Critical Swimming Speed and Metabolic Activity as Predictors of Ecologically Relevant Behaviour in Juvenile Rainbow Trout (*Onchorhynchus mykiss*)

by

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Abstract:

Swimming ability in fish is vital for survival since it relates to behaviours such as avoiding predators, capturing prey, migration, and reproduction. Critical swimming speed is the most commonly used measure to quantify swimming ability, and so it is often assumed to be a good indicator of behaviour. Due to the relationship between metabolic activity and swim performance, it is possible that aerobic and anaerobic metabolism influence behaviour in fish. This study sought to examine the potential link between critical swimming speed and ecologically relevant behaviour, and to determine the extent that aerobic and anaerobic metabolism play in determining behaviour. Our results suggest that critical swimming speed is not related to predator avoidance or prey capture behaviour in a lab setting, but that burst swimming speed may be related to these behaviours. Aerobic and anaerobic metabolic activity were related to predator avoidance and prey capture behaviour. Since metabolism influences swim performance by providing fuel and determining recovery capability, it is possible that metabolism is a more accurate predictor of predator avoidance and prey capture ability.

Preface:

This thesis represents an original work by Kyle C. Dehaan. Dr Keith Tierney contributed to the development of the research question and the experimental methods, as well as the final document. The work was conducted in accordance with animal ethics as outline in AUP#022.

Acknowledgements:

First, I would like to thank my supervisor Keith Tierney for the opportunity to conduct this research, as well as for the support and guidance he provided throughout my degree. I would also like to thank Ted Allison for taking the time to be on my committee and providing his expertise and guidance on this project, as well as to Colleen St Clair for her time as my arm's length examiner. I would also like to thank Natasha Klappstein and Isabelle Adatia for their time and work reviewing the behaviour video. Thank you to Greg Goss and his lab members for supplying the fish for this work, and for all their help throughout this project. Next, I'd like to acknowledge NSERC for providing funding throughout the course of this degree. I'd like to thank the University of Alberta for providing the opportunity to pursue this degree, as well as all the staff who were essential to the completion of this degree. I would like to thank all my family and friends who have supported me throughout this degree. Lastly, a special thank you to Shay, who saw me through this degree.

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Glossary of Terms:

AC Absolute control

AS Aerobic scope

CBZ Carbamazepine

CF Caffeine

CHP Chlorpyrifos

CS Citrate synthase

COX Cytochrome C oxidase

DMSO Dimethyl sulfoxide

LDH Lactate dehydrogenase

MMR Maximum metabolic rate

PCP Pentachlorophenol

RMR Routine metabolic rate

TBT Tributyltin

Chapter 1: Introduction

1.1 Background of Swim Performance

Swimming ability in fish is vital for migration, avoiding predators, capturing prey, spawning, and maintaining position in the water column (Reidy et al. 2000; Plaut 2001). When swimming is impaired, a fish has reduced fitness indicating there is a selective pressure on swimming ability traits and the behaviours related to them. Locomotor ability, and the swimming strategy employed (sprint, endurance, or generalist), intuitively relates to a species' behavioural phenotype in relation to 'starvation-predation risk trade-off'. Furthermore, behavioural alterations may be useful indicators of "ecological death" (Nassef et al. 2010) since a fish with impaired predator avoidance or feeding behaviour is unlikely to survive and reproduce.

Salmonids depend heavily upon endurance swimming (Taylor and McPhail 1985) and fast start bursts (Webb 1975) for various aspects of survival. The intense locomotor demands faced by salmonids require high metabolic capacity for fuel utilization and recovery. Unlike some species, salmonids perform well at both endurance and sprint swimming (Reidy et al. 2000), and typically require high aerobic and anaerobic capacities in order to fuel red and white muscle respectively, but also to recover from exhaustive exercise (Goolish 1991). Survival skills such as the ability to escape predators and capture prey are dependent upon locomotion, and so these behaviours are likely linked to the metabolic capacity as well.

To better understand the energy demands of anadromous salmonid genera such as *Oncorhynchus* and *Salmo* during migration, and to determine water flow speed exiting dams and culverts, there was a need to quantify the swimming performance in fish (Brett 1964). Currently, swimming performance has been characterized into three areas depending on speed and endurance. Sustained swimming refers to steady, aerobically fueled swimming at relatively low speeds that can be maintained indefinitely without fatigue, or simply greater than 200 minutes (Hoar 1981). This type of swimming would be expected

during foraging or station holding. Conversely, burst swimming is anaerobically fueled high speed swimming maintained for under 10 seconds, and results in fatigue (Hoar 1981). These bouts of burst swimming are employed during predator escape, prey capture, or navigating areas of high flow during migrations. Finally, prolonged swimming refers to the range of speeds between sustained and burst swimming that results in fatigue (Hoar 1981). This is mostly aerobically fueled, but near the point of fatigue there is anaerobic contribution from white muscle. In a natural environment, prolonged swimming is often impossible to distinguish from sustained swimming (Plaut 2001) since determination of fatigue is not possible.

The most common test used to quantify swim performance is the incremental velocity test, or critical swimming test which generates U_{crit} values. This test involves incremental water velocity increases over set time intervals until the fish fatigues. The speed at which the fish fatigues is used to calculate the U_{crit} value. U_{crit} is a measure of the maximum prolonged swimming speed and has been used to compare swim performance across species. While useful in certain applications, the ubiquitous application of U_{crit} for all fishes is debatable since there are major differences in life history traits, behaviour phenotypes, and metabolic physiology across fish species.

1.2 Background of Metabolism

Locomotion is dependent on the integration of different systems, including red and white muscle, and cardiorespiratory tissues. During intense exercise, energy stores within white muscle such as glycogen and phosphocreatine deplete, and lactate builds up due to glycolysis (Wood 1991). With aerobically fueled red muscle, energy supply matches demand, waste buildup is balanced by removal, and as such muscle activity can be maintained (Wood 1991). To quantify the scope of aerobic activity, physiologists have employed aerobic scope measurements, which is the difference between minimum metabolic rate and maximum metabolic rate. Minimum metabolic rate, often termed standard metabolic rate (SMR) or routine metabolic rate (RMR) is the oxygen consumption of a non-feeding

animal that is needed to maintain basal function, while maximum metabolic rate (MMR) is the oxygen consumption during peak activity (Allen et al. 2016). Aerobic scope (AS) is therefore intimately linked to peak performance of cardiac and red muscle under intense exercise.

Fatigue following intense exercise may be due to the buildup of metabolic wastes, but also from the depletion of energy stores in the white muscle. Approximately 20% of lactate from white muscle in rainbow trout (*Onchorhynchus mykiss*) enters the blood to be utilized as fuel for metabolic oxidation in tissues such as the liver, heart, and red muscle (Milligan and Girard 1993). However, there is also evidence for a high capacity of *in situ* white muscle glycogenesis (Milligan and Girard 1993) as a way of replenishing glycogen stores following exhaustive exercise. One of the primary anaerobic metabolic enzymes necessary for metabolic recovery is lactate dehydrogenase (LDH) which catalyzes the final step in glycolysis, the oxidation of pyruvate to lactate. This is important under anaerobic conditions since it regenerates an NAD+ molecule needed for glycolysis to continue generating ATP. In doing so, lactate dehydrogenase prolongs activity and delays muscle fatigue by allowing glycolysis to continue.

Despite producing less energy than the citric acid cycle, glycolysis may be more vital to fish than it is for mammals since it is common for fish muscle to be hypoxic (Orrego et al. 2011), and so lactate dehydrogenase activity may have a greater importance to fish. Lactate dehydrogenase also functions during the recovery period following exhaustive exercise since it is responsible for the conversion of lactate back into pyruvate, which is then used to regenerate ATP and glycogen stores through aerobic oxidation pathways (Gleeson 1996). Pyruvate is used to create acetyl-CoA under aerobic conditions, which can then enter the Citric Acid Cycle to produce ATP and reducing equivalents needed to regenerate glycogen stores. There is the possibility for a physiological trade-off between increasing the amount of stored energy for white muscle function, and the ability of white muscle to recover energy stores quicker. A larger store of energy would allow for greater anaerobic activity, but it would then

require more time to replenish the energy store, whereas recovering energy stores quicker following fatigue would allow for quick repeated bouts of activity.

Mitochondrial oxidation of fuel to produce ATP makes up the majority of energy production in aerobic red muscle (Battersby and Moyes 1998). Citrate synthase (CS) plays a large role in aerobic energy production since it is the first pace setting enzyme of the citric acid cycle. While the citric acid cycle produces ATP, the electron transport chain is the primary energy production system in mitochondria and is intrinsically linked to the citric acid cycle through the reliance on intermediates such as NADH and $FADH_2$. Cytochrome C oxidase (COX) is the final enzyme in the ETC located in the mitochondrial membrane. Both citrate synthase and cytochrome C oxidase are used as markers of aerobic capacity (Orrego et al. 2011; Zak et al. 2017). These pathways are also vital in the restoration of energy stores following exhaustive exercise when oxygen levels are adequate as they generate the necessary ATP and reducing equivalents needed for glycogenesis.

In order to fuel high levels of activity, active foragers such as rainbow trout could be expected to have a large aerobic scope and higher levels of citrate synthase and cytochrome C oxidase activity (Morash et al. 2014). Since these enzymes are vital in the production of large quantities of ATP, it would follow that they would be more active in fish that are actively foraging. Alternatively, sit and wait predators may require higher aerobic capacity because of faster recovery from exhaustive exercise, which is fueled by aerobic metabolism (Metcalfe et al. 2016). Increased vigilance for predators likely comes with a metabolic cost due to the energy needed to maintain high vigilance, and the loss of foraging (Domenici 2010). Also, the brain is highly energetically expensive to maintain, and so increasing brain activity required for active vigilance would be energetically expensive (Killen et al. 2015). For post-exhaustive exercise, maintaining predator vigilance could delay recovery of energy stores in the muscle and thus limit a fish's ability to engage in multiple burst swim escapes. Furthermore, by being actively vigilant, a fish is no longer capable of active foraging which further reduces energy stores.

1.3 Background of Behaviour

Loss of foraging potential is one of the major costs associated with increased predator vigilance and factors into the decision to engage in predator avoidance behaviour (Killen et al. 2015). Fleeing too early or too often would mean the balance of 'starvation-predation risk trade-off' is skewed towards avoiding predation, and a fish could be facing starvation risk. Conversely, fleeing too late shifts the balance away from avoiding predation and leaves an individual at risk of being prey upon. This is of particular concern to active foraging opportunistic omnivores such as rainbow trout because they may be exposed to both aquatic and airborne predators while they forage.

When an alarm signal (chemical or physical) is detected, fish typically respond by fleeing (Lawrence and Smith 1989), freezing (Brown and Godin 1997), increased shelter use (Brown and Smith 1998), or shoaling (Brown and Laland 2003). Fleeing from a physical attack implies the risk of capture is greater than the benefit of saving energy or the cost of reduced foraging (Brown and Smith 1997). Fish may freeze rather than flee from a physical attack, but this increases the risk of being captured and may be pre-emptive rather than reactionary. The presence of chemical alarm cues (eg. predator kairomones or conspecific signals) elicits a different response, typically due to a lack of visual stimuli of a predator. In such cases, fish typically find shelter or group together to reduce the risk of predation.

Escaping from a predator has been related to sprint swimming (Domenici 2010; Bellinger et al. 2014), since it is beneficial to rapidly accelerate away from the predator strike. Similarly, different foraging strategies utilize various swimming types. Ambush predators employing a sit and wait feeding strategy, such as northern pike (*Esox lucius*), could be expected to rely more heavily on burst swimming to capture prey (Goolish 1991) than an active forager that is consistently searching for prey. Rainbow trout fall into the latter type, and as such would be expected to rely on prolonged swimming, with the prey capture attempt involving a burst swim.

Rainbow trout make the decision to capture prey, similar to that of avoiding predators, based on visual and olfactory information, as well as previous experience with the prey. Naïve fish, such as hatchery raised fish released into the wild, have been found to consume inanimate objects and stones that resemble pellet food (Brown et al.), and typically eat a smaller range of prey compared to wild counterparts (Brown et al.). Successful prey capture ability, following prey recognition, then relies on locomotor ability, such as maneuverability, acceleration, and strike accuracy to successfully capture prey (Higham 2007). Maneuverability and acceleration directly relate to a fish's ability to move in their environment, while strike accuracy depends on the speed of the fish and the preys' movement pattern.

Predator avoidance and prey capture behaviour can be examined by using endpoints that have importance in an ecological context. Since there are various types of responses to predator strikes, the endpoints used seek to quantify an aspect of the type of response. In order to examine fleeing response, it is common to measure the distance moved from the point of attack (Dannewitz 2001), while the time it takes to react to the predator strike and the time spent fleeing or frozen following a strike are commonly used for both fleeing and frozen responses (Brown and Godin 1997; Petersson and Järvi 2006). Prey recognition is commonly quantified by the time until the first strike (Nassef et al. 2010; Brown et al.). To examine turning ability and maneuverability, the turning radii, angles, and rate may be measured (Domenici 2001). Acceleration is measured either in separate burst swim tests or by calculation during a predator strike. Lastly, strike accuracy can be determined by the number of successful strikes relative to unsuccessful strikes or by the location of the prey relative to the location of the fish's mouth during a successful strike (Higham 2007).

Previous work on swim performance has often made assumptions regarding the ecological relevance of measurements such as U_{crit} (Farrell et al. 1998; Plaut 2001). Most of the work on swim performance has looked at comparing metrics across different species (Webb 1975; Webb 1978; Webb 1988; McDonald et al. 1998), examining how swim performance changes under various conditions

(Gallaugher et al. 1995; Farrell et al. 1998), or looking at underlying physiological factors that contribute to swim performance (Wood 1991; Kieffer et al. 1998; Reidy et al. 2000). Considering the lack of work directly examining how individual changes in U_{crit} may be linked to changes in ecologically relevant behaviour, we should be cautious in making conclusions regarding the extent to which locomotor ability impacts behaviour.

Swim performance and behavioural traits are largely variable, with inter-individual variation often being larger than between species variation. Metabolic traits such as differences in fuel supply, metabolic enzyme activity, and waste removal capacities could play into the variation in swim performance and behaviour. Since U_{crit} has been the most commonly used measure of swim performance, it has been assumed to be a good predictor of ecologically relevant behaviour. I hypothesized that a change in U_{crit} would lead to a likewise change in predator escape velocity and prey capture ability, and that higher rates of metabolic enzyme activity would be related to faster predator escape velocity and prey capture ability. In order to test this, I will use intraperitoneal injections of compounds known to affect swim performance and analyze the individual level changes in U_{crit} and behaviour before and after injection.

Chapter 2: Methods

Fish Housing:

Juvenile rainbow trout were obtained from Raven Brood Trout station in Caroline, AB, Canada. Daily care was provided by the University of Alberta Science Animal Support Services. Fish were fed pellet rations twice daily, with live *Daphnia magna* and frozen brine shrimp being added 7 days prior to the first experiment and continued through to the end of trials to acclimate fish to prey. Holding temperature for fish was maintained at 13°C, which is within the optimum temperature range for rainbow trout (Ineno et al. 2005).

Swim Performance Protocol:

To obtain a strong view of individuals' swim performance, I performed an incremental velocity test (measure of mostly aerobic performance), followed by a constant acceleration test (measure of mostly anaerobic performance), with a 20 min rest period between the swims. Swim tests were then repeated following injection of an experimental compound to instigate a change in U_{crit}. Fish were withheld food for 24 hours prior to the first swim, at which point they were acclimated to the swim chamber for 30 min at 13°C, with a water velocity of approximately 5cm/s prior to the first velocity increase. Swim tests were conducted according to the methods outlined by Brett (Brett 1964) with some modifications. Time increments for the U_{crit} tests were set to 20 min rather than the 60 min intervals in Brett's original methodology to shorten the time of each test, without affecting the U_{crit} performance (Hammer 1995). Velocity was increased by approximately 3cm/s at each step until fatigue. The U_{burst} test was then conducted which involved approximately 5cm/s increases every 1 min, starting at approximately 21cm/s. An electric gate at the back of the swim chamber was used to provide 3V pulses to encourage the fish to swim, thus reducing the potential for behavioural rather than physiological fatigue. Fatigue for both tests was determined as the point in time where a fish was incapable of continuing to swim against the flow. U_{crit} and U_{burst} values were calculated as follows:

$$U_{crit} = V_f + \left[\left(\frac{T_f}{T_i}\right)\right] * V_i \qquad U_{burst} = V_f + \left[\left(\frac{T_f}{T_i}\right)\right] * V_i$$

Where V_f is the velocity of the final completed increment;

V_i is the velocity of increment change;

T_f is the time of fatigue;

T_i is the time increment

Experimental Compounds and Injection Methods:

Following the pre-injection trials, the fish were allowed to recover for 24 hours in a 7L aerated tank. Individuals were then anaesthetized in buffered MS-222 and injected intraperitoneally with the assigned test compound. From our Animal Use Protocol AUP#022, approval for 15 fish per treatment group was received. Due to unforeseen circumstances the experiment was terminated early, with 12 fish used for absolute control (AC) and injection control dimethyl sulfoxide solvent (DMSO), 11 fish for carbamazepine (CBZ), chlorpyrifos (CHP), pentachlorophenol (PCP), and tributyltin (TBT), and 9 fish for caffeine (CF). Preliminary experimental injections began at one tenth the 96-hour LC50 values for various teleost fish for each of the treatments to ensure that sublethal concentrations were used. When mortality was observed, the dose was reduced by 50% until mortality was not observed. The final injection doses are outlined in Table 1. Injections were done 48-hours after the pre-injection swims, and post-injection swims for carbamazepine, chlorpyrifos, dimethyl sulfoxide solvent, and tributyltin were conducted 24-hours after injection. Pentachlorophenol-injected fish were swam 48-hours after injection, while caffeine-injected fish were swam 1-hour after injection. These differences were due to pharmacokinetics and metabolization of the various compounds.

Table 1. List of treatment groups and estimated mode of action on swim performance.

Treatment Group	Dose/Concentration	Mode of action
Absolute Control (AC)	N/A	N/A
Carbamazepine (CBZ)	1µg/g	Neuronal inhibitor; expected to decrease *Ucrit* and *Uburst*
Caffeine (CF)	4000µg/g	Adenosine receptor antagonist; expected to increase *Ucrit* and *Uburst*
Chlorpyrifos (CHP)	50µg/g	Acetylcholinesterase inhibitor; expected to decrease *Ucrit* and *Uburst*
Dimethyl sulfoxide (DMSO)	100µg/g	N/A
Pentachlorophenol (PCP)	2µg/g	Aerobic metabolism inhibitor; expected to decrease *Ucrit* and *Uburst*
Tributyltin (TBT)	0.4µg/g	Anaerobic metabolism inhibitor; expected to decrease *Ucrit* and *Uburst*

Metabolic Rate and Aerobic Scope:

Metabolic rate measurements were taken for the pre-injection and injection trials. Routine metabolic rate was measured prior to the swim test by placing the fish in a sealed 7L Plexiglas test tank and measurements were taken for 10 minutes with an oxygen probe. The fish were then returned to the test tank following the swim tests and maximum metabolic rate was measured similarly to routine metabolic rate. Routine and maximum metabolic rates were calculated by using the shallowest slope for routine metabolic rate and the steepest slope for maximum metabolic rate and corrected for water displacement in the tank by the individual fish, as well as for oxygen use by the probe. Aerobic scope was then calculated as the difference between maximum and routine metabolic rates.

Behavioural Assays:

The behavioural assays were conducted in the 7L plexiglass tanks at 13°C and video recorded using a lateral-facing CCTV camera. They consisted of a 10 min period of routine behaviour, followed by the mock predator strike with a 10 min response period, and lastly, the introduction of 10 live *Daphnia magna* with 10 min of prey capture activity. The fish were acclimated to live prey for a minimum of 7

days prior to testing to eliminate the bold/shy responses to a novel food source (Ware 1971). The simulated predator consisted of a model duck head attached to a lever that allowed the beak to break the water surface and be immediately removed. A second predator strike was used if a fish was not facing the direction of the first strike. These trials were conducted entirely behind a curtain to prevent the fish from being exposed to outside stimuli and to ensure the predator is kept from sight until its introduction. Prey was introduced with a plastic pipette from behind the curtain.

Tissue Collection and Enzyme Assays:

Following the injection swim and behaviour trials, fish were euthanized with a lethal dose of buffered MS-222 and muscle and liver tissue were collected. Muscle tissue was excised from the lateral portion of the fish, placed into three microfuge tubes and flash frozen with liquid nitrogen. Whole liver was collected and flash frozen in liquid nitrogen. Tissue was then stored at -80°C until enzyme assays were conducted. Citrate synthase, lactate dehydrogenase, and cytochrome C oxidase assays were conducted following the methodology outlined in SigmaAldrich (ON, Canada) assay kits for each enzyme (kit numbers MAK193, MAK066, CYTOCOX1, respectively).

Citrate Synthase:

First, 20g of muscle and liver tissue were homogenized in 200µL of assay buffer, centrifuged at 10,000 g at 4°C and the soluble fraction was used for the assay. This assay was performed in a 96-well plate samples were diluted by a factor of two.

Lactate Dehydrogenase:

For the lactate dehydrogenase assays, 10g of liver and muscle tissue was homogenized in 200µL of assay buffer, centrifuged at 10,000 g at 4°C. The soluble fraction was separated and diluted by a factor of 20 for the assay.

Cytochrome C Oxidase:

Similar to the citrate synthase assays, for the cytochrome C oxidase assays 20g of muscle tissue was homogenized in 200µL of buffer, centrifuged at 10,000 g at 4°C and the soluble fraction was used

for the assay. The cytochrome C oxidase assays were performed in 1mL cuvettes, rather than a 96-well plate due to issues with scaling down the volumes for the plate.

Bradford Assays:

In order to quantify the level of enzyme activity per gram of protein, I used a Bradford Assay for each group of enzyme trials. Assays were conducted according to Quick Start Bradford Protein Assay (Bio-Rad Laboratories, CA, USA) instructions. This involved serial dilutions of bovine serum to produce a standard curve and using the soluble fraction from the enzyme trials as my sample volumes.

Statistical Analysis:

A two-way repeated measures ANOVA was used to determine the effect of treatment on U_{crit} and U_{burst}. Predator escape velocity (PEV) was calculated by measuring the distance travelled during fleeing response in pixels, converting it to centimeters, and dividing by the time in seconds of the response. Due to PEV failing the assumptions of the ANOVA, a one-way ANOVA's on ranks was used. Latency to first capture (LoC) was measured as the time in seconds until the first prey capture was attempted. Fish that failed to make a capture attempt during the five-minute interval were assigned the maximum time to be included in a two-way repeated measures ANOVA. The proportion of fish that responded to the predator strike, the proportion of fish requiring a second strike, and the proportion of fish that attempted a prey strike were analyzed by assigning a 1 to fish that did not respond, and a 2 to fish that did respond. Inter-rater reliability (IRR) was calculated as the percent agreement between the two raters for a 5% range of a subset of values for predator escape velocity, latency of capture, and time spent moving during each phase. The IRR values indicated acceptable level of agreement among raters, with 0.75 (PEV), 0.875 (LoC), and 0.75 (time spent moving).

Lactate and glucose levels were calculated per gram of fish for each individual. Resting metabolic rate, maximum metabolic rate, and aerobic scope were analyzed with a two-way repeated measures ANOVA. Enzyme activity rates were calculated as percent of the average dimethyl sulfoxide

solvent control value per individual. A one-way ANOVA was used to assess possible differences between treatments for each of the variables. The metabolic enzyme activity rates were log10 transformed in order to normalize the data. Spearman correlation analyses were used to determine the extent of association between percent change in resting metabolic rate, maximum metabolic rate, and aerobic scope, or percent of control enzyme activity and the percent change in behavioural endpoints.

Chapter 3: Results

Swim Performance:

Following the injections U_{crit} was significantly altered ($F_{6,63}$= 8.85, p = <0.001) in a manner consistent with the expectations for each compound (Fig. 1). There was no significant change in U_{crit} of the absolute control fish, indicating that the repeated swims did not include a training effect. Injections of dimethyl sulfoxide solvent reduced U_{crit} by 0.635BL/s (t= 2.28, p= 0.03) potentially due to the injection technique rather than an effect of the dimethyl sulfoxide solvent. Caffeine injections increased U_{crit} by 0.906BL/s (t= 2.65, p= 0.01) while injections of carbamazepine (MD= 0.973, t= 3.02, p= 0.004), chlorpyrifos (MD= 0.858, t= 2.66, p= 0.010), pentachlorophenol (MD= 1.62, t= 5.55, p= <0.001), and tributyltin (MD= 1.82, t= 5.95, p= <0.001) all reduced U_{crit}, indicating that aerobically fueled swim performance can be effectively altered through various physiological pathways. In contrast, U_{burst} was not affected by any of the treatments (Fig.2). Since U_{burst} is primarily fueled by anaerobic metabolism, it is possible that the compounds imposed a higher cost to aerobic metabolic capacity which lead to U_{crit} being affected without affecting U_{burst}.

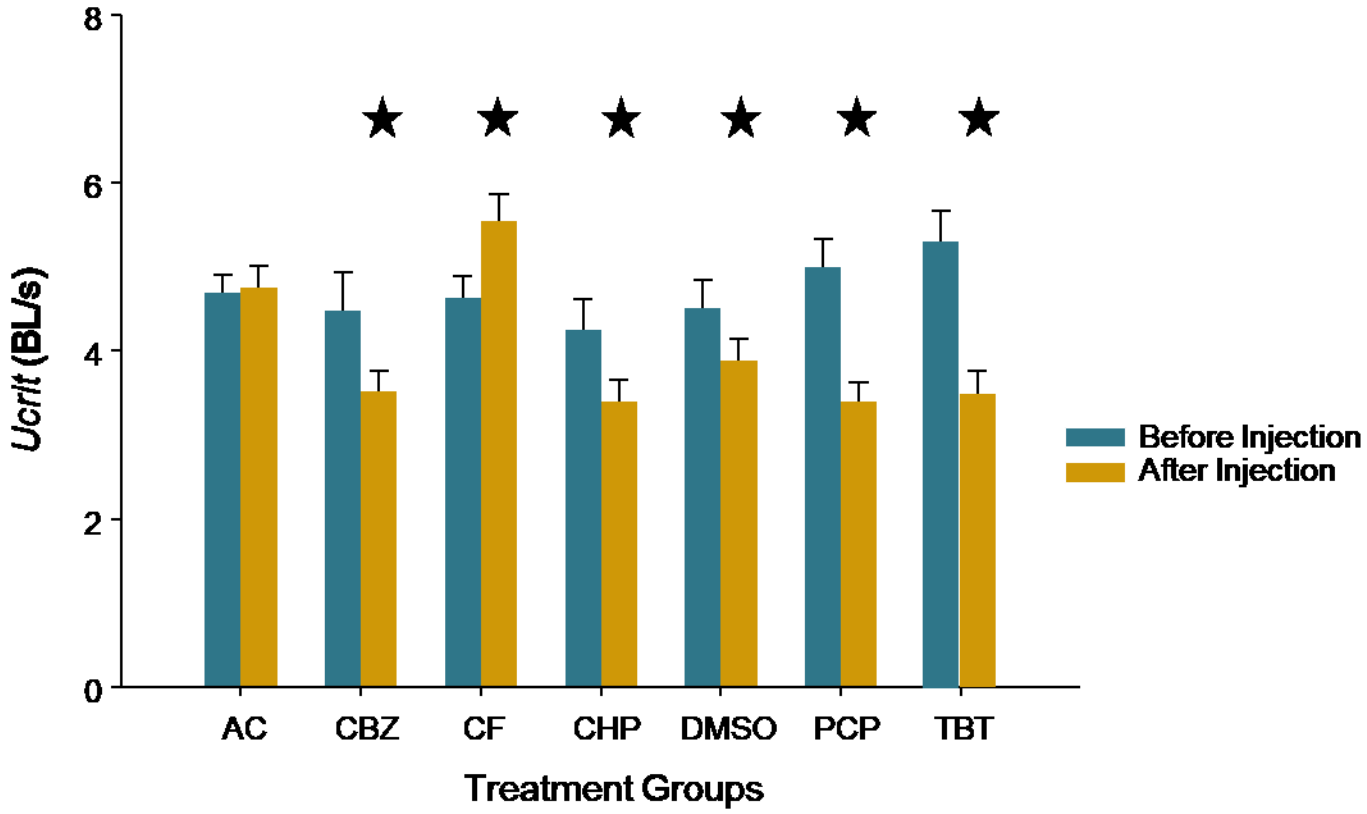

Figure 1. Mean U_{crit} values in body lengths/ second (BL/s) before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

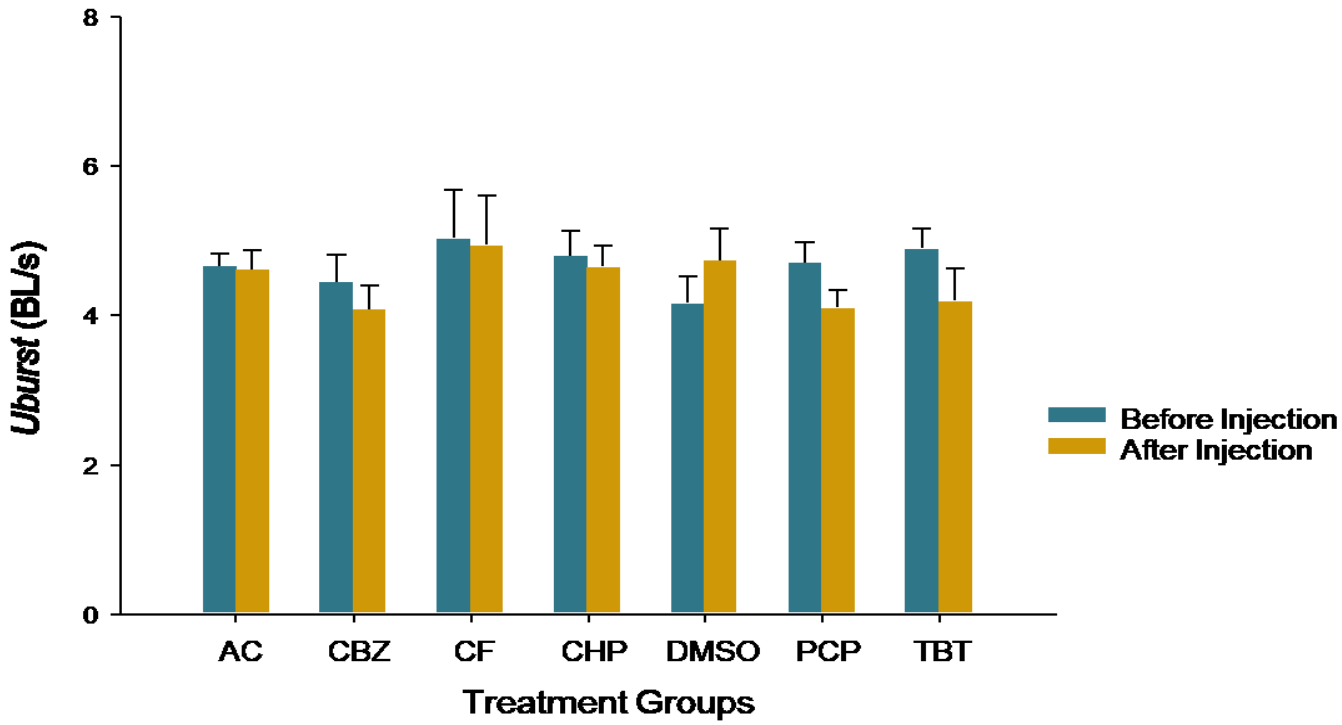

Figure 2. Mean U_{burst} values in body lengths/ second (BL/s) before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

Metabolic Capacity

Aerobic Scope:

To determine if metabolic rate impacts behaviour, I examined resting metabolic rate, maximum metabolic rate, and aerobic scope of each fish prior to and following injection of the experimental compounds. However, aerobic scope did not change within treatments following injection, nor were there any differences between treatment groups (Fig. 3c). Similarly, resting and maximum metabolic rate also showed no differences within or between groups (Fig. 3a, Fig. 3b). Despite not being statistically significant, pentachlorophenol treated fish had the greatest decrease in aerobic scope following injection due to the lowest resting metabolic rate among groups, while caffeine and tributyltin treated fish saw large rises in maximum metabolic rate leading to the highest aerobic scope following

injection. These results are likely due to the effect of the compounds on aerobic metabolism. The other treatment groups did not see significant changes in aerobic scope likely because the compounds are neuroactive (carbamazepine and chlorpyrifos) or thought to be inert (dimethyl sulfoxide solvent).

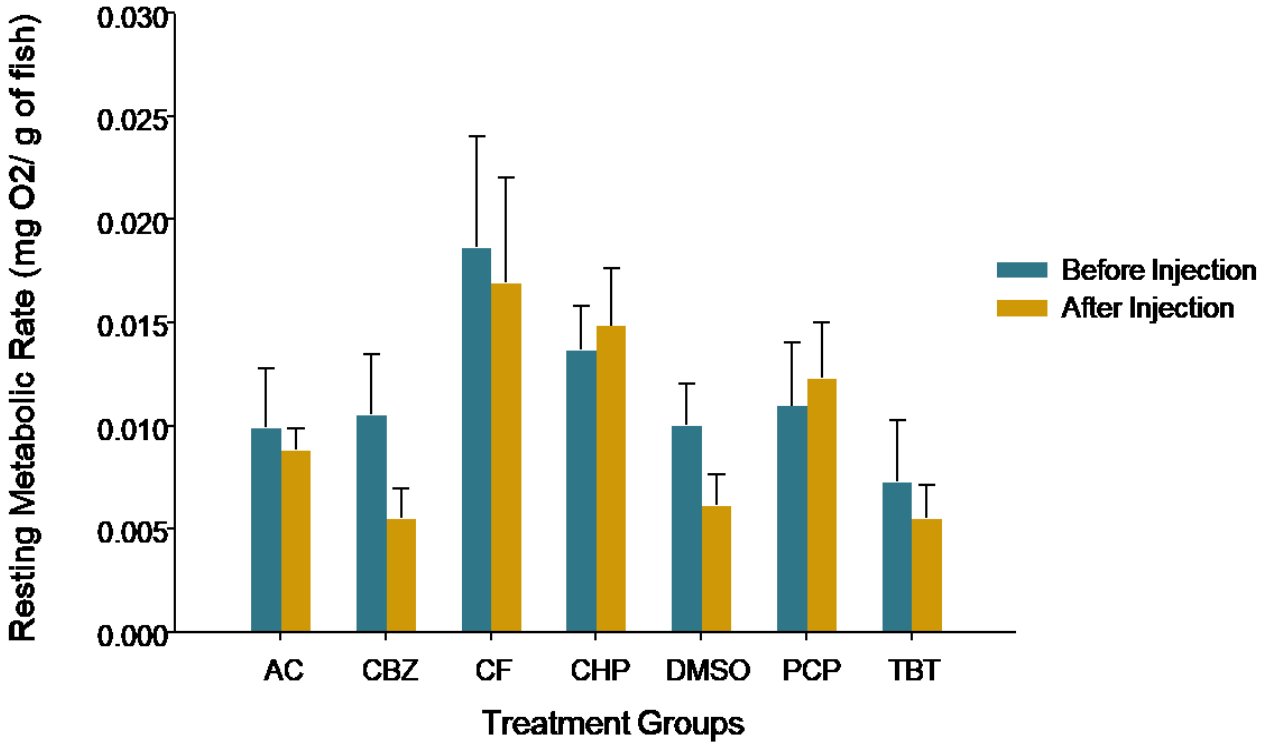

Figure 3a. Mean resting metabolic rate (RMR) before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

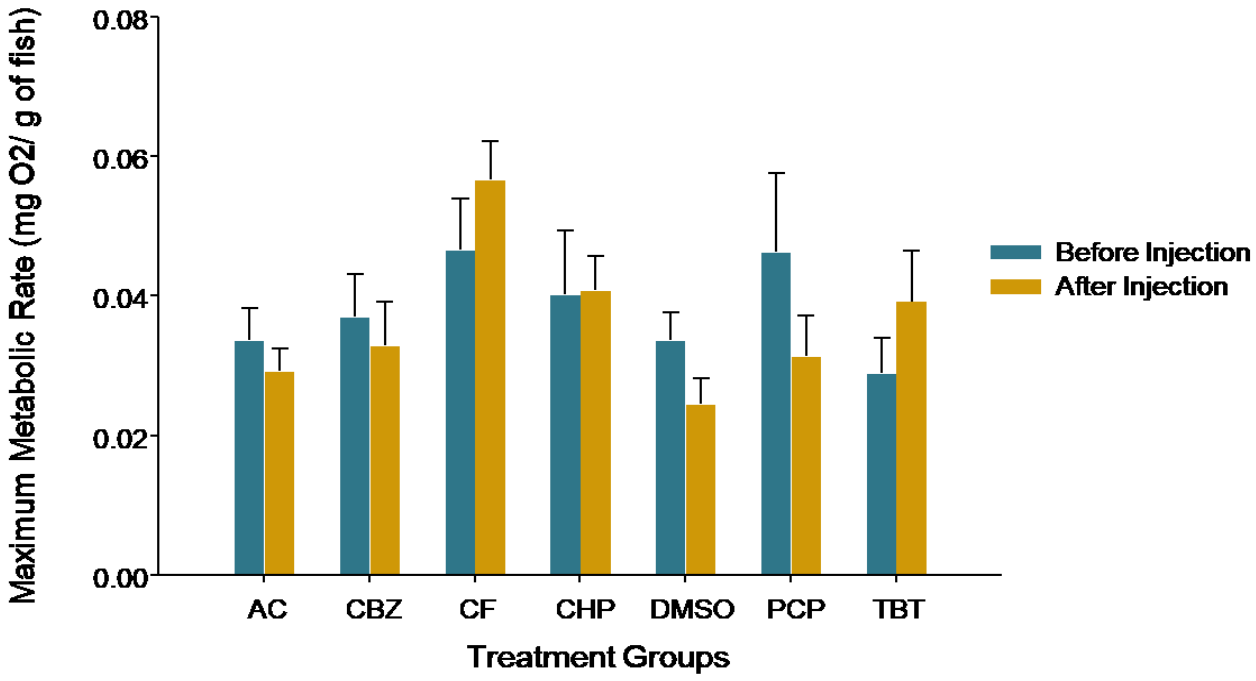

Figure 3b. Mean maximum metabolic rate (MMR) before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

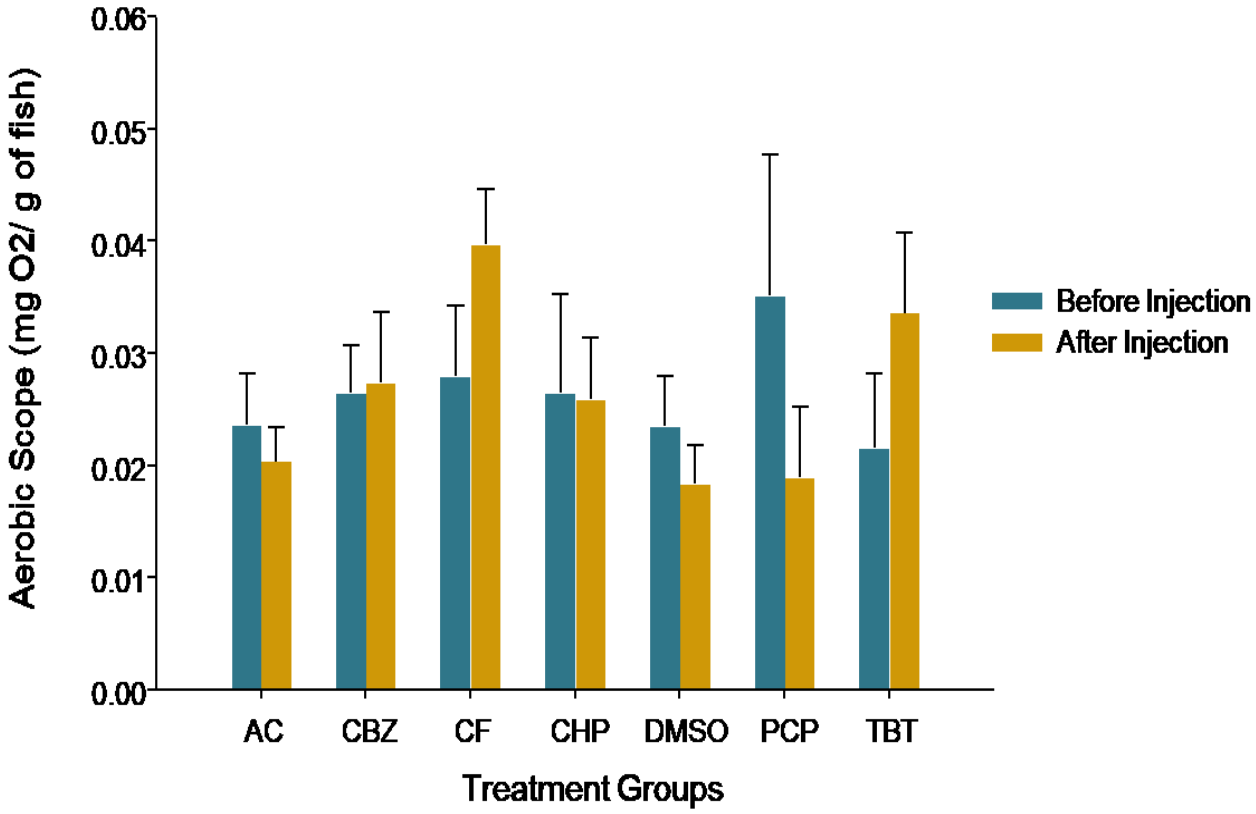

Figure 3c. Mean aerobic scope (AS) before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

Lactate and Glucose:

Plasma lactate and glucose per gram of fish did not differ between treatment groups (Fig. 4). Since lactate concentration in the blood only represents approximately 20% of total lactate produced, it is possible that lactate leakage was unaffected by the treatments used and did not represent differences in anaerobic capacity. Similarly, glucose has been found to be regenerated *in situ* in muscle from lactate, rather than through the Cori cycle in the liver, and so the lack of differences between treatments is not surprising.

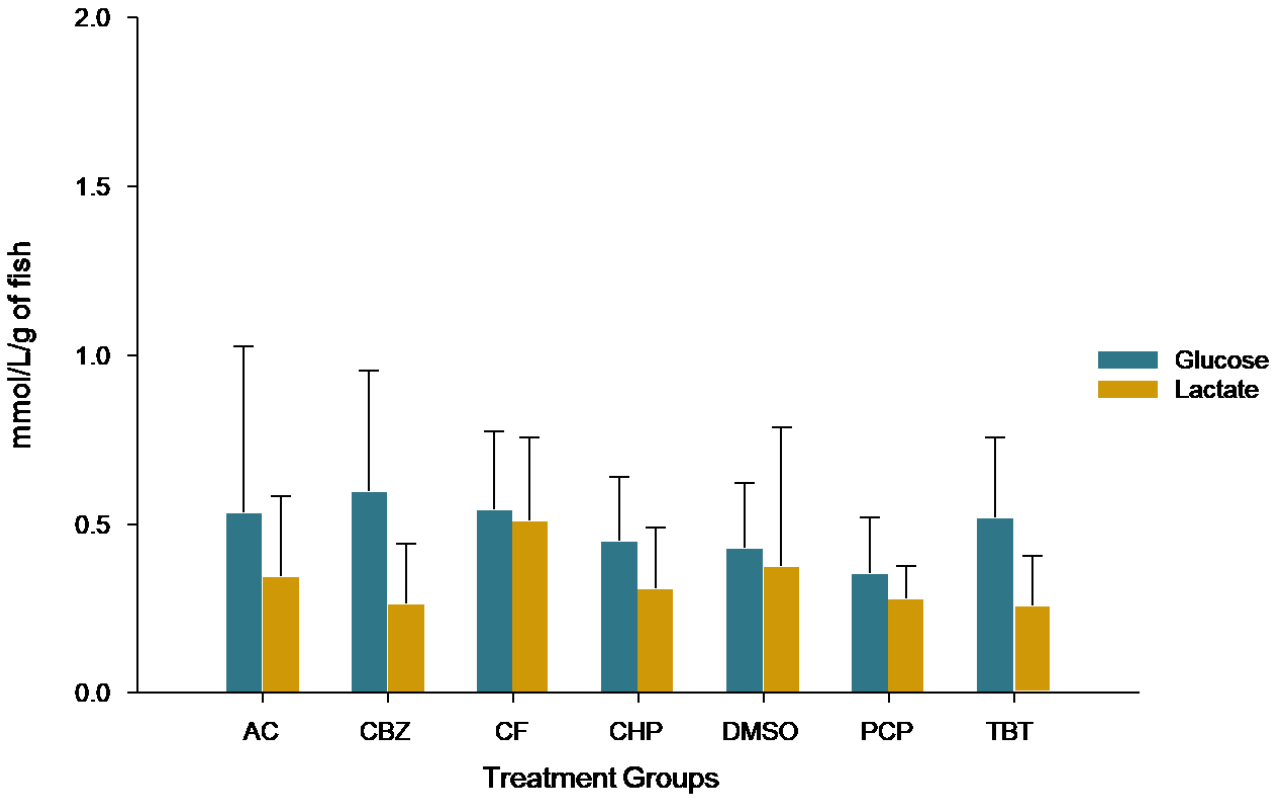

Figure 4. Mean plasma glucose and lactate concentration per gram of fish before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

Metabolic Enzymes:

Overall, citrate synthase activity in the liver and muscle as a percent of dimethyl sulfoxide solvent control differed across treatment groups ($F_{5, 39}$=11.5, p=<0.001 and $F_{5, 42}$=8.00, p=<0.001, respectively). Pentachlorophenol and tributyltin treated fish had reduced activity in the liver and muscle, carbamazepine, caffeine, and chlorpyrifos treated fish had increased activity compared to control in the liver, and carbamazepine and chlorpyrifos treated fish had increased activity in the muscle (Fig. 5a, Fig. 5b). The differences in citrate synthase activity do not appear to follow trends in swim performance or metabolic rate, so it is likely that these differences are the result of direct or indirect action of the

compounds. Pentachlorophenol and tributyltin have known inhibitory effects on various aspects of metabolism, while carbamazepine, caffeine, and chlorpyrifos are neuroactive compounds. It is possible that the effects of carbamazepine, caffeine, and chlorpyrifos imposed a higher energetic cost on the fish, which was compensated by higher citrate synthase activity.

Citrate synthase functions in aerobic energy production, but also in the recovery of energy stores following exhaustive exercise. In conjunction with citrate synthase, lactate dehydrogenase is vital to the recovery process by providing the substrate for citrate synthase. Lactate dehydrogenase activity in the liver and muscle as a percent of dimethyl sulfoxide solvent control was different across treatment groups ($F_{5, 45}$=7.84, p=<0.001 and $F_{5, 52}$=5.40, p=<0.001, respectively). Absolute control, pentachlorophenol, and tributyltin treatment groups had reduced lactate dehydrogenase activity, while carbamazepine and chlorpyrifos treated fish had increased lactate dehydrogenase activity as compared to the dimethyl sulfoxide solvent control (Fig. 5c, Fig. 5d). However, the changes in lactate dehydrogenase activity were lower than those of citrate synthase activity, particularly in the muscle tissue. This could indicate that activity of these enzymes in the muscle is more important to energy production and recovery, with citrate synthase activity being required at much greater levels than lactate dehydrogenase.

Overall, cytochrome C oxidase activity in the muscle did not differ across treatment groups (Fig. 5e), despite the differences in citrate synthase and lactate dehydrogenase activity between treatment groups. However, absolute control and carbamazepine treated fish had greatly increased cytochrome C oxidase activity, while pentachlorophenol treated fish had reduced activity as compared to the dimethyl sulfoxide solvent control. Since citrate synthase and cytochrome C oxidase are functionally linked in regard to energy production, it follows that changes in one would necessitate a change in the other. Tributyltin treated fish, however, had an increase in cytochrome C oxidase activity but a decrease in citrate synthase activity as compared to dimethyl sulfoxide solvent control. This can likely be attributed

to the complexity of the toxic effects tributyltin has on fish, since it has been found to impact numerous aspects of metabolic, cell, and membrane physiology.

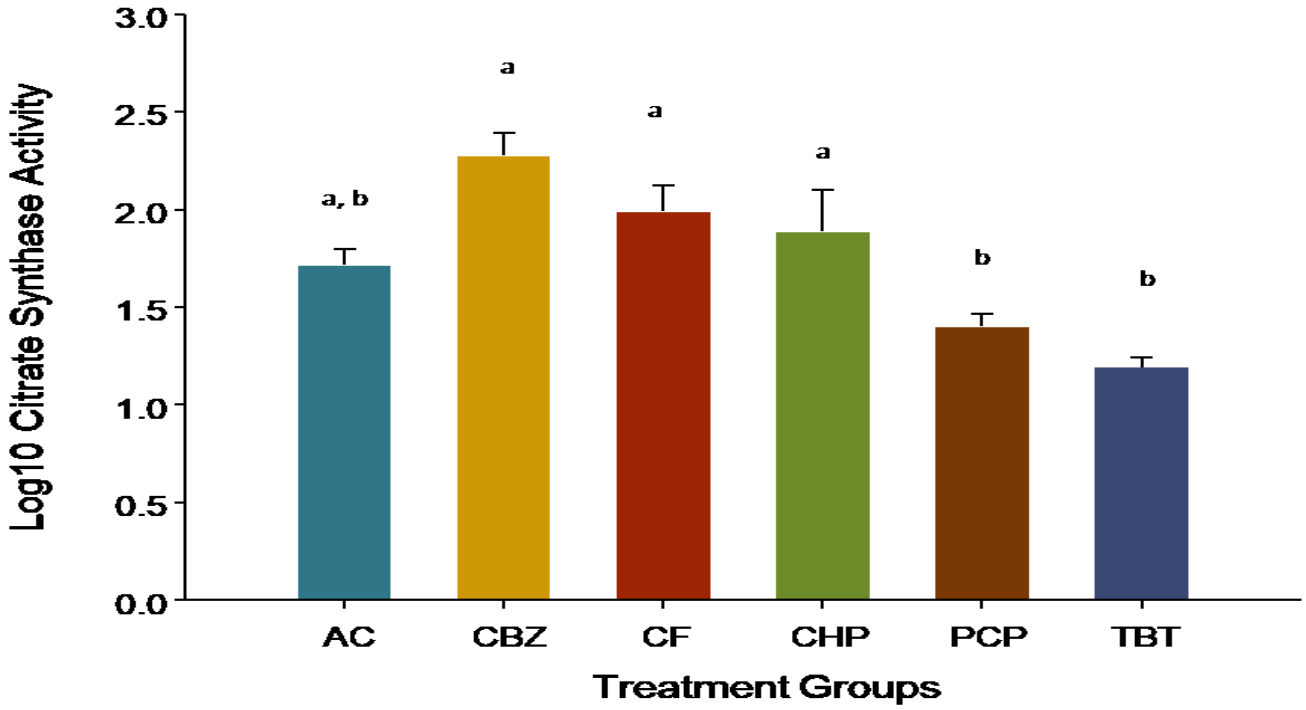

Figure 5a. Log10 of citrate synthase (CS) activity in the liver as a percentage of dimethyl sulfoxide solvent control. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, PCP for pentachlorophenol, and TBT for tributyltin.

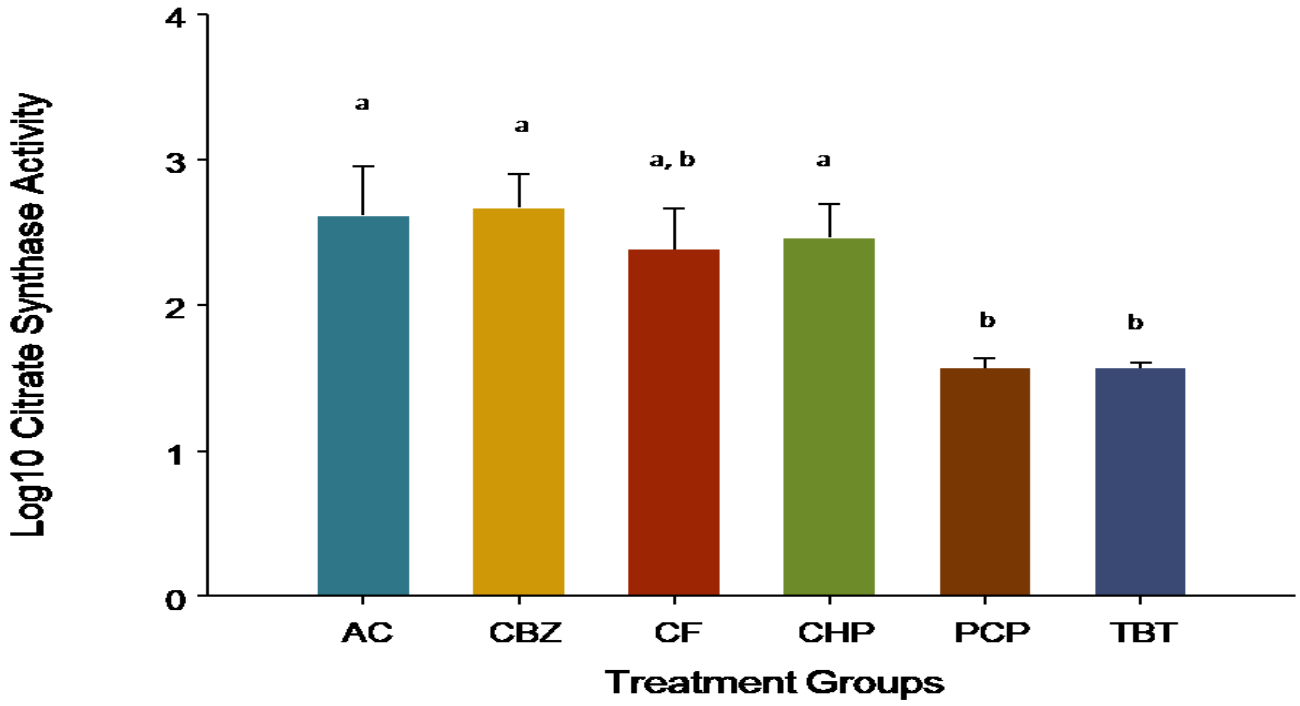

Figure 5b. Log10 of citrate synthase (CS) activity in the muscle as a percentage of dimethyl sulfoxide solvent control. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, PCP for pentachlorophenol, and TBT for tributyltin.

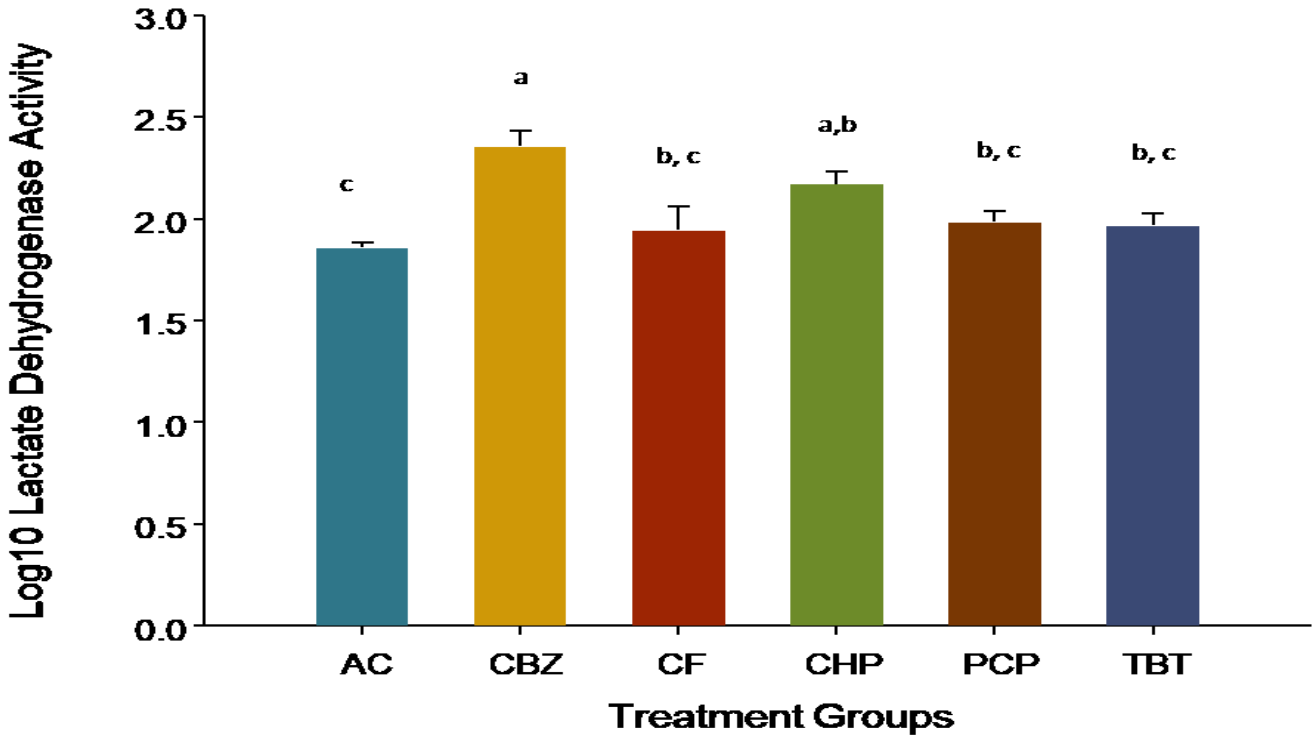

Figure 5c. Log10 of lactate dehydrogenase (LDH) activity in the liver as a percentage of dimethyl sulfoxide solvent control. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, PCP for pentachlorophenol, and TBT for tributyltin.

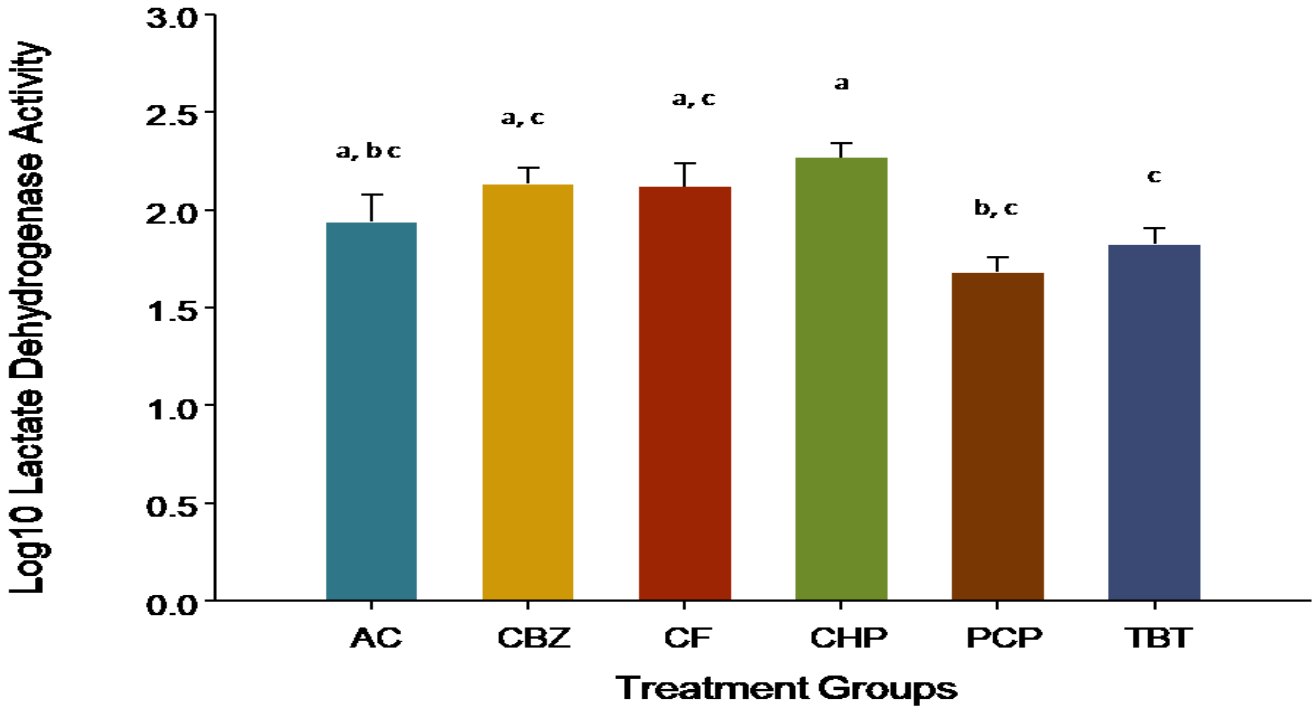

Figure 5d. Log10 of lactate dehydrogenase (LDH) activity in the muscle as a percentage of dimethyl sulfoxide solvent control. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, PCP for pentachlorophenol, and TBT for tributyltin.

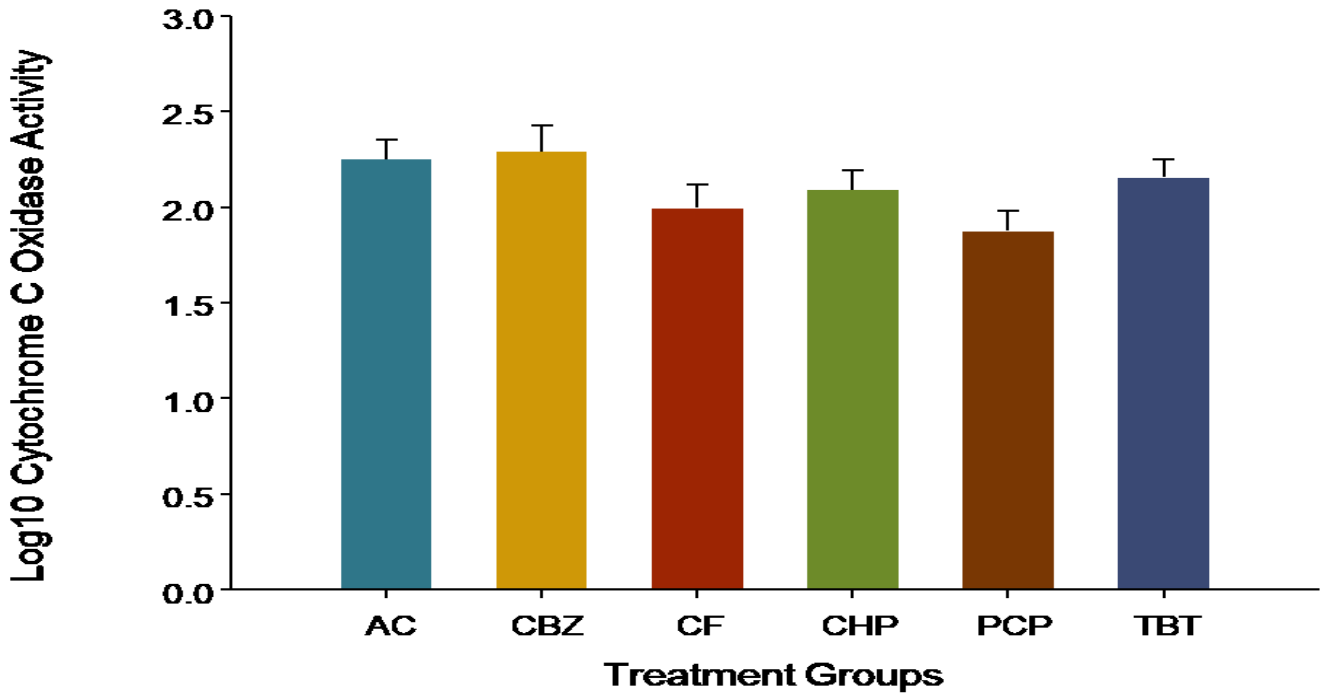

Figure 5e. Log10 cytochrome C oxidase (COX) activity in the liver as a percent of dimethyl sulfoxide solvent control. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, PCP for pentachlorophenol, and TBT for tributyltin.

Predator Avoidance:

Predator escape velocity did not change with treatment of any of the compounds (Fig. 6). Since the response to predator strikes is generally a sprint swim away from the point of attack, it follows that a lack of change in U_{burst} would translate to a lack of change in predator escape velocity. To further examine predator avoidance, I examined the proportion of fish that responded to strikes, as well as the proportion of fish that required multiple strikes to elicit a response before and following injection. There were no between or within group differences (Fig. S1 and Fig. S2). However, five possible behavioural phenotypes were present. Chlorpyrifos treated fish did not change in their response to the predator nor the number of multiple strikes needed to elicit a response. Absolute control fish saw a reduction in the proportion of fish requiring multiple strikes to elicit a response while the proportion of fish responding

to the predator did not change. Fish treated with tributyltin did not show a change in response proportion but following injection there was a greater number of fish requiring multiple strikes. Chlorpyrifos and caffeine treated fish saw a reduction in the response proportion despite no change in proportion of multiple strikes required. Lastly, dimethyl sulfoxide solvent and pentachlorophenol treated fish had an increase in the proportion of fish responding to the predator despite no changes to the proportion of fish requiring multiple strikes. These differences could be the result of inherent behavioural variation, or due to the variety of effects from the compounds (metabolic vs neuroactive), or a combination of both.

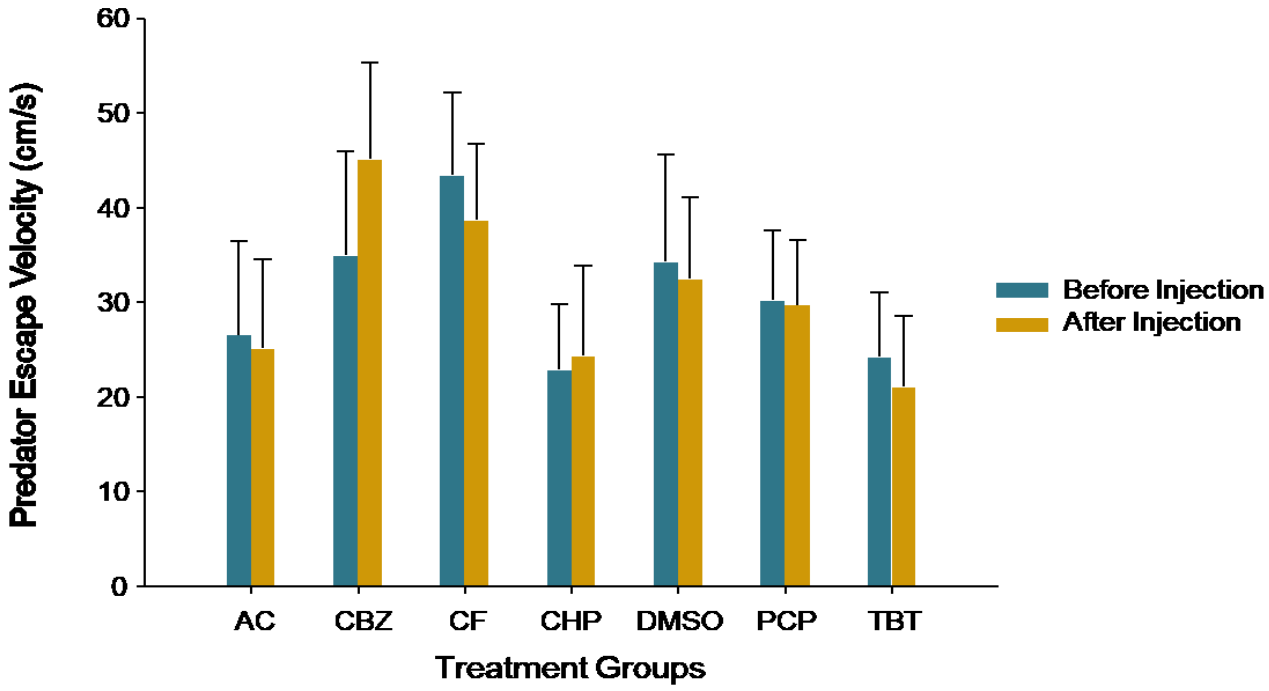

Figure 6. Mean predator escape velocity (PEV) before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

Prey Capture:

Latency to first capture attempt decreased in the post-injection trials ($F_{6, 65}$=12.9, p=<0.001). Absolute control fish had a reduction in latency of capture of 85.7s (t=2.00, p=0.05), caffeine treated fish of 96.3s (t=2.11, p=0.04), pentachlorophenol treated fish of 85.3s (t=2.06, p=0.04), and tributyltin of 141s (t=3.41, p=0.001) (Fig. 7). There are no ubiquitous trends following the changes in U_{crit}, metabolic rate, or metabolic enzyme activity. However, this may suggest multiple avenues of effect. There could be an interplay of hunger, metabolic differences, and acclimation to handling in the after-injection trials that lead to these decreases. The before-injection trials would have involved multiple new situations (swim chamber, behaviour chamber) as well as handling that the fish would not have been acclimated to. This could have led to a high level of stress initially and reduced appetite. Also, since fish were fasted 24 hours prior to the first trial, and not fed during the 3 to 4-day experiment, it is possible that increased hunger in the after-injection trial caused the decrease in latency.

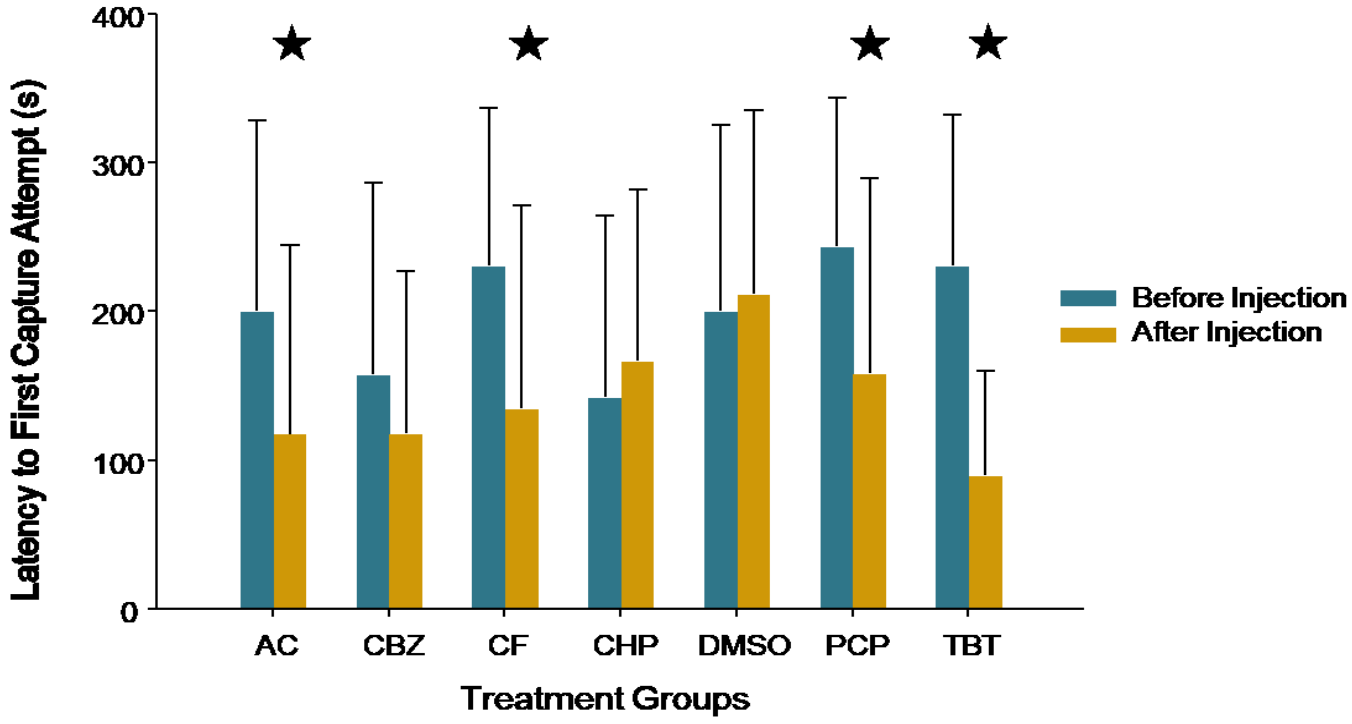

Figure 7. Mean latencies of capture before and after injections of experimental compounds. AC for absolute control, CBZ for carbamazepine, CF for caffeine, CHP for chlorpyrifos, DMSO for dimethyl sulfoxide solvent control, PCP for pentachlorophenol, and TBT for tributyltin.

Integrating Swim Performance, Metabolism, and Behaviour

Examining the relationships between swim performance and behaviour, I found no evidence of U_{crit} being related to any of the behavioural endpoints, regardless of treatment group. However, percent change in U_{burst} was positively related to percent change in latency of capture for caffeine and tributyltin treated fish (Fig. 8a and Fig. 8b). This means that as U_{burst} values following injection increased relative to the before injection values, so did the latencies of capture, indicating that faster sprint swimming speed may result in longer latency of capture. Since prey capture attempts involve a burst swim toward the prey, it is possible that following the swim tests the fish with the highest U_{burst} values following injection would prioritize recovery over prey capture.

In order to determine what extent metabolism influences behaviour, I performed Spearman correlation analysis on percent change in aerobic scope, resting metabolic rate, and maximum metabolic rate. I found that percent change in aerobic scope was positively related to percent change in time spent moving in routine phase for chlorpyrifos treated fish (Fig. 9a). This could indicate that a higher aerobic metabolic capacity increases the recovery speed following exhaustive exercise, and so fish could resume routine swimming activity quicker. Similarly, percent change in maximum metabolic rate was positively related to percent change in latency of capture in pentachlorophenol treated fish (Fig. 9b). Due to the effects of PCP, fish could have been respiring more while not producing ATP, which would cause a longer recovery of energy stores.

Similar to metabolic rate, percent changes in citrate synthase and lactate dehydrogenase activity in the liver, and cytochrome C oxidase were positively related to predator escape velocity in chlorpyrifos fish (Fig. 10). Since CS and COX activity are responsible for the majority of energy

generation, it follows that PEV would be linked to the activity of these enzymes. PEV is a burst swim response to a perceived predator strike and as such would rely on rapid energy mobilization in the muscle. Percent changes in citrate synthase activity in the muscle and cytochrome C oxidase activity were negatively related to latency of capture in pentachlorophenol treated fish (Fig. 11). Due to the inhibitory affects of PCP on oxidative phosphorylation, it is possible that PCP treated fish had an impaired ability to generate ATP which in turn lead to greater need to eat.

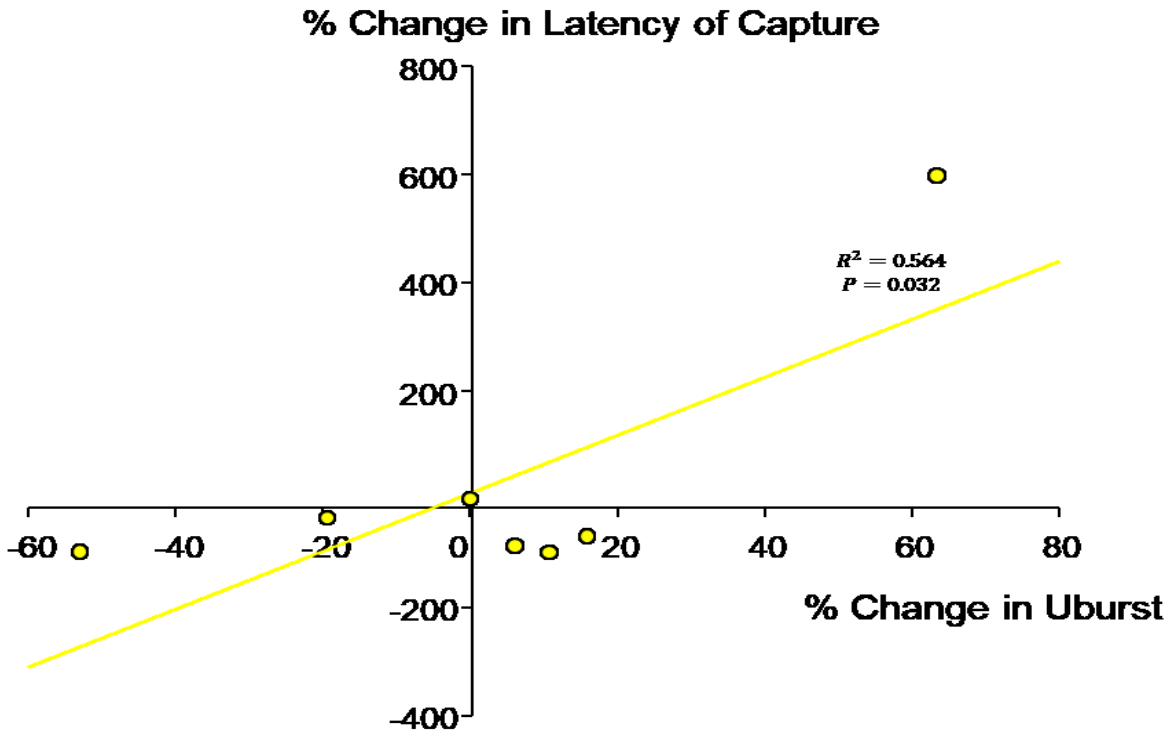

Figure 8a. Relationship between percent change in U_{burst} values and percent change in latency of capture in caffeine treated fish.

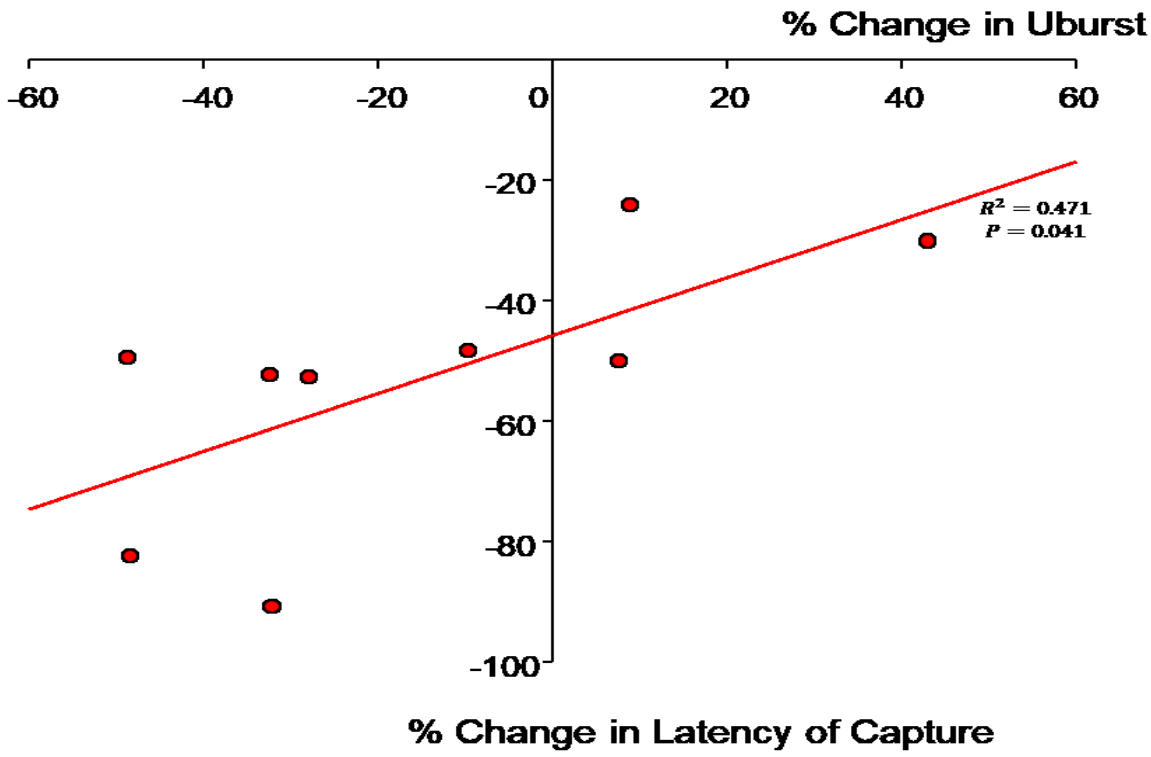

Figure 8b. Relationship between percent change in U_{burst} values and percent change in latency of capture in tributyltin treated fish.

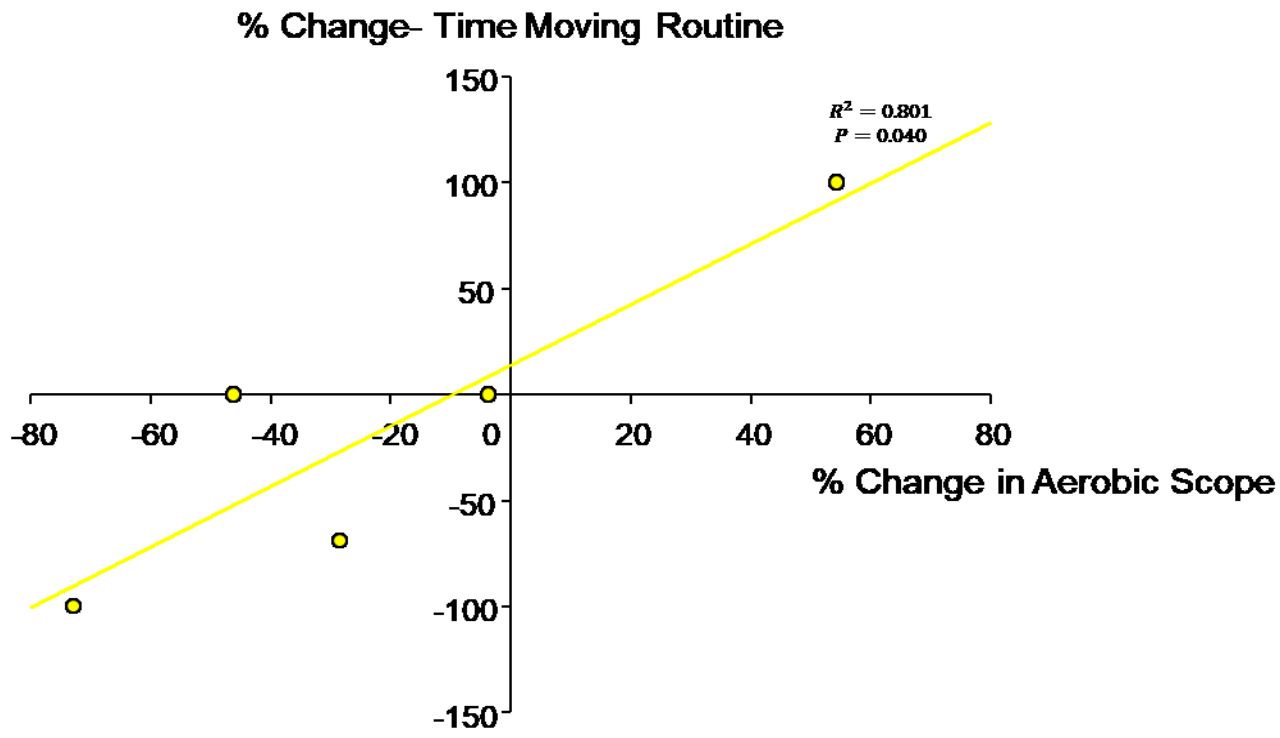

Figure 9a. Relationship between percent change in aerobic scope and percent change in time spent moving during the routine phase in chlorpyrifos treated fish.

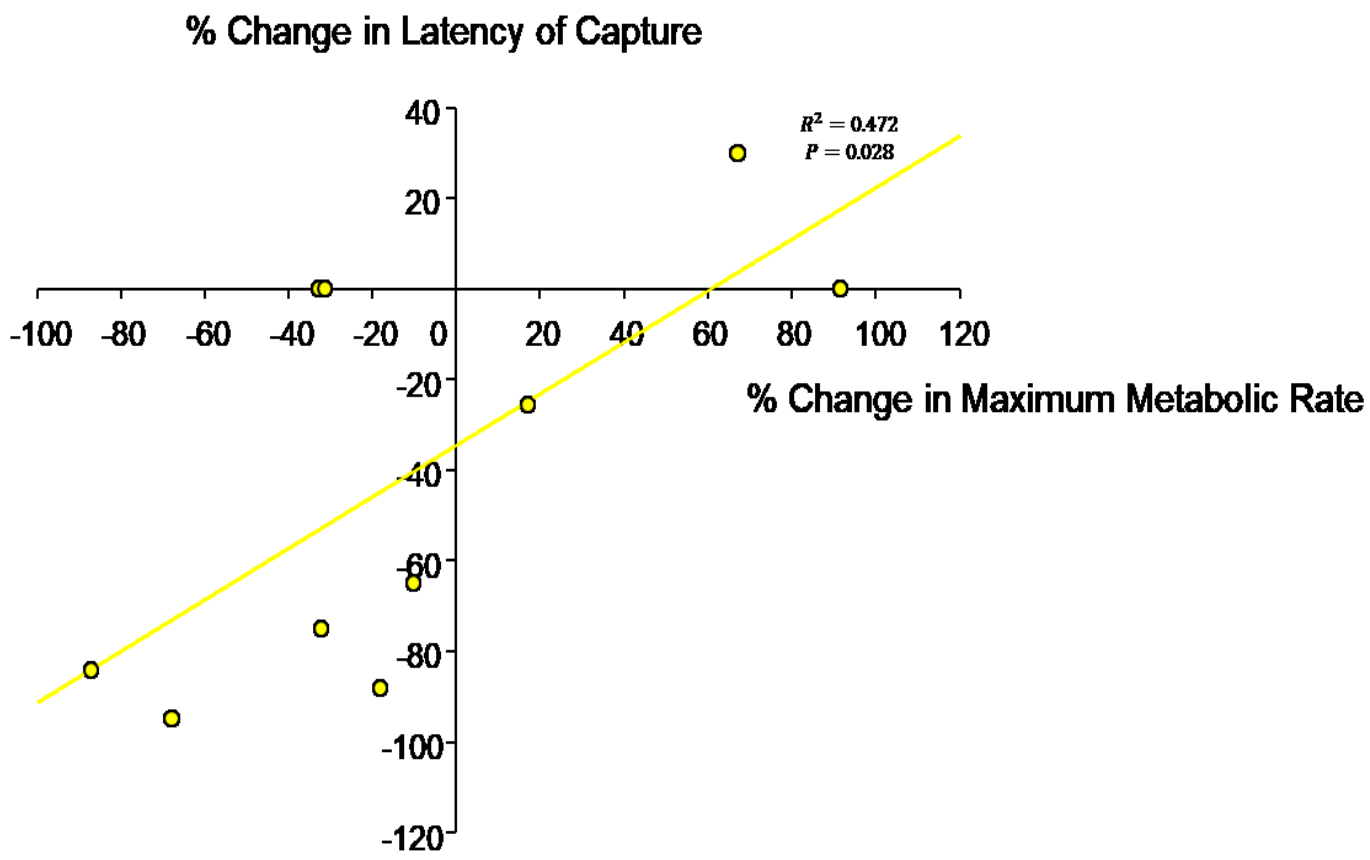

Figure 9b. Relationship between percent change in maximum metabolic rate and percent change in latency of capture in pentachlorophenol treated fish.

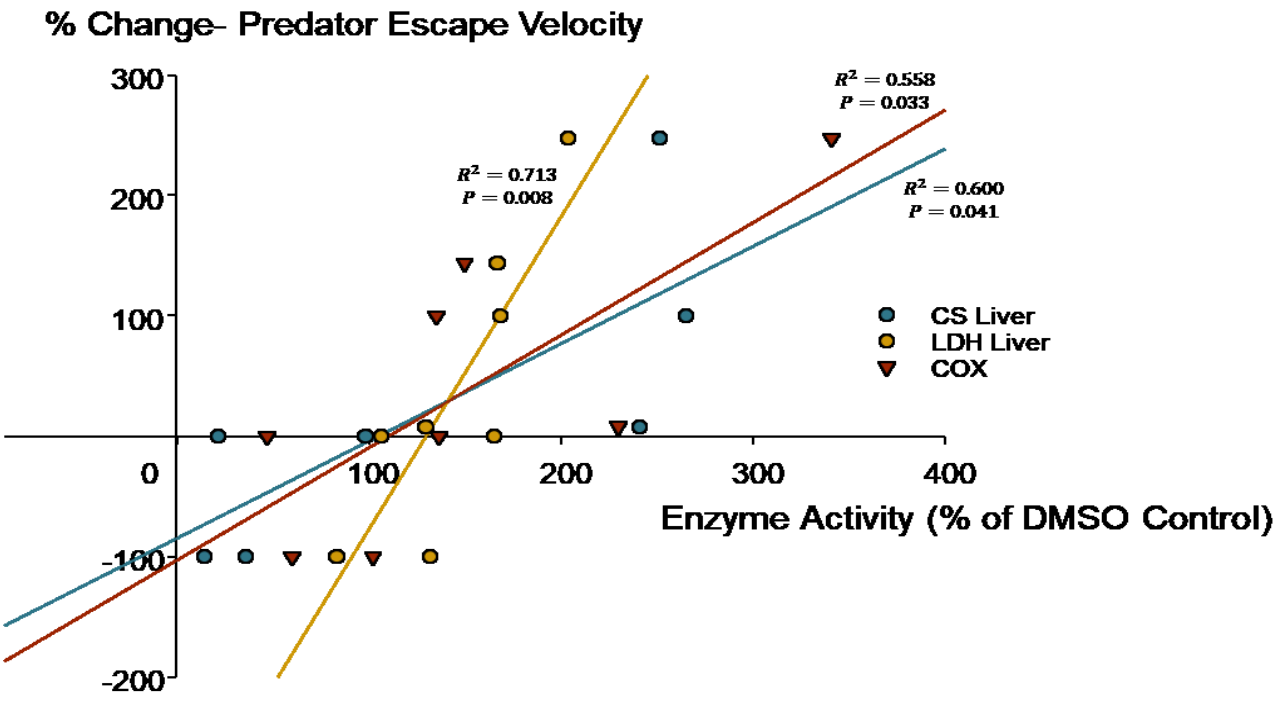

Figure 10. Relationship between percent change in enzyme activity and percent change in predator escape velocity in chlorpyrifos treated fish. CS for citrate synthase, LDH for lactate dehydrogenase, and COX for cytochrome C oxidase.

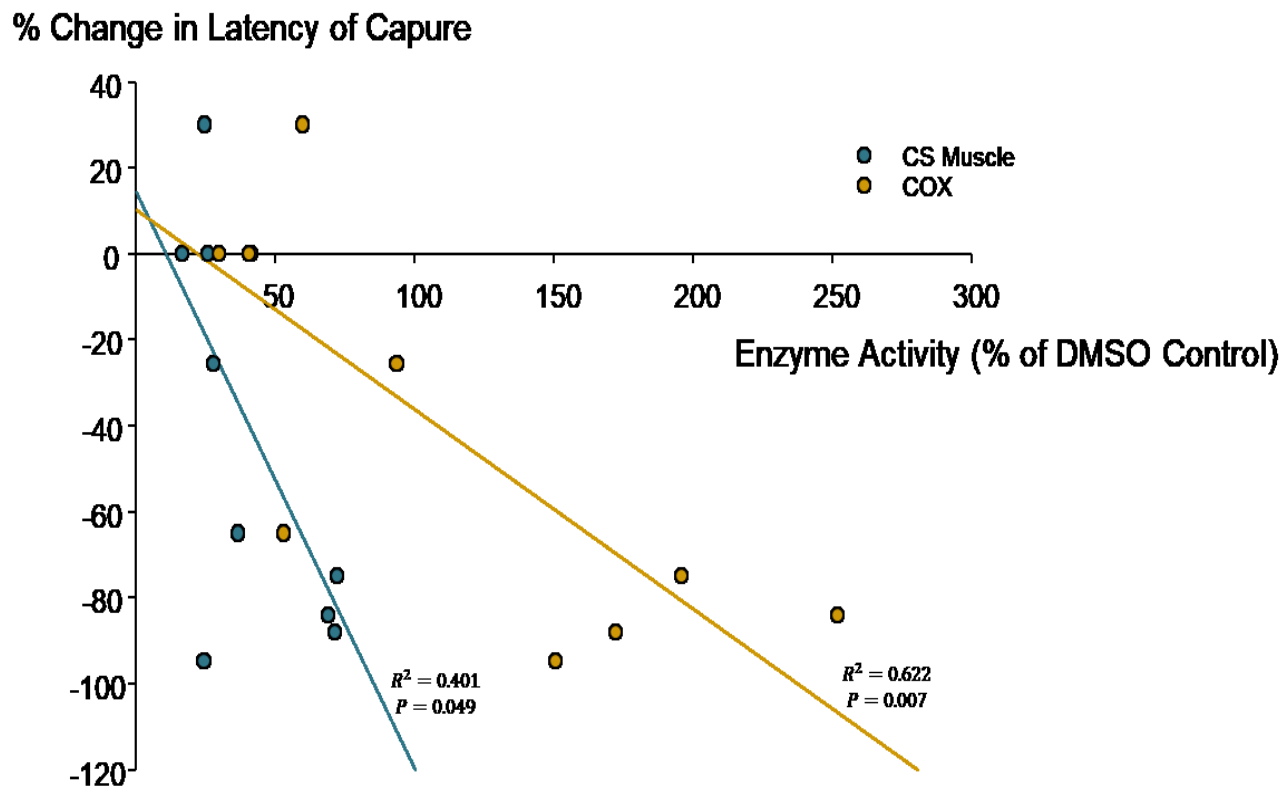

Figure 11. Relationship between percent change in enzyme activity and percent change in latency of capture in pentachlorophenol treated fish. CS for citrate synthase and COX for cytochrome C oxidase.

Chapter 4: Discussion

Swim Performance and Behaviour

Currently, the relationship between U_{crit} and ecologically relevant behaviour in fish is inferential at best with few, if any, studies attempting to directly look at the possible relationship. The purpose of this study was to examine this possible relationship, by analyzing differences in swimming ability and behavioural responses. Overall, I did not find evidence that predator avoidance or prey capture behaviour can be predicted by changes in U_{crit}. While these behaviours are locomotor dependent, they are complex and likely rely on more than swimming ability alone. It is also important to note that this study was conducted on hatchery raised trout, which may show different responses compared to wild counterparts.

In my study, U_{crit} but not U_{burst} was found to be altered by the compounds used, with carbamazepine, chlorpyrifos, dimethyl sulfoxide solvent, pentachlorophenol, and tributyltin significantly reducing U_{crit}, and caffeine increasing it. The changes were as expected, except those due to dimethyl sulfoxide, which is generally considered to be an inert solvent and as such is widely used for non-polar compounds. Tributyltin was the only treatment group to significantly reduce U_{burst}, likely due to the effects of tributyltin on anaerobic metabolism. The range of U_{crit} values for my fish were about 2BL/s to 7BL/s, while the U_{burst} values of my fish ranged from about 2BL/s to 10BL/s which is in agreement with previous work on rainbow trout (Farrell 2008; Bellinger et al. 2014; Berli et al. 2014; Osachoff et al. 2014) under various conditions.

Carbamazepine is a known voltage gated sodium channel blocker (Kuo et al. 1997; Kuo 1998) with possible effects on acetylcholine release and metabolism (Mizuno et al. 2000), and inhibition of serotonin (Tanahashi et al. 2012). Due to slowing the propagation of impulses at the neuromuscular junction, it follows that swim performance would be adversely affected. In a previous study on Japanese medaka (*Oryzias latipes*), chronic carbamazepine treatment was found to increase the time to eat prey, as well as decrease swimming speed (Nassef et al. 2010). However, the decrease in swimming speed was only found after 8 days of treatment, while the increase in time to capture prey was seen after 6 days, which indicates that swimming speed wasn't likely the cause of reduced prey capture ability. My results did not indicate an effect on anaerobically fueled sprint style swimming from carbamazepine, which is contrary to the previous study, but this could be the result of different treatment vectors, exposure times, and species differences. Due to the various effects of carbamazepine, it is possible that the drug elicited different effects on locomotion and behaviour, without those two being directly related.

Few studies have examined the effects of caffeine on swim performance in fish, but it has been found to cause locomotory enhancement at low to medium doses increased swimming speed and distance travelled (Gupta et al. 2014). This coincides with my findings regarding U_{crit}, but it is impossible

to make direct comparisons due to the lack of studies measuring U_{crit} and U_{burst} following caffeine exposure. Caffeine has also been found to induce higher levels of anxiety in fish (Egan et al. 2009) if in high doses, but does not affect cognitive performance in low to medium doses (Santos et al. 2016). In my study, fish treated with caffeine did not show a significant change in predator escape velocity, proportion of fish responding to predator strikes, proportion of fish requiring multiple strikes, or proportion of fish attempting prey capture.

The acetylcholinesterase inhibitor chlorpyrifos has been found to reduce U_{crit} in salmonids (Tierney et al. 2007) by stopping the breakdown of acetylcholine, which impairs neuromuscular function. Both endurance and burst swim performance has been found to be adversely affected by chlorpyrifos (Tierney et al. 2007), however my study only found evidence of U_{crit} being affected. This is consistent with the acetylcholinesterase inhibition effects of chlorpyrifos (Rice et al. 1997), which results in tetanus-like symptoms on muscle fibre firing. Chlorpyrifos has previously been found to alter feeding behaviour in Atlantic salmon (*Salma salar*) by reducing the number of prey strikes, as well as increasing the time to first strike (Sandahl et al. 2005). My results contradict this study, with no significant change in latency to capture. While my study did not examine the activity of acetylcholinesterase in the muscle or brain, it is possible that the swimming performance effects found in my fish were the result of a build-up of acetylcholine in the muscle, but the dose of chlorpyrifos may not have been high enough to induce behavioural impairment. Furthermore, my fish were injected with treatments rather than exposed in the water, which may also account for the differences.

Pentachlorophenol has been found to have an adverse effect on aerobic metabolism by uncoupling oxidative phosphorylation from ATP synthesis (Gagnon 2002) without affecting ATP synthase (Weinbach 1954). One study (Farrell et al. 1998) found that pentachlorophenol did not significantly impact repeated U_{crit} in sockeye salmon (*Onchorhynchus nerka*), which conflicts with my findings. It is possible that mature fish are less sensitive to the toxic effects of pentachlorophenol than the juvenile

rainbow trout used in my study. Differences in exposure methods and concentrations used may also result in the differences seen.

Lastly, in my study tributyltin was found to reduce U_{crit} and U_{burst}, which could be the result of lactate dehydrogenase inhibition (Rurangwa et al. 2002; Gronczewska et al. 2004) or disruption of ATPase (Fent 1996), or a combination of both. As far as I can find, there have been no studies examining the effects of tributyltin on swim performance in adults, however, in zebrafish (*Danio rerio*) larvae were found to have drastically reduced swimming speed and distance travelled when exposed to tributyltin (Liang et al. 2017). Latency to capture was significantly lower after injection, but this is likely an artifact resulting from the increase in fish responding to prey. All individuals attempted to capture prey following injection, whereas only 75% of fish attempted to capture prey prior to injection.

Considering that the treatments used altered U_{crit}, I expected to find predator escape velocity to be likewise altered. However, there were no changes in predator escape velocity between groups or within groups following the injections. Furthermore, there were no trends across treatments regarding proportion of predator response or proportion of multiple strikes needed to elicit a response. Carbamazepine treated fish had a decrease in predator response proportion which seems to be consistent with the reduced U_{crit}, but caffeine treated fish also saw a decrease in predator response proportion despite having increased U_{crit}. Furthermore, the proportion of fish responding did not change within chlorpyrifos and tributyltin treatment groups, while dimethyl sulfoxide solvent and pentachlorophenol treated fish had an increase in the predator response proportion. Since the fish used in this study were hatchery bred and raised, their behavioural responses likely differ from wild fish (Alvarez and Nicieza 2003; Petersson and Järvi 2006), which have been found to flee more readily from predators. This could indicate that swim speeds were not the determining factor in predator interactions, but rather predator recognition and decision making in relation to perceived risk may have been more important.

Similar to the anti-predator behaviour results, latency to first capture attempt did not relate to changes in U_{crit}, but rather coincided with the proportion of fish attempting a capture. The decision to capture prey seems to be more complex than the variables examined in this study and may also be influenced by individual boldness/shyness behavioural phenotypes, which would indicate that there is a high degree of variation inherent within the population. The absolute control, caffeine, pentachlorophenol, and tributyltin groups all saw increases in the proportion of prey capture and a likewise decrease in the latency of first capture attempt in the after trials, while carbamazepine, chlorpyrifos, and dimethyl sulfoxide solvent did not show any changes. Since fish were fasted for the duration of the experiment, it is possible that the fish were simply hungrier during the post-injection trials and therefore more willing to capture prey. Also, considering the metabolic impacts of caffeine, pentachlorophenol, and tributyltin, it is possible that they had a higher energetic cost during exercise which led to decreased blood glucose and muscle glycogen stores leading to increased willingness to feed.

With pentachlorophenol and tributyltin, no cognitive alterations have been reported, unlike carbamazepine (Nassef et al. 2010; Calcagno et al. 2016), caffeine (Tran et al. 2017), and chlorpyrifos (Bonansea et al. 2016). My results did not coincide with the reported anxiolytic effects of carbamazepine, the anxiogenic effects of caffeine, or the hyperactivity effects of chlorpyrifos. This could be a result of the compounds eliciting different effects based on exposure route, dose, exposure time, and species. However, since all five treatments did significantly affect swim performance, it is likely that alterations in U_{crit} do not necessarily imply that predator avoidance behaviour or prey capture ability will be significantly altered. The interplay between swimming physiology and ecologically relevant behaviour in rainbow trout is too complex to expect a single metric such as U_{crit} to be an accurate predictor of behavioural responses. Furthermore, since the fish were captive bred, their behavioural responses may

not be comparable to wild conspecifics. Also, the amount of handling, the use of a size-limited test chamber, and the challenge of recreating a predator strike could impact the behavioural responses.

Metabolism and Behaviour

Despite the differences in enzyme activity, we did not detect any differences in aerobic scope following injections, nor between treatments. Intraspecific variation in metabolic rate, particularly resting metabolic rate has been found to be large even after controlling for temperature, sex, age, and body mass (Metcalfe et al. 2016). There was a common trend of increased aerobic scope across all treatments during the second trial which likely provided the increased oxygen for the rise in citrate synthase and cytochrome C oxidase activity. Furthermore, caffeine treated fish saw the largest increase in aerobic scope (Fig.3), which coincides with the drugs effects on hyperactivity (Gupta et al. 2014). Aerobic scope is the difference between minimal and maximum oxygen consumption (Soofiani and Priede), and it is possible that my oxygen consumption measurements were less sensitive than enzyme activity.

When compared to previous metabolic rate and aerobic scope measurements (Wilson et al. 1994; Zhang et al.), my values were larger by approximately a factor of 10. Due to the small size of our fish compared to water volume of the swim chamber, the method used for determination of aerobic scope was less accurate than traditional methods. Fish needed to be transferred from the swim chamber to the 7L test chamber following fatigue, which prevented the determination of oxygen consumption during maximum swimming speed and likely caused increased stress. Despite this limitation, it still gives us useful information regarding metabolic rate following treatment of the compounds since the method was consistent throughout the experiment.

Similar to aerobic scope, there were no differences in plasma glucose or lactate per gram of fish. Plasma glucose and lactate are commonly used as indicators of stresss (Martinez-Porchas and Martinez-Cordova 2009), but are also related to aerobic and anaerobic metabolism. Lactate concentration in the

blood alone is not a reliable measure anaerobic capacity, however, since only about 20% of the total lactate produced by the muscle is cleared to the blood (Pagnotta and Milligan). Fish are likely able to store lactate for *in situ* glycogenesis (Girard and Milligan 1992), and rainbow trout have been found to maintain a constant lactate clearance over a range of metabolic rates (Weber 1991). Blood glucose is also not the most reliable, since during exhaustive exercise fish would be rapidly consuming glucose and so it is possible that no change would be detected (Martinez-Porchas and Martinez-Cordova 2009). Furthermore, since the Cori Cycle is negligible in fish (Milligan and McDonald 1988; Milligan 1996), lactate is not being converted to glucose in the liver in large quantities. Nutritional status, diet, age, sex, and glycogen stores could all affect the mobilization of glucose. To get a more accurate picture of the aerobic and anaerobic capacity of fish, lactate content in the muscle tissue should be measured, rather than in the blood, and glucose and lactate concentrations should be used in conjunction with other endpoints.

Metabolic enzyme activity is another common endpoint used to determine differences in aerobic and anaerobic metabolic capacity in fishes. Compared to the dimethyl sulfoxide solvent control, carbamazepine, caffeine, and chlorpyrifos treated fish had increased levels of citrate synthase activity in the liver and muscle, while pentachlorophenol and tributyltin treated fish had decreased levels in liver and muscle. Since citrate synthase is an important rate limiting enzyme in the citric acid cycle, it is vital to energy production. Given the neuromuscular effects of carbamazepine (Gao and Chuang 1992; Kuo 1998) and chlorpyrifos (Rice et al. 1997), and the hyperactivity effects of caffeine (Cruz et al. 2017), it is possible that these groups increased citrate synthase activity as a way to compensate for the larger energy expenditure in the muscle. In contrast, pentachlorophenol has previously been found to uncouple oxidative phosphorylation from ATP synthesis (Weinbach 1954), which has lead to increases in lactate dehydrogenase and cytochrome C oxidase activity in the liver, higher lactate concentrations, and higher oxygen consumption rates in pink snapper (Gagnon 2002). This was not found in my study, which

showed low lactate dehydrogenase and cytochrome C oxidase activity, and similar lactate and oxygen consumption as control fish. However, lower concentration used in my treatments, as well as a difference in species and temperature used (rainbow trout at 13°C in my study compared to pink snapper (*Pagrus auratus*) at 21°C in Gagnon 2002), it is difficult to compare the results. The fish used in my study may have had an inherently lower respiration rate due to the lower acclimation temperature.

Given that tributyltin has been found to be an lactate dehydrogenase inhibitor, it was unexpected that the tributyltin treated fish did not have reduced lactate dehydrogenase compared to control. It is possible that injection of tributyltin not the most ideal route of entry for the toxic effects to be observed. Dosing the water, which is a common method of treatment (Greco et al. 2007; Liang et al. 2017), with transport of tributyltin across the gills may have resulted in greater inhibition of lactate dehydrogenase activity. Pentachlorophenol treated fish however, were found to have muscle lactate dehydrogenase activity about 50% that of dimethyl sulfoxide solvent control and was significantly less than chlorpyrifos treated fish that had activity in the muscle about 200% of dimethyl sulfoxide solvent control. As stated earlier, chlorpyrifos treated fish were likely expending more energy but being overall less effective at swimming due to the effects of chlorpyrifos on the muscle. This could have caused the stimulation of lactate dehydrogenase activity to compensate for the energy deficit.

Both aerobic and anaerobic metabolic pathways are suggested to influence the ability of fish to escape predators (Rasmussen et al. 2011), since the activity of citrate synthase, cytochrome C oxidase, and lactate dehydrogenase would influence an individuals energy production capability and use, as well as allowing for quick recovery of glycogen reserves following bursts of activities. Chlorpyrifos treated fish were the only group to have a relationship between enzyme activity and predator escape velocity, which could be a symptom of the low sample size within the treatment groups.

Metabolic activity, behaviour, and locomotion are intrinsically linked in a complex manner. Changes in one aspect does not inherently mean the others will change by the same degree or in a predictable manner. The influence of a multitude of factors means predicting changes in behaviour by looking purely at metabolic activity is insufficient. Behavioural changes may be caused by deviations in metabolic activity, to a degree, but individuals will strive to maintain homeostasis through compensatory methods. Since many of the behaviours commonly examined in fish are vital to their survival, it is likewise important to maintain a range of normality. In this way, despite undergoing alterations in metabolism, individuals can still escape predators or capture food.

Conclusions and Future Directions:

Behavioural phenotypes in animals are the result of a complex interplay among a variety of physiological factors. From my study, it is difficult to draw definitive conclusions since there was a high degree of variability among a small sample size of individuals. However, overall it appears that U_{crit} should not be used alone as an indicator of behavioural changes. By examining aspects of locomotion that are relevant to the behaviour in question, we can get a more accurate picture of the relationship between swim performance and behaviour. The behaviours examined in this study were more reliant on burst type swimming, and it appears that both aerobic and anaerobic metabolic capacities are more important for the quick recovery of metabolic fuels for anaerobic swimming.

In the future, it would be beneficial to extend this research to a larger number of fish while also altering the methods to derive more concrete results. The use of a smaller swim chamber would allow a more accurate depiction of oxygen consumption and aerobic scope, while examining the glycogen, phosphocreatine, and lactate content in the white muscle would give us a better view of fuel use and waste production. Lastly, the behaviour assays could be designed in a way that reduced variability by having a larger test tank to allow full range of movement, as well as altering the predator apparatus to ensure the point of strike consistently elicits a response.

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Supplemental Data:

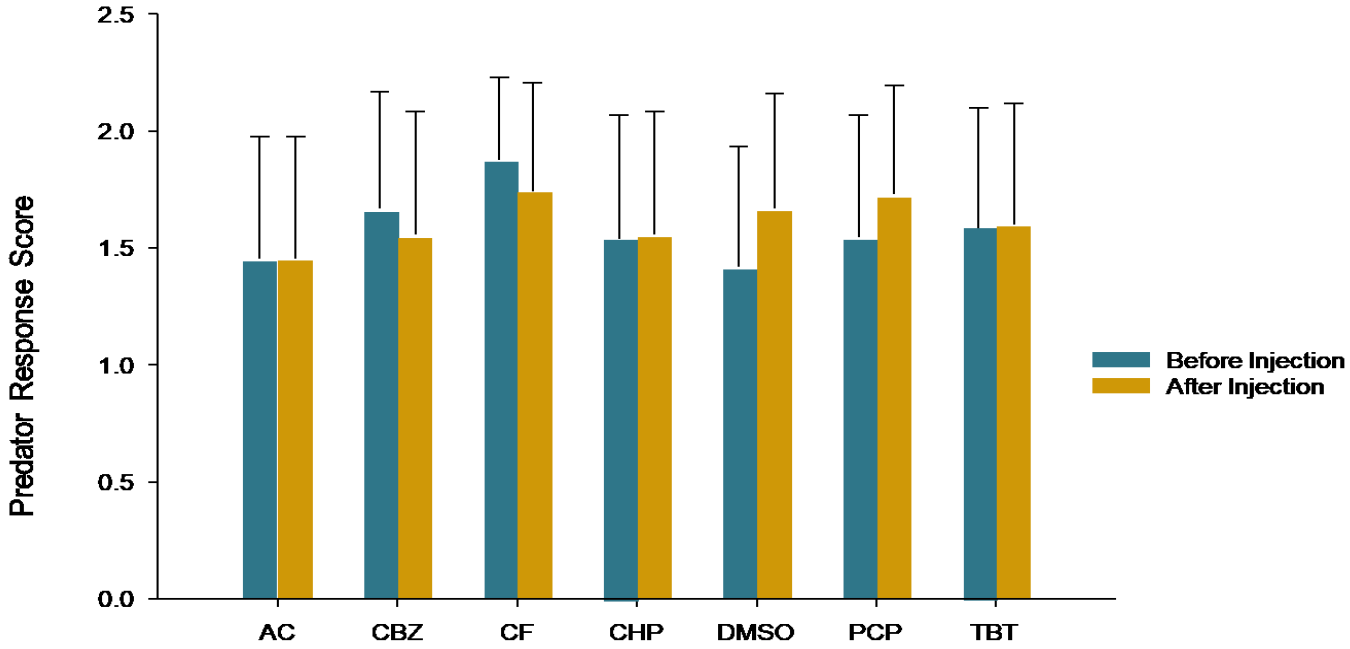

Figure S1. Proportion of changes in predator response before and after injections of experimental compounds. 1 was assigned to fish that did not respond to the predator, and 2 was assigned to fish that did respond to the predator.

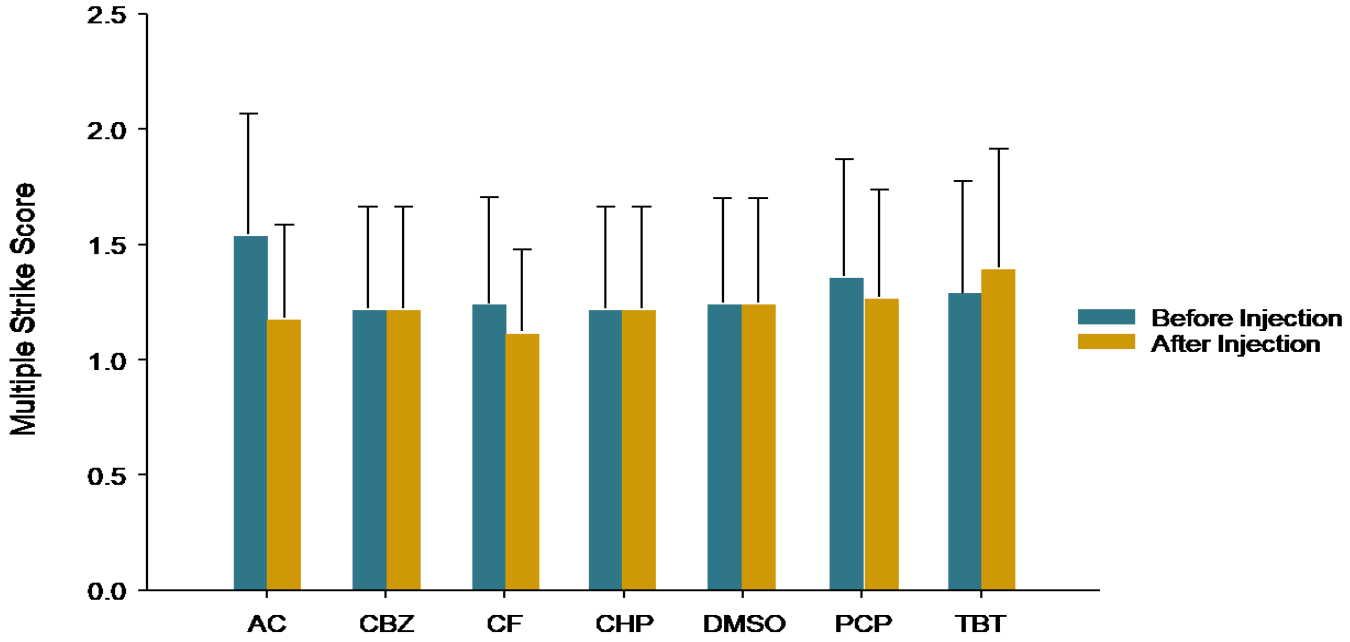

Figure S2. Proportion of changes in multiple predator strikes used to elicit a response before and after injections of experimental compounds. 1 was assigned to fish that did not respond to the predator, and 2 was assigned to fish that did respond to the predator.

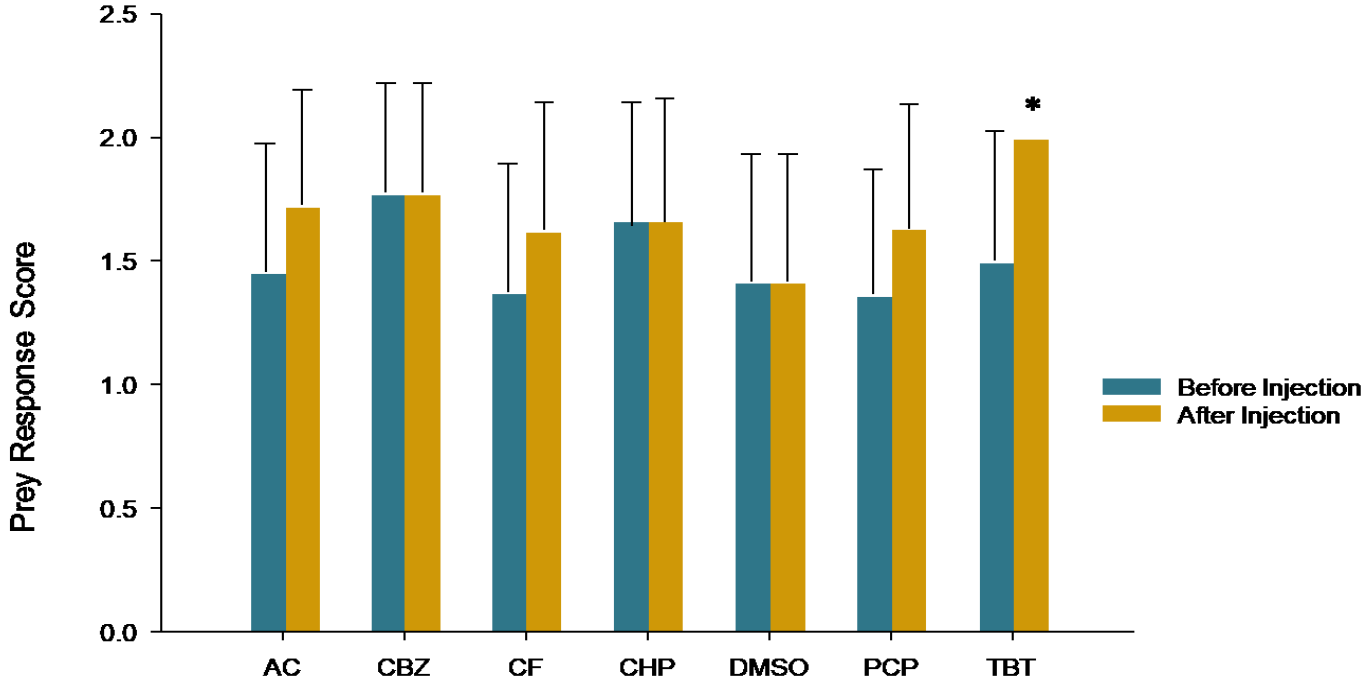

Figure S3. Proportion of change in prey capture attempt before and after injections of experimental compounds. 1 was assigned to fish that did not respond to the predator, and 2 was assigned to fish that did respond to the predator.