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Life stage dependent responses to the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), provide insight into glucose homeostasis and metabolism in the sea lamprey (*Petromyzon marinus*)

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ABSTRACT

The primary method of sea lamprey (*Petromyzon marinus*) control in the Great Lakes is the treatment of streams and rivers with the pesticide 3-trifluoromethyl-4-nitrophenol (TFM), which targets larval sea lamprey. However, less is known about the effects of TFM on other stages of the sea lamprey's complex life cycle. The goal of this study was to determine how TFM affected internal energy stores, metabolites, and ion balance in larval, juvenile (parasitic) and adult sea lamprey. The larvae were more tolerant to TFM than the adults, with a 2-fold higher 12 h TFM LC_{50} and a 1.5-fold higher $LC_{99.9}$. Acute (3 h) exposure of the larvae, parasites and adults to their respective 12 h TFM $LC_{99.9}$ led to marked reductions in glycogen and phosphocreatine in the adult brain, with lesser or no effect in the larvae and parasites. Increased lactate in the brain, at less than the expected stoichiometry, suggested that it was exported to the blood. Kidney glycogen declined after TFM exposure, suggesting that this organ plays an important role in glucose homeostasis. TFM-induced disturbances to ion balance were minimal. In conclusion, TFM perturbs energy metabolism in all major stages of the sea lamprey life cycle in a similar fashion, but the adults appear to be the most sensitive. Thus, the adult stage could be a viable and effective target for TFM treatment, particularly when used in combination with other existing and emerging strategies of sea lamprey control.

1. Introduction

The integrated pest management of sea lamprey (*Petromyzon marinus*) in the Great Lakes uses a combination of barriers and traps to prevent adult sea lamprey from reaching their spawning grounds, with ongoing research efforts aimed at developing chemical attractants and/or repellants to improve the efficiency of these methods (Li et al., 2007; Mclaughlin et al., 2007). Despite much progress in this area, the primary method of sea lamprey control continues to be the application of the chemical 3-trifluoromethyl-4-nitrophenol (TFM) to nursery streams containing multiple generations of burrow-dwelling larval sea lamprey (Hubert, 2003).

TFM exerts its toxicity by uncoupling mitochondrial oxidative phosphorylation (Niblett and Ballantyne, 1976; Birceanu et al., 2011), leading to impaired ATP production and an increased reliance on anaerobic metabolism to generate ATP (Wilkie et al., 2007; Birceanu et al., 2009, 2014). This results in marked reductions in glycogen and phosphocreatine (PCr) levels in various tissues (the brain is especially vulnerable), as these anaerobic energy reserves are rapidly consumed to make-up for the shortfall in ATP supply (Birceanu et al., 2009; Clifford et al., 2012). Recent studies on non-target fishes such as rainbow trout (*Oncorhynchus mykiss*) have indicated that they respond to toxic concentrations of TFM similarly to the sea lamprey (Birceanu et al., 2011, 2014). The greater tolerance of rainbow trout and other non-target fishes to TFM is due to a greater capacity to detoxify the lampricide, through its biotransformation to TFM-glucuronide (Lech and Statham, 1975; Kane et al., 1993, 1994). As a result, the median lethal concentration (LC_{50}) and the $LC_{99.9}$ of TFM are approximately 3–5 times higher than in most non-target fishes (Boogaard et al., 2003; McDonald and Kolar, 2007). Although Applegate et al. (1961) noted that the post-metamorphic juvenile and adult sea lampreys were more sensitive to TFM, it is somewhat surprising that we have little

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additional knowledge about the effects of this lampricide on other stages of the sea lamprey's complex life cycle.

With their relatively sedentary life style and tendency to congregate in large numbers in streams, larval sea lampreys are the target of TFM applications. However, with the advent of chemical attractants and repellants (Li et al., 2007; Imre et al., 2010, 2014; Wagner et al., 2011) to direct adult sea lamprey into traps or even specific regions of a stream, it may be possible to use TFM to target the adults during their spawning migrations and increase the overall effectiveness of sea lamprey control efforts. Similar approaches might also be used to target post-metamorphic juvenile lamprey on their downstream migrations to the Great Lakes, where they prey upon top predators such as lake trout (*Salvelinus namaycush*), white fish (*Coregonus clupeaformis*) and other economically important game and commercial fish species (McLeod et al., 2011; Moody et al., 2011). However, the effectiveness of targeting other stages in the sea lamprey life cycle will depend on a better understanding of how TFM affects the physiology of juvenile and adult sea lamprey. To date, it is unclear whether the post-metamorphic sea lamprey respond to TFM in a similar manner to the larval stage.

The metamorphosing period of the sea lamprey life cycle is characterized by major changes in internal and external body structure, including extensive re-organization and changes in the fine architecture of the gills (Youson, 1980; Bartels and Potter, 2004; Reis-Santos et al., 2008). Changes in the structure of the kidneys, and the loss of bile ducts in the liver also occur during metamorphosis (Youson, 1980, 2003), along with changes in the metabolic make-up of the animals, including an increased capacity to deaminate amino acids during the parasitic phase, when they are ingesting large quantities of protein-rich blood (Wilkie et al., 2006). Marked reductions in lipid stores also take place due to the prolonged non-trophic periods that accompany metamorphosis in juvenile sea lamprey, and in the adults that migrate upstream (O'Boyle and Beamish, 1977; Beamish et al., 1979). Taken together, these physiological alterations may impact the sea lamprey's sensitivity to TFM following metamorphosis.

The goal of the present study was to compare the TFM-induced responses of the juvenile and the adult sea lamprey to those of the larvae, with a particular focus on the effects of TFM on glycogen and glucose homeostasis. Unlike earlier studies, in which larval sea lamprey were exposed to their 12 h TFM LC_{50}, here we characterized how exposure to the 12 h $LC_{99.9}$ affected ion homeostasis and energy reserves, including glycogen, ATP and PCr in the brain, liver, kidney and muscle at each life stage. The 12 h $LC_{99.9}$ was chosen to more accurately reflect the doses of TFM that the sea lamprey would encounter during actual field applications, in which TFM is typically at 1.3-1.5 times this value (McDonald and Kolar, 2007; Scholefield et al., 2008).

2. Materials and methods

2.1. Experimental animals and holding

Larval sea lamprey (*P. marinus*; 1.9 ± 0.2 g, 85–150 mm) were provided courtesy of the Hammond Bay Biological Station (HBBS), United States Geological Survey (USGS, Millersburg, Michigan), and shipped to Wilfrid Laurier University in plastic bags filled with 20–30 l of oxygen saturated water. Parasitic juvenile sea lamprey (134.4 ± 8.8 g, 30–50 cm) were captured in Lake Huron by commercial fishermen, shipped and held at the HBBS for no more than 3 weeks, and then shipped to Wilfrid Laurier University in an identical manner. Pre-spawn, adult sea lamprey (226.5 ± 8.1 g, 40–55 cm) were captured in traps on the Humber River, Toronto during their upstream migration in April-May, and shipped to Wilfrid Laurier University in a 400 l fish hauler containing well-aerated water, courtesy of Mr. R. McDonald, Sea Lamprey Control Center, Fisheries and Oceans Canada (DFO; Sault St. Marie, Ontario, Canada). The larval sea lamprey were held in 110 l holding tanks continuously receiving aerated fresh water (pH ~ 8.0; titratable alkalinity ~ 200 mg CaCO3 l^{-1}; hardness ~ 450 mg CaCO3 l^{-1}; temperature 10–13 °C), at a rate of 1–2 l min^{-1}, and lined with sand (4–5 cm deep), to provide the larvae with burrowing substrate. Free-swimming parasitic juveniles and adult sea lamprey were held in 100–500 l holding tanks continuously receiving the same water at a rate of 3–5 l per minute. All animals were held under a 12 h light:12 h dark photoperiod. Baker's yeast (2 g yeast per larva; Holmes and Youson, 1994; Wilkie et al., 1999) was used to feed larval sea lamprey once a week, but it was not possible to feed the large parasitic lamprey due to an inability to get fish that were large enough for them to feed upon, and the adults do not feed during this terminal phase of their life cycle (Larsen, 1980). The larval lamprey were held in the lab for a minimum of 1 month before experiments, but were not fed one week prior to the beginning of the experiments. The juvenile parasitic and adult sea lamprey were held at HBBS and by the DFO, respectively, for 2–4 weeks before being shipped to Wilfrid Laurier University, where they were also held for approximately 2–4 weeks before experiments. 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 protocols

2.2.1. Determination of the acute toxicity of TFM

To determine the toxicity of TFM to larval, juvenile and adult sea lamprey in Wilfrid Laurier well-water, two acute toxicity experiments were conducted by exposing the larvae to nominal TFM concentrations of 0.0, 1.0, 3.0, 5.0, 7.0, 10.0 and 20.0 mg l^{-1} for 12 h and the adults to nominal TFM concentrations of 0.0, 0.25, 0.5 1.0, 2.0 5.0, 10.0 and 25.0 mg l^{-1} for 12 h. We were unable to obtain sufficient numbers of parasitic juvenile lamprey to perform acute toxicity experiments because they are very difficult to capture during this lake-dwelling phase of their life cycle. Accordingly, experiments on the parasitic juvenile sea lamprey were limited to physiological studies (see below). Prior to the start of each toxicity test, the larval and adult sea lamprey were acclimated to their respective exposure containers for 12 h, in groups of 5–6 at each test concentration. Because sea lamprey are negatively phototactic (Rovainen and Schieber, 1975), the containers were darkened and covered to reduce light exposure (Wilkie et al., 1999). At regular time intervals (1 h), the number of surviving and dead animals was tabulated and used to calculate the LC_{50} for TFM, or the median lethal concentration of TFM that killed 50% of the animals, and the $LC_{99.9}$, which was the concentration of TFM that killed 99.9% of the animals during the 12 h toxicity experiment. Field formulation TFM (Clariant SFC GmbH, Frankfurt, Germany) was used for all experiments [35% active ingredient dissolved in isopropanol; provided courtesy of the Sea Lamprey Control Centre, Department of Fisheries and Oceans (DFO) Canada, Sault Ste. Marie, ON], and TFM exposure concentrations were verified using precision TFM standards provided by DFO on a plate spectrophotometer (SpectraMax 190, Molecular Devices, CA), at a wavelength of 395 nm.

2.2.2. Effects of TFM on lamprey metabolism and plasma ions

To test the hypothesis that the severity of TFM-induced physiological disturbances reflect life stage sensitivity, the effects of TFM on high energy phosphagens and glycogen, along with metabolites (lactate), were measured in different tissues (brain, liver, kidney and muscle) of the animals following 0 (control), 1, 2 and 3 h of exposure to the predetermined TFM 12 h $LC_{99.9}$ (nominal concentration of 7.6 mg l^{-1} for larvae and 5.0 mg l^{-1} for juvenile parasitic and adult sea lamprey). Potential ionic disturbances were also evaluated by measuring plasma ion concentrations (Na^+, Cl^-, Ca^{2+}), as well as whole body water content.

Individual larvae were placed into separate, darkened 850 ml containers containing aquarium cotton (2 g per container; Wilkie et al., 1999) as burrowing substrate, to calm the animals (thigmokinesis: