Effects of Fixed- and Variable-Time Schedules of Mirror Presentations on the Swimming Behaviour of *Betta splendens*

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Abstract

The effects of response-independent mirror presentation schedules on the swimming behaviour of *Betta splendens* were studied in two experiments. In experiment 1, four fish received alternating baseline (no mirror) and fixed-time (FT) 2-min or variable-time (VT) 2-min mirror presentation conditions. Two fish consistently showed increased rates of mirror-side lap-swimming (MSLS; a back-and-forth swimming pattern) and decreased distance from the mirror during the inter-stimulus intervals (ISIs) of FT and VT sessions, and during baseline sessions that followed FT and VT phases. Data from one fish indicated that a VT schedule might increase both proximity to the mirror and MSLS rates that have decreased on FT. Therefore, in experiment 2 three fish from experiment 1 received alternating FT and VT conditions. Proximity to the mirror and MSLS rates increased during VT phases relative to FT phases in two fish, though effects were small and did not occur across all alternations.

Additional findings were that MSLS during ISIs tended to increase within FT phases, that MSLS generally occurred either at a steady rate within ISIs or showed a scalloped effect, that mirror presentations produced approach to the mirror side that persisted during ISIs and subsequent baseline phases, and that mirror-side distance during the mirror presentations was less than during the ISIs.

Although the findings were not consistent across all fish, they were replicated a number of times within at least two of the fish. The findings are discussed in terms of adventitious operant conditioning, respondent conditioning, and the behaviour systems approach. The results of this study increase the generality of response-independent schedule effects on locomotive behaviour.

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Dedication

to Daniela

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Chapter 1 - Introduction

Repeated stimulus presentations can induce a variety of behaviours in animals, even when delivery is response-independent – that is, delivery occurs regardless of the animal's activity. Schedules of response-independent food delivery induce distinctive patterns of locomotion in pigeons. This effect can be observed at a range of inter-stimulus intervals (ISIs) (Innis, Simmelhag-Grant, & Staddon, 1983) regardless of whether ISIs are of fixed or variable length (Staddon & Simmelhag, 1971). Schedule-induced locomotion has also been observed in rats (Silva and Timberlake, 2005), hamsters (Anderson & Shettleworth, 1977), and humans (Muller, Crow, and Cheney, 1979).

In a seminal study (Skinner, 1948), pigeons in an experimental chamber exhibited various stereotyped behaviours during response-independent food schedules. One of the conditioned behaviours (turning "counter-clockwise about the cage, making two or three turns between reinforcements") was locomotive; the rest consisted primarily of the pigeon moving its head in various ways. Skinner concluded that operant conditioning had occurred due to adventitious reinforcement: food "accidentally" followed various behaviours, which thereby become slightly more likely to precede the next food delivery, and so on.

Results from a subsequent study (Staddon & Simmelhag, 1971) led the authors to de-emphasize the role of operant conditioning in favour of Pavlovian or respondent conditioning and adjunctive behaviour – i.e., behaviour that is neither operant nor respondent. Sixteen behaviour categories were identified and recorded in pigeons on response-independent food schedules; three categories involved locomotion. The authors concluded that behaviours occurring within a few seconds following food consumption were probably adjunctive. The authors acknowledged that some behaviours that became predominant toward the ends of ISIs may have been operantly conditioned through adventitious reinforcement, as Skinner suggested, but these *terminal* behaviours were often displaced by pecking that appeared to be temporally (i.e. respondently) conditioned.

The form and strength of schedule-induced behaviour are influenced both by schedule variability and by the presence of a stimulus (e.g. changing keylight brightness) that predicts food delivery (Matthews, Bordi, & Depollo, 1995). Pigeons received food on fixed-time (FT) or variable-time (VT) schedules, both of which consisted of response-independent stimulus presentations. In FT schedules the intervals between presentations were fixed; in VT schedules the intervals between presentations varied randomly. Pacing along the chamber walls occurred during both schedule types, and thus depended only on the intermittent presentation of food. Pacing accounted for a larger proportion of ISIs during unsignalled (i.e. no predictive keylight) VT sessions than during unsignalled FT sessions.

Timberlake and Lucas (1985) performed nine related experiments as systematic replications of the Skinner (1948) and Staddon and Simmelhag (1971) studies. Although Timberlake and Lucas reproduced the general finding that stereotyped behaviours could be induced by response-independent schedules, the authors rejected Skinner's "superstition" (i.e. accidental reinforcement of arbitrary behaviour) explanation for the phenomenon for two reasons. First, the behaviours that emerged were highly uniform across subjects, making it implausible that food deliveries "captured" whatever behaviour happened to precede them. Second, the same set of behaviours tended to be induced despite the manipulations Timberlake and Lucas made to increase or decrease the probabilities that they would be accidentally reinforced.

Timberlake and Lucas also rejected the stimulus-substitution (respondent conditioning) explanation given by Staddon and Simmelhag for the dominance of pecking as a terminal behaviour in their study. Timberlake and Lucas obtained very little pecking in most of their experiments. Since pecking is an unconditioned response to grain delivery, it would presumably be the conditioned response that would occur if temporal conditioning were taking place.

Timberlake and Lucas instead proposed a behaviour systems approach (BSA) to explain their results. According to the BSA, much of the behaviour induced by response-independent food schedules is elicited by those schedules and influenced by ecologically relevant aspects of the experimental situation (Timberlake, 1983). The elicited behaviour is pre-organized into modules that are functional in the pigeon's natural environment. This organization is evident in the way that the pigeons tend to engage in different behaviours at different times during ISIs, and in the fact that certain behaviours are likely to follow other behaviours, as found by Staddon and Simmelhag (1971).

Timberlake and Lucas tested the BSA by moving the food-delivery mechanism from the wall of the chamber to the centre of the floor. Response-independent schedules presented in this new arrangement caused 3 out of 4 experimentally naive pigeons to spend roughly between 25% and 75% of their time walking and head bobbing while oriented towards the centre of the chamber - behaviour that was rarely seen in the preceding experiments and that was similar to natural ground foraging behaviour by pigeons.

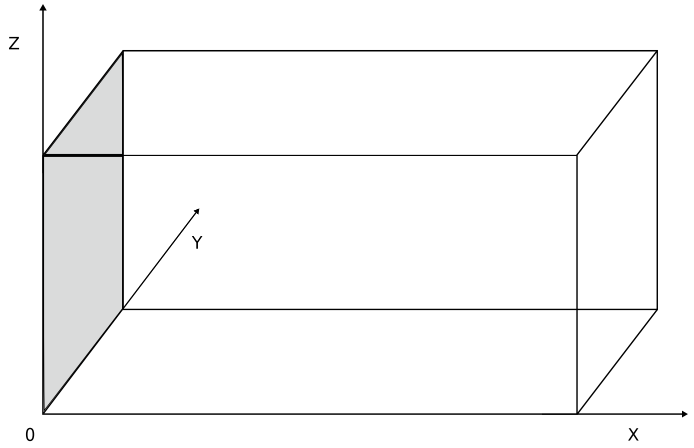
Video Tracking of Stereotyped Swimming by Betta splendens

In the studies described thus far, locomotion was measured either by direct human observation (Skinner, 1948; Staddon & Simmelhag, 1971) or using pressure-sensitive plates in the experimental chamber floor (Matthews et al., 1990). Computerized video tracking systems permit automated measurement of animal locomotion with high precision and accuracy (Lind, Vinther, Hemmingsen, & Hansen, 2005). Such systems also enable analyses such as the creation of virtual targets and automated response shaping (Pear & Legris, 1987), and the computerized classification of locomotive patterns without advance specification of the classes or number of classes (Kinsner, Cheung, Cannons, Pear, & Martin, 2006).

Martin et al. (2003) used a computerized video-tracking system to study swimming by *Betta splendens* before, during, and after response-independent mirror presentations. Bettas, also called Siamese fighting fish are a species of freshwater aquarium fish native to Southeast Asia. The sight of a conspecific elicits strongly agonistic behaviour in males. The aggressive response includes distinctive somatic and locomotive components and is reliably elicited by a mirror image (Simpson, 1968).

In the context of Martin et al.'s (2003) rectangular prism-shaped experimental tank, *X* coordinates indicated distance from one of the short walls where a mirror was presented, *Y* coordinates indicated distance from the long wall closest to the tracking cameras, and *Z* coordinates indicated distance from the bottom of the tank (see Figure 1). Mirror presentation was controlled by turning on or off a light bulb behind a two-way mirror placed against the outside of a tank wall. The mirror was reflective when the light was off, and non-reflective when the light was on.

Figure 1. *Experimental tank coordinates, Martin et al. (2003).*



After receiving two baseline sessions (i.e. no mirror presentations) and two sessions of FT 10-min mirror presentations, a majority of the fish showed increased rates of a stereotyped swimming pattern during the return to baseline sessions. Specifically, the fish swam back and forth along the *X*-axis of the tank, covering approximately 90% of the available *X* coordinate range before turning around and swimming the same distance in the opposite direction. A larger proportion of time was allocated to stereotyped swimming during return to baseline than during the ISIs in the FT sessions.

The Martin et al. (2003) study used 10-minute ISIs and 10-minute mirror periods. These parameters resulted in stereotyped swimming in a majority of fish when used in 8-hour sessions. Briefer ISIs and mirror periods would allow delivery of a comparable number of mirror presentations during shorter sessions, but the range of schedule values that effectively induce swimming patterns is unknown. The results of studies using response-dependent mirror presentations may provide some indications.

On an FI schedule, the first instance of a specified response after a fixed period of time following the previous reinforcement is reinforced. Bettas are sensitive to interval differences in the range of 0.5 min to 4 min on fixed-interval (FI) schedules (Higa & Simm, 2004). Wait time and break point, two measures of the tendency for animals on FI schedules to accelerate responding towards the end of intervals, increased as FI values (30 s, 60 s, 120 s, and 240 s values were used) increased in the experiment.

The duration of mirror presentations to bettas as a consequence for swimming through a small tunnel has been varied among 5, 10, 20, and 40 seconds (Hogan, Kleist, & Hutchings, 1970). All of these durations maintained approximately equal rates of responding. This result may not generalize to response-independent mirror-presentation schedules, however, since the tunnel was arranged such that reinforced responses positioned the fish to see their mirror images immediately. During response-independent presentations a fish may not be positioned to see the mirror as soon as it appears, making it possible that brief presentations would go unnoticed.

Statement of the Problem

Attempts to explain the effects of response-independent schedules in terms of established learning principles have stimulated experimentation for decades. Recent work in this area (e.g. Silva & Timberlake, 1999; Silva & Timberlake, 2005) has focused on the BSA, which emphasizes species-specific pre-organized behaviour modules that are adapted to the animal's natural environment. Most work on response-independent schedules has been done using food with pigeons and rats, therefore studying an aquatic, territorial species and a non-food stimulus provides an opportunity to extend the generality of response-independent schedule effects, and to critically evaluate explanations, including the BSA, for the observed behaviours.

Although Martin et al. (2003) observed increased back-and-forth swimming by *Betta splendens* following an FT schedule of mirror presentations, limited experimental control was demonstrated. I therefore performed an experiment to study the effects of response-independent schedules of mirror presentations on the swimming behaviour of *Betta splendens* using FT and VT schedules. In a second experiment I followed up on an unexpected finding from experiment 1, and examined the effect of mirror presentation predictability on habituation of aggressive display to the mirror.

Experiment 1

The purpose of Experiment 1 was to study the effects of FT and VT schedules of mirror presentation on swimming patterns during ISIs and baseline sessions.

Chapter 2 - Method

Subjects and Setting

Four experimentally naive male *Betta splendens* fish were purchased from a local pet store. The fish were selected on the basis of erecting gill-covers within approximately 3 s of being presented with a pocket mirror. The fish were housed individually, with no view of each other or other bettas. Home tanks were 20 L rectangular prisms, with gravel floors and two plastic plants. The fish were fed daily following experimental sessions. Home tank water temperature was kept at 26 +/- 1 °C.

The experimental tank dimensions (L x W x D) were 40 x 40 x 20 cm. The tank's length and width were designed to create a bottom surface area that filled the fields of view of the video tracking cameras (see Apparatus). During sessions the tank was filled with heated (26.5 °C - selected to match home tank temperature) water to a depth of 10 cm, creating a total water volume of 16 L. After each session the tank was pumped empty, cleaned, and refilled with tap water aged at least three days for dechlorination. The area underneath and immediately surrounding the experimental tank was covered with non-glossy white plastic sheets to facilitate video tracking (see Apparatus).

The fish were transported to and from the experimental tank with the house lights off and the mirror presentation box light on. Data acquisition began when the house lights were turned on. The session room was not entered during data acquisition.

The experimental tank was made of clear glass and the sides were not normally covered during sessions. Extraneous objects were removed from the session room, but it is possible that the fish could distinguish the views outside of the non-mirror/light box walls of the tank. The room's door, painted orange with a black frame, was approximately

2 m away from one of the tank corners opposite to the mirror.

Apparatus

Data were collected using a video-tracking system (VTS). Two cameras positioned above the experimental tank conveyed video signals to a computer system that measured each fish's position in the tank 10 times per second. This sampling frequency was adequate to avoid aliasing (i.e. incorrectly recording rapid movement as slower movement) given the average length of the fish and their estimated maximum swimming speed (Barry, 2003). A video signal from one of the cameras was recorded on a PC using a video capture card.

Position was recorded in terms of X, Y, and Z coordinates expressed in millimetres. Accuracy was tested by placing a 1-cm diameter circular test target at each of the tank's corners in turn, both on the tank floor, and also raised to 10 cm (i.e. the level of the water's surface). The maximum difference between VTS measurements and physical measurements (i.e. using a measuring tape) in terms of the X and Z (i.e. the horizontal) coordinates (the only spatial coordinates used in this study) at any target point was approximately 2%. For example, a target placed

200 mm from the x axis was measured at an x coordinate of 204 mm.

The system occasionally recorded a loss in tracking instead of the target's position. Tracking losses occurred in 0.05% of all records, an average of 16.97 lost records (i.e. 1.7 seconds) per session.

One tank wall was adjacent to a mirror presentation box. The box contained a one-way mirror (40 cm long x 15 cm high) along the tank wall and two compact fluorescent light bulbs, each rated by the manufacturer at 15 W and 1000 lm. When the bulbs were on, light passed through a 2 mm thick styrene diffuser panel and through the one-way mirror, making it non-reflective. When the bulbs were off, the interior of the box was dark and the mirror was reflective. The VTS program controlled the flow of electrical power to the bulbs.

Procedure

Each fish received a daily one-hour session conducted at approximately the same time each day. Start times occasionally varied by up to 30 min from the typical time.

The experiment began with baseline and FT conditions (see below for schedule details) that alternated starting with baseline. Fish 1 received 14 alternations, fish 2 received 4 alternations, fish 3 received 8 alternations, and fish 4 received 6 alternations (Table 1). Fish 1 and 4 also received a VT condition (see below) alternated with baseline, beginning with VT immediately after the last alternation to baseline from the first portion of the experiment. Fish 1 received 4 alternations in the VT/ baseline portion, and fish 4 received 5 alternations in the VT/baseline portion.

Table 1

Sequence and Duration of Phases, Experiment 1

Condition	Fish 1	Fish 2	Fish 3	Fish 4
	Number of Sessions			
A	18	5	16	7
B	2	3	2	2
A	14	5	9	10
B	2	6	2	2
A	5	6	9	3
B	2		2	2
A	7		6	3
B	2		2	
A	8		20	
B	2			
A	9			
B	2			
A	10			
B	2			
A	9			
C	2			2
A	10			5
C	2			2
A	8			7
C				2
A				4

Note. A = Baseline, B = Fixed-Time, C = Variable-Time

In baseline sessions the light box remained on (no mirror presentations) at all times. Baseline phases generally continued until the mirror-side lap swimming (MSLS; see Measures, below) rate had decreased relative to the phase's early sessions, and was stable or trending downwards. Each subject's first baseline phase continued until lap-swimming rates on all four tank sides were stable or trending downwards.

An FT 2-minute schedule with 2-minute mirror durations was used in FT phases. These values resulted in 15 mirror presentations per one-hour session. As the first subject to show stable baseline performance, fish 2 was the first to receive the FT condition. Its response to the mirror decreased greatly during the third FT session, after which it was returned to baseline. All other FT and VT phases (for fish 1, 3, and 4) in Experiment 1 were therefore restricted to two days.

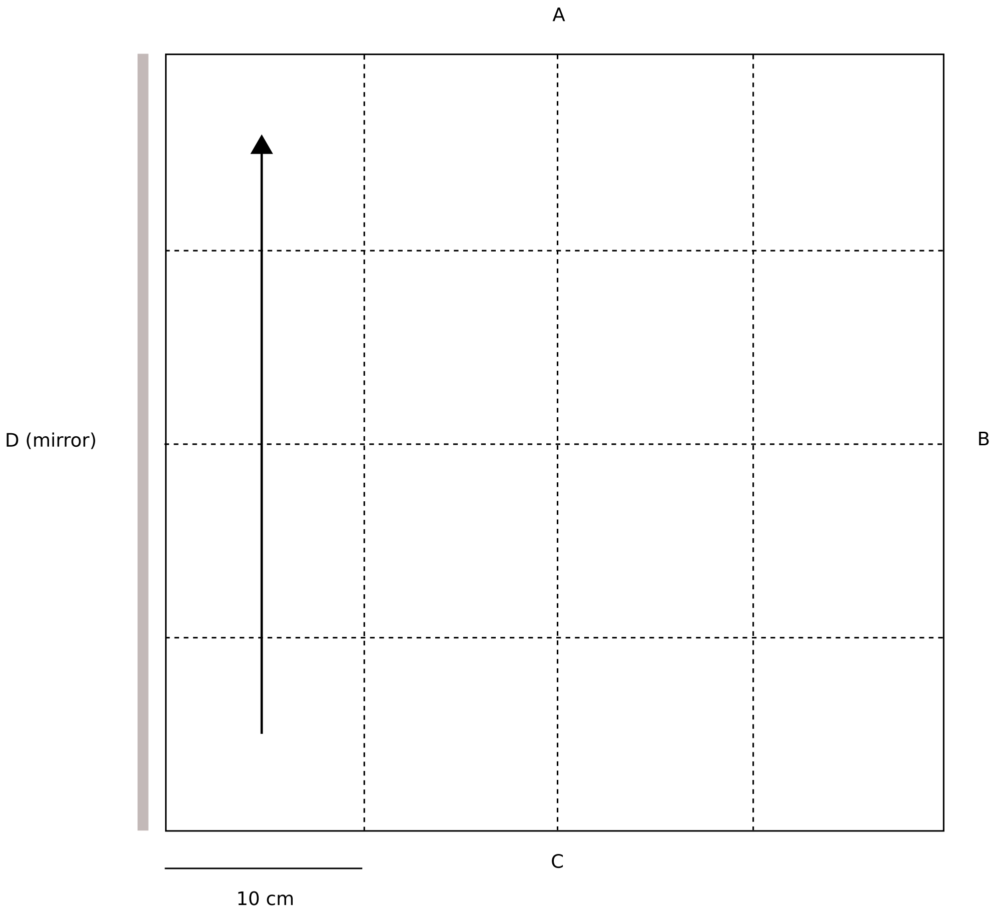
A VT 2-minutes schedule with 2-minute mirror durations was used in VT phases. ISI variability was implemented by a computer program that tested a probability (0.0083) once per second, yielding a geometric probability distribution with a minimum 1 s ISI. VT sessions terminated after 15 mirror presentations, in order to equate the number of mirror presentations with those in the FT sessions.

Measures

Data were analyzed by measuring the experimental tank as a grid of 16 cubes, each 10 cm x 10 cm x 10 cm (Figure

2). Cubes in the grid extended from the tank bottom to the water's surface. When swimming along a tank wall, the fish tended to stay within a few centimetres of the glass.

Figure 2. *Experimental tank grid diagram.*



A lap of swimming along a tank wall was defined as moving directly from any corner cube of the tank to either of the nearest (i.e. non-diagonal) corner cubes, passing only through cubes adjacent to a tank wall. MSLS rate, the primary dependent variable, was calculated as the number of laps swum at the mirror side divided by either the amount of session time that the mirror was not present (for MSLS rates during ISIs or baseline sessions), or by the amount of session time that the mirror was present (for MSLS rates during mirror periods).

Mean distance from the mirror per session was calculated from the spatiotemporal record.

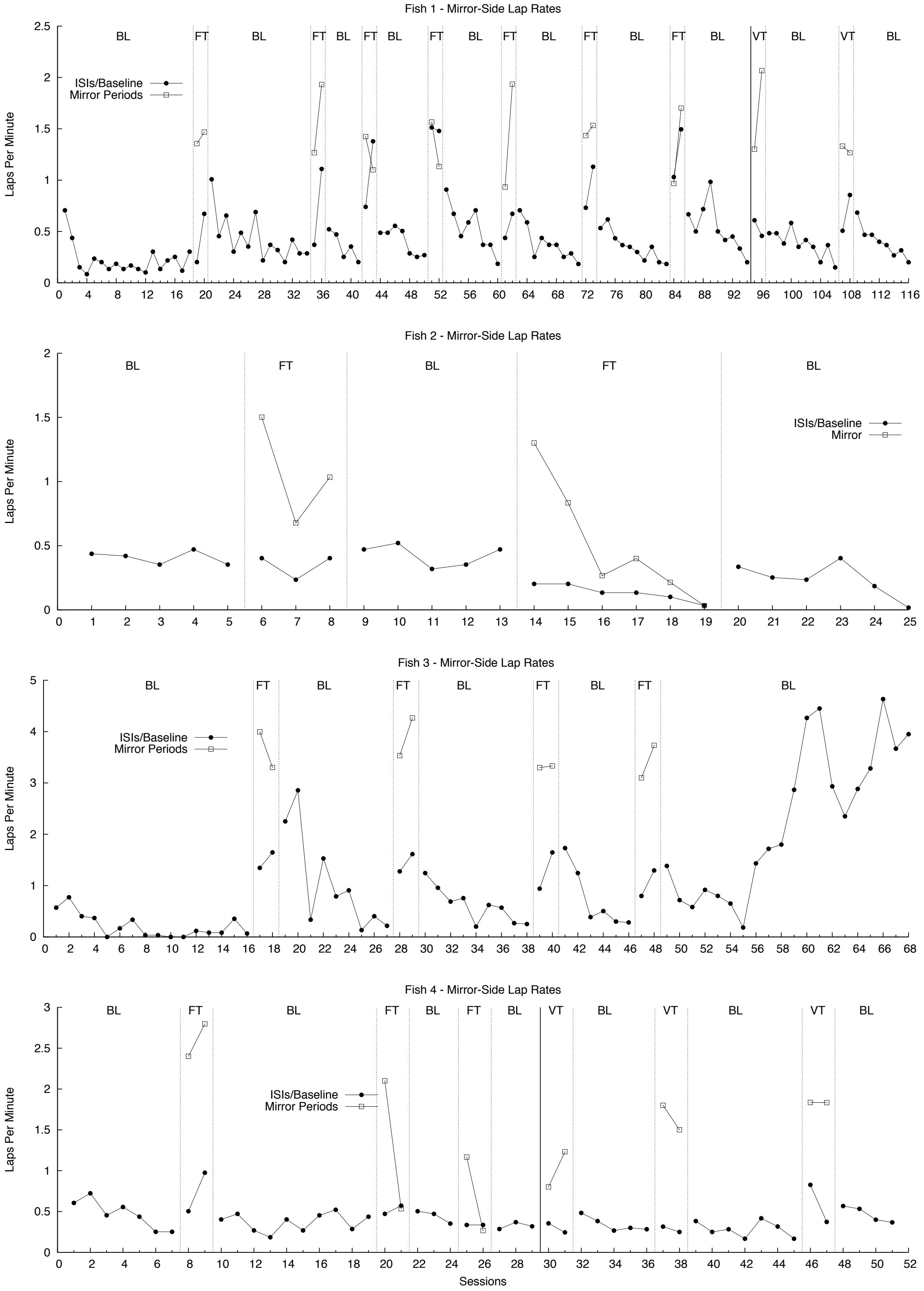
Chapter 3 - Results

MSLS Rates Across Phases

Figure 3 presents MSLS rates for all four fish.

Mirror periods. MSLS rates were reliably higher during mirror presentations than during baseline sessions and with very few exceptions during the ISIs (Figure 3). Fish 1's rates were higher during ISIs on the 2nd days of its 3rd and 4th FT phases, and on the first day of its 7th FT phase. Fish 2's rates during both mirror presentations and ISIs decreased during its 2nd FT phase until on the 6th day both were nearly zero (0.03 laps per minute). Fish 4's rates were lower during mirror presentations than during ISIs on day two of its 2nd and 3rd FT phases.

Figure 3. *MSLS rates, experiment 1.*



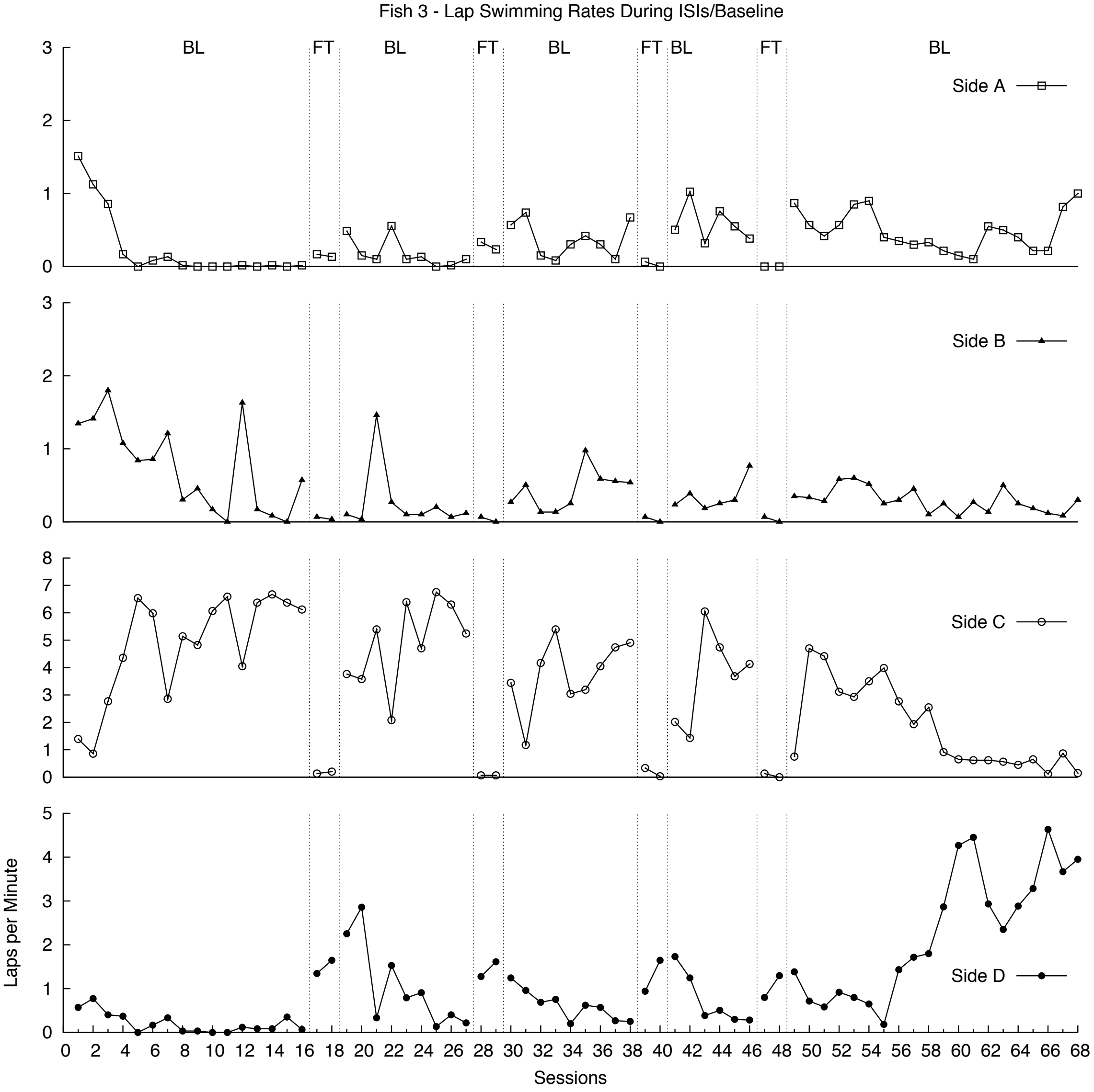
For fish 2 – the only fish to have more than two sessions of FT during any phase - there was a reliable decrease in MSLS rates across sessions and across phases. No other fish showed this decrease across phases.

Baseline sessions. Baseline MSLS rates following FT or VT phases tended to start out high and then to decrease. MSLS rates were generally higher during baseline sessions that closely followed FT or VT phases than during baseline sessions that closely preceded FT or VT phases. Often the MSLS rate on the first baseline session was higher than the rate during the ISIs of the previous FT or VT phase.

In Fish 1 and 3, these increases occurred upon every return to baseline, lasted two or more sessions, and were generally 200% to 300% or more. Fish 4 showed the effect inconsistently in that slight MSLS rate increases occurred following the first two FT phases, and following the first two VT phases. MSLS rate increases following Fish 4's final VT session were similar in proportion and duration to the increases seen in Fish 1 and 3. Fish 2, however, did not show the same effect.

MSLS rates generally decreased within baseline phases for Fish 1, 3, and 4. Part way through Fish 3's final baseline phase, however, its rate increased over six consecutive sessions and remained elevated for the remainder of the experiment. No environmental changes were identified that might have caused this increase, but all fish showed preferences for certain sides of the experimental tank, and these preferences sometimes changed. For example, Fish 3 increased its activity at side C during the first baseline phase, mainly at the expense of activity at sides A and B (Figure 4). Lap-swimming at side C decreased in the final baseline phase as lap-swimming at side D increased.

Figure 4. *Lap-swimming rates, all sides, experiment 1.*



ISIs. MSLS rates during ISIs at the beginning of an FT or VT phase tended to increase over MSLS rates at the end of the previous baseline phase. Two fish showed reliable MSLS rate increases during ISIs relative to preceding baseline levels when FT or VT was introduced. For fish 1 this result was seen in 6 out of 7 FT phases and 2 out of 2

VT phases. Fish 1's rate during day 2 (session 20) of its 1st FT phase was higher than baseline level, but its rate during day 1 (session 19) was not. Fish 3 showed the same result in 4 out of 4 FT phases. Fish 4 showed elevated rates during the ISIs of its first FT phase (sessions 8 and 9) and on day 1 of its third VT phase (session 46). Fish 2 did not show elevated MSLS rates during the ISIs of any FT session.

MSLS Rates Within FT Phases

MSLS rates during ISIs tended to increase within FT phases - the rates were higher on day 2 than on day 1. This was seen in 6 out of 7 FT phases for fish 1, and 4 out of 4 FT phases for fish 3. Fish 4 nearly doubled its rate from day 1 to day 2 during its first FT phase, showed a smaller increase during its second FT phase, and swam at equal rates on the two days of its third and final FT phase. Fish 4's decreasing trend across FT phases during ISIs corresponded to decreasing lap rates during mirror presentations. Fish 2 showed no within-phase trend during its first FT phase, and a decreasing trend during its second FT phase.

MSLS Rates Within ISIs

Figures 5 through 8 present cumulative mirror-side laps swum during ISIs of FT sessions. Since rates generally ranged from 0 to 2 laps per minute, cumulative records for individual 2-min ISIs provide limited information about when during the ISIs laps tended to occur. Each plot in Figures 5 through 8 therefore aggregates the laps from all 15 ISIs for each session into a single 120-s interval. For example, if a fish completed a lap during the 30th second of the 3rd, 5th, and 10th ISIs of a particular session, the cumulative value displayed would increment by three laps at that time value.

MSLS generally occurred either a steady rate within ISIs (i.e. the cumulative record stayed close to the slope of the sessional rate, shown as a dotted line in Figures 5 through 8), or at a relatively low rate for the first portion of the ISIs and at a higher rate during the latter portion. The latter type of performance is evident in plots where most or all of the area between the dotted sessional rate line and the cumulative record lies below the dotted line. In only two sessions (fish 2, session 15, Figure 6; and fish 4, session 16, Figure 8) was the majority of this area above the dotted line.

Figure 5. *Cumulative mirror-side laps, fish 1, experiment 1.*

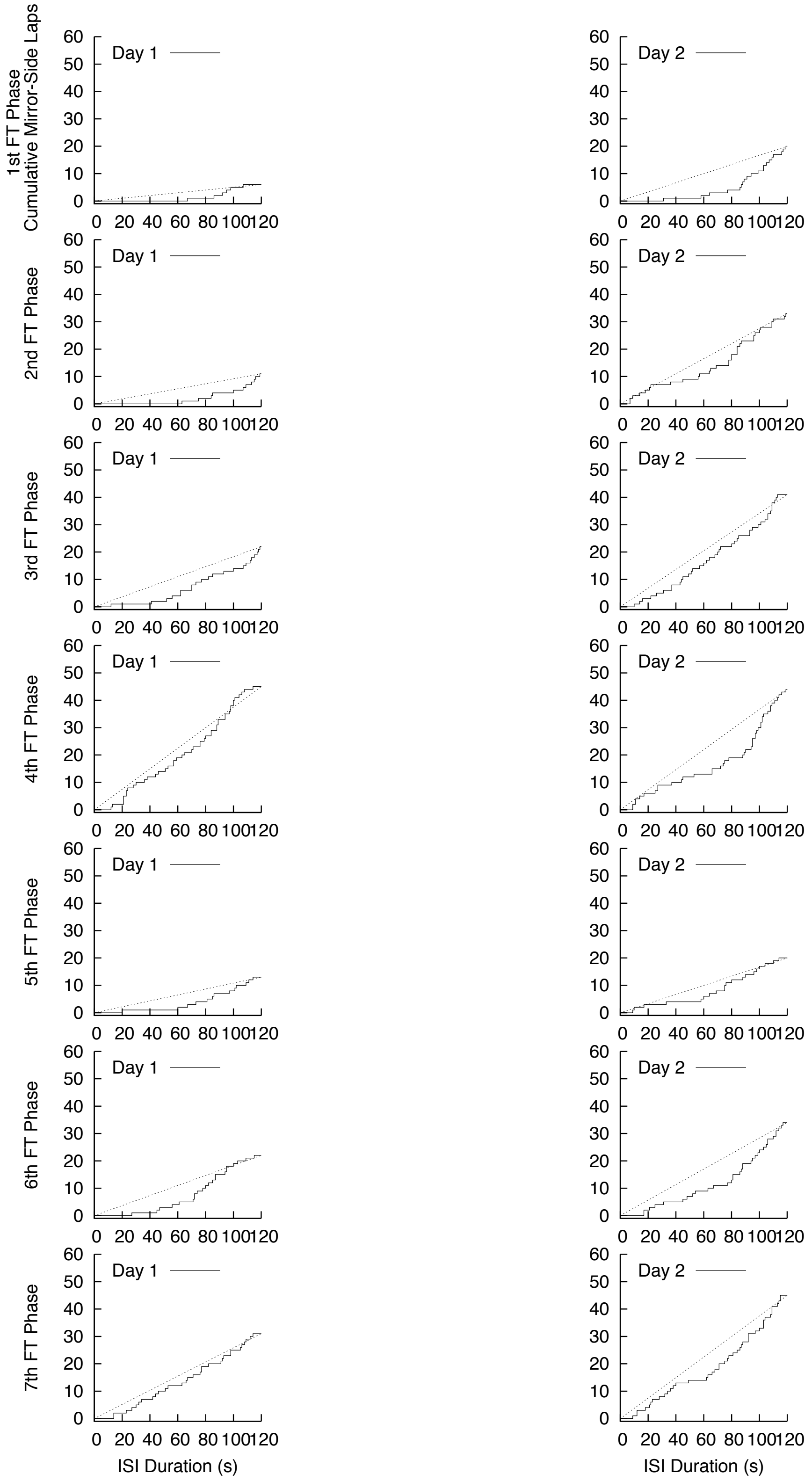


Figure 6. *Cumulative mirror-side laps, fish 2, experiment 1.*

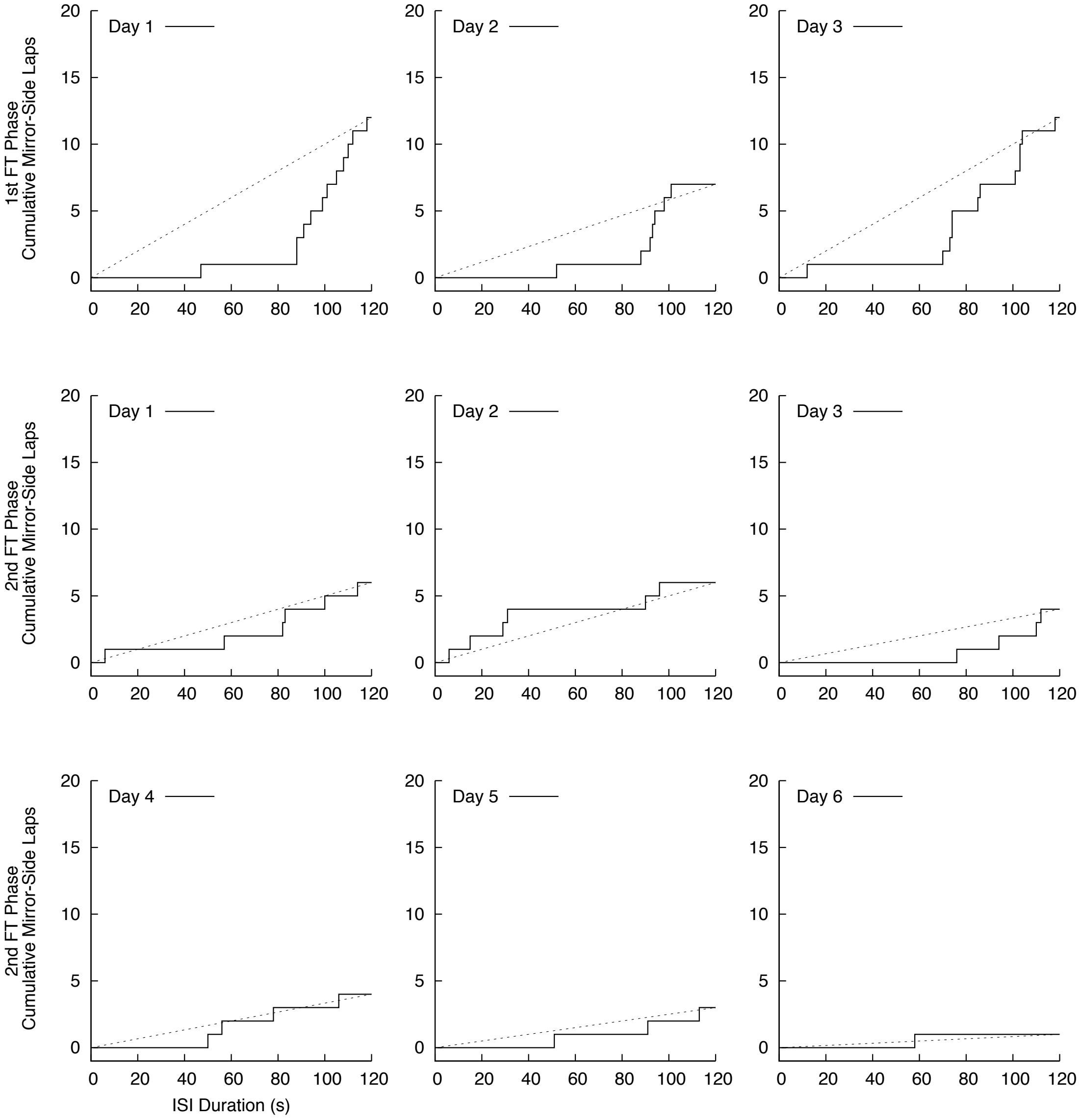


Figure 7. *Cumulative mirror-side laps, fish 3, experiment 1.*

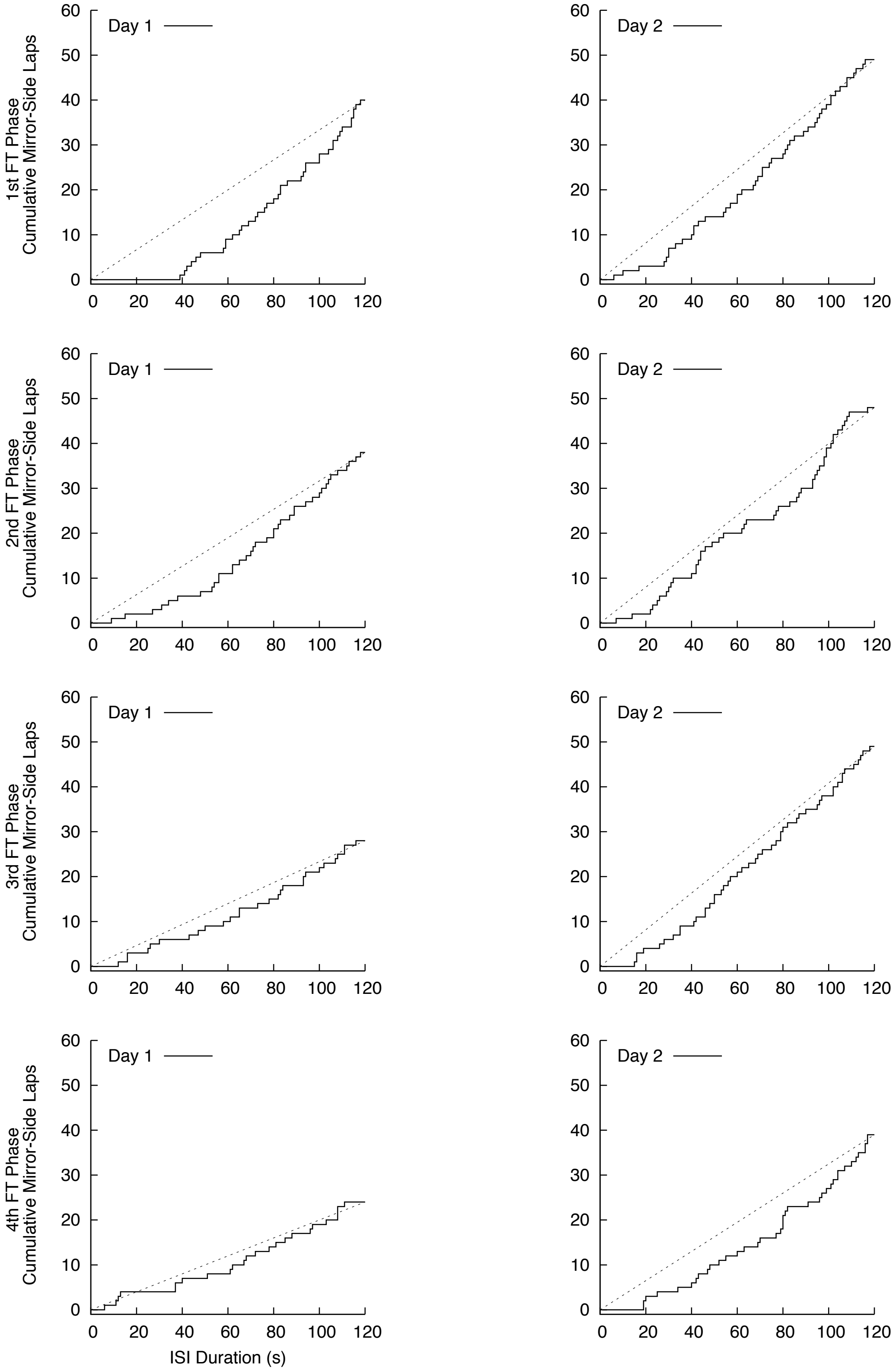
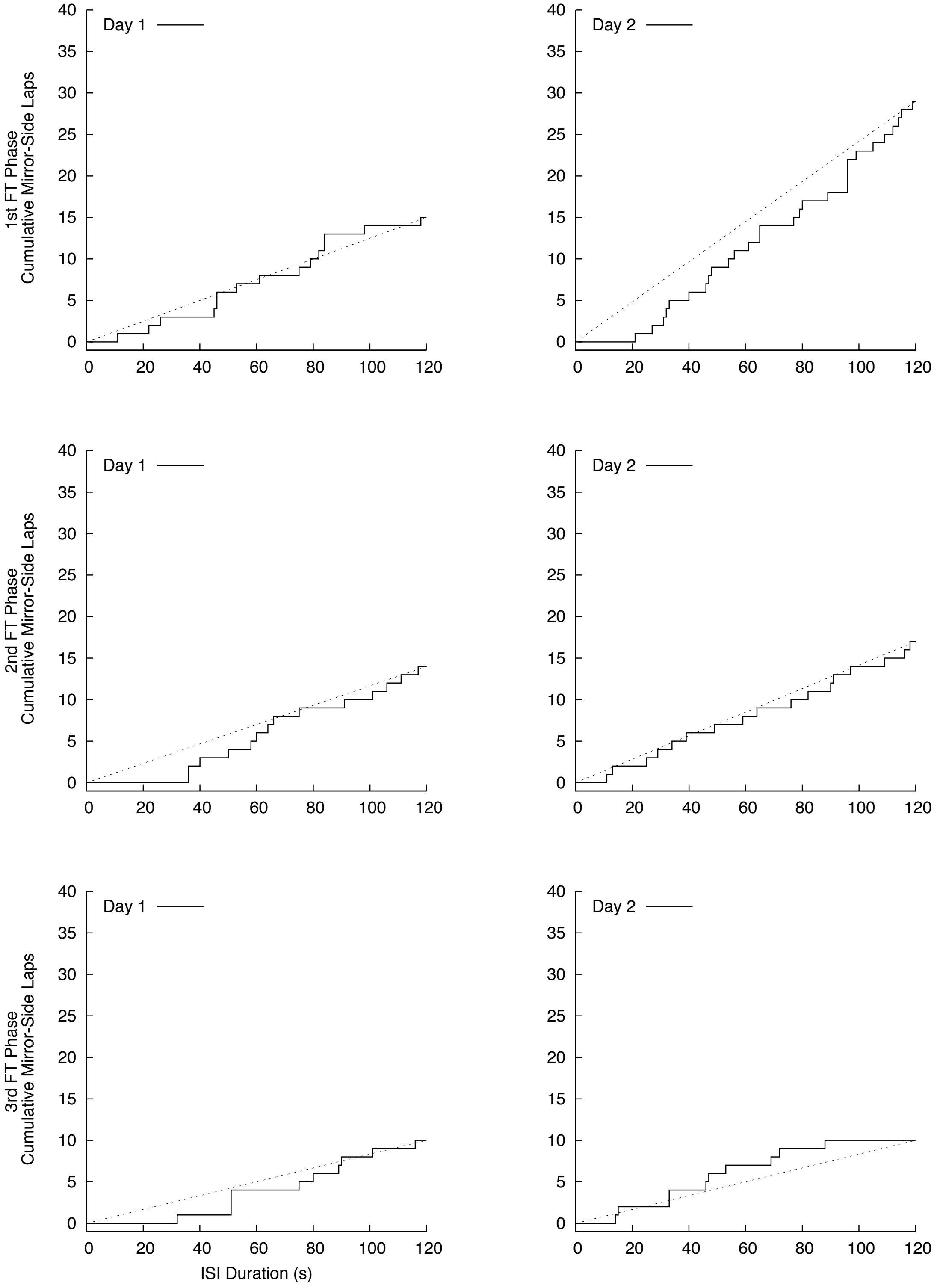


Figure 8. *Cumulative mirror-side laps, fish 4, experiment 1.*



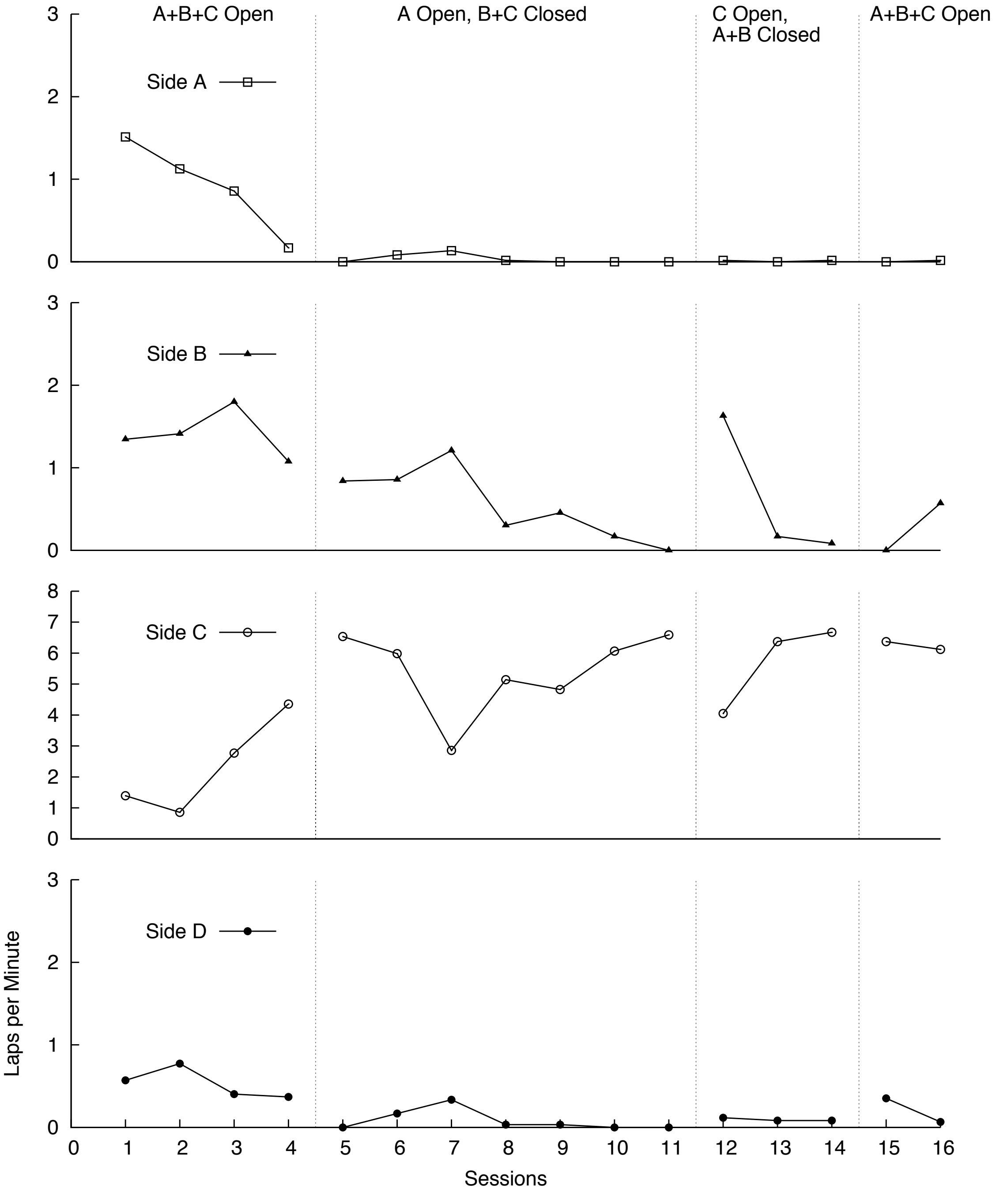
Side Coverings for Fish 3

All fish swam laps at each of the four sides of the experimental tank during baseline. Before the experiment began it was unknown where lap-swimming rates might change during or following an FT phase. Fish 1's first baseline phase was therefore continued until rates at all sides were reasonably stable, resulting in a phase duration of 16 days.

A technique for increasing or decreasing swimming at the non-mirror sides during the baseline condition could be useful for reducing the time required to observe stable lap-swimming rates. On the possibility that the fish were influenced by what they could see outside of the tank, I experimented briefly with covering sides A, B, or C by placing white plastic sheeting outside the tank during fish 3's first baseline phase.

After 4 sessions without coverings (phase 1; Figure 9), sides B and C were covered while A remained open (phase 2). In phase 3 (sessions 12 through 14), side C was uncovered, while A and B were covered. Finally sides A, B, and C were all uncovered (sessions 15 and 16).

Figure 9. *MSLS rates with side-coverings, fish 3, experiment 1.*

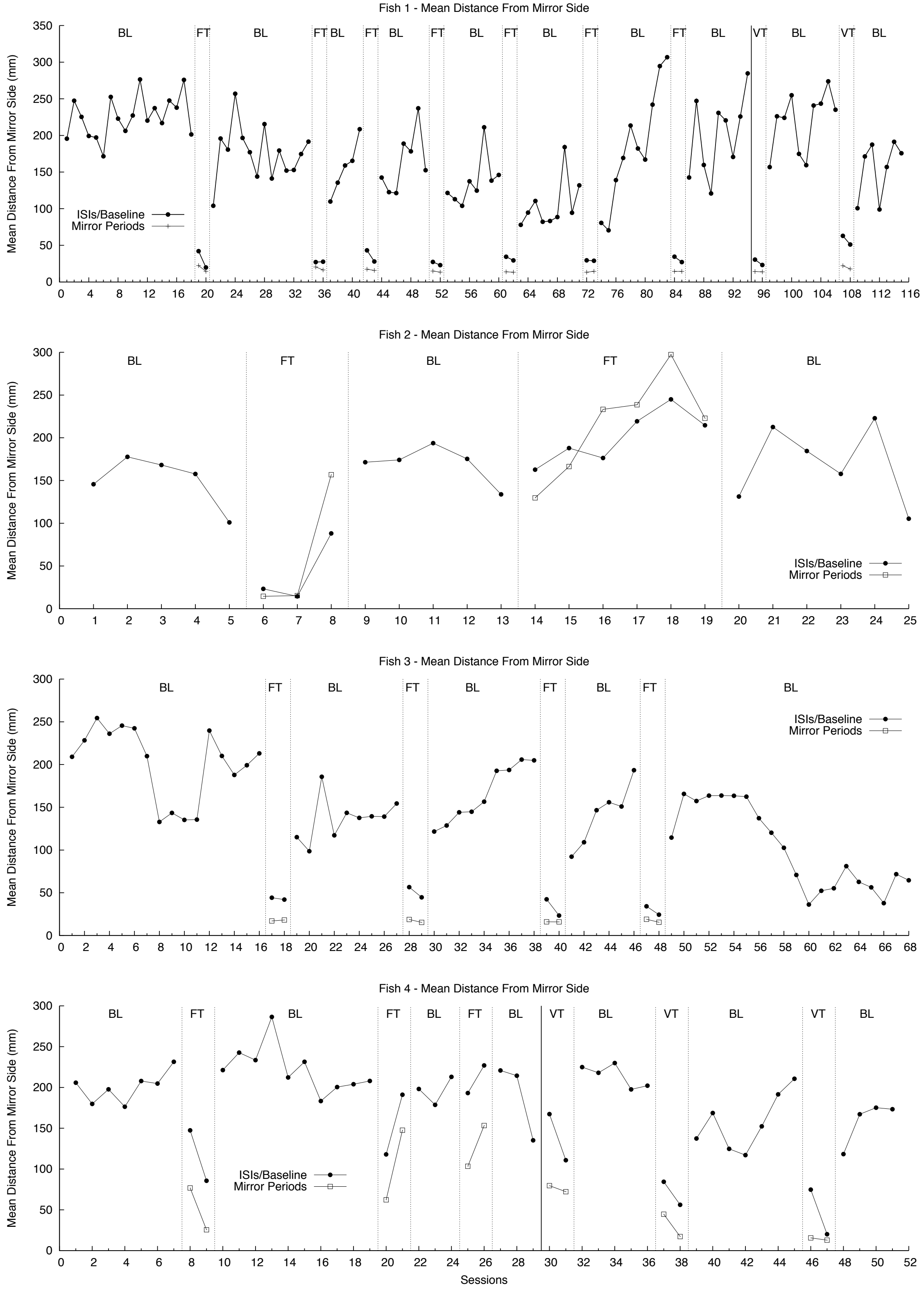


These manipulations were limited in terms of phase length and combinations of covered and uncovered sides, but the results suggested that side covering had little effect on fish 3's lap-swimming rates. Leaving a single side uncovered did not increase lap-swimming from a low rate (side A in phase 2), nor did it produce a sustained decrease in lap-swimming from a high rate (side C in phase 3). Covering a previously open side appeared to have no effect on side A rates (between phases 2 and 3) or side C rates (between phases 1 and 2) cases. Rate changes at side B suggest some effect of side coverings, but the effect was judged insufficient to justify using the coverings as a control technique.

Distance from Mirror Side

Figure 10 presents mean distance from the mirror for each session for all four fish. Overall, mirror presentations produced approach to the mirror side that persisted during ISIs and subsequent baseline phases, although this effect diminished over time for two fish. Thus, distance from the mirror followed MSLS rates fairly closely.

Figure 10. *Mean distance from mirror side, experiment 1.*



Mirror periods. For all fish, distance from the mirror side was reliably lower during mirror presentations than during the baseline sessions and with almost no exceptions during the ISIs.

Baseline sessions. There was a tendency in three out of the four fish for baseline mirror-side distance following the FT sessions after the first and the VT sessions to start out low and then to increase.

ISIs. In general, mirror-side distance during the ISIs was higher than during the mirror presentations. The fish generally showed mirror-side distance decreases during ISIs relative to preceding baseline levels when FT or VT was introduced.

Chapter 4 - Discussion

The key finding of this experiment was that response-independent schedules of mirror presentations generally increased rates of back-and-forth lap-swimming by *Betta splendens* during ISIs and subsequent baseline sessions, relative to early sessions in baseline phases. Although these findings were not consistent across all fish, they were replicated a number of times within at least two of the fish. What learning process or processes might account for these findings? Two possibilities are operant and respondent conditioning.

The observed MSLS increases may have been due to operant conditioning, through the process of adventitious reinforcement. Mirror presentations often reinforce swimming behaviour that precedes them for both males (Thompson, 1963) and females (Elcoro, Silva, & Lattal, 2008) of this species. All fish in this experiment spent a majority of their time near one of the tank walls, and often swam back and forth while close to a wall. This behaviour would likely have occurred prior to some mirror presentations and may have been adventitiously reinforced by them.

This explanation is consistent with the MSLS increases during ISIs as well as during returns to baseline, and with MSLS increases within FT phases. Adventitious reinforcement could also account for some of the variability within and between fish. One difficulty with this explanation is that one might expect to have seen a stronger scallop within intervals during FT sessions. That is, since only laps swum near the ends of ISIs were consequated by mirror presentations, lap-swimming should have been low early in ISIs, and accelerated toward the 120 s mark. In fact, MSLS rates tended to increase slightly within sessions for fish 1, 2 (during the first FT phase only) and 3, but this aspect of performance did not become more pronounced over time (Figures 5, 6, and 7, respectively).

Future experiments could test the contribution of adventitious reinforcement directly, using methods similar to those employed by Timberlake and Lucas (1985). For example, the VTS could be programmed to identify instances of lap-swimming and only present the mirror if the behaviour has not occurred within the preceding 10 s. Any MSLS increase during ISIs using this procedure would be the result of some process other than adventitious reinforcement.

Could the MSLS increases have been due to respondent conditioning? This explanation requires that lap-swimming occurred as an unconditioned response either to mirror presentation or to mirror withdrawal. Elicitation of lap-swimming by the removal of a conspecific's image is plausible from an ecological perspective, because an intruder's retreat from one part of a defender's territory might often be followed by intrusion at some other area. Elicitation by mirror withdrawal implies that MSLS should have been highest at the beginning of ISIs, however, and this was not the case (Figures 5 through 8). Furthermore, respondent conditioning occurs most readily when a previously neutral stimulus precedes and overlaps with the unconditioned elicitor. In FT sessions the stimulus that most reliably preceded mirror withdrawal was 120 s of mirror presentation - a stimulus that never occurred in the return-to-baseline condition, when MSLS was nevertheless elevated.

The possibility that MSLS occurred as an unconditioned response to the mirror's presence is supported by the fact that all fish showed elevated MSLS rates during mirror periods, though this effect diminished over time for fish 2 and 4. Possible conditioned stimuli (CS) include the light panel and time (temporal conditioning), or more accurately, physiological correlates of the passage of time. One problem for seemingly any CS, however, is that mirror presentations reliably elicited several other responses, including gill-cover erection and sideways undulation (Simpson, 1968), yet during unrecorded observations of hundreds of sessions, neither of these behaviours was ever seen to occur except in the mirror's presence. It's unclear why only MSLS would condition, since in a previous experiment on respondent conditioning with *Betta splendens* (Thompson & Sturm, 1965) all components of the agonistic display conditioned to a light that preceded mirror presentations.

Explaining increased MSLS as temporal conditioning faces a challenge similar to that faced by an operant conditioning explanation: the CR should presumably be strongest near the ends of ISIs. Although fish 1 and 3 showed slight increases of MSLS rates within ISIs, rates were often highest after only 20 or 30 s of the interval had elapsed (Figures 5 and 7, respectively). One would also expect that switching from FT phases to VT phases would diminish or eliminate a temporally conditioned MSLS increase. Although fish 1's MSLS rates during its first VT phase were lower than during the preceding FT phase, the rates increased during the second VT phase relative to the first - a result difficult to explain as temporal conditioning (Figure 3).

MSLS may have increased as an indirect effect of increased proximity to that side. An image of a conspecific elicits approach in *Betta splendens*, and bettas readily learn to spend more time in areas where images of conspecifics have previously appeared (Bronstein, 1986). Whatever the relative contributions of operant and respondent conditioning to a conditioned place preference, it could account for the MSLS increases in the present experiment, since all fish tended to swim back and forth at the side to which they were closest.

A conditioned place preference may not account for at least one feature of the results, however. Fish 1's MSLS rate during ISIs increased on the 2nd day relative to the 1st day during 6 out of 7 FT phases (Figure 3). Fish 3's MSLS rate during ISIs increased on the 2nd day during 4 out of 4 FT phases (Figure 3). While it's true that fish 1 and 3 also decreased their distances from the mirror on the 2nd day of each FT phase (Figure 10) the changes in distance were not more than 1 or 2 cm. In other words, both fish were sufficiently close to the mirror side relative to the other three sides during all FT sessions that it is difficult to see how proximity to that side alone can account for the reliable within-phase increases in MSLS rates.

To explain their findings, Timberlake and Lucas (1985) de-emphasized both operant and respondent conditioning in favour of BSA. According to BSA, food delivery activates behavioural modes comprised of modules of unconditioned reflexes. A rat's feeding system, for example, consists of modules in the following modes: a general search mode, a focal search mode, a handling/consumption mode, and a post-food focal search mode. If *Betta splendens* have a comparable system related to territory defense, it is plausible that the system includes a focal search mode that could be conditioned to stimuli that predict another male's imminent appearance in a particular location. The system might also include a general search mode that is active when less information is available about the location or time of intrusion.

A key prediction of BSA is that CS-US interval length may influence CR development. Long CS-US intervals may condition a general search mode, shorter intervals may condition a focal search mode, and very short intervals may condition a handling/consumption mode. The responses that occur as a result of the conditioning procedure are those that are appropriate to whichever mode has been conditioned. An important way to test this prediction in a future experiment using bettas would be to add a stimulus brightness ramp, as used by Matthews et al. (1990) or Silva and Timberlake (1998). During ISIs in an FT schedule, the light panel (or another light) would increment in brightness at regular intervals, e.g. every 30 s. BSA would be supported if different swimming patterns, reflecting general versus focal search modes, came to be controlled by different levels of brightness.

Some behaviour induced by response-independent schedules has been described as neither operant nor respondent, but attributed to a third category called adjunctive behaviour. The clearest evidence that behaviour is adjunctive involves demonstrating that it is highly unlikely to be either operant or respondent. This experiment did not rule out both of these processes, so there is no clear evidence that the MSLS rates increases were adjunctive.

This experiment provided limited information about the effects of schedule variability. Schedule variability had no clear effect on lap-swimming rates during ISIs, though it appeared to decrease them in fish 1 (Figure 3). In fish 4, the VT schedule appeared to increase proximity to the mirror side (Figure 10) and increase MSLS rates during mirror periods, relative to behaviour during the FT schedule (Figure 3). These increases were not replicated within or across fish, however.

The main finding of increased MSLS during ISIs and baseline sessions was seen only in fish 1 and 3 (Figure 3). The failure of fish 2 and 4 to show a clear effect of response-independent schedules on MSLS rates might be explained as a by-product of habituation of the agonistic response to the mirror. This explanation is not strongly supported, however, by the only available measure of agonistic response: mean distance from the mirror. Although fish 2's MSLS rates were lowest during its 2nd FT phase (Figure 3), when mean distance from the mirror was highest (Figure 10), MSLS rates during ISIs were not elevated above baseline level during sessions 6 and 7, when mean distance was very low. And although fish 4's MSLS rates decreased over three FT phases (Figure 3) while mean distance increased (Figure 10), mean distance then decreased over three VT phases while MSLS rates remained low during 2 out of 3 of these phases.

During the first baseline phase, all four fish swam laps at the light panel side at lower mean rates than they swam laps at any of the other three sides. If the light had the effect of depressing lap swimming in proximity to it, this effect would have competed with the effect of intermittent mirror presentations. Alternative mirror delivery systems that employ a shutter rather than a light box could be used in the future. The effect of the light panel independent of the mirror could also be tested experimentally by alternating its location.

Experiment 2

An interesting finding in Experiment 1 was that one fish – Fish 4 – showed increases in mean distance from the mirror side over three FT phases, both during mirror presentations and during ISIs. Its mean distance from the mirror side then decreased across three VT phases. The changes in mean distance coincided with informal observations that the fish's gill-cover erections decreased in frequency during the second and third FT phases, and then increased in frequency during VT phases. The primary purpose of Experiment 2 was to compare the effects of FT and VT schedules on distance from the mirror, to evaluate whether schedule variability influences aggressive response to the mirror image.

A secondary purpose of Experiment 2 was to examine MSLS rates during extended phases of FT and VT schedules, since this measure also decreased across FT phases in fish 4, and then increased across several VT phases.

Chapter 5 - Method

Subjects, Setting, and Materials

Fish 1, 3, and 4 from Experiment 1 served. All aspects of the setting, apparatus, schedules and measures were the same as for Experiment 1.

Design

Daily one-hour sessions for Experiment 2 commenced the day following the final day of Experiment 1 for each fish. Experiment 2 consisted of FT and VT conditions that alternated starting with FT. Fish 1 received two alternations, fish 3 received four alternations, and fish 4 received three alterations (Table 2). I planned to continue sessions in the first FT phase until each fish's average distance from the mirror side increased to typical level of Experiment 1 baseline phases, i.e. 150 to 250 mm away. But because fish 1 and 3 showed little change in average distance, particularly during mirror presentations, over two and three weeks respectively, the VT schedule was introduced in order to see whether it would disrupt the behaviour shown in the first FT phase. With the exception of the first FT phase, phases generally continued until relatively stable performance across sessions was demonstrated.

Table 2

Sequence and Duration of Phases, Experiment 2

Condition	Fish 1	Fish 3	Fish 4
		Number of Sessions	
A	15	19	18
B	11	12	11
A	9	16	13
B		24	9
A		13	

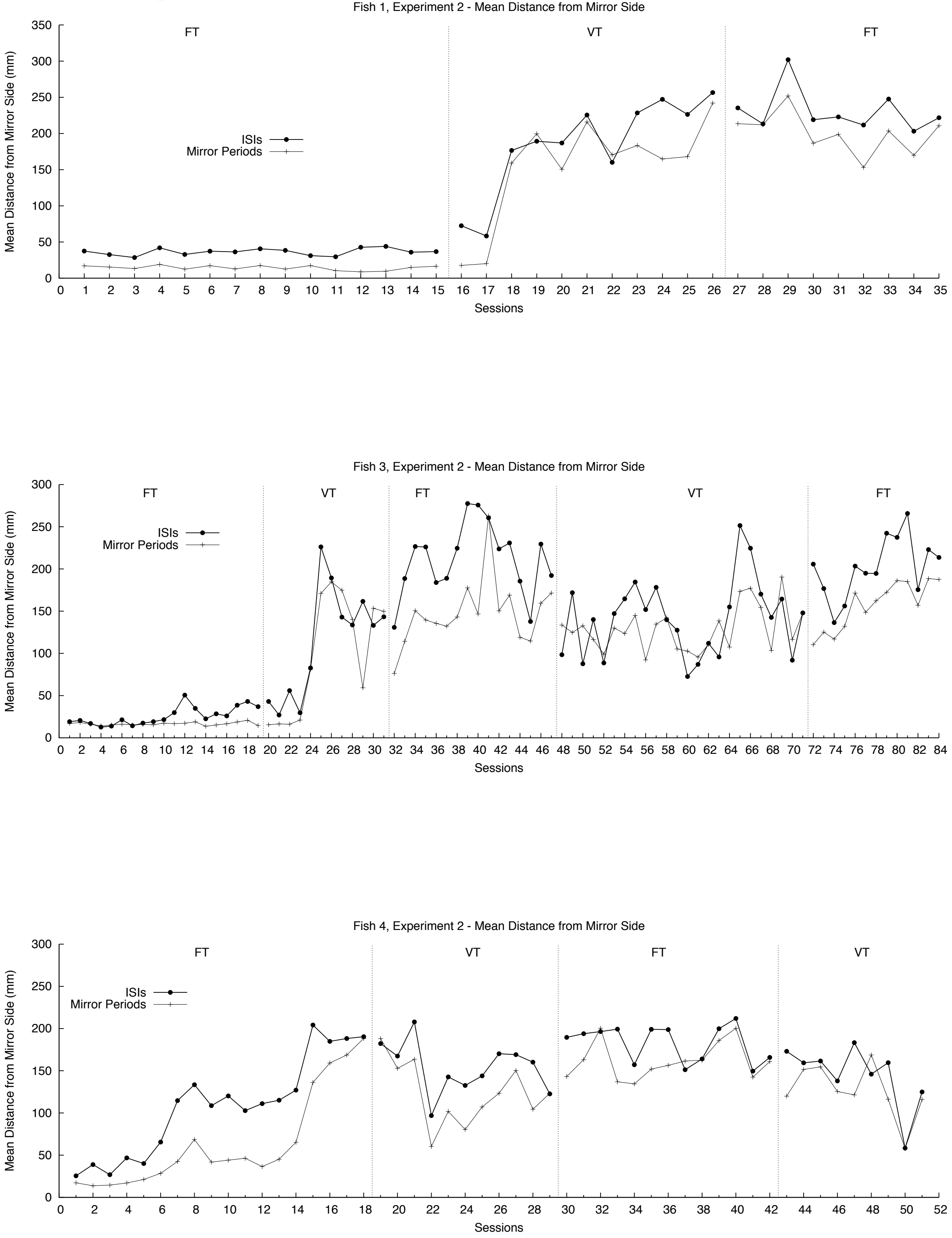
Note. A = Fixed-Time, B = Variable-Time

Chapter 6 - Results

Distance from Mirror

All fish were generally closer to the mirror wall when the mirror was present than when it was absent during both FT and VT (Figure 11). Overall, however, there was little consistency between or within fish with regard to distance from the mirror as a function of schedule variability.

Figure 11. *Mean distance from mirror side, experiment 2.*



Fish 1 showed an immediate increase in mean distance during ISIs after the change from FT to VT. In the third session after the introduction of VT, Fish 1’s distance during both ISIs and mirror periods increased several fold relative to the preceding FT phase. Reinstating the FT schedule at session 27 produced no apparent change in mean distance.

Fish 3 showed little change in mean distance after the introduction of VT, particularly during mirror periods, until the 5th session after VT was first introduced. When FT was reinstated mean distance dropped slightly during mirror periods, but then returned to approximately the level at which the previous phase ended. Mean distance during mirror periods changed little over the remaining phases.

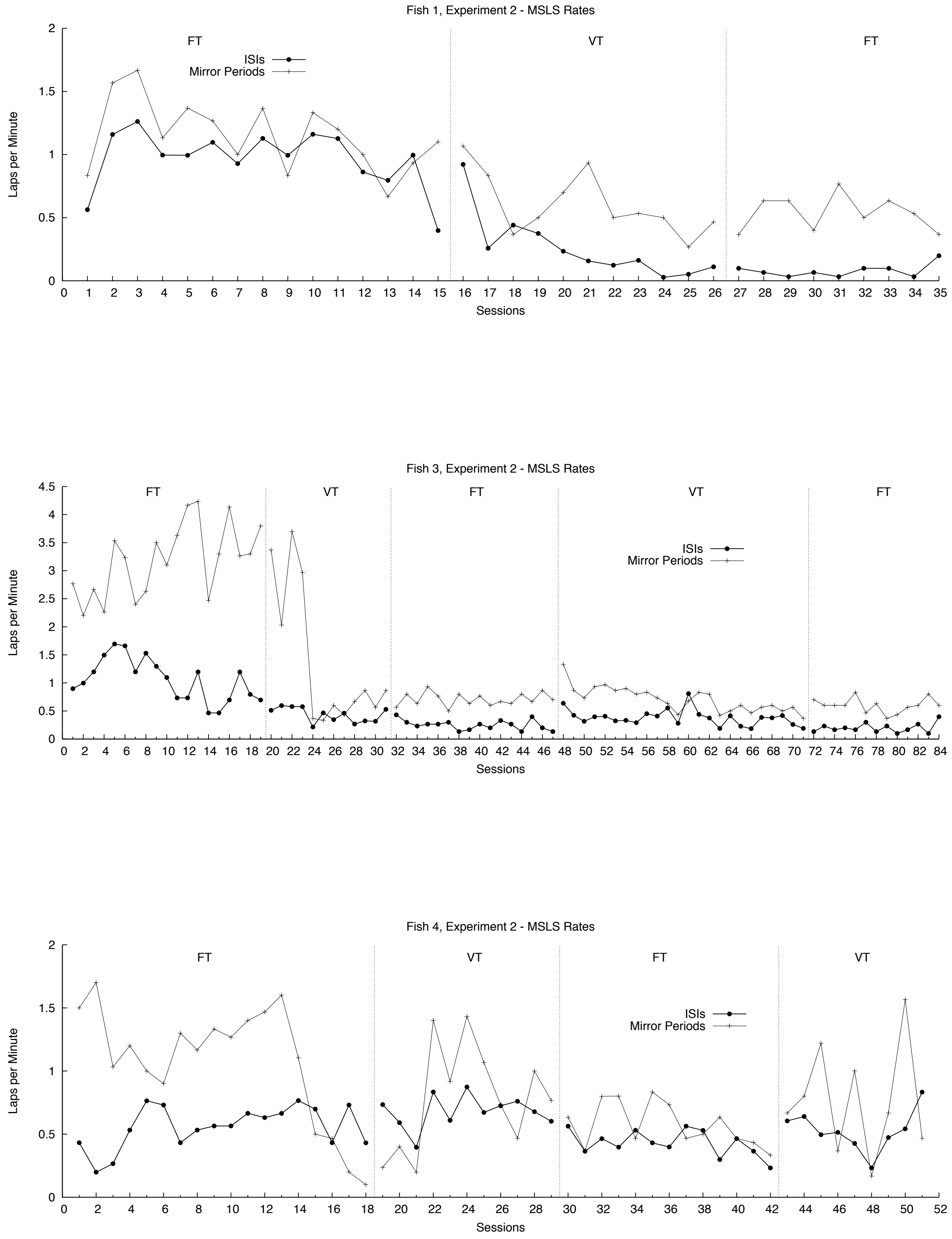
Fish 3's distance during ISIs appeared elevated in the 2nd and 3rd FT phases relative to the two VT phases, and relative to the mean distance during mirror periods in all FT phases. Mean distance was greater during ISIs than during mirror periods in 78.95% (15/19) of sessions in the 1st FT phase, 93.75% (15/16) of sessions in the 2nd FT phase, and 100% (13/13) of sessions in the 3rd FT phase, versus 58.33% (7/12) in the 1st VT phase and 54.17% (13/24) in the 2nd VT phase.

Fish 4 generally swam closer to the mirror side during ISIs and mirror periods in the two VT phases than in the 2nd FT phase or the last four sessions of the first FT phase. For this fish, as for the other fish, the mean distance was less during mirror presentations than during ISIs in most sessions (43/51, 84.31%); however, this difference was less during the last 5 or 6 sessions of the last two phases than during the other sessions in Experiment 2. Thus, introducing VT after FT tended to decrease distance from the mirror wall, and vice versa.

MSLS Rates

There was little consistency within or between fish regarding the effect of schedule variability on MSLS rates (Figure 12).

Figure 12. *MSLS rates, experiment 2.*



Fish 1's MSLS rates during ISIs and mirror periods both diminished throughout the three phases and no clear difference was observed between FT and VT conditions.

Fish 3's MSLS rates decreased during the first VT phase, especially during mirror periods, and this difference persisted through all subsequent phases. The drop in MSLS during mirror periods between sessions 23 and 24 coincided with a large increase in mean distance from the mirror (Figure 11). MSLS rates were slightly elevated in the 2nd VT phase relative to the preceding and following FT phases, but there were many overlapping data points between conditions.

Fish 4's MSLS rates during mirror periods in the two VT phases were generally higher and more variable between sessions than in the 2nd FT phase or in the last four sessions of the 1st FT phase. MSLS rates during ISIs showed little change after VT was first introduced, but declined during the 2nd FT phase and increased following the change to the 2nd VT phase. There were many points of overlap between conditions both during ISIs and during mirror periods.

Chapter 7 - Discussion

A major goal of Experiment 2 was to explore the generality of a result from Experiment 1, where switching from FT to VT phases (alternated with a no-mirror baseline condition) appeared to reinstate one fish's aggressive response to the mirror. This goal was partially achieved, in that the VT schedule appeared to produce lower average distances from the mirror than the FT schedule in 2 of 3 fish. Differences between phases were small even for fish 3 and 4, with multiple points of overlap in the plotted distance data.

As in Experiment 1, Fish 4's distance from the mirror side decreased during the VT schedule relative to FT both during mirror presentations and ISIs. Fish 3's result in Experiment 2 differed in that an effect of schedule variability was seen clearly, or at least more clearly, only during ISIs. The possibility that schedule variability could have relatively independent effects on behaviour during mirror and non-mirror periods merits further research.

The effect of schedule variability on MSLS rates was similar to the effect on mean distance from the mirror: two fish (3 and 4) showed small differences in rates between VT and FT during their final three phases. MSLS rates were generally higher when mean distance from the mirror was less, a relationship also observed in Experiment 1.

Experiment 2 was limited by the use of a single measure of agonistic response to the mirror, namely average distance from the mirror side of the tank. Approach to an opponent, or mirror image, is an integral part of the agonistic response (Simpson, 1968) and other measures of approach have been used successfully to study habituation in *Betta splendens* (Bronstein, 1994). Nevertheless rates and/or durations of gill-cover erections would also be of interest, though in this experiment the quality of recorded video images was too low to permit reliable measurement of this behaviour.

Computer classification of spatiotemporal data is an intriguing possibility for future studies. Kinsner et al. (2006) demonstrated that computing the variance fractal dimension trajectory of VTS measurements of Betta swimming behaviour permits automated classification of swimming patterns. Additional research is needed to show that the classes identified using this approach correspond to human-observable locomotive behaviours, e.g. aggressive responses to the mirror. Once this is shown, however, it may be fruitful to retrospectively analyze the data collected in the present experiments.

Chapter 8 - General Discussion

In experiment 1, response-independent mirror presentation schedules increased lap-swimming rates and proximity to the mirror during ISIs and subsequent baseline sessions, relative to early sessions in baseline phases. This finding adds to the interspecies generality of response-independent schedule effects. Perhaps more importantly, it suggests that *Betta splendens* are a useful species with which to continue critically evaluating alternative explanations for behaviours induced by response-independent schedules. Although the present findings did not conclusively support or rule out particular learning processes as explanations, they point the way to future experiments that may do so.

In experiment 2, a VT schedule increased lap-swimming rates and proximity to the mirror relative to an FT schedule. The increase was small and replication should be attempted. Nevertheless this result is important because if VT reinstates agonistic display after a period of FT, the reinstatement may be an example of dishabituation. In that case, this would be the first clear demonstration of dishabituation with this species. Demonstrating dishabituation is important because it is one of the defining characteristics of habituation and may distinguish it from other forms of response decrement, e.g. effector fatigue. A reliable technique for reinstating agonistic display to a mirror also has a practical implication for researchers working with bettas, who could use presentation variability as a way to extend periods of effective responding in their subjects.

Taken together, the present experiments increased our knowledge of schedule-induced behaviour. Schedule-induced stereotypy is common in captive animals, and is often detrimental to their welfare (Mason, 1991). A variety of human behavioural excesses may also be schedule-induced, including locomotion (Muller, Crow, and Cheney, 1979) and some repetitive behaviours displayed by persons with autism and developmental disabilities (Lerman, Iwata, Zarcone, & Ringdahl, 1994). Results may therefore inform future efforts to study and treat these behaviours.

References

Anderson, M., & Shettleworth, S. (1977). Behavioral adaptation to fixed-interval and fixed-time food delivery in golden hamsters. *Journal of the Experimental Analysis of Behavior, 25*, 33-49.

Barry, R. L. (2003). *Multifractal characterization for classification of network traffic*. Master's thesis, University of Manitoba.

Bronstein, P. M. (1986). Socially mediated learning in male *Betta splendens*. *Journal of Comparative Psychology, 100*(3), 279-284.

Bronstein, P. M. (1994). On the predictability, sensitization, and habituation of aggression in male bettas (*Betta splendens*). *Journal of Comparative Psychology, 108*(1), 45-57.

Elcoro, M., Silva, S. P., & Lattal, K. A. (2008). Visual reinforcement in the female Siamese fighting fish, *Betta splendens*. *Journal of the Experimental Analysis of Behavior, 90*(1), 53-60.

Higa, J. J., & Simm, L. A. (2004). Interval timing in Siamese fighting fish (*Betta splendens*). *Behavioural Processes, 67*(3), 501-509.

Hogan, J. A., Kleist, S., & Hutchings, C. (1970). Display and food as reinforcers in the Siamese fighting fish (*Betta splendens*). *Journal of Comparative and Physiological Psychology, 70*, 351-357.

Innis, N. K., Simmelhag-Grant, V. L., & Staddon, J. E. R. (1983). Behavior induced by periodic food delivery: the effects of interfood interval. *Journal of the Experimental Analysis of Behavior, 39*(2), 309-322.

Kinsner, W., Cheung, V., Cannons, K., Pear, J. J., & Martin, T. L. (2006). Signal classification through multifractal analysis and complex domain neural networks. *IEEE Transactions: Systems, Man, and Cybernetics, Part C, 36*(2), 196-203.

Lerman, D. C., Iwata, B. A., Zarcone, J. R., & Ringdahl, J. (1994). Assessment of stereotypic and self-injurious behavior as adjunctive responses. *Journal of Applied Behavior Analysis, 27*(4), 715-728.

Lind, N. M., Vinther, M., Hemmingsen, R. P., & Hansen, A. K. (2005). Validation of a digital video tracking system for recording pig locomotor behaviour. *Journal of Neuroscience Methods, 143*(2), 123-132.

Mason, G. J. (1991). Stereotypies: a critical review. *Animal Behaviour, 41*(6), 1015-1037.

Martin, T. L., Schledewitz, K., Chan, W., & Pear, J. J.

(2003, May). Swimming Patterns During Habituation of Agonistic Display in *Betta splendens*. In S. P. da Silva (Chair), *Behavioral Processes in Siamese Fighting Fish*. Symposium conducted at the 29th Annual Convention of the International Association for Behavior Analysis, San Francisco, CA.

Matthews, J. J., Bordi, F., & Depollo, D. (1990). Schedule-induced kinesic and taxic behavioral stereotypy in the pigeon. *Journal of Experimental Psychology: Animal Behavior Processes, 16*(4), 335-344.

Muller, P. G., Crow, R. E., & Cheney, C. D. (1979). Schedule-induced locomotor activity in humans. *Journal of the Experimental Analysis of Behavior, 31*(1), 83-90.

Pear, J. J., & Legris, J. A. (1987). Shaping by automated tracking of an arbitrary operant response. *Journal of the Experimental Analysis of Behavior, 47*(2), 241-247.

Silva, K., & Timberlake, W. (1998). The organization and temporal properties of appetitive behavior in rats. *Animal Learning & Behavior, 26*(2), 182-195.

Silva, K. M., & Timberlake, W. (1999). Rats' behavior during an interfood clock is altered by the temporal pattern of interfood stimuli. *Learning and Motivation,* *30*(2), 183-200.

Silva, K. M., & Timberlake, W. (2005). A behavior systems view of the organization of multiple responses during a partially or continuously reinforced interfood clock. *Learning & Behavior, 33*(1), 99-110.

Simpson, M. J. A. (1968). The display of the Siamese fighting fish, *Betta splendens*. *Animal Behaviour Monographs, 1*(1), 1-73.

Skinner, B. F. (1992). 'Superstition' in the pigeon. 1948. *Journal of Experimental Psychology, General, 121*(3), 273-274.

Staddon, J. E. R., & Simmelhag, V. (1971). The "superstition" experiment: a reexamination of its implications for the principles of adaptive behavior. *Psychological Review, 78*(1), 3-43.

Thompson, T., & Sturm, T. (1965). Classical conditioning of aggressive display in Siamese fighting fish. *Journal of the Experimental Analysis of Behavior, 8*(6), 347-403.

Thompson, T. I. (1963). Visual reinforcement in Siamese fighting fish. *Science, 141*(3575), 55-57.

Timberlake, W. (1983). The functional organization of appetitive behavior: behavior systems and learning.

Advances in Analysis of Behavior, 3, 177-221.

Timberlake, W., & Lucas, G. A. (1985). The basis of superstitious behavior: chance contingency, stimulus substitution, or appetitive behavior. *Journal of the Experimental Analysis of Behavior, 44*(3), 279-299.