Ecotoxicology and Environmental Safety 75 (2012) 142–150

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Swim performance and energy homeostasis in spottail shiner (*Notropis hudsonius*) collected downstream of a uranium mill

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ARTICLE INFO

Article history:
Received 6 April 2011
Received in revised form
26 August 2011
Accepted 3 September 2011
Available online 23 September 2011

Keywords:
Uranium milling effluent
Spottail shiner
Swim performance
Metabolism
Critical swim
Speed
Energy homeostasis

ABSTRACT

The Key Lake uranium milling operation (Saskatchewan, Canada) releases complex effluent into the local watershed. The objective of the current study was to investigate whether fish from an effluent-receiving waterbody exhibited differences in swimming performance and energy homeostasis compared to fish from a local reference site. Juvenile spottail shiner (*Notropis hudsonius*) were collected from a lake downstream of the uranium mill, and compared to fish collected from a nearby reference lake. Critical swimming speed (U_{crit}; fatigue velocity), tail beat frequency, and tail amplitude did not differ significantly when comparing fish collected from the exposure lake and reference lake. Captured shiner used in swim tests were considered fatigued, and metabolic endpoints were compared between this group and non-fatigued fish, which were treated similarly but not subjected to swim tests. In both non-fatigued and fatigued shiner, liver glycogen was significantly greater in fish collected from the exposure lake compared to the reference lake. However, it is unclear if this effect, and others related to condition, were the result of contaminant exposure or other environmental factors. While there were no differences in plasma lactate, hematocrit or liver triglycerides in non-fatigued fish between sites, only fatigued reference fish had increased lactate and hematocrit and decreased triglycerides. In non-fatigued fish, plasma glucose did not significantly differ between sites, but significantly decreased after swimming only in fish from the exposure lake. In summary, shiner from the exposure site demonstrated similar swim endurance and possessed greater energy stores despite metabolic alterations compared to shiner from the reference site. Therefore, because fish collected downstream of the uranium mill operation had similar swimming ability as fish from the reference lake, U_{crit} test results presented here may not reflect or be indicative of metabolic effects of complex effluent exposure.

1. Introduction

Integrated physiological traits in fish, such as swimming performance and metabolic status can provide more biologically insightful information associated with contaminant exposure than traditional environmental and morphometric endpoints (Rajotte and Couture, 2002; McKenzie et al., 2007). Fish survival in the wild strongly depends on swimming ability as it relates to activities such as feeding, predator evasion, migration and mating (Plaut, 2001). To characterize swimming performance a number of protocols based on Brett's critical swim speed (U_{crit}; Brett, 1964) have been developed. This endpoint has provided an ecologically relevant assessment of swim endurance in many fish species (reviewed by Plaut, 2001). In addition to swim endurance, swim motion characteristics, such as tail beat frequency and tail beat amplitude, can be used to quantify the manner in which a fish swims and its relation to metabolism (Bainbridge, 1958; Videler and Wardle, 1978; Steinhausen et al., 2005; Tudorache et al., 2010).

Maximal oxygen consumption in salmonids occurs at or immediately prior to U_{crit}, suggesting cardiorespiratory performance also plays a key role in determining swimming performance (Farrell, 2007). This phenomenon could be true for other fish groups as well. Some fish species maximize oxygen carrying capacity by increasing hematocrit during activity or in hypoxic environments (Butler et al., 1992; Gallaugher et al., 1995; Robb and Abrahams, 2003; Sandblom and Axelsson, 2007). Similarly, cardiac output and cardiac morphology have been shown to strongly influence swimming ability (Claireaux et al., 2005; Farrell, 2007). During slow or routine swimming, and for swimming speeds up to approximately 80 percent U_{crit}, intramuscular aerobic metabolism is the predominant metabolic mechanism used to power swimming (Webb, 1971). Triglycerides account for

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doi:10.1016/j.ecoenv.2011.09.002

the bulk of oxidizable substrates fueling aerobic swimming, although protein can serve as an alternate fuel source once lipid pools are exhausted (reviewed by Moyes and West, 1995). At speeds greater than 80 percent U_{crit}, or burst swimming, most energy is derived from anaerobically metabolized intramuscular glycogen. As a result, lactate is generated in muscle during burst swimming, which enters systemic circulation and serves as a measurable endpoint of anaerobic metabolic activity.

Tissue aerobic and anaerobic capacity can be characterized by assessing the activity of rate-limiting enzymes involved in metabolic pathways. Citrate synthase (CS) is a key enzyme in the citric acid cycle, and is considered an indicator of tissue aerobic ability (Rajotte and Couture, 2002; Lemos et al., 2003). Alternatively, β-hydroxyacyl coenzyme A dehydrogenase (HOAD) is involved in β-oxidation of lipids, the activity of which can be indicative of tissue lipolytic ability (Londraville and Duvall, 2002; Rajotte and Couture, 2002). While these enzymes are primarily involved in aerobic metabolism some anaerobic activity could play a role in routine swimming (Moyes et al., 1992; Rajotte and Couture, 2002). The acute physiological stress response is also closely related to metabolic responses during swimming. Catecholamines and cortisol are released as a part of this response and cause metabolic and cardiorespiratory changes (Jobling, 1994). This leads to increased hematocrit, plasma lactate, and mobilized glycogen stores, which may coincide with exercise-related changes in metabolism.

Critical swim speed is a sensitive endpoint that has been shown to be affected by many environmental contaminants, including ammonia (reviewed by McKenzie et al., 2003), dissolved metals (Wilson and Wood, 1992; Beaumont et al., 1995; Alsop et al., 1999; McGeer et al., 2000; Rajotte and Couture, 2002; Taylor et al., 2004), and complex mixtures such as coal ash (Hopkins et al., 2003), crude oil fractions (Kennedy and Farrell, 2006) or urban river systems (McKenzie et al., 2007). Some environmental contaminants have the ability to cause subtle developmental effects in juvenile or adult life stages that could significantly reduce swimming and/or cardiovascular ability. For example, selenium is known to cause cardiovascular and morphological terata in maternally exposed fish larvae (Holm et al., 2005; Muscatello et al., 2006). Morphological deformities such as spinal curvatures could also impair swim motion characteristics (tail beat frequency or tail beat amplitude) while cardiovascular abnormalities could impair oxygen delivery to aerobic tissues.

Chronic trace element exposure is reported to alter the acute stress response and energy homeostasis in some northern fish species. For example, wild yellow perch (*Perca flavescens*) from metal-contaminated lakes have reduced muscle enzyme activities (CS, HOAD, lactate dehydrogenase; Rajotte and Couture, 2002; Audet and Couture, 2003; Couture and Rajotte, 2003; Couture and Pyle, 2008), altered seasonal energetic stores (Levesque et al., 2002) and impaired cortisol production (Brodeur et al., 1997; Laflamme et al., 2000; Levesque et al., 2002). Previous studies reported that northern pike (*Esox lucius*) and burbot (*Lota lota*) downstream of the Key Lake uranium mill (northern Saskatchewan, Canada) have higher triglyceride and higher glycogen stores than fish from reference lakes (Bennett and Janz, 2007; Kelly and Janz, 2008). No difference was observed in prey triglyceride levels, but fish collected from the exposure site had significantly lower parasite abundance, which offers a possible partial explanation for the observed increased energy stores (Kelly and Janz, 2008). Characterizing the swimming performance and energy homeostasis in fish downstream of the Key Lake uranium mill could thus provide insight into these previously observed differences in bioenergetic endpoints.

The objective of this study was to investigate the potential effects of chronic exposure to metal milling effluent on swimming performance and energy homeostasis in a wild minnow species, spottail shiner (*Notropis hudsonius*). To evaluate these effects, shiner were collected from a lake downstream of the Key Lake uranium mill and from a nearby ecologically similar reference lake. Fish from each lake were either swam to fatigue using on-site incremental velocity (U_{crit}) tests, or withheld from U_{crit} tests (non-fatigued). In addition to swim performance, trace element body burdens, basic biometrics (fork length, mass, condition factor, hepatosomatic index), biochemical measures of energy stores (liver and muscle glycogen and triglycerides), metabolic pathway endpoints (hematocrit, plasma glucose and lactate, and muscle metabolic enzyme activity), and cardiovascular structures (bulbus arteriosus, ventricle, dorsal aorta diameter) were compared between fish collected from each lake.

2. Materials and methods

2.1. Study site, trace element analysis, and fish collection

The Key Lake uranium mill is located in northern Saskatchewan, Canada (57°13′N, 105°38′W) and has been discharging treated mill effluent for approximately 30 years into a small local drainage called the David Creek drainage system (for map see Muscatello et al., 2006). In 2006, this mill discharged approximately 135,130 m^3 treated effluent/month (which is known to contain variable, but generally elevated concentrations of arsenic, molybdenum, nickel, selenium, uranium, thallium, ammonia, and organics; Golder Associates, 2008). The exposure site (Delta Lake) is part of the David Creek drainage system located 10 km downstream of effluent release, where effluent makes up approximately 28 percent lake water volume (Golder Associates, 2008). The reference site (Yeoung Lake) is located approximately 7 km southeast of the mill site within a separate drainage system, and was chosen for its hydroecological similarity to Delta Lake (Phibbs et al., 2011).

General water chemistry (pH, conductivity, total dissolved solids, salinity, and temperature) were assessed on-site using a multi-parameter YSI probe (six series—YSI Inc., Yellow Springs, OH). Temperature was measured on three separate days during fish captures. All other basic water chemistry parameters in the present study were based on a single sample taken during fish collection. Juvenile spottail shiner were collected from different locations in both lakes in mid-June 2009 using beach seines. Fish were kept in holding nets in resident lakes for up to 48 h. On the day of swim tests, shiner were transported approximately 10 km from resident lakes to the experiment site in coolers containing fresh aerated lake water. Water temperature was maintained below 15 °C. All fish were treated in accordance with protocols approved by the University of Saskatchewan Animal Research Ethics Board and the Canadian Council on Animal Care guidelines on experimental animal care and use.

2.2. Swim performance tests

Immediately prior to swim tests shiner were removed randomly from holding coolers and weighed. Critical swim speed was evaluated in individual shiner using a LoligoSystems Mini Swim Tunnel (LoligoSystems, Tjele, Denmark) with circulating fresh oxygenated water from the corresponding lake. Circulating water temperature was maintained at 12 °C, which reflected the average ambient lake temperature at the time of collection. The swim tunnel apparatus was filled with approximately 50 L of circulating lake water. Shiner were acclimated in the swim chamber for 45 minutes at low flow (approximately 1 body length/s). This acclimation period was selected based on logistical considerations, primarily limited time and resources in the field. During swim tests, water velocity was increased stepwise in 5 min increments by 5 cm/s (*i.e.* velocity increased approximately 1 body length/increment). Fish were considered fatigued once they could no longer remove themselves from the downstream swim tunnel grate, despite efforts to continue swimming against the tunnel current. This protocol was directly adapted from the protocols of Dussault et al. (2008) and Kaufmann (1990), which were modified to accommodate early life-stage and/or small bodied fish in small swim tunnels. Critical swim speed was calculated as: $U_{crit}=V_p+((t_f/t_i)\times V_i)$, where: V_i is the velocity increase per increment; V_p is the final velocity swam; t_i is the increment time length; t_f is the duration of the last velocity increment until fatigue. Because fish occupied greater than 5 percent volume of the swim tunnel, a solid blocking coefficient was calculated for each fish and applied to final U_{crit} values (Bell and Terhune, 1970).

Swim motion was recorded concurrently with a high speed camera (250 frames/s) to enable measurement of tail beat frequency and tail amplitude using Midas 2.0 imaging software. Both endpoints were calculated at the highest completed velocity interval during U_{crit} testing. Tail beat frequency was analyzed using Adobe Premiere Elements 2.0, while video frames for tail amplitude analysis were selected using this software and then exported for analysis with Image-Pro