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The effects of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) on fuel stores and ion balance in a non-target fish, the rainbow trout (*Oncorhynchus mykiss*)☆

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ABSTRACT

The pesticide 3-trifluoromethyl-4-nitrophenol (TFM) is used to control sea lamprey (*Petromyzon marinus*) populations in the Great Lakes through its application to nursery streams containing larval sea lampreys. TFM uncouples oxidative phosphorylation, impairing mitochondrial ATP production in sea lampreys and rainbow trout (*Oncorhynchus mykiss*). However, little else is known about its sub-lethal effects on non-target aquatic species. The present study tested the hypotheses that TFM exposure in hard water leads to (i) marked depletion of energy stores in metabolically active tissues (brain, muscle, kidney, liver) and (ii) disruption of active ion transport across the gill, adversely affecting electrolyte homeostasis in trout. Exposure of trout to 11.0 mg l^{-1} TFM (12-h LC_{50}) led to increases in muscle TFM and TFM-glucuronide concentrations, peaking at 9 h and 12 h, respectively. Muscle and brain glycogen was reduced by 50%, while kidney and muscle lactate increased with TFM exposure. Kidney ATP and phosphocreatine decreased by 50% and 70%, respectively. TFM exposure caused no changes in whole body ion (Na^+, Cl^-, Ca^{2+}, K^+) concentrations, gill Na^+/K^+ ATPase activity, or unidirectional Na^+ movements across the gills. We conclude that TFM causes a mismatch between ATP supply and demand in trout, leading to increased reliance on glycolysis, but it does not have physiologically relevant effects on ion balance in hard water.

1. Introduction

The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is used to control sea lamprey (*Petromyzon marinus*) populations in the Great Lakes, where it is applied to nursery streams containing larval sea lampreys (Smith and Tibbles, 1980; Bills et al., 2003; Boogaard et al., 2003; McDonald and Kolar, 2007). TFM is the major component in the integrated pest management program of the Great Lakes Fisheries Commission, which was established in 1955 as a partnership between Canada and the United States to coordinate fisheries research and management, and to control sea lamprey populations in this region (Great Lakes Fishery Commission, 2011). The use of TFM has contributed to the restoration of fisheries that were decimated in the mid-20th century due to the combined effects of overfishing and lamprey predation (i.e. parasitism; Lowry, 1970; Christie et al., 2003; McDonald and Kolar, 2007). Treatments with TFM have proven effective mainly because of the specificity of TFM for the larval lampreys (Applegate and King, 1962; Lech and Costrini, 1972; Lech and Statham, 1975) and the relatively sedentary life style of these animals, which mainly restricts them to streams and rivers in this life stage. Despite its success in sea lamprey control, little is known about the potential physiological effects that TFM has on non-target fishes (McDonald and Kolar, 2007).

It is known that the concentrations of TFM tolerated by most fishes are 3–5 times higher than that required to kill larval sea lampreys (Applegate and King, 1962; Bills et al., 2003; Boogaard et al., 2003). This tolerance is related to the greater capacity of most non-target fishes to

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biotransform TFM to TFM-glucuronide via the process of glucuronidation (Olson and Marking, 1973; Lech, 1974; Lech and Statham, 1975; Kane et al., 1993, 1994), making TFM more water soluble and easier to excrete via renal pathways or the gastrointestinal tract (Clarke et al., 1991).

However, there is some evidence that suggests that TFM exposure can have negative effects on non-target fish species. Christie and Battle (1963) demonstrated that TFM can damage the gills in trout (*Oncorhynchus mykiss*) and larval sea lampreys, but Mallatt et al. (1994) detected no changes in gill ultrastructure in trout exposed to their respective 9-h TFM LC_{100} (TFM concentration that is lethal to 100% of the trout over a 9 h exposure period). Kane et al. (1993) reported that bullfrog (*Rana catesbiana*) tadpoles were approximately 13 times more sensitive to TFM than adults due to a lower glucuronidation capacity in the tadpole phase (larval LC_{50} = 0.95 mg l^{-1} vs. adult LC_{50} = 12.99 mg l^{-1}).

Evidence that TFM exerts its toxic effects by creating a shortfall in ATP supply in sea lampreys was provided by Wilkie et al. (2007a), who observed significant decreases in plasma glucose and whole body phosphocreatine (PCr) levels in larval lamprey exposed to TFM (12-h LC_{50} = 2.0 mg l^{-1}). In addition, Birceanu et al. (2009) and Clifford et al. (2012) reported that as the exposure time increased, ATP and glycogen levels in the brains and in the livers of larval sea lampreys were reduced in a step-wise fashion following exposure to sub-lethal (12-h LC_{50}) and lethal (12-h $LC_{99.9}$) concentrations of TFM, respectively. Recently, Birceanu et al. (2011) used isolated liver mitochondria to demonstrate that TFM causes such shortfalls in ATP supply by impairing oxidative phosphorylation in both rainbow trout and sea lampreys. This would subsequently force the fish to rely more on their glycogen supplies and anaerobic glycolysis in order to maintain the ATP demand in the body. Viant et al. (2001) suggested that TFM tolerance could be related to capacity for sustained anaerobic glycolysis in two marine molluscs, limpets (*Lottia gigantean*) and abalone (*Haliotis rufescens*).

While the proximate mechanism of TFM toxicity is the same in rainbow trout and sea lampreys, it is the trout's high glucuronidation capacity (Olson and Marking, 1973; Lech, 1974; Lech and Statham, 1975) that likely prevents the buildup of free-TFM to levels that significantly impair mitochondrial function, and thus aerobic ATP production. Under these conditions, the fish would be forced to rely more on their anaerobic energy stores (i.e. glycogen, phosphocreatine) to compensate for the shortfall in ATP. This shortage of ATP in the body could also indirectly affect ion homeostasis by reducing the ATP supply to ATP-dependent ion pumps in the kidneys and in the gills. Thus, the overarching goal of the present study was to determine if TFM exposure is interfering with ATP supply in rainbow trout, and whether or not such disturbances also impair ion homeostasis.

To test the hypothesis that TFM toxicity results in a mismatch between ATP supply and ATP demand in the trout, we exposed the fish to their respective 12-h TFM LC_{50}, and measured changes in tissue (brain, liver, muscle, kidney) glycogen, ATP, phosphocreatine (PCr) and lactate over the 12 h exposure period, which approximates the length of time that a typical TFM treatment lasts in the field (B. Stephens, DFO — Sea Lamprey Control Center, pers. comm.). The rates of Na^+ uptake, gill Na^+/K^+ ATPase activity, plasma ion (Na^+, Cl^-) and whole body ion (Na^+, Cl^-, Ca^{2+} and K^+) concentrations, and muscle tissue water were also quantified in rainbow trout, following exposure to TFM to determine if the lampricide interfered with gill-mediated ion exchange.

2. Material and methods

2.1. Experimental animals and holding

Rainbow trout (*O. mykiss*, Salmonidae; mass = 5–10 g; N = 62 for the metabolite experiments; mass = 30 ± 2.8 g; N = 48 for the ion flux experiments) were purchased from Rainbow Springs Hatchery (Thamesford, ON, Canada) and held in 120 l polyethylene tanks receiving hard Wilfrid Laurier University well water on a flow-through basis (pH ~ 8.0; titratable alkalinity ~ 200 mg $CaCO_3$ l^{-1}; hardness ~ 450 mg $CaCO_3$ l^{-1}; $[Na^+]$ ~ 1.1 mmol l^{-1}; temperature ~ 10–13 °C). The fish were held under a 12 h light:12 h dark photoperiod, and fed 3 times per week with ground 3.0 commercial floating pellets for the small fish and whole pellets for the larger fish (Corey Feed Mills, Fredericton, NB, Canada). The animals were held in the laboratory for at least 2 weeks before experiments commenced, and were starved for 72 h prior to the experiments, to decrease the amount of ammonia that could accumulate in the water during the 12-h static TFM exposure period. All experiments and fish husbandry were approved by the Wilfrid Laurier University Animal Care Committee and followed Canadian Council of Animal Care guidelines.

2.2. Experimental protocol

2.2.1. Determination of the acute toxicity of TFM

To determine the 12-h LC_{50} of TFM in Wilfrid Laurier well water for rainbow trout, a range finder experiment was conducted by exposing trout to either control conditions (no TFM) or to nominal TFM concentrations of 8.0, 10.0, 12.0, 16.0, 20.0 and 25.0 mg l^{-1}, for 12 h, in 15 l of well water. All fish were acclimated to their respective treatment containers for 12 h prior to the addition of the chemical. A total of 5 fish were exposed to each concentration, at pH 8.14 ± 0.03. The experiments were conducted in the dark, since TFM is sensitive to photodegradation (Carey and Fox, 1981; Hubert, 2003) and the containers were placed in a flow-through tank supplied with well water to ensure the temperature remained constant during the exposure. For unidirectional ion flux experiments, larger rainbow trout (30 ± 2.8 g) were exposed to their respective TFM 12-h LC_{100} in the same well water to determine if higher TFM exposure concentrations interfered with unidirectional Na^+ movements (influx, efflux, net flux) across the gill. These experiments were therefore preceded by a second range finder experiment during which the trout (N = 10 per concentration) were exposed to the same nominal concentrations as above (measured [TFM] = 8.0, 9.7, 11.6, 13.9, 17.8 and 27.1 mg l^{-1}, plus non-exposed controls) to determine the LC_{100} of TFM in the same water.

Field formulation TFM (35% active ingredient in isopropanol; Clariant SFC Gmbh Werk Griesheim, Germany) was used for all range finder and TFM exposure experiments and provided courtesy of the Sea Lamprey Control Center, Fisheries and Oceans Canada (Department of Fisheries and Oceans [DFO]; Sault Ste. Marie, ON, Canada). To verify water TFM concentrations, the absorbance of water samples was measured at a wavelength of 395 nm using a 96-well plate spectrophotometer (SpectraMax 190, Molecular Devices, CA, USA), and using the precision TFM standards and the Standard Operating Procedures (Instrument Operating Procedure, IOP012.3, DFO, Sault Ste. Marie, ON, Canada) provided by the Sea Lamprey Control Center and modified for the 96-well plate spectrophotometer (SpectraMax 190, Molecular Devices, CA, USA) used for the current measurements.

2.2.2. TFM accumulation and its effects on energy stores, metabolites and whole body ions

To test the hypothesis that TFM toxicity was associated with an energy imbalance in rainbow trout, the effects of TFM on fuel stores [ATP, PCr, glycogen and glucose] and lactate were measured in the liver, brain, muscle and blood at different intervals (1, 3, 6, 9, 12 h) during exposure to the pre-determined 12-h LC_{50} of TFM (nominal [TFM] = 11.0 mg l^{-1}, n = 8 at each time point in TFM-exposed fish; n = 11 controls with n = 5 sampled at the beginning of the experiment and 6 sampled 12 h later). Each of the fish (control and TFM-exposed trout) were contained in static 1.0 l containers filled with 1.0 l of Wilfrid Laurier aerated well water and left to acclimate for 12–24 h prior to the beginning of the experiment. Immediately prior to the addition of TFM to the treatment containers, approximately 75% of the water was replaced with fresh well water in all containers, including the controls. At each sample interval (0, 1, 3, 6, 9, 12 h), sub-sets of live fish were