11-2015

Uncovering adaptive versus acclimatized alterations in standard metabolic rate in Brown Bullhead (Ameiurus nebulosus)

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TITLE: Uncovering adaptive versus acclimatized alterations in Standard Metabolic Rate in Brown Bullhead (*Ameiurus nebulosus)*.

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ABSTRACT

Standard metabolic rates (SMR) were measured in Brown Bullheads collected from two locations of the Detroit River, North America, representative of highly contaminated and uncontaminated areas. Measurements of SMR were completed within 10 d of fish collections (acute trials), for fish held in a common pond environment for 1 year (clearance trials) and for F1 generation fish raised in the pond environment (F1 study). SMRs were significantly higher (26%) in fish from the contaminated area during acute trials. Both populations showed large decreases in SMR (49 to 52 %) following clearance, however, differences between populations were still evident. There were no significant differences in SMRs between populations for F1 fish. This study demonstrates that Detroit River Brown Bullheads from contaminated areas have higher metabolic rates than fish from clean locations and this metabolic effect is retained for long durations after fish are placed in a common environment. The loss of metabolic differences in F1 offspring indicates that the observed differences in SMR were acclimation based and not adaptive or related to maternal effects.

Key Words: Brown Bullhead, Standard metabolic rate, pollution adaptation, bioenergetics

INTRODUCTION

Standard metabolic rate (SMR) is an important component of the energy budget and represents the minimal energy required to sustain physiological function excluding consumption, digestion and activity (Enders *et al.* 2006). Since the SMR defines the baseline for the scope of somatic growth and reproduction in fish (Adam and Breck 1990), SMR may have strong linkages to animal fitness and can be subject to selective pressures under different environments and chronic stressors/interactions (Fitzgibbon *et al.* 2007; Burton *et al.* 2011; Killen et al. 2013). Further, SMRs are known to vary within populations as a result of intrinsic and extrinsic factors (Norin and Malte 2012). Inherent factors such as genotype, maternal effects, early development conditions and behavioural traits have all been shown to influence the energetic maintenance costs of conspecifics (Burton et. al. 2011). Extrinsic conditions are also known to cause changes in fish SMR including social interactions, seasonal shifts (Beamish 1964; Sloman *et al.* 2000; Chipps *et al.* 2000), photoperiod (Biswas and Takeuchi 2002), habitat (Millidine *et al.* 2006), fish density (Reid *et al.* 2011), feeding activity, water quality (e.g. low dissolved oxygen concentrations and pH) (Ginnekena and Thillart 2009; Fromm 1980; Cech et al. 1985) and contaminant exposure. Despite these interactions, the common bioenergetic modelling approach (e.g. Wisconsin Model) treats all individuals of a species equally and provides little opportunity for adjustment to intrinsic or extrinsic variables described above. In order to improve the accuracy of bioenergetic modelling applications it is necessary to improve our understanding of extrinsic factor/SMR relationships by comparing SMRs among populations of fish under different environmental conditions.

One extrinsic factor that has been shown to directly influence metabolic rate of fish is exposure to toxic contaminants. Exposure to metals, pesticides, polycyclic aromatic

hydrocarbons (PAHs) and persistent organic pollutants (POPs), and the subsequent metabolic effects (e.g. change in O_2 consumption rates) in fish have been well documented (Heath 1987; Handy and DePledge 1999). For example, Waiwood and Beamish (1978) observed that for a given swimming speed and pH, Rainbow Trout (*Oncorhynchus mykiss*) in water dosed with copper (25 and 40 µg/L) exhibited higher oxygen consumption rates than controls. Exposure to PAHs has also been shown to affect metabolic rates of Mummichog (*Fundulus heteroclitus*), whereby fish exposed to PAHs in their diet (900 ng/g Σ-PAH) demonstrated a 13% increase in oxygen consumption rate relative to controls (Merten 2005). In other studies, both Largemouth Bass (*Micropterus salmoides*) and Rainbow Trout O_2 consumption rates significantly increased following exposure to elevated pesticide concentrations of dieldrin and DDT respectively (Lunn et al. 1976; Beyers et al. 1999). The above studies indicate that contaminant exposures have a bioenergetic cost on fish. Whether this effect is the result of energy allocation related to an acclimated response to the stressor (Jobling 1994; Barton 2002) or the result of a direct interaction between the chemical and a biochemical pathway regulating fish metabolism (i.e. a toxic consequence of the exposure; Basha et al. 1984; Ali et al. 1993; Willet et al. 2001; Richter et al. 2011) is unknown. Long-term exposure to chemical stressors in the environment may also contribute to natural selection in exposed fish populations resulting in heritable differences in fish/stressor responses (Meyer and Di Giulio 2002, 2003; Breckels and Neff 2010; Wirgin et al. 2011). Adaptive responses and/or maternal effects to fish metabolic rate would be expected to contribute to population differences in energy metabolism of fish, observable in offspring reared outside of the environment of parental capture.

To date, most investigations studying the effects of contaminants on fish SMR have been performed under laboratory conditions (Macleod and Pessah 1973; Heath 1987; Beyers et al.

1999). There exists limited information about how the SMRs of natural fish populations respond to long-term (multi-generation) exposures to mixtures of toxic contaminants in the field. In the present study, intraspecific variation of SMR was determined in two relatively isolated populations (Soderberg 2013) of Brown Bullhead (*Ameiurus nebulosus*) inhabiting clean and contaminated areas within the Detroit River. This three part study was developed to contrast SMR in the two populations of fish i) immediately following capture from their natural environment (acute study), ii) following a long-term acclimation of field captured individuals to a clean aquaculture environment (clearance study) and iii) in F1 offspring derived from each population (F1 study). This permitted an examination of inter-population differences in SMR and attributing such responses to acclimation or heritable response/maternal effects.

MATERIALS and METHODS

Site description and fish collections

The Detroit River is a connecting channel within the Huron-Erie corridor of the Laurentian Great Lakes. In 1987 the river was designated as a Great Lakes Area of Concern by the International Joint Commission owing to a series of beneficial use impairments, many being related to toxic contaminants in water and sediments (Green et al. 2010). Previous sediment surveys of the Detroit River have reported widespread and elevated concentrations of PAHs, PCBs, organochlorine pesticides and metals (e.g. copper, mercury, cadmium, lead, nickel and zinc) within depositional zones along the south east channel of the river (e.g. Trenton Channel) compared to less contaminated upstream locations (e.g. Peche Island) (Kashian et al. 2008; Drouillard et al. 2006; Szalinska et al. 2007; Szalinska et al. 2013). Contamination of the lower downstream reach is believed to be a long-term legacy phenomena associated with a hundred

104 years of intense population growth and industrial activities within the region (Kauss and Hamdy
105 1985, UGLCCS 1988).

106 Sampling of Brown Bullheads was conducted in the above two regions, with Peche Island
107 (42˚20’42.29” N and 82˚55’39.30” W) representative of the clean location and Trenton Channel
108 (42˚10’49.01” N and 83˚09’09.42”W) representative of contaminant areas. In order to eliminate
109 environmental factors that may affect between-population SMR comparisons in this study,
110 sampling locations were carefully chosen to ensure that habitat between the two sites were
111 similar. This included sampling fish from sites with similar depths, cover, current, temperature,
112 pH, dissolved oxygen concentrations, substrate, and submergent macrophtye community (i.e.
113 habitat structure) for acute trials and maintaining fish under identical pond environments for
114 clearance trials.

115 Despite inhabiting the same system, genetic evidence suggests that fish from these two
116 locations are reproductively isolated from one another (Soderberg 2013). Fish from these two
117 areas also display large differences in tissue-accumulated contaminant concentrations (Leadley et
118 al. 1998; Farwell et al. 2013). Measurements of SMR were conducted in three main study trials
119 to contrast metabolic rate of fish derived from clean and contaminated sites. The first study
120 (Acute SMR) involved measurement of SMRs in fish from clean and contaminated sites shortly
121 (within 3-10 d of collection) following their capture from the field. The second study (Cleared
122 SMR) involved measurement of SMRs in fish from the two locations after holding the fish in
123 mesocosm ponds at an aquaculture facility for a period of 1 year. The third study (F1 SMR)
124 involved measurement of SMR in offspring of the cleared fish used in study 2. Experimental
125 conditions for each sub-study are outlined in greater detail below. All studies described were

126 performed following ethical review and approval from the local animal care committee at the
127 University of Windsor in compliance with the Canadian Council for Animal Care guidelines.
128 Detroit River Brown Bullheads were collected from July through September 2009 to
129 generate fish for long-term holding for Cleared and F1 studies. Fish were collected using a 5
130 meter single boom electrofishing vessel equipped with a 5 kW generator. Two bow netters
131 retrieved stunned fish as they appeared and the collected fish were immediately transferred to
132 onboard aerated live wells for positive identification and recovery. A total of 50 Brown
133 Bullheads with a wet mass ranging from 163 - 495 g, were collected from Trenton Channel
134 followed by 53 Brown Bullheads from Peche Island with a wet mass ranging from 113 – 495 g.
135 Fish from each sampling location were rapidly transferred to a nearby fish farm in Essex, Ontario
136 where they were released into separate earthen ponds (mesocosms) following a 30-minute
137 acclimation period. These semi natural mesocosms each measured approximately 140 m^3 (L 12.5
138 m x W 7.5 m x D 1.5 m) and were supplied with continuous 24h aeration year round. The
139 bullheads were held in the mesocosms for a period of one year and allowed to spawn naturally
140 the following spring.

141 Between July and October 2010 both river locations were re-sampled in order to collect
142 bullheads for the acute SMR study. Post reproductive Bullheads were collected as described
143 above and then quickly transferred to indoor holding facilities. A total of 21 bullheads with a wet
144 mass ranging from 119-495 g were collected from Trenton Channel and 23 bullheads with a wet
145 mass ranging from 113-399 g were collected from Peche Island. Fish were held in tanks as
146 detailed below and SMR measurements were conducted on each individual in the acute trial
147 following a brief tank acclimation of 24-48h to ensure clearance of the gastrointestinal (GI) tract.

148 *Respirometry and SMR Determination*

All SMR measurements were performed using a single chamber intermittent flow respirometer (Loligo Systems; DAQ-PAC-G1S) controlled through AutoResp™ 1. The typical configuration for the respirometer requires the fish be placed in a closed respirometer chamber which is then immersed in an ambient tank where two small recirculation pumps move water through the system during the computer controlled flush and measurement periods. This configuration was modified with the addition of a 950 L freshwater tank in combination with the ambient tank. The extra tank provided a location to connect inline heating and chilling units as well as a place for air stone placement (i.e. aeration) away from the ambient tank. A submersible water pump was used to provide a continuous flow of fresh water to the ambient tank where the respirometer chamber was located. This set up ensured a more stable system in terms of maintaining saturated dissolved oxygen concentrations, temperature and overall water quality while also minimizing vibration and noise.

To obtain reliable estimates of oxygen consumption rate, three sizes of respirometry chambers were used in the study. A large sized chamber (volume = 2924 mL) was used for bullheads in the size range of 200+ mm, a medium sized chamber (volume = 1133 mL) was used for bullheads in the size range of 150 – 200 mm and a small chamber (volume = 196 mL) was used for bullheads that were less than 100 mm (mainly trial 3; F1 offspring fish). Prior to placement in the respirometry chamber, fish were sedated by immersion in an aerated 20 L pail with MS-222 (50 mg/L) buffered with sodium bicarbonate. Once the fish was unresponsive, it was removed and measured for total length (mm), mass (g) and volume (L; via water displacement). The data on fish mass and volume was entered into the respirometry software, the respirometer was set to a constant flush cycle and the fish was placed in the chamber and allowed to recover. When the fish demonstrated signs of recovery, all air bubbles were removed

from the chamber and the respirometry computer program was initialized. Flush and measurement cycles (typically 400 and 375 seconds respectively) were adjusted to ensure that oxygen levels remained near saturation in the chamber during the measurement cycle when the chamber was sealed. SMR measurements were taken for each fish over a 12-24 h period. All measurements were taken under low light conditions by covering the ambient tank. Since the respirometer used was a single chamber design, only one fish SMR was recorded at a time. In order to correct for oxygen consumption that occurs from the natural build-up of microbial biomass within the respirometer system (Grottum and Sigholt 1998; Clark et al. 2013) blank measurements (i.e. trial runs with no fish) were conducted at the end of each study run. The resulting mean O_2 consumption value of blanks was subtracted from the fish O_2 consumption value of each study trial.

Across fish, heightened oxygen consumption rates were commonly observed during the first 4 h after initiating the AutoResp™ 1 computer program. This was interpreted as stress associated with handling, sedation recovery and initial response of fish to confinement. As a general rule, all readings from the first 4h of measurement were censored from consideration in the evaluation of SMR. Fish O_2 consumption profiles typically show variable periods in the rate of O_2 consumption as opposed to a steady consumption rate (Steffensen 1989). For the calculation of SMR, only measurements that occurred within the 25^{th} and 75^{th} percentile during the post 4h measurement period were considered. This was done to exclude spontaneous periods of high or low O_2 consumption. The high values were attributed to acute periods of routine metabolism (i.e. spontaneous activity) (Steffenson 1989). Abnormally low values were attributed to either a brief change in the metabolic rate of the fish (e.g. hypometabolism, hypoventilation) or irregular sensor readings resulting in abnormally low and invalid O_2 consumption

measurements (Clarke et al. 2013). Selected respirograms, reflecting raw O_2 consumption measurements with time for an individual fish are presented in Figure 1. Only measurements post 4 h of experiment initiation (left of vertical line) and within the 25-75 percentile distribution (horizontal dashed lines) were used to generate a mean SMR value for each fish used in the studies.

Study 1 (Acute SMR)

Following field collections, fish designated for the acute SMR study were transferred to a 7000 L indoor tank equipped with a recirculating bio-filtration unit that included supplemental aeration and three 120 Watt UV sterilizers. The holding tank water temperature was equivalent to the river temperature. Acute fish were brought from the field in batches, placed in coolers containing water from their site of capture. The coolers were then suspended in the holding tanks, and tank water was slowly added over of period 2 h. When the water was fully renewed in the cooler, it was fully immersed allowing the fish to swim freely out of the cooler. The exact same procedure was used in the case of field and pond held fish which were being collected over the same time frame.

Submerged open-ended 600 mm by 76 mm potable PVC pipes were added to the tanks as refuges for fish and the ambient tank was covered with an opaque lid to reduce overhead light and eliminate external room shadows. Following acclimation, the SMR of fish of both clean and contaminated origin were measured at 23˚C (12-24 hr trial runs) using the respirometer as described above. The acute SMR trials were started immediately following the initial 48 hr fast and conducted on individual fish from July 27 through November 1 2010. Depending upon collection success on a particular sampling day, an unavoidable time lag occurred between the

time of collection for some bullheads and the start of their SMR measurements (i.e. some fish remained in the holding tank longer than others). Most fish were assessed within 5 days of collection from the field to a maximum of 10 days. The SMR of each fish was measured only once.

Study 2 (Cleared SMR)

Fish collected for the long-term holding investigation were placed in two separate outdoor ~140 m3 earthen ponds. The fish were kept in the uncontaminated mesocosms for a period of one year (July 2009 to July 2010) under natural photoperiod and temperature conditions. The ponds were supplied with continuous aeration year round and fish were provisioned with a maintenance ration of fish pellets recommended for cool water fish (Martin Mills Inc.). Bullheads were not fed pellet rations when pond temperature fell below 8˚C.

Basic water quality conditions were periodically assessed within each of the ponds to ensure dissolved O_2, pH, and temperature were within acceptable ranges for the health of the fish and to ensure that water quality between the ponds remained within similar ranges. Basic water quality parameters were measured *in situ* using a Hydrolab Surveyor 3/ Reporter multiparameter water quality logging system.

The removal of these fish from their original sources of contaminant exposure (i.e. sample locations) and subsequent yearlong holding into an uncontaminated system allowed accumulated contaminants in the fish to be depurated. Consequently Study 2 bullheads are hereafter referred to as "cleared" in reference to the long-term contaminant depuration of these fish in the holding ponds (for a description of reductions in persistent organic pollutant burdens measured in pond held fish see Farwell et al. 2012 and data in Table 1).

Following the holding period, the bullheads were removed from both ponds and transferred to a 7000 L indoor tank (identical to those used in Study 1) according to the same procedure used in acute trials. Water temperatures were maintained at 23ºC during measurement and all fish were acclimated to the new tank for 72 hours before SMR measurements commenced. Similar to Study 1, all fish were fasted for 48 h prior to placement in the chamber and individual measurements were taken over a 12-24 h period. Fish from different treatments were measured in an alternating fashion across SMR trials periodically interrupted for completing acute SMR (Study 1) trials as fish were collected. SMR measurements for all Study 2 fish commenced July 27 2010 and were completed November 1 2010.

Study 3 (F1 SMR)

The study 2 fish residing in the two treatment ponds spawned naturally during the spring of 2010. Schools of young bullhead, hereafter designated as F1 fish, were observed in both ponds during late June 2010. The F1 fish were allowed to grow out in each of the parental ponds and were not sampled until they reached a mean mass of ~20 g. During the grow out period, the F1 fish were provided Silver Cup® starter feed in addition to the ration provided to adult fish, as well as naturally occurring forage . In mid-August, 17 F1 fish from the Peche Island pond and 8 fish from the Trenton Channel Pond were collected and transferred to the 7000 L indoor tanks (water temperature of 23ºC) used for holding fish in Study 1 and 2 during SMR measurements. The low numbers of F1 fish retrieved from the ponds were not a result of low survivorship but a result of other projects making use of these F1 fish for other research trials. The SMR measurements of individual fish followed the same protocol as described for the previous Study 1 and 2 with the exception of utilizing a smaller respirometry chamber (volume = 196 mL) to

increase the sensitivity of the respirometry measurements. A total of 25 measurements were taken for F1 fish from the two treatment groups between August 17 and September 30 2011.

Data Analysis

Prior to analysis, assumptions of data normality and heteroscedacitity were tested using Shapiro-Wilk normality test and Levene's Test for homogeneity of variance. Non-normal data were log transformed and re-tested to ensure assumptions of analysis of variance were met. A general linear model (GLM) was used to test treatment differences as well as all combinations of treatment interactions in a 2 x 3 design whereby:

Log SMR = log BW + Population + Treatment + Log BW * Population + Log BW * Treatment + Treatment * Population + Log BW * Treatment * Population + Constant

In the above model, BW is fish body mass (g) measured for each fish tested, population is a categorical variable corresponding to fish origin (or parental origin) and treatment is categorical variable corresponding to the three experimental trials: Acute, Cleared or F1 trials. Following initial model evaluation it was observed that body mass had a highly significant effect on SMR ($F_{1,85} = 27.67$; $p<0.001$) as expected from known allometric relationships between body size and fish metabolic rate. However, all interaction terms involving body mass were found to be non-significant (Population * BW, $F_{1,85} = 1.375$, $p >0.2$; Treatment * BW, $F_{2,85} = 1.072$, $p>0.3$ and Population * Treatment * BW, $F_{2,85} = 0.792$, $p>0.4$). This indicated that each population and treatment exhibited similar allometry of SMR with respect to body size. Subsequently, analysis of covariance was performed using log BW as a co-variate within the 2 x 3 factorial design by removing the interaction terms involving BW according to:

Log SMR = log BW + Population + Treatment + Treatment * Population + Constant

In this case, the R^2 of the second model showed only a small decrease in explanatory power (R^2 of model 1 was 0.89 while the R^2 of model 2 was 0.87) and both model I and II exhibited similar Akaiki Information Criteria (AIC) values at -221.3 and -221.9, respectively. Given the large size differences between treatments, especially since F1 fish which were much smaller than acute or cleared fish, Model II was considered the more appropriate method for testing main effects. Following interpretation of the main effects, a posteriori tests (Tukeys HSD) were used to examine differences between SMR for each combination of population and treatment. All statistical tests were completed using SYSTAT 13 statistical software. For data summary purposes, SMRs were size corrected to a standard 200 g fish based on the slope generated for log BW (-0.336) from model 2 such that:

$$SMR_{SS} = \frac{\log(-0.336 \cdot BW)}{\log(-0.336 \cdot 200)} \cdot SMR$$

Throughout the text means and standard errors (SE) are reported for variables that exhibited normal distribution, while geometric means and 95% confidence intervals are reported for variables that exhibited log normal distributions

RESULTS

Bullhead mortalities soon after the transfer to the outdoor mesocosms were observed in both ponds (TR = 2 fish and PI = 1 fish) and attributed to collection and transfer stress. No bullhead deaths were recorded in the facility's large holding tanks during the pre SMR holding period, but two Trenton Channel bullheads (TR) deaths occurred during the SMR trials in the respirometer chamber due to a flush pump failure. Data from these trials were excluded.

Bullheads in each pond began accepting pellet food within 7 days following the transfer from their respective river locations. Pond water quality remained within acceptable guidelines (CCME 1999) throughout the holding period (DO > 6 mg $\cdot L^{-1}$, pH 7.2 to 7.6). Pond temperatures increased and decreased naturally following seasonal progression. In order to avoid excessive pond warming during peak summer months, aerators were placed in the shallow areas of the ponds to avoid complete mixing of the entire pond thereby allowing bottom waters to remain cooler. Winter aeration allowed for the exchange of gases preventing potential winterkill (Lynch and Norland 2001). No winter mortalities were noted for either pond.

Data on body size, sample numbers and raw standard metabolic rate (uncorrected for fish size) are presented in Table 1. The GLM Model II fit to the data explained 87% of the variation across treatments and populations. There were highly significant differences between SMRs for the two populations ($F_{1,90} = 12.7$; $p<0.001$); highly significant differences between the three treatments ($F_{2,90} = 145.4$; $p<0.001$) and significant differences among SMRs for the treatment x population interaction ($F_{2,90} = 6.5$; $p<0.01$). In order to demonstrate GLM Model II fits, uncorrected SMR data are expressed against fish body mass for each experimental group in Figure 2 along with predictions generated from the GLM Model II. GLM model predictions were strongest for Peche Island Acute and Cleared trials, while data for F1 showed poorer allometric response, mainly due to the limited size range of fish tested in that trial. Model predictions also tended to underestimate SMR for Trenton Channel acute and cleared fish relative to actual measurements (Figure 2).

Figure 3 presents a comparison of size corrected SMR data for PI and TC fish from each of the three experimental studies. For Study 1 (Acute SMR_{SS}), the mean and 95% confidence interval SMRss of PI fish was 80.7 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$ (95% CI: 73.5 to 88.5 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$) and

was significantly ($p<0.001$; Tukey's HSD) lower than TC fish (100.7 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$; 95% CI: 95.0 to 106.8 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$). This corresponded to a between population difference in SMR_{ss} of 19.9%. In Study 2 (Cleared SMR_{ss}), the mean and 95% CI SMR_{ss} of PI fish (38.5 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$; 95%CI: 36.0 to 41.1 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$) was significantly lower (Tukey's HSD; $p<0.001$) than TC fish (50.1 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$; 95% CI: 43.1 to 58.3 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$). In this case, the between population difference in SMR was 23.2%. Notably, the SMR of cleared fish from each population showed a decrease in SMR values compared to acute fish from each population. PI fish showed a 52.3% decrease in SMR of cleared fish relative to acute fish. Similarly, TC fish exhibited a 50.2% drop in SMR of cleared compared to acute fish. For Study 3 (PI and TC F1 SMR_{SS},), the mean and 95% CI SMR_{SS} for TC F1 offspring (69.6 mg O_2oxygen$\cdot kg^{-1} \cdot hr^{-1}$; 95%CI: 60.8 to 79.7 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$) was not significantly different ($p>0.9$; Tukey's HSD) from PI F1 fish (73.5 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$; 95%CI: 67.5 to 80.0 mg $O_2 \cdot kg^{-1} \cdot hr^{-1}$). However, F1 fish did show generally higher size corrected SMRss relative to cleared fish. The PI acclimated fish had significantly ($p<0.01$; Tukey's HSD) lower SMR_{SS} compared to PI F1 fish, but were not significantly different ($p>0.05$; Tukey's HSD) from TC F1 fish. TC cleared fish were not significantly ($p>0.9$; Tukey's HSD) different from TC F1 fish or PI F1 fish ($p>0.6$; Tukey's HSD). In contrast, acute fish from each treatment group were significantly higher than fish across all other groups from both cleared and acute trials. These observations provide a general indication that cleared and F1 fish approached one another with respect to overall SMR_{SS} compared to acute fish which consistently showed the highest size corrected SMR_{SS} measurements.

DISCUSSION

The results of Study 1 (Acute SMR) are consistent with the initial hypothesis that Brown Bullheads collected from contaminated regions within the Detroit River exhibit increased SMR_{SS} compared to fish collected from less contaminated areas. Of interest was that the significant difference in SMR_{SS} between the two populations persisted following a one year period where fish from the two populations were placed in a common clean low/stress environment (Study 2 cleared SMR). Whether or not the between population differences in SMR_{SS} represent permanent changes to SMR of examined fish or if between population differences in SMR are capable of being lost after longer holding periods is not known.

The two acute collection locations were chosen to represent gradients in river contamination for pollutants such as PAHs known to contribute to altered metabolic rates of fish (Heath 1987; Handy and DePledge 1999; Merten 2005). Sediment contamination for organic and metal contaminants at TC has been known to be enriched relative to upstream areas of the Detroit River since at least the 1970's and the sediment chemistry related to PCBs, PAHs, OC-pesticides, mercury and metals was shown to have been stable for the past 10 years (Drouillard et al. 2006; Szalinska et al. 2013). These differences in sediment chemistry translate into different chemical exposures for biota within the system. PCBs and PAHs in caged mussels sampled from the TC and PI (Gewurtz et al. 2002; Drouillard et al. 2013) showed notably higher contamination at TC compared to the up river reference location in the vicinity of PI. Past studies further reported that bullheads from PI and TC exhibited significant differences in contaminant exposures to PCBs, PAHs and organochlorine compounds (e.g. DDD, DDE, DDT, heptachlor; chlorinated benzenes) (Leadley et al. 1998, 1999). Notably, Farwell et al (2012) measured PCB residues in eggs generated by a subset of acute and cleared TC and PI fish used concurrently with the present study. The data from the above study is presented in Table 1. Results show that

373 eggs from acute TC fish contained 7.6 fold higher total PCB concentrations compared to PI fish
374 ova. Following one year of clearing in the mesocosms, both fish populations decreased their
375 PCB concentrations by approximately 52 and 49% for TC and PI fish, respectively. However,
376 cleared TC fish in that study still contained higher concentrations than PI fish post clearing.
377 Unfortunately, we did not measure PCBs in F1 fish from this study. However, F1 fish of similar
378 size from an alternate population (derived from Bay of Quinte, Lake Ontario) reared in the same
379 ponds under the same conditions were analyzed. The alternate population F1 fish had sum PCB
380 concentrations that were 50% and 30% lower than acute and cleared fish, respectively (Table 1).
381 Thus, the somewhat higher SMR_{SS} of F1 fish relative to cleared fish is not likely a result of PCBs
382 levels in F1's.

383 In general, the between population differences in SMR_{SS} observed for acute and cleared
384 fish from the present research is consistent with the magnitude of SMR alterations shown to be
385 induced under laboratory conditions after exposing fish to various contaminants. For example,
386 Merten et al. (2005) exposed Mummichog to a gradient of PAH contaminated food and observed
387 a significant increase in Mummichog SMRs following exposure (120+ days) to a diet
388 contaminated with PAHs (2800 ng/g w/w). Conversely, the Mummichog SMRs were depressed
389 at 10% concentrations (840 ng/g w/w) compared to control fish. Beyer et al. (1999) observed a
390 similar result in Largemouth Bass (*Micropterus salmoides*) where routine metabolic rates
391 initially decreased following an acute (1-4 day) exposure to the pesticide dieldrin but increased
392 significantly over time (16 days) with increasing exposure concentrations. Similar responses
393 have also been shown to occur in fish following exposure to metals. For example, the SMR of
394 fathead minnows and golden shiners both decreased following an acute exposure (24h) to
395 cadmium and copper, where longer exposures (>96h) resulted in elevated SMRs in both species

(Pistole et al. 2008; Peles et al. 2012). In the above case, the SMR of golden shiners exposed to the two treatments concentrations of 200 and 500 $\mu g \cdot L^{-1}$ Cd for 96 h increased by 65% compared to the control fish (Peles et al. 2012). These studies and several others support a metabolic cost for fish chronically exposed to multiple chemical stressors in the environment (Hopkins et al. 1999, Calow and Sibly 1990; Calow 1991; Rowe, 2003).

However, not all potentially toxic chemicals encountered by fish in the natural environment result in altered SMRs. For example, despite laboratory studies demonstrating a 16.7 percent increase in the SMR of Mosquitofish exposed to 100 µg/L of mercury for 48hrs (Tatara et al. 2001), no metabolic rate differences were observed in Mosquitofish with elevated body burdens of mercury and sampled from pre Hg dosed mesocosms compared to an uncontaminated reference population (Hopkins et al. 2003). In another study, Lake Chubsuckers (*Erimyzon sucetta*) exposed to coal ash–polluted sediments for 4 months had significantly elevated body burdens of Se, Sr, and V but no detectable differences in SMR, although increased mortality and significantly reduced growth rates demonstrated a bioenergetics cost experienced by exposed populations (Hopkins et al. 2000).

Following the clearance period in outdoor mesocosms, both TC and PI fish showed decreases in their SMR_{SS} (52% and 49 % respectively) relative to acute fish from the same respective population. Notably, these within population differences in SMR_{SS} pre- and post-clearance were more than 2 fold greater than the between population differences observed in either study. Differences in SMR_{SS} between acute and cleared fish from the same populations were observed even though both groups exhibited similar size ranges and were measured at the same time owing to the staggered collection of fish used in acclimation trials and fish used for acute trials. This implies that a much broader set of extrinsic factors were influencing the SMR

of fish, beyond those of tissue burdens of POPs compounds such as measured for PCBs (Farewell et al. 2012). Conditions other than exposure to environmental contaminants such as changes in diet and diet abundance, the presence and complexity of in-stream structural habitat, water quality (e.g. dissolved O_2 concentrations), presence of conspecifics and social effects such as aggression and dominance have all been correlated with SMR in fish (Cech 1985; Lahti *et al*. 2002; Millidine et al. 2009; Biro and Stamps 2010; Burton et al. 2011).

Certainly a quiescent pond environment with ample food and change in diet that includes conditions of low predation risk, limited shelter and only the presence of conspecifics in a limited space represents a significant environmental change compared to the riverine system where the cleared fish were collected. Based on the wide range of extrinsic factors that are known to affect SMRs in fish, it is plausible that a non-adaptive response to the change in the environment may be in large part responsible for the decline in SMR that was observed in both cleared TC and PI populations. For example, a case for altered SMR based on the habitat complexity provided through artificial cover was demonstrated by Millidine et al. (2006), where the addition of shelter led to a 31% reduction in the mean SMR of Atlantic salmon parr (*Salmo salar*) compared to parr that were measured without shelter. In another more recent study, phenotypic plasticity in SMR has been shown to occur in Guppies (*Poecilia reticulate*) in response to a change in environment, particularly in the presence and absence of predator cues (Handelsman et al. 2013).

Support for this non-adaptive response argument from the present research was that the population differences in SMR_{SS} were found to be lost in Study 3 which involved rearing F1 offspring from TC and PI populations in a common environment. To our knowledge this is the first study to investigate the change in standard metabolic rates of fish populations following

442 acclimation to a common environment and in F1 offspring generated from the two populations.
443 This enables rejection of the hypothesis that the population differences measured in SMR_{SS}
444 generated in study 1 and 2 reflect a local adaptation of fish from the two collection regions.
445 Adaptation to chemical exposure has been demonstrated to occur across other traits in Brown
446 Bullhead (Williams 2014) and other fish species (Rowe 2003; Hopkins et al. 2003). For example,
447 fish collected from waters near cotton fields in Mississippi with a long exposure history of
448 treatment with chlorinated hydrocarbon pesticides exhibited a marked resistance to DDT
449 compared with fish sampled from areas with no past exposure to these chemicals (Bradleigh et
450 al. 1963). Study three also rules out maternal factors (either maternal offloading of contaminants
451 or other maternal factors) as a potential modifier of SMR in the study system.

452 The results of this investigation demonstrate that fish from contaminated environments in
453 the Detroit River maintain elevated SMRs in comparison to fish inhabiting less contaminated
454 sites within the same riverine system. These metabolic effects persist in fish after removing them
455 from the contaminated environment over periods as long as one year. The results support a
456 general acclimation syndrome response (Selye 1956; modified by Beyer et al. 1999), whereby
457 the energy expenditure in an organism changes over time in order to compensate for the effects
458 of an encountered stressor. Following this conceptual progression, altered SMRs and/or other
459 metabolic costs expenditures (e.g. specific dynamic action) occur during the resistance stage in
460 the syndrome where physiological compensation for the effects of the stressor(s) becomes part of
461 the daily bioenergetic cost of living for the exposed animal (Beyer et al. 1999). However, it is
462 clear from this study that SMR as a physiological parameter is highly sensitive to a wide array of
463 extrinsic factors. Notably the change of environment during clearing trials would appear to have
464 had a large effect on SMR measurements, and thus the exact nature of the stressor(s) and stressor

interactions responsible for population differences in bullhead SMRs cannot be directly identified.

Given that the location differences in SMR are not inherited attributes, and assuming the observed within and between population effects are potentially additive, the scope for variation in SMR among Detroit River bullheads ranges from 19.9 to 23.2% related to pollutant exposures and 50.3 to 52.3% differences attributed to environment. Sherwood et al. (2000) demonstrated yellow perch from metal polluted lakes had 3 fold lower annual growth increments owing to elevated energetic costs of polluted fish. However, in this case, the increased metabolic costs also included additional foraging costs due to pollutant induced reductions in prey. Notably, differences in SMR resulting from a combination of environment and pollution effects were observed to approach the magnitude of activity multipliers commonly used to convert SMR to a routine metabolic rate (RMR) estimate in bioenergetic models (Hanson et al. 1997; Brigs and Post 1997). Commonly, any differences in routine metabolic rate of fish as measured by in-situ methods are interpreted to largely represent differences in fish activity (see Sherwood et al. 2000). However, these observations support a growing body evidence that substantive intraspecific variation in SMRs exist within and among fish populations of the same species and more importantly that such differences can be sustained within a connected aquatic system (Burton et al. 2011). The results have implications for bioenergetics modelling applications where all individuals of a species are treated similarly with respect to SMR. Additional research aimed at generating a scope for environment or pollutant-induced SMR shifts in other species, similar to the scope of activity concept, would be useful to further expand the accuracy of fish bioenergetics models.

Acknowledgement—We thank Mike Burtnyk, Michelle Farwell and Linda Söderberg for help with field collections and Mike Thibert for laboratory assistance. The present study was funded by a National Sciences and Engineering Research Council of Canada Strategic Project Grant and the University of Windsor. All research was conducted in accordance with the principles and guidelines of the Canadian Council on Animal Care.

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Table 1. Body size, standard metabolic rates (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$) and sum PCB concentrations (µg/g lipid) in brown bullheads from different experimental trials

Trial	Population	Body Mass ± SE (g)	N	SMR (95%)[1] (mg $O_2 \cdot kg^{-1} \cdot h^{-1}$)	Sum PCBs ± SE (µ/g Lipid)
Acute	Peche Island	262.9 ± 18.3	23	80.7 (69.5 - 87.1)	0.94 ± 0.04[2]
	Trenton Channel	256.1 ± 22.8	21	97.9 (91.0 - 105.3)	7.14 ± 0.50[2]
Cleared	Peche Island	320.7 ± 24.2	15	35.6 (33.3 - 38.1)	0.61± 0.09[2]
	Trenton Channel	273.5 ± 21.6	11	47.6 (40.8 - 55.5)	2.65± 0.162
F1	Peche Island	46.8 ± 8.8	18	106.6 (93.9 - 121.2)	NA
	Trenton Channel	35.2 ± 5.8	8	107.2 (91.7 - 125.4)	NA
	F1 from fish ponds		48		0.75± 0.07[3]

721 Figure Captions

722 Figure 1. Raw standard metabolic rate (SMR) readings for 3 selected Brown Bullhead
723 treatments (acute, cleared and F1 groups) over time. Vertical line designates first 4 h period
724 which was censored from SMR calculations. Horizontal solid lines represents 4-24 h mean of
725 non-censored values, dashed horiztonal lines present 25% and 75% quartiles used to cenosor
726 outlier readings.

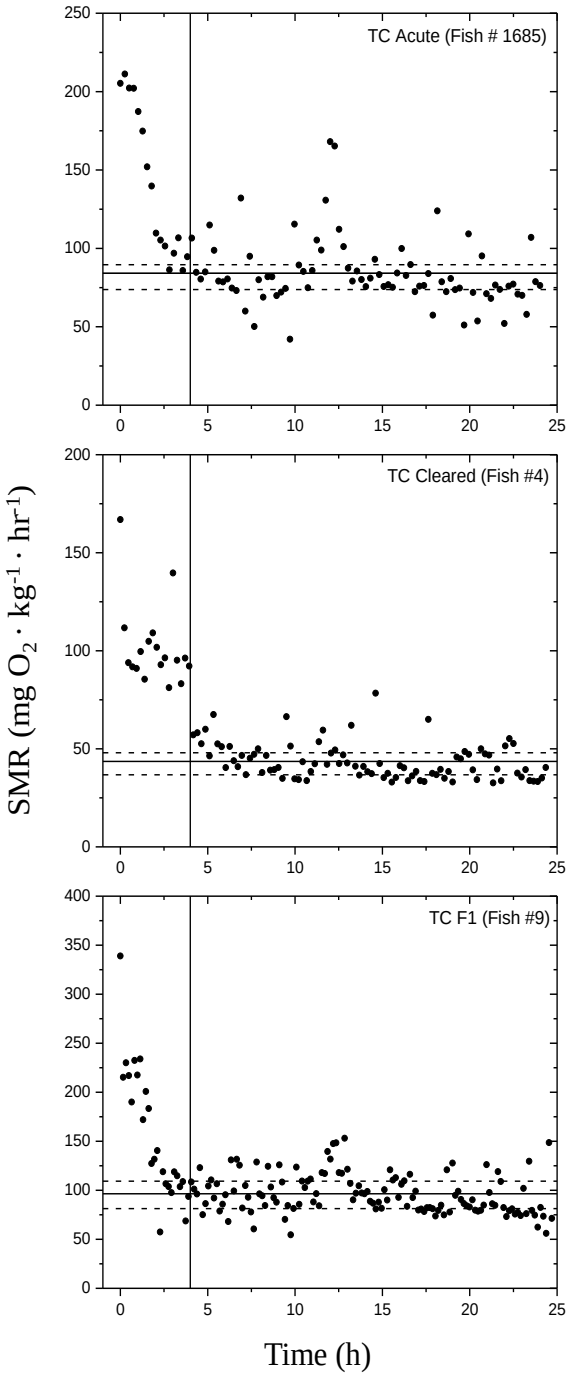
727

728 Figure 2. Standard metabolic rates measured in individual brown bullheads as a function of
729 body mass in each experimental trial. Top, middle and lower graphs represent acute, acclimated
730 and F1 experiments. Square symbols (■) present data for Peche Island fish, open circles (○)
731 refer to data for Trenton Channel fish. Solid line is the GLM predicted RMR for Peche Island
732 fish in a given experimental trial. Dashed line is the GLM predicted RMR for Trenton Channel
733 fish in a given trial.

734

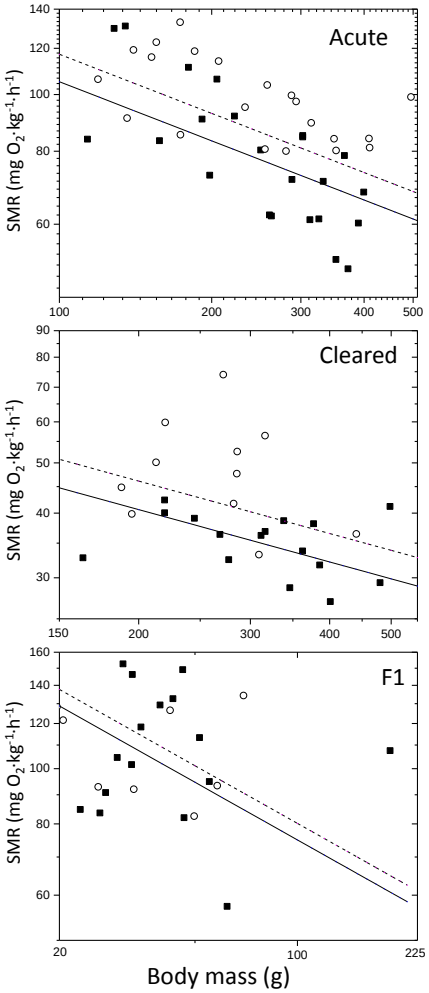
735 Figure 3. Geometric mean size standardized resting metabolic rates of brown bullheads for
736 different populations and treatments. Error bars denote 95% confidence intervals around
737 geometric mean. Hollow bars are Peche Island Fish, thatched bars are Trenton Channel fish.
738 Columns that have different letters are significantly different from one another ($p<0.05$; Tukey's
739 HSD).

740



741

742 Figure 1



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744

745 Figure 2

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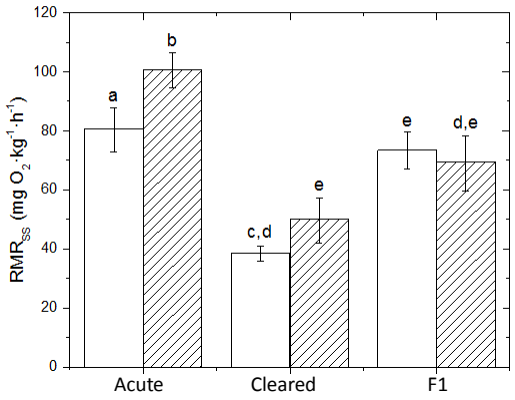

747

748 Figure 3

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