABs with regards to the door and the water pump nozzles),

(c) the uncontrolled stimulation produced by the water pumps activation/operation, and (d) the

use of a door between compartments instead of a hurdle. Further research is needed to explore

the effects of manipulating these factors on the establishment of discriminated and non-

discriminated free-avoidance phenomena. Moreover, in the case of the spatial and temporal

configuration of the air stones and CABs, future studies could test if certain behavioural

phenomena that are rarely studied with aversive stimulation could be reproduced using WFs,

namely sign- and goal-tracking effects (Boakes, 1977; Hearst & Jenkins, 1974; Silva et al.,

1992).

Similarly, a combined analysis of the different types of data led to tentative interpretations of the major and unexpected outcomes of Experiment 2, which included: (a) a dramatic increase in frequency of escape during the first session (escape bursts); (b) the progressive rather than immediate decline of escape throughout the experiment; (c) the resurgence of escape bursts immediately after a change of condition occurred; (c) the frequent exposure of fish to several consecutive WF periods without crossing; and (d) the development of swimming patterns across fish that were related to specific areas of the experimental tank. The proposed explanations of these findings were overall related to the effects of uncontrolled sources of reinforcement (e.g., the intrinsic delay between the pump activation and WFs reaching their full strength, or conditioned reinforcement functions of pump-related stimuli carried over from Experiment 1) and the development of alternative behaviour (e.g., door-corner swimming could have allowed the fish to reduce their exposure to high-intensity WFs). Thus, further research could not only explore the role of these factors in regard to the absence of free-operant avoidance and positive punishment phenomena, but could also confirm whether a series of behavioural phenomena such as extinction bursts, spontaneous recovery, escape from punishment, and conditioned reinforcement can be systematically replicated using a non-conventional form of stimulation, such as WFs.

Providing an explanation of the psychobiological mechanisms by which WFs affect the behaviour of bettas was beyond the purpose of this dissertation. However, it is expected that further research will explore the proposed notion that the unconditioned aversive function of WFs demonstrated in these experiments is related to territorial, bubble-nesting, or breeding activities, and/or could be associated with predatory risks or the presence of intruders. Such efforts to incorporate biological and ecological aspects of bettas into the understanding of the

behavioural functions of WFs could also extend the scope of the interpretations for the

unpredicted outcomes, especially the lack of free-operant avoidance. Behaviour analysis has

greatly benefited from incorporating information about the experimental animal's characteristics

(e.g., its ecology and behaviour in natural settings) into an inclusive framework that allows for

the functional interpretation of laboratory outcomes and ultimately, to a more comprehensive

understanding of behavioural and learning phenomena (e.g., research on predispositions and

constraints in learning that has interpreted controversial phenomena such as *misbehaviour* –

Breland & Breland, 1961; *autoshaping* – Brown & Jenkins, 1968; *superstitious behaviour* –

Skinner, 1948; and *taste aversion* – Garcia & Koelling, 1966 – see Timberlake & Lucas, 1989

and Timberlake, 1990 for a review of these studies). This is the case, for instance, of the

behavioural systems approach, also known as *biological behaviorism* (Timberlake, 1993, 1999,

2001; Timberlake & Lucas, 1989), which assumes that behavioural processes are supported and

constrained by organized and interrelated regulatory systems, such as feeding or defense, and

that learning occurs within the context of those already functioning systems (Timberlake, 2001;

Timberlake & Lucas, 1989). With this perspective, an important aspect to consider when

interpreting the outcomes of the studies with the WF paradigm is that male bettas are highly

territorial fish, especially in isolation (Monsives et al., 2009). Specifically, the procedure used

here involved daily removal of an isolated subject from its individual home tank (i.e., its

territory) and its introduction into an environment that is, to a certain degree, unfamiliar (e.g.,

between sessions, the experimental tank is cleaned and has its water changed). It is possible that

fish react to this situation similarly to how they would naturally respond when entering the

territories of conspecifics and/or establishing their own territory in a novel environment (e.g.,

displaying activities of exploration, inspection, and/or defense). Taking this into consideration,

betta adjustment to the contingencies programmed in the present experiments could have been

supported or constrained by the behavioural interactions triggered by the inherent characteristics

of the apparatuses and procedures. For instance, it is plausible that the size of the shuttle tank and

its compartments may have an effect on the swimming trajectories, time allocations, and crossing

patterns of bettas, especially given that it is uncertain to what extent their size corresponds to the

area that a male would typically defend in the wild (there appears to be no information in the

literature on the specific dimensions of a typical male betta's territory in the wild). Similarly,

uncontrolled mirror reflections occurring in the tank's glass walls could have elicited territorial

or defensive behaviour; it is precisely to these types of behaviours that lap-swimming (Martin,

2010), one of the stereotypical swimming patterns observed repeatedly throughout the present

studies, belongs.

Interesting research questions arise from these considerations regarding the ecology and

biology of bettas and the characteristics of the WF paradigm. For instance, male and female

bettas have different territorial behaviour in natural settings; males construct bubble nests and

entice females to spawn there, then chase them out of their territories (Jaroensutasinee &

Jaroensutasinee, 2003). Since females have not yet been tested in the WF experimental

paradigm, future studies should examine whether they show the same behavioural patterns

observed in the present studies, including sensitivity to WFs, overreliance on escape behaviour,

and low incidence of avoidance (e.g., if the aversive function of WFs is related to territorial,

bubble-nesting, and breeding activities it is possible that such stimulus is not as aversive for

females). Similarly, other studies could test if free-avoidance can be established by varying the

size of the experimental tank and/or changing the required response from one that involves

displacement between compartments to one that allows the fish to remain in the same area (e.g.,

contacts with a virtual target, as in previous studies of the spatiotemporal program – Sales, 2014;

pressing a paddle or lever – Hogan & Rozin, 1962; Longo & Bitterman, 1959; or biting a

manipulandum – Strength, McCoy, & Hull, 1981). Finally, the WF paradigm could be used to

extend the findings of previous research that has reported physiological and behavioural

differences between wild and domesticated variants of bettas (e.g., aggressiveness levels and

coping styles; Verbeek et al., 2007, 2008).

Regardless of the fact that further research is needed to confirm the mechanisms by which

WFs affect bettas, it appears that the WF paradigm offers the possibility of studying aversive

control phenomena in the absence of a pain component, which is not always involved in

naturally occurring aversive interactions. This is a promising feature of the paradigm in view of

its accordance with principles endorsed by worldwide agencies that regulate humane animal

experimentation (refinement, reduction, and replacement – e.g., Canadian Council on Animal

Care, 2007; U.S. National Research Council, 2011); namely, the refining of procedures to

minimize or eliminate pain and distress, and to enhance animal well-being (e.g., since the

paradigm requires fish to exercise, it may actually be beneficial to their health and well-being,

which may be explored in future studies). Accordingly, the WF paradigm could be particularly

valuable in: (a) research on experimental models of anxiety and depression, in which shock-

based procedures have been criticized because they entail a pain component that does not seem

to have a counterpart in these emotional phenomena in humans (Brodie & Boren, 1959; Barker et

al., 2010; McQuade, Creton, & Stanford, 1999); (b) the assessment of emotional responses and

welfare of fish through the use of preference/avoidance tests (Millot, Cerqueira, Castanheira,

Øverli, Martins, & Oliveira, 2014); and, more generally, (c) the ongoing debate regarding fishes'

capacity to experience negative affective states (e.g., pain and stress – Braithwaite & Ebbesson, 2014; Huntingford et al., 2006; Rose et al., 2014).

In a broader scope, the WF paradigm facilitates future research on the basic processes of aversive control, which has declined substantially over the past decades. This is primarily a consequence of over-reliance on shock-based procedures which, notwithstanding their role in the success of preeminent behaviour analytic research programs during the past century, have been increasingly criticized on ethical, practical, and ecological validity grounds. This situation has resulted in considerable efforts being directed towards finding alternatives that permit the continued study of the principles of behaviour controlled by aversive stimuli. These efforts are critical considering that most of what is currently known about basic processes of aversive control was established with a single stimulus (electric shock). Thus, broadening the generality of such findings requires testing of well-studied facts utilizing different forms of stimulation that are more ecologically valid. The present research contributed to these efforts by demonstrating the feasibility of a distinct ecologically valid stimulus – WFs – that bettas are likely to encounter in their natural environment.

Nevertheless, the fact that free-operant avoidance and punishment could not be replicated is of particular relevance when considering the outcomes of previous research that implemented non-shock procedures. The findings of these studies are overall inconsistent regarding the potential of different stimuli to control behaviour under aversive contingencies, and to produce the same patterns of behaviour observed extensively and reliably with shocks. As a result, some researchers have proposed that inherent characteristics of the stimulation, including the "degree of aversiveness" (McAdie et al., 1993, p. 224) could explain the differences in behavioural outcomes produced by shock and non-shock procedures. However, the fact that some of the

studies with alternative stimuli have indeed demonstrated free-operant avoidance and

punishment using a wide range of experimental paradigms (e.g., bright light – Barker et al.,

2010; wind – Rohles, 1965; artificial gravity – Clark et al., 1973; pinching of the tail – Brodie &

Boren, 1959; pressurized air – Spealman, 1978; and loud noise – McAdie et al., 1993) supports

the notion that procedural aspects are responsible for the lack of replication of some negative

reinforcing and punishment phenomena using WFs, especially considering that an escape

contingency involving this stimulus did produce clear and reliable behavioural control. In other

words, it is more parsimonious to assume that WFs can control betta free-operant avoidance

behaviour given the right "tuning" (Timberlake, 1999, p. 259) of the procedures, apparatuses,

and parameters, than to assume that they cannot. This particularly seems to be the case given

how little is known about the mechanisms by which WFs affect bettas, including the biological

and ecological relevance of this stimulus for the species. The development of other highly

effective operant devices, such as levers for rats and pecking keys for pigeons, that enabled the

discovery of some behavioural principles, resulted from an iterative process of refining the

experimental apparatus and procedures to the characteristics of the experimental animals (e.g.,

the foraging behaviour of rats). As Timberlake (1999) noted:

> An unusually well-documented example of the importance of tuning was provided by
> Skinner (1938, 1959) in discussing his invention of the lever press as the predominant
> operant response in rats. He left an extensive account of successive changes in his
> apparatus and measurement procedures as he struggled to make the response easier to
> measure and more reliable (Skinner 1938, 1959; Timberlake, 1990)… It seems evident
> that the final design of Skinner's lever reflected a careful tuning to the foraging repertoire
> of the rat. Tuning, then, is a singular testimony to the ability of experimental
> psychologists to fit tasks to their subjects, a critical contributor to the development of
> functional laws and theories, and one of the best methods for exploring the perceptual-
> motor and regulatory organization of an organism. Arguably, every effective
> experimental procedure and laboratory apparatus has been carefully tuned by previous
> experimenters to facilitate and clarify the results of their manipulations. If a manipulation
> does not make good contact with the animal's perceptual-motor organization and

regulatory processes, there is little chance the data will be sufficiently regular to be interpreted in terms of a fundamental law. (pp. 259 - 260)

The application of this working hypothesis, regarding the need for tuning the characteristics of the WF device to the characteristics of bettas, may guide further research that will allow for the identification of the factors responsible for the replication of well-established behavioural phenomena (or the non-replication of those phenomena), continue expanding the potential of the spatiotemporal analysis research program, and, more importantly, bring renewed attention to the area of aversive control, which "seems to be in jeopardy" (Catania, 2008, p. 114) despite its unquestionable relevance to experimental and applied behaviour analysis.

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Appendix

Spreadsheet designed for manual scoring of behaviours displayed during CABs and non-CABs periods in Experiment 1.

Date: Condition: Session No.:

Video time (min:sec)	CABs											non-CABs															
	C	IC	TA	SU	SA	AppC	TD	SAW	AG	NS	Other	C	AG	AppAS	SBAS	AGDWF	SU	SA	AppD	AppN	SBTD	HW	LSLW-R	LSSW-R	LSLW-I	LSSW-I	Other
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Additional Observations: