DETERMINATION OF STANDARD AND FIELD METABOLIC RATES IN TWO GREAT LAKES INVADING FISH SPECIES: ROUND GOBY (NEOGOBIUS MELANOSTOMUS) AND TUBENOSE GOBY (PROTERORHINUS SEMILUNARIS)

Jessica Anna O'Neil

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DETERMINATION OF STANDARD AND FIELD METABOLIC RATES IN TWO GREAT LAKES INVADING FISH SPECIES: ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) AND TUBENOSE GOBY (*PROTERORHINUS SEMILUNARIS*)

by

Jessica O'Neil

A Thesis
Submitted to the Faculty of Graduate Studies through the Great Lakes Institute for Environmental Research in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2013

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DETERMINATION OF STANDARD AND FIELD METABOLIC RATES IN TWO GREAT LAKES INVADING FISH SPECIES: ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) AND TUBENOSE GOBY (*PROTERORHINUS SEMILUNARIS*)

by

Jessica O'Neil

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Ontario Ministry of Natural Resources

31 July 2013
DECLARATION OF CO-AUTHORSHIP AND PREVIOUS PUBLICATION

CO-AUTHORSHIP STATEMENT

I hereby declare that this thesis incorporates material that is a result of joint research under the supervision of Dr. Ken G. Drouillard (University of Windsor) and Dr. Timothy B. Johnson (Ontario Ministry of Natural Resources). Chapter 3 contains material from an article entitled: "Validation of rapid assimilation of PCBs following IP dosing in the round goby (Neogobius melanostomus)", published in Bulletin of Environmental Contamination and Toxicology (BECT). This article was co-authored by O'Neil J., Johnson, T.B. and Drouillard, K.G.

In all chapters, the primary contributions, main ideas, field work, experimental designs and data analysis were performed by the author. The contribution of co-authors was primarily through the revision of manuscript drafts and assistance with data interpretation.

This thesis includes one original paper that has been previously published in a peer reviewed journal, as follows:


I certify that the above material describes work completed during my registration as graduate student at the University of Windsor.

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ABSTRACT

Although both round (*Neogobius melanostomus*) and tubenose (*Proterorhinus semilunaris*) gobies were introduced to the Great Lakes around 1990, their differential range expansion post-invasion remains poorly understood. Metabolic rates of round and tubenose gobies were evaluated to determine the influence of physiological tolerance on invasive species success. Standard metabolic rates (SMR) of round and tubenose gobies were measured across temperatures using intermittent flow respirometry. Tubenose gobies showed higher SMR, reaching metabolic optima at lower temperatures (23°C) and exhibiting elevated stress responses at high temperatures. Field metabolic rates (FMR) of round gobies from three Great Lakes populations were also compared using chemical tracers. Although genetic differences between round goby populations have been documented, similar FMRs were seen among populations. These results suggest that SMR is a useful approach to compare differences in temperature dependent physiological tolerance of invasive species, and that FMR can be used to evaluate metabolic costs among wild populations.
DEDICATION

To my mother, father, sister and Nonna
ACKNOWLEDGEMENTS

I would like to thank my advisors, Dr. Ken Drouillard and Dr. Tim Johnson for giving me the opportunity to expand my knowledge and feeding my love to learn. I would like to thank Todd Leadley for his invaluable help with experimental set-up and maintenance as well as Anne McLeod for the valuable discussions I shared throughout the process of my learning experience. I would also like to thank the other members of my committee, Dr. Dennis Higgs and Dr. Aaron Fisk for their support and encouragement as I faced challenges with my research and Dr. Nargis Ismail for her patience and willingness to help me in the laboratory throughout the course of my research. Finally I give special thanks to my mom, dad, sister and Nonna for their unending support, love and encouragement throughout the entire duration of my education.

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CHAPTER 1

GENERAL INTRODUCTION

Introduction

The term alien invasive species (AIS) refers to a successfully reproducing, harmful species that exists in an environment where it previously did not (Mills et al. 1993). In contrast, an alien species refers to a species that exists in an environment where it previously did not, but does not appear to cause harm (Mooney and Hobbs, 2000). Biological invasions can negatively affect both terrestrial and aquatic ecosystems by disrupting food webs (Ng et al. 2008), altering nutrient cycling (Bunnell et al. 2005) and pollutant transfer (Morrison et al. 2000), and reducing the biodiversity of native populations (French and Jude, 2001; Vanderploeg et al. 2002). Human-mediated species transport has resulted in the establishment of over 180 alien species in the Great Lakes ecosystem alone (Holeck et al. 2004; Kornis and Vander Zanden, 2010). Ricciardi and MacIsaac (2000) described a pattern of invasion unique to the Great Lakes ecosystem: approximately 70% of the introduced species discovered since 1985 originated from the Black, Caspian and Azov Seas (Ponto-Caspian region). This pattern of invasion derives not only from the climatically matched habitats found in the Great Lakes and the Ponto-Caspian regions, but also from the tolerance of Ponto-Caspian biota to habitats of variable water levels and salinities (Ricciardi and MacIsaac, 2000; Kocovsky et al. 2011).

In 1990, two Ponto-Caspian invasive fish species, the round goby *(Neogobius melanostomus)* and the tubenose goby *(Proterorhinus semilunaris)*,
were found in the St. Clair River of the Great Lakes basin (Jude et al. 1992). The importance of the St. Clair River to AIS establishment stems from its role as a navigation channel, permitting access of maritime and overseas vessels to the upper Great Lakes (Jude et al. 1992). Although these two invaders entered the Great Lakes around the same time via ballast water discharge from foreign vessels, the range expansion of each species post-invasion has been significantly different.

The rapid range expansion of the round goby throughout the Great Lakes has defined this species as a more successful AIS compared to the tubenose goby (Vanderploeg et al. 2002; Kocovsky et al. 2011). Within the first 5 years of its discovery in the St. Clair River (Jude et al. 1992), the round goby had spread throughout all of the Great Lakes (Marsden et al. 1996). In contrast, the expansion of the tubenose goby has been less extensive, and introduced individuals have shown a tendency to reside in a small area close to where they were believed to have originally been introduced. Until 2001, tubenose goby range expansion was thought to be geographically confined to Lake St. Clair and the western basin of Lake Erie; only within the last decade have tubenose gobies been reported in western Lake Superior and Lake Ontario (Vanderploeg et al. 2002; Fuller et al. 2013). Based on the differential spread and impacts round and tubenose gobies have had on the ecosystem, most invasion biologists would classify the round goby as an alien invasive species and the tubenose goby as an alien species (H.J. MacIsaac, personal communication). Stepien et al. (2005) examined mitochondrial cytochrome b genes of round and tubenose gobies from
the Great Lakes basin and reported 83% sequence homology between tubenose goby and the most common fresh water round goby cytochrome B haplotype. Moreover, an evaluation of mitochondrial DNA control regions sequenced by Dillon and Stepien (2001) revealed a higher level of genetic variability in round gobies compared to tubenose gobies. More recent research has found that the genetic diversity of round gobies differs across their invaded range (Brown and Stepien, 2009). The most genetically distinct round goby populations occur in Lake Huron and Lake Ontario, with Lake Huron's round goby populations showing the lowest amount of genetic diversity (Brown and Stepien, 2009). Although previous studies suggest that the invasion success of round and tubenose gobies may be positively correlated to population levels of genetic variability, the basis for the differential range expansion of these two closely related Great Lakes invaders remains poorly understood (Dougherty et al. 1996; Dillon and Stepien 2001).

Genetic variation, feeding ecology differences and differences in physiological performance represent potential explanations for the differences in invasive range and success of these closely related invading species. In 1989, Ehrlich proposed that successful invasive species possess a suite of characteristics including: (1) high genetic variability, (2) an association with humans, (3) abundance in their native range, (4) wide feeding niche, (5) short generation time, (6) larger size than most related species, (7) fertilized females are able to colonize alone and (8) the ability to function under a wide variety of physical conditions (physiological tolerance). The list of characteristics favourable
for invasive species was expanded by Lodge (1993) to include the following habitat traits: early succession, climatically matched to invaded environment, low native species diversity, absence of predators and frequent disturbance. A common hypothesis is that successful invasive species display exceptional physiological tolerance to environmental stressors of their new habitat (Braby and Somero, 2006). Undoubtedly, all aquatic invasive species possess at least one of the traits outlined by Ehrlich (1989) and Lodge (1993), however few case studies lend support to the claim that physiological tolerance is a pre-requisite for the successful invasion of an aquatic habitat (McMahon, 2002).

Many studies have found that temperature is a limiting factor influencing habitats where ectothermic species may reside. Braby and Somero (2006) evaluated the influence of temperature on the distribution patterns of three species of blue mussels (Mytilus species) in the North Pacific. Their research found that the native species (Mytilus trossulus), was more tolerant to low temperatures than the other two hybrid blue mussel species Mytilus edulis and Mytilus galloprovincialis. However, the invasive species, M. galloprovincialis was more tolerant to high water temperatures than both the native species and remaining hybrid. Overall, Braby and Somero (2006) found that the temperature tolerance of the invasive blue mussel (M. galloprovincialis) influenced its range distribution and could be used to predict the future invasion success of this species in the North Pacific and other areas. In another study on hull fouling communities in Bodega Harbor, California, (Sorte et al. 2010) found that introduced species were more tolerant of higher temperatures than native
If invasive species are more tolerant to higher temperatures in general, Sorte et al. (2010) predicted heightened consequences for native populations as global water temperatures continue to rise as a result of climate change. Rahel et al. (2008) predicted that native fish populations of the Laurentian Great Lakes will face a similar threat from invasive fish species as global warming increases water temperature and as human mediated transport facilitates the bypass of traditional biogeographic barriers when species are transported beyond their native ranges (Carlton and Geller, 1993).

Based on the case studies of the aquatic invasive species outlined above, one possible reason for the differential range expansion of round and tubenose gobies may be explained by differences in the physiological performance (metabolic efficiency) of these species. It has been suggested that the metabolic capacity of round gobies exceeds that of native species in regions of establishment, thereby making it a fierce competitor (Cross and Rawding, 2009). However, few studies have evaluated the metabolic efficiency of round gobies across a temperature gradient (Lee and Johnson, 2005) and studies concerning most aspects of physiology in the elusive tubenose goby are absent. Many studies on invasive species attempt to assess a species' probability for range expansion by examining range distribution in its native habitat and estimating physiological tolerance through exposure to extreme environmental attributes across their native range. However, direct empirical measurements of physiological efficiency, such as standard metabolic rate (SMR) (Lee and Johnson, 2005) and field metabolic rate (FMR) (Paterson et al. 2007; Drouillard
et al. 2009) are preferred due to limitations in model estimates. Furthermore, understanding the physiological efficiency of a species may provide a more accurate metric to make predictions about biotic resistance, predator-prey interactions, and global warming induced changes in species assembly (Rahel et al. 2008).

A simplified energy mass balance as been popularised by the Wisconsin model (Kitchell, 1983; Hanson et al. 1995):

\[
\text{Energy Consumed by Feeding} = \text{Total Metabolism} + \text{Waste} + \text{Gain}
\]

\[
C = SMR + A + SDA + U + G \quad \text{(Equation 1.1)}
\]

where \( SMR \) is the organism's standard metabolic rate (kJ·d\(^{-1}\)); \( A \) is the costs of activity (kJ·d\(^{-1}\)); \( SDA \) is the specific dynamic action (kJ·d\(^{-1}\)); \( U \) is the loss of energy to waste (fecal egestion or urinary excretion; kJ·d\(^{-1}\)) and \( G \) is the growth of the organism (kJ·d\(^{-1}\)). In practice, the SMR of a species is most commonly analyzed through the use of respirometry, which directly measures an organism's rate of oxygen usage under resting and fasted conditions. The temperature and size dependence of SMR for round gobies was measured by Lee and Johnson (2005) and used to develop the first bioenergetic model for this species. However, the use of respirometry has limitations because it only provides a short-term measure of SMR under artificial conditions and fails to incorporate the acclimation capacity and metabolic efficiency of an individual over seasonal temperature cycles (Lee and Johnson, 2005; Paterson et al. 2007). Through the use of a chemical tracer depuration approach, an organism's FMR can be estimated (Drouillard et al. 2009). In this method, animals are dosed via
intraperitoneal (IP) injection with non-environmental polychlorinated-biphenyl (PCB) performance reference compounds (PRCs) (Raeside et al. 2009) and the elimination of each PRC is tracked through time under free-living conditions. Research has shown that the main route of chemical elimination in fish occurs via loss across their gill surfaces during respiration and can be evaluated by Equation 1.2 (Drouillard et al. 2009; Paterson et al. 2010):

\[
Q_V = \frac{FMR}{D_{O_2} \cdot \bar{C}_{O_2} \cdot E_{O_2}} 
\]  

(Equation 1.2)

where \( FMR \) is the organism’s field metabolic rate (kJ·g\(^{-1}\) body weight/d); \( D_{O_2} \) is the oxycalorific coefficient for converting oxygen respired to energy of fish (14.30 kJ·g\(^{-1}\) O\(_2\)) (Norstrom et al. 1976); \( \bar{C}_{O_2} \) is the weighted average concentration of dissolved oxygen in water over time (g O\(_2\)·mL\(^{-1}\) water); and \( E_{O_2} \) is the oxygen exchange efficiency across the gills (0.6; unitless). Thus, for hydrophobic organic chemicals chemical elimination \( (k_{total}) \) can be modelled according to the following equation:

\[
k_{total} = \frac{E_W Q_V}{BCF} 
\]  

(Equation 1.3)

where \( Q_V \) is the gill ventilation rate (mL·g\(^{-1}\)·d\(^{-1}\)), \( E_W \) is the chemical exchange efficiency across the gills (0.54, unitless), and \( BCF \) is the biota/water equilibrium partition coefficient; \( BCF \) is approximately equal to the fraction of lipid \( (X_{lipid}) \) \times octanol water partition coefficient of the chemical \( (K_{OW}) \) (Drouillard et al. 2009). Finally, combining Equation 1.2 and Equation 1.3 allows for the determination of an organism’s FMR:
To the best of my knowledge, few studies have investigated how physiological tolerance, such as metabolic efficiency across temperatures (Paterson et al. 2007, Drouillard et al. 2009), contributes to invasion risk and range expansion. The research presented in this thesis examines the metabolic efficiency of round and tubenose gobies from populations in the Great Lakes. In the second chapter of my thesis, respirometry techniques were used to evaluate the SMRs of round and tubenose gobies from Lake St. Clair across a temperature gradient. The SMRs of both species are compared to evaluate whether differences in the metabolic rate of each species contributes to their realized differential range distribution. Based on the differences in range expansion of round and tubenose gobies, I test the hypothesis that round gobies exhibit increased metabolic rate, as indicated by lower SMRs compared to the less successful tubenose goby. In the third chapter of my thesis, I validate the use of IP injections for chemical depuration studies of round gobies. Round gobies from Lake St. Clair were injected with a 14-congener PRC-PCB tracer mixture and allowed to depurate chemical on a compressed time scale between 0.25 and 8 days. I evaluate the time required to complete chemical assimilation of an IP dose by resolving the shortest sampling time where native and IP injected congeners achieve similar tissue distribution in small fish. Finally, the fourth chapter of my thesis explores the use of a chemical tracer depuration approach to compare the FMRs of round gobies from three lake populations:
Lake St. Clair, Lake Huron and Lake Ontario during a 97 day period of rapid
temperature change. The three lakes differ in water temperature characteristics
(Table 1.1). Specifically, I test the hypothesis that FMRs and temperature
acclimation capacity varies between round goby populations, such that Lake St.
Clair and Lake Ontario fish will show greater similarities in FMR response
compared to fish from Lake Huron, the least genetically diverse and most
genetically distinct population (Brown and Stepien, 2009). This hypothesis was
generated under the assumption that genetic characteristics of round goby sub-
populations are matched with environmental temperature characteristics in their
respective invasive ranges.

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*Canadian Journal of Fisheries and Aquatic Sciences.* 59: 1209-1228.
Table 1.1: Summary of mean ± SE and range of open water temperatures for Lake St. Clair, Lake Huron and Lake Ontario between January 2011 and December 2011. Data for Lake Huron and Lake Ontario surface water temperatures were obtained from the Great Lakes Information Network (2013) and Lake St. Clair data was obtained from the Great Lakes Institute for Environmental Research database.

<table>
<thead>
<tr>
<th>Lake Population</th>
<th>Mean ± SE (°C)</th>
<th>Temperature Range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake St. Clair</td>
<td>10.99 ± 0.42</td>
<td>2.49-26.2</td>
</tr>
<tr>
<td>Lake Huron</td>
<td>8.76 ± 0.35</td>
<td>0.93-19.97</td>
</tr>
<tr>
<td>Lake Ontario</td>
<td>10.00 ± 0.38</td>
<td>1.77-22.38</td>
</tr>
</tbody>
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CHAPTER 2

DETERMINATION OF STANDARD METABOLIC RATES IN TWO RECENT GREAT LAKES INVADERS: ROUND (*NEOGOBIOUS MELANOSTOMUS*) AND TUBENOSE (*PROTERORHINUS SEMILUNARIS*) GOBY

**Introduction**

In 1990, two Ponto-Caspian alien invasive fish species, the round goby (*Neogobius melanostomus*) and the tubenose goby (*Proterorhinus semilunaris*), were found in the St. Clair River of the Great Lakes basin (Jude et al. 1992). Although these two species entered the Great Lakes around the same time via ballast water discharge from foreign vessels, the range expansion of each species post-invasion has shown major differences. Since their introduction, round gobies have established throughout the entire Great Lakes basin (Vanderploeg et al. 2002; Kocovsky et al. 2011). In contrast, expansion of the tubenose goby has been confined to Lake St. Clair and the western basin of Lake Erie for much of its invasion history. Only within the last decade have tubenose gobies been reported in western Lake Superior and Lake Ontario (Vanderploeg et al. 2002; Fuller et. al 2013). Although well documented, the basis for the differential range expansion of these two closely related invaders (Dougherty et al. 1996; Dillon and Stepien, 2001; Stepien et al. 2005) remains poorly understood. Round and tubenose gobies therefore present a model system for evaluating how life history traits and differences in phenotypic characteristics relate to alien invasive species’ potential for distribution (Dougherty et al. 1996; Stepien et al. 2005).
The range distribution of a species may be influenced by a combination of many ecological, historical and physiological factors, however little is known about the limiting role of physiological tolerance in governing range distribution (Ehrlich, 1989; Lodge, 1993; Braby and Somero, 2006). Although a common hypothesis is that successful invasive species display exceptional physiological tolerance to stressors of their new habitat (Braby and Somero, 2006), little empirical support exists for the claim ( McMahon, 2002). Since many studies have found that temperature is a factor influencing habitats where ectothermic species may reside (Braby and Somero, 2006; Sorte et al. 2010), differences in physiological processes leading to acclimation strategies and temperature stress responses of round and tubenose gobies may ultimately predict their range expansion. However, most studies attempting to document potential invasive species range expansion base their estimates of physiological tolerance on temperature conditions across the native range of the invader (Braby and Somero, 2006; Sorte et al. 2010). This presumes that temperature, as opposed to other abiotic stressors and/or biological interactions, acts as the major regulator of the native range distribution of the species. Round gobies are a eurythermal species with tolerance to water temperatures ranging from -1°C to 30°C (Ng and Gray, 2011), with a range of 23°C-26°C being the optimal temperature for growth (Lee and Johnson, 2005). It has also been suggested that the metabolic capacity of round gobies exceeds that of native species in regions of establishment, thereby making it a dominant competitor for food resources (Cross and Rawding, 2009). Although a large body of research exists describing
the physiological tolerance (Lee and Johnson, 2005), reproductive behaviour (Corkum et al. 1998; Meunier et al. 2009), and life history traits (Jude et al. 1992) of round gobies, research on tubenose gobies is generally lacking.

In this study, the standard metabolic rates (SMRs) of round and tubenose gobies from Lake St. Clair were evaluated and compared across several temperatures using intermittent flow respirometry. The main objective of this research was to test the hypothesis that round gobies have greater metabolic efficiency, manifested as a reduced standard metabolic rate across temperatures, compared to tubenose gobies.

Materials and Methods

Sample collection and fish husbandry

Intermittent flow respirometry was used to calculate the SMRs of 27 round and 16 tubenose gobies of masses 5.9 ± 0.320g (mean ± SE) and 3.267 ± 0.290g (mean ± SE), respectively. Fish were collected by beach seine and minnow traps from 2 locations in the Detroit River (42°20'14.8452"N, 82°54'58.1466"W and 42°18'25.0776"N, 83°4'31.1376"W) between July 2012 and October 2012. Prior to the initiation of the experiment, individuals were held in flow-through aquaria under ambient environmental temperature and photoperiod in the aquatic facility at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, ON, Canada). The temperature range experiences between the acclimation and each subsequent experimental period was 5°C. Water quality was monitored weekly by measuring pH, dissolved oxygen, temperature, conductivity and oxidation/reduction potential (Hydrolab,
Campbell Scientific Corp., Edmonton, AB, Canada). Fish were placed in static holding aquaria equipped with submersible Top Fin® aquarium heaters and acclimated to each experimental temperature for at least 72 hours before respirometry trials were initiated. This research was conducted with approval from the University of Windsor’s Animal Care Committee.

**Respirometry system specifications**

Intermittent respirometry, which uses AutoResp™ Version 2.0 software (Loligo® Systems, Denmark) to combine principles from closed and flow-through respirometry, was used to measure the SMRs of round and tubenose gobies. The respirometry system consisted of a 114L polyethylene ambient holding tank housing the respirometer, and a separate 416L temperature controlled reservoir. The respirometry system re-circulated aerated water between the ambient holding tank and temperature controlled reservoir to maintain the oxygen saturation of water. The overall setup of the respirometer consisted of a submersible glass respirometry chamber (D007, 33mm diameter, total length 100mm, Qubit Systems Inc, Kingston, ON, Canada) with ports for recirculation, 2 submersible aquarium pumps, a submersible galvanic oxygen probe housed within a vessel (MINI-DO, Loligo® Systems, Denmark), connective tubing and a central relay system (DAQ-M, Loligo® Systems, Denmark). The glass chamber contained one set of ports for recirculating water within the chamber during periods of SMR measurement and a second set of ports for intermittently flushing water inside the glass chamber with water from the ambient holding tank before initiating the next measuring period. AutoResp™ software provided automated
system control and data collection of SMR measurements over each 24 h trial. Standard metabolic rate measurements proceeded sequentially in a loop of three automated stages: measuring period, flush period, and wait period. During the measuring period, the flush pump was off and water was recirculated within the glass chamber. The oxygen probe detected declining levels of oxygen as a function of time, which was then translated into fish SMR by AutoResp™ through linear regression analysis. After the measuring period, the flush pump would flush water from the glass chamber and replenish it with oxygen-saturated water from the ambient holding tank. When the flush period was complete the automated loop ended with a wait period, which was necessary to account for a lag in the system between the flush period and subsequent measurements.

Using respirometry to measure oxygen consumption

Prior to the initiation of a respirometry trial, each fish was netted from the holding aquarium where it had acclimated and fasted for at least 72 hours, then placed in a bucket with the same water and anaesthetized using clove oil (40mg·L⁻¹). Each individual was measured for total length (mm), standard length (mm), weight (g) and volume (mL; by water displacement). After biometric data were collected, the respirometer was inspected for air bubbles within tubing, the respirometry chamber itself, and the oxygen probe vessel before measurements were initiated. If air bubbles were present in the chamber, they were released by disconnecting and re-connecting pieces of the apparatus. After ensuring all bubbles were removed, the individual was placed into the respirometry chamber and measurements taken over a 24 h period. Respirometry measurements were
taken in the following order: 10°C, 18°C, 23°C, 26°C and 30°C. The reservoir was equipped with a chiller for 10°C and 18°C treatments and a heater for 23°C, 26°C and 30°C treatments to achieve ambient temperatures in the holding tank. Before the initiation of a new temperature treatment, the oxygen probe was recalibrated by placing it first in oxygen saturated water and adjusting the meter reading, then rinsing with distilled water, and finally placing it in oxygen free water and adjusting the meter reading (MINI-DO, Loligo® Systems). In order to observe a decline in the concentration of oxygen in the respirometry chamber, a measuring period of 300 seconds, a flush period of 100 seconds and a wait period of 30 seconds were used for 10°C, 18°C, 23°C and 26°C temperature treatments. Due to the increased stress and oxygen consumption of fish under the 30°C temperature treatment, the measuring, flush, and wait periods were adjusted to 120 seconds, 90 seconds, and 20 seconds respectively to prevent mortality. Readings were taken under dark conditions to limit photoperiod/oxygen consumption interactions and minimize disturbance of fish by reducing visual stimuli during the course of measurements. The 114L polyethylene ambient holding tank was covered with a tight fitting lid prior to the initiation of measurements and also had 2 small viewing windows which allowed us to make observations without disturbing the fish.

Initial respirometry trials were conducted as separate trials using 3 individual round and tubenose gobies per temperature. Due to the limited availability of tubenose gobies, 1 individual in the 26°C treatment, and 3 individuals in the 30°C treatment were used more than once; no round gobies
were used more than once across temperature treatments. This study was repeated (study 2) across each of the temperature treatments with 2 separate individual round gobies and 3 blanks per temperature immediately following the first study. Blank trials were performed using a 3.8g glass volumetric flask stopper as a fish surrogate to account for microbial oxygen demand present within the system. Blank oxygen consumption trials appeared to drift with an increase in temperature. After demonstrating equivalent responses of round gobies from both studies, the blank trials were used to adjust data from all round and tubenose goby SMR measurements.

**Data Analysis**

Respirometry data for round and tubenose goby trials in studies 1 and 2, as well as blank trials were censored to eliminate the first 4 hours of respirometry measurements. During the first 4 hours of measurement, the oxygen consumption of a fish may be artificially elevated or reduced due to stress from handling, confinement to the respirometry chamber, and sedation with clove oil. After censoring the first 4h of data, the mean SMR_{total} was calculated for each individual at each temperature treatment as a measurement of mean oxygen consumption rates through time. The linear regressions for all SMR_{total} measurements had $r^2$ values > 0.78, thereby indicating a strong relationship between the decline in oxygen levels as a function of time for all trials. SMR_{total} may include periods of brief activity by individuals within chambers and therefore may overestimate the true metabolic rate minimum at a given temperature trial. To estimate the minimum SMR_{min}, a baseline oxygen consumption rate was
established for each individual's SMR trial to determine a measure of the oxygen consumption minima experienced by a given individual during the 24 h measurement period (See Figure 2.1).

The oxygen consumption baseline was used to establish periods of time where multiple measurement points corresponded to the minimum oxygen consumption for an individual fish (Cech et al. 1994; Mesa et al. 2013). Each respirometry trial was subjected to the following criteria when determining the placement of the baseline: a minimum of 4 consecutive measurement points (corresponding to 1 hour of consecutive measurement) were used per segment and a minimum of 3 repeating segments (corresponding to 3 total hours of measurement) were present. These two criteria ensured signal stability and repeatability throughout the duration of the trial. After determining SMR$_{\text{total}}$ and SMR$_{\text{min}}$, the SMR values were then adjusted for blank signals at each temperature to correct for oxygen consumption in the system occurring independent of fish within the chambers.

Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used for statistical analyses and completed using the R statistical computing program (R Core Team 2012) and SYSTAT (Systat Software Inc. 2008). Significance levels of $\alpha < 0.05$ were used as criteria for significant differences for all analyses. Prior to ANOVA, a Shapiro-Wilk test was performed to test data for normality ($p < 0.05$), resulting in a log transformation of the data set ($p > 0.05$) in order to conform to ANOVA assumptions. Chauvenet's criterion was used to evaluate the data set for the presence of outliers. Predicted round
goby SMR$_{\text{min}}$ values were determined using the equation presented by Lee and Johnson (2005) for round gobies:

$$SMR_{\text{pred}} = 0.94 \cdot W^{-0.157} \cdot e^{(0.061\cdot T)} \quad \text{(Equation 2.1)}$$

where SMR$_{\text{pred}}$ is the round goby basal metabolic rate (mg oxygen $\cdot$ g$^{-1}$ body weight $\cdot$ day$^{-1}$) predicted from the Lee and Johnson (2005) model, $W$ is the weight of the fish (g), and $T$ is the water temperature ($^\circ$C). Individual body weights and temperatures from experimental trials were used in conjunction with Equation 2.1 to compare measured round goby SMR$_{\text{min}}$ values with Lee and Johnson's (2005) model predictions. After evaluating the fit of the Lee and Johnson model to experimental trials for round goby data, the allometric portion of the model was used to size adjust measured round goby SMR$_{\text{min}}$ values to the mean size of tubenose gobies measured in the equivalent temperature treatments. The adjustment was performed as follows:

$$SMR_{\text{adj}(RG)x} = \frac{W_{TG}^{-0.157}}{W_{RG}^{-0.157}} \cdot SMR_{\text{Min}(RG)x} \quad \text{(Equation 2.2)}$$

where SMR$_{\text{adj}(RG)x}$ is the blank and body weight adjusted SMR$_{\text{min}}$ (mg oxygen $\cdot$ g$^{-1}$ $\cdot$ d$^{-1}$) for an individual round goby, $W_{\text{TG}}$ is the average weight (g) of tubenose gobies measured for a given temperature treatment, $W_{\text{RG}}$ is the average weight (g) of round gobies measured for a given temperature treatment, and SMR$_{\text{min}(RG)x}$ is the blank adjusted baseline SMR determined for an individual round goby trial. This adjustment enabled scaling individual round goby metabolic rate data to a mean body size equivalent of the smaller tubenose gobies enabling comparison.
of SMR$_{\text{min}}$ without bias due to fish size.

**Results**

Water quality exhibited minor week-to-week variation, but all parameters remained within non-stressful ranges for captive rearing of fish (See Table 2.2). Only one round goby died during this study at the 30°C temperature trial. This death was likely due to the increased stress and oxygen usage of the fish at the 30°C treatment. As a consequence, the respirometer's flush, wait and measurement periods were reduced from 100, 30 and 300 seconds to 90, 20 and 120 seconds respectively for the remaining experimental trials to prevent future mortalities. Respirometry data from the affected individual was not included in subsequent analysis. The measuring periods for individual respirometry trials ranged between 16 and 24 hours and averaged 19.1±1.8h (mean ± SD) after censoring the data for the first 4 h of trial initiation. There was no visible evidence for formation of biofilms in the respirometry system and reservoir over the course of the study, although blank readings indicated oxygen demand by the system independent of fish. Blank signal response for oxygen consumption was also observed to increase with temperature (Figure 2.2).

Unlike measurements taken with fish, oxygen consumption profiles from blanks did not show elevated oxygen consumption during the first 4 h of the trial nor did they show pronounced activity peaks during the measurement period (Figure 2.1). Although the blank signal response was relatively high, respirometry trials containing fish had significantly higher SMR$_{\text{total}}$ values compared to blanks (Figure 2.3, ANOVA, p < 0.05). ANCOVA was used to test for differences
between raw $\text{SMR}_{\text{total}}$ and $\text{SMR}_{\text{min}}$ values for round gobies from study 1 and 2 after adjusting for temperature as the covariate. Prior to running the ANCOVA, the study day x temperature interaction term was found to be non-significant (ANOVA, $p > 0.05$), enabling temperature to be adjusted as the covariate. ANCOVA revealed no significant differences ($p > 0.05$) between raw $\text{SMR}_{\text{total}}$ and $\text{SMR}_{\text{min}}$ values for round gobies from study 1 and 2. Given the similarities in raw round goby SMR measurements between the two studies, blank system signals were assumed to be representative for all measurements taken during the two studies. All subsequent data were blank corrected by subtracting the average blank signal at a given temperature treatment from each $\text{SMR}_{\text{total}}$ or $\text{SMR}_{\text{min}}$ of fish tested at the same temperature. Blank adjusted round goby $\text{SMR}_{\text{total}}$ and $\text{SMR}_{\text{min}}$ values are presented in Table 2.1.

Chauvenet's criterion was used to reject one very high outlier measurement at the 10°C treatment, a round goby with a $\text{SMR}_{\text{total}}$ of 6.24 mg O$_2$·g$^{-1}$·day$^{-1}$. Round goby $\text{SMR}_{\text{total}}$ and $\text{SMR}_{\text{min}}$ were significantly dependent on temperature ($p < 0.05$; ANOVA). The $\text{SMR}_{\text{min}}$ and $\text{SMR}_{\text{total}}$ of round gobies were also compared with predicted SMR values ($\text{SMR}_{\text{pred}}$), which were generated from the Lee and Johnson model (Equation 2.1; Figure 2.4). The ANOVA on log transformed data indicated significant ($p < 0.05$) differences between round goby $\text{SMR}_{\text{min}}$ and $\text{SMR}_{\text{pred}}$, as well as a significant interaction ($p < 0.05$) between $\text{SMR}_{\text{group}}$ (i.e. $\text{SMR}_{\text{min}}$, $\text{SMR}_{\text{tot}}$ and $\text{SMR}_{\text{pred}}$) and temperature. These results indicate differences in the temperature dependence of SMR between model predictions and observed measurements. Based on Figure 2.4, differences
between SMR\textsubscript{min} and SMR\textsubscript{pred} were greatest at the elevated temperature treatments (26°C and 30°C). Prior to ANCOVA, the group x temperature interaction term was tested for the 26°C and 30°C temperature treatments and found to be non-significant (ANOVA, p > 0.05). After adjusting for temperature as the covariate, ANCOVA revealed significant (p < 0.05) differences between round goby SMR\textsubscript{min} and SMR\textsubscript{pred} values. When the ANOVA was restricted to the 10°C and 18°C temperature treatments, the group x temperature interaction term was also found non-significant (p > 0.05). However, using temperature as the covariate ANCOVA revealed non-significant (p > 0.05) differences between round goby SMR\textsubscript{min} and SMR\textsubscript{pred} at lower temperature treatments.

Similar to round goby, SMR\textsubscript{total} and SMR\textsubscript{min} of tubenose goby were significantly dependant on temperature (ANOVA, p < 0.05) (Table 2.1). An ANOVA was used to compare the sizes of round and tubenose gobies and revealed significant differences (p < 0.05) in fish size between species, particularly at the 10 °C, 18 °C, 23 °C, and 26 °C treatments. To remove bias related to different sizes of fish, Equation 2.2 was used to size adjust the SMR\textsubscript{min} and SMR\textsubscript{total} of round gobies to the equivalent of the mean body mass for tubenose gobies at each temperature treatment. The allometric adjustment increased the oxygen consumption rate (SMR\textsubscript{adj}(RG)x) of round goby by an average of 11.52 ± 1.02% over the measured values given the larger sizes of round gobies. A comparison of size adjusted round goby SMR\textsubscript{min} and tubenose goby SMR\textsubscript{min} values is provided in Figures 2.5 and 2.7. Mean SMR\textsubscript{min} values of tubenose gobies were generally higher than round gobies except for the 30°C
treatment where measurements were highly variable among individuals of both species. ANOVA indicated a non-significant ($p > 0.05$) temperature x species interaction term, indicating similar temperature dependence of $\text{SMR}_{\text{min}}$ in round and tubenose gobies. After correcting for temperature as a covariate, $\text{SMR}_{\text{min}}$ was not found to be significantly different (ANCOVA, $p > 0.05$) between species. A comparison of size adjusted round goby $\text{SMR}_{\text{total}}$ and tubenose goby $\text{SMR}_{\text{total}}$ is presented in Figures 2.6 and 2.7. As observed for $\text{SMR}_{\text{min}}$, the general ANOVA indicated a non-significant ($p > 0.05$) temperature x species interaction term. Following correction for temperature as a covariate, ANCOVA revealed significant ($p < 0.05$) differences between $\text{SMR}_{\text{total}}$ for round and tubenose gobies. This relationship can be further understood by comparing each species' ratio of $\text{SMR}_{\text{total}}/\text{SMR}_{\text{min}}$. For round gobies the average ratio of $\text{SMR}_{\text{total}}/\text{SMR}_{\text{min}}$ was $1.57\pm0.18 \text{ mg O}_2\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ (mean ± SE) and for tubenose gobies the ratio was $1.69\pm0.35 \text{ mg O}_2\cdot\text{g}^{-1}\cdot\text{day}^{-1}$ (mean ± SE), across temperature treatments.

**Discussion**

The results of the present study were consistent with other studies examining the temperature dependence of SMRs of fish (Cech et al. 1994; Lee and Johnson, 2005; Clarke and Johnston; 1999; Mesa et al. 2013). Measurements of round goby SMR's were also generally consistent with measurements reported by Lee and Johnson (2005) at the lower temperature treatments but were significantly higher than the Lee and Johnson model predicted values at the higher temperatures. These discrepancies are likely due to differences in experimental protocols regarding the duration of acclimation.
Lee and Johnson (2005) used a 10 d acclimation period between temperature treatments while a shorter 72 h time period was used in the present study. Temperature acclimation in fish and other ectotherms requires production of new proteins optimized to the temperature conditions present (Schmidt-Nielsen 1990). It is likely that the longer acclimation periods used between temperature treatments by Lee and Johnson permitted protein turnover to occur and provided a more appropriate measurement of the acclimated metabolic rate of individual fish. The acclimation bias at the lower temperature treatments was not evident because my experiment was initiated with winter fish, which were well acclimated to lower temperatures. As such, the SMR measurements for round and tubenose gobies at high temperatures may be higher than for a fully acclimated fish.

The differences in sizes between round and tubenose gobies required size standardization before comparing SMRs between species. The size adjusted SMR_{min} of round and tubenose gobies were not found to differ significantly after adjusting for temperature, although the statistical test results approached the significance criteria. In general, tubenose gobies exhibited higher SMR_{min} values than round gobies at most temperatures except for the 30°C treatment. Closer inspection of Figure 2.5 reveals a metabolic peak for tubenose goby SMR_{min} occurring at 23°C and a drop in the average SMR_{min} at higher temperatures. High individual variation in SMR measurements at these temperatures precluded statistical detection of the above trends. In contrast to the tubenose goby data, average round goby SMR_{min} values continued to increase with temperature up to the maximum temperature treatment. The above observations are consistent
with those of Lee and Johnson (2005) who demonstrated exponential increases in round goby SMR with temperature up to 27°C. The latter authors further showed that maximum food consumption rates of round gobies decreased sharply after 26°C indicating the on-set of temperature stress. These observations suggest that tubenose goby had reached their metabolic optima at a lower temperature compared to round gobies and were likely experiencing thermal stress at the 26 and 30°C temperature treatments.

The comparison of size adjusted SMR\textsubscript{total} between species revealed significant differences in metabolic rate. In this case, tubenose gobies demonstrated higher average SMR\textsubscript{total} values at all temperature treatments. The difference in patterns among species comparisons for SMR\textsubscript{min} and SMR\textsubscript{total} are hypothesized to be related to activity of fish within the respirometer chambers. The results imply greater activity by tubenose goby while present in the respirometry chambers that may be indicative of an overall elevated stress response compared to round gobies. The reasoning behind the interpretation of peaks in oxygen consumption as activity within the respirometry chamber hinges on the critical assumptions of intermittent flow-through respirometry. In intermittent flow-through respirometry the conditions, behaviours and environment of the respirometry chamber are controlled by an automated system and are therefore assumed to be consistent across each 24-hour trial. However, changes in oxygen consumption may also be related to circadian activity and species specific behaviour. Previous research has documented the influence of diel cycle on oxygen consumption in atipa (\textit{Hoplosternum littorale}) (Boujard et al.
1990) and river puffer fish (Takifugu obscurus) (Kim et al. 1997) and round gobies have been known to feed at night through the use of their lateral line system (Janssen and Jude, 2001). Taken together, my interpretations of SMR_{min} (true standard metabolic rate) and SMR_{total} (standard metabolic rate with brief periods of activity) point to differences in metabolic efficiency and temperature tolerance between the two goby species. Tubenose gobies have higher metabolic rates at low temperatures and achieve a metabolic optimum at a lower temperature than round gobies. At higher temperatures, total metabolic rate of tubenose goby increases over that of round goby due to elevated activity associated with a heat stress response. Such trends would be expected between species with different temperature optima and breadth of thermal tolerance (Ford et al. 2004).

Neilson and Stepien (2009) presented the native range distribution of round and tubenose goby populations in the Ponto-Caspian region. Their data indicated co-existence of round and tubenose goby populations along the 45ºN latitude. However, round gobies were distributed over greater latitudes at both the northern and southern (50ºN and 40ºN latitude) range. These results suggest that round gobies not only possess a higher temperature preference but also a greater range of thermal tolerance. The results from the present research are consistent with the hypothesis that differences in the metabolic response of round and tubenose goby to temperature may partially account for differences in the range expansion experienced by the two species following entry into the Great Lakes. Furthermore, previous research has evaluated the relationship
between competitive ability and metabolic rate in closely related species of trout (*Salvelinus* spp.) (Warnock and Rasmussen, 2013) and sculpin (*Gymnoanthus tricuspid* and *Myoxocephalus* spp.) (Seth et al. 2013). Both studies found that increased competitive ability was demonstrated by species with increased resting metabolic rate. Although my study found that round gobies had reduced SMR$_{total}$ compared to tubenose gobies, the results from Warnock and Rasmussen (2013) and Seth et al. (2013) suggest that round gobies, based on their increased aggressive nature and suspected competitive ability (Corkum et al. 2004), would have increased SMR$_{total}$ values. Future research should aim to explore the competitive ability of round and tubenose gobies and its relationship to metabolic rates.

Round and tubenose goby also differ in many other traits including feeding ecology (Jude et al. 1992) size (Corkum et al. 1998; Vanderploeg et al. 2002; Jude et al. 1992), growth rates (Corkum et al. 1998; Vanderploeg et al. 2002; Jude et al. 1992) and reproductive strategy (Corkum et al. 1998; MacInnis and Corkum, 2000; Meunier et al. 2009) that can influence differences in range distribution. Ray and Corkum (2001) suggested that while round gobies exhibit high site fidelity, their ability to tolerate diverse habitats has contributed to their establishment success (Charlebois *et al.* 1997; Ray and Corkum, 2001). Tubenose gobies prefer still water habitats compared to the structurally complex benthic habitats frequently occupied by round gobies (Jude et al. 1992; Kocovsky et al. 2011). With regards to morphology, tubenose gobies are significantly smaller at the first age of reproduction compared to round gobies (Corkum et al.
Finally, round gobies possess more diverse reproductive strategies. Male round gobies have an alternative breeding male/sneaker male reproductive strategy (MacInnis and Corkum, 2000) and female round gobies can spawn more than once per season (MacInnis and Corkum, 2000; Meunier et al. 2009). Although round gobies have a higher number of reproductive events, their fecundity is generally lower than that of similar sized fish (MacInnis and Corkum, 2000). To date, no alternative reproductive strategy has been documented for the tubenose goby, where females appear to only spawn once per season (Corkum et al. 1998). In addition, male round gobies also display nest guarding behaviour, which confers a higher rate of offspring survival (Meunier et al. 2009). Although the reproductive strategies of round gobies appear to greatly influence their reproductive success, the prime determinant of differential invasion success in round and tubenose gobies is unclear. Future research should investigate the life history, feeding ecology and reproductive strategies of tubenose gobies to evaluate whether significant differences in these characteristics exist between species.

Overall, this study has improved our understanding of the influence of temperature on the metabolic rate of round and tubenose gobies. To my knowledge, this study is the first to present respirometry measurements on tubenose goby for comparison with the more well studied round goby. Tubenose gobies exhibited higher standard metabolic rates compared to round gobies at most temperatures except for the 30°C treatment. At the latter temperature, the total metabolic rate (SMR_{total}) of tubenose gobies exceeded those of round gobies.
gobies suggestive of acute stress occurring in the tubenose goby compared to a lower stress response by round goby even though both species were outside their temperature optima. The metabolic efficiency of a species, as described by $\text{SMR}_{\text{min}}$ and $\text{SMR}_{\text{total}}$, over a range of temperatures may provide a useful metric to compare and contrast the acute stress response and temperature tolerance of closely related species pairs. Continued research on tubenose gobies is required to better understand physiological tolerance to temperatures (at lower and higher temperatures than used in the present study), allometric scaling coefficients, the response of this species to other stressors such as low dissolved oxygen and suspended solids, preferred habitat characteristics, diet preference, and digestion efficiencies.

**References**


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Meunier, B, Yavno, S, Ahmed, S, Corkum, LD. (2009). First Documentation of Spawning and Nest Guarding in the Laboratory by the Invasive Fish, The


Table 2.1: Blank adjusted round and tubenose goby individual and mean ± SE (mg O\(_2\) · g\(^{-1}\) · day\(^{-1}\)) SMR\(_{\text{total}}\) and SMR\(_{\text{min}}\) by temperature treatments. RG = round goby, TG = tubenose goby. Individuals are grouped according temperature treatment (10°C, 18°C, 23°C, 26°C or 30°C). Actual measured temperatures are also presented.

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<td>Actual Temp (°C)</td>
<td>Group Mean Temp. and Range (°C)</td>
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<td>SMR&lt;sub&gt;total&lt;/sub&gt; Group Mean ± SE (mg O&lt;sub&gt;2&lt;/sub&gt; · g&lt;sup&gt;-1&lt;/sup&gt; · day&lt;sup&gt;-1&lt;/sup&gt;)</td>
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Table 2.2: Weekly water quality parameters measured in the respirometry chamber between January 17, 2013 to May 13, 2013. Parameters include temperature (°C), specific conductivity (SPC) (mS/cm), dissolved oxygen (mg/L), pH and oxidation/reduction potential (mV).

<table>
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<th>Date</th>
<th>Temperature (°C)</th>
<th>SPC (mS/cm)</th>
<th>Dissolved Oxygen (mg/L)</th>
<th>pH</th>
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Figure 2.1: Respirogram comparing the oxygen consumption profile of a round goby (triangle symbols) and a blank trial (dots) as a function of time. Both trials were conducted at a treatment temperature of 20ºC. In the round goby trial, circled segments A, B and C indicate baseline minimum oxygen consumption values used to calculate mean SMR$_{\text{min}}$ and the dashed line represents the estimated baseline used to calculate SMR$_{\text{min}}$. 
Figure 2.2: Relationship between blank trial log (O₂ consumption) (mg O₂·g⁻¹·day⁻¹) and temperature (°C). Blank respirometry trials used a glass stopper weighing 3.8g to determine the background levels of oxygen usage by the respirometry chamber in the absence of a fish. Three blank respirometry trials were conducted across experimental temperatures and used to correct measured round and tubenose goby oxygen consumption values.
Figure 2.3: Comparison of non-blank adjusted standard metabolic rate minimums (SMR_min) (mean mg O_2·g^{-1}·day^{-1} ± SE) of round gobies from study 1, round gobies from study 2, and blank respirometry trials from study 2 across temperatures.
Figure 2.4: Comparison of different measures of standard metabolic rate (mean mg O₂ · g⁻¹ · d⁻¹ ± SE) in round gobies across temperature treatments (ºC). SMR(min) and SMR(tot) values were measured, while SMR(pred) values were generated from model parameters presented in Lee and Johnson (2005).
Figure 2.5: Minimum standard metabolic rates (mean mg O₂·g⁻¹·d⁻¹ ± SE) for size-adjusted round gobies and tubenose gobies across temperature treatments (°C). Round goby minimum standard metabolic rate SMR(adj(RG)x) was size-adjusted to account for the larger size of round gobies compared to tubenose gobies using Equation 2.2, which was adapted from Lee and Johnson (2005).
Figure 2.6: Comparison of mean ± SE total standard metabolic rates (mg O$_2$·g$^{-1}$·d$^{-1}$) in size-adjusted round gobies and mean total standard metabolic rates in tubenose gobies across temperature treatments (°C). Round goby standard metabolic rate totals SMR(adj(RG)x) were size-adjusted to account for the larger size of round gobies compared to tubenose gobies using Equation 2.2, which was adapted from Lee and Johnson (2005).
Figure 2.7: Contribution of SMR(min) to SMR(total) values for round (top) and tubenose (bottom) gobies across temperatures(°C). Round goby SMR(min) and SMR(total) values were size-adjusted to account for the larger size of round gobies compared to tubenose gobies using Equation 2.2, which was adapted from Lee and Johnson (2005). SMR(min) values are presented in white and SMR(total) values are presented in black. Bars overlap to illustrate the contribution of SMR(min) to SMR(total) in each species.
CHAPTER 3

VALIDATION OF RAPID ASSIMILATION OF PCBS FOLLOWING IP DOSING IN THE ROUND GOBY (NEOGOBIUS MELANOSTOMUS)¹.

Introduction

Research documenting toxicity, toxicokinetics and use of organic contaminants as chemical tracers requires careful control and precision of the dose received by study organisms in order to distinguish between treatments with statistical rigor (Paterson et al. 2007). Past studies have dosed fish with hydrophobic organochlorines in several ways including: dietary exposure to spiked food (Fisk et al. 1998; Gutjahr-Gobell et al. 1999; Andersson et al. 2001), intraperitoneal injection with oil carriers (Brown et al. 1998; Andersson et al. 2001; Paterson et al. 2007), oral administration with an oil or gelatin carrier (Sijm et al. 1993) and implantation with silastic capsules (Andersson et al. 2001). Sijm et al. (1993) demonstrated that the retention characteristics, chemical uptake efficiencies and kinetics of PCBs dosed to fish can be dependent upon the method of dosage. However, the choice of dosing method will vary depending on the study objectives and goals.

Dietary exposure arguably best mimics the natural route of PCB uptake in

¹ This chapter is a result of joint research under the supervision of Dr. Ken G. Drouillard (University of Windsor) and Dr. Timothy B. Johnson (Ontario Ministry of Natural Resources). Chapter 3 contains material from an article entitled: ”Validation of rapid assimilation of PCBS following IP dosing in the round goby (Neogobius melanostomus)”, published in Bulletin of Environmental Contamination and Toxicology (BECT). This article was co-authored by O'Neil J., Johnson, T.B. and Drouillard, K.G. In this chapter, the primary contributions, main ideas, field work, experimental designs and data analysis were performed by the author. The contribution of co-authors was primarily through the revision of manuscript drafts and assistance with data interpretation.
the aquatic food web and is considered the best method for dosing animals to predict the toxicological response of an organism in its natural habitat (Andersson et al. 2001). However, when dietary exposures are performed with communally held fish under laboratory conditions, individual differences in fish feeding rates and access to food can contribute to high variation in the received dose (Metcalfe, 1986; Andersson et al. 2001). Dietary exposure also takes more time to achieve a target tissue concentration given that fish consume only between 1 to 3% of their body weight of food per day under maintenance conditions (Allen and Wootton, 1982). In contrast, intraperitoneal (IP) injection allows a more uniform dose to be administered to each individual (Brown et al. 1998). Furthermore, IP injections facilitate exposure of an organism to a target contaminant concentration over shorter time periods to better mimic acute effects (Gutjahr-Gobell et al. 1999). Little information is available concerning the time required for fish to assimilate hydrophobic chemicals into their tissues following IP injection and whether or not the tissue distribution of chemical following IP dosing is equivalent to dietary exposure methods. Andersson et al. (2001) investigated the latter and demonstrated that an IP injection of 20 PCB congeners in Zebrafish (Danio rerio) resulted in an equivalent chemical distribution to tissues after 7 days when compared to fish dosed by food. Unfortunately, the above study only compared the two dosing techniques at a single sampling point and it is not known if shorter times following IP dosing would yield comparable results.

The purpose of this study was to examine the assimilation of non-
environmental PCBs in round gobies (*Neogobius melanostomus*) after IP injection and compare tissue distribution of the dose to the distribution of PCBs bioaccumulated by the same fish through natural exposures (“native” PCBs). The round goby is a successful invasive species that entered the Great Lakes ecosystem via ballast water discharge (Jude et al. 1992; Ricciardi and MacIsaac, 2000). Round gobies are a benthic-dwelling species, but unlike other teleost fish lack a swim bladder (Jude et al. 1992). As a result, these fish are in close contact with contaminated sediments and are often characterized by a high bioaccumulation of PCBs (Vanderploeg et al. 2002). By using native PCBs as an internal control, the shortest sampling time where native and IP-dosed congeners achieve similar tissue distribution in fish was used to evaluate the time required to complete chemical assimilation of the IP dose.

**Materials and Methods**

**Sample collection and fish husbandry**

Round gobies averaging 5.975 ±0.778g (mean ± SE) were sampled from the Detroit River (42°18’25.04"N, 83°4’31.1"W). Fish were collected between May 23, 2012 and June 22, 2012, and held in a flow through holding tank prior to the start of the experiment. Fish were held under ambient environmental temperature and photoperiod in the aquatic facility at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, ON, Canada). Water quality was monitored daily by measuring pH, dissolved oxygen, temperature, conductivity, and oxidation/reduction potential (Hydrolab, Campbell Scientific Corp., Edmonton, AB, Canada). Throughout the experiment the water
temperature averaged 25.174 ± 0.177 °C (mean ± SE).

**IP injection with PRC-PCB congener mixture**

Environmentally rare PCBs, henceforth designated as performance reference compounds (PRCs), were selected as dosing chemicals such that they could be analytically distinguished from native (environmentally accumulated) PCBs (Raeside et al. 2009). The dosing mixture contained the following PCBs (IUPAC #s): 13, 21, 23, 43, 62, 89, 57, 68, 112, 125, 166, 204 and 205 derived from individual standards of neat chemical (AccuStandard, New Haven, CT, USA). This mixture was initially dissolved in hexane and then diluted in sunflower oil to a nominal dose of 40ng/μL for PCBs 13, 23, 43, 62, 89, 68, 112, 125, 166, 204, 205; 50ng/mL for PCB 21; and 200ng/mL for PCB 57. The log Kow values for the PRC compounds ranged between 5.06 and 8.0 (Hawker and Connell, 1988) 88 . Experimental fish were weighed, sexed, measured for total length and given an IP injection using a 10μL Hamilton MICROLITER® Syringe. Dosing occurred on Day 1 of the experiment (July 19, 2012) after fish were given a minimum of 3.5 weeks acclimation to experimental conditions. Target tissue concentrations for individual PRCs in fish tissue were 20ng/g for PCBs 6, 13, 23, 43, 62, 89, 68, 112, 125, 166, 204, 205; 25ng/g for PCB 21; and 100ng/g for PCB 57. Each fish was weighed prior to dosing and the injected volume (between 3 and 10 uL of dosing oil) was altered per fish to achieve the target nominal dose. Control fish were sham-dosed with an equivalent volume of sunflower oil containing no PRCs. After injection, experimental fish were placed in two 10-gallon flow-through glass aquaria containing 12 individuals per tank. Control fish
were placed in an identical 10-gallon flow through glass aquarium containing 6 fish. All tanks received ambient water from the Detroit River and the tank renewal time was one hour. Throughout the course of the experiment, fish were fed *ad libitum* Premium Soft Krill Pellet from Angels Plus (Olean, NY, USA). Three experimental fish were sampled on days 0.25, 2, 4, and 8 of the experiment, and 3 control fish were sampled on days 0.25 and 8. Upon collection, fish were euthanized via anaesthetic overdose and processed immediately. Fish were weighed, sexed, and total/standard lengths were measured. Each individual was dissected to isolate a sample of dorsal muscle from the whole body sample. Liver, brain, and the eggs of females were removed for another study. The remaining whole body carcass and dorsal muscle samples were collected for each individual, homogenized, and placed in hexane rinsed aluminum tins. Tissue samples were stored in a freezer at -23°C until chemical analysis.

*Procedure for tissue analysis of PCB content*

Each sample was analyzed for moisture, lipid and PCB content as per Daley et al. (2009). Briefly, 0.5g of tissue was ground with a mortar and pestle using 15g of activated sodium sulfate and packed into glass columns containing 25mL of a 50:50 (v/v) dichloromethane: hexane. Each column was spiked with 50µL of PCB34 as a recovery standard and left for 1 hour. Following elution, an additional 15mL of 50:50 dichloromethane:hexane was added to and eluted from the columns and sample extracts were concentrated to ~2mL by vacuum evaporation. Extracts were brought back to a volume of 10mL using hexane. One mL of the 10mL extract was removed for gravimetric determination of neutral
lipids (Drouillard et al. 2004). The remaining volume was concentrated to ~2mL and Florisil cleanup was performed as per Lazar et al. (1992) except that the first Florisil fraction (containing PCBs) used 50mL hexane. The cleaned-up extract was collected and reduced via vacuum evaporation to <1mL and brought to a final volume of 1mL in isooctane. The final extract was analyzed for individual PCBs by gas chromatography electron capture detection (GC-ECD) as per Lazar et al. (1992). For each set of 6 samples, an in-house reference homogenate (Detroit River carp), method blank, external PCB standard (Quebec Ministry of Environment Congener mix; AccuStandard, NewHaven, CT, USA), and PCB 34 recovery standard was analyzed. The average recovery of the internal standard was 86.9 ± 4.9% (mean ± SD), and the range was 78-102%. Individual PCB concentrations in samples were corrected for PCB 34 recovery prior to analysis. The recovery of individual PCB congeners for the in house reference homogenate were within two standard deviations of the mean laboratory control database values from the Great Lakes Institute of Environmental Research accredited organic analytical laboratory.

Data Analysis

The received dose was contrasted against the nominal dose by expressing a target dose fraction calculated by multiplying the carcass concentration of each PRC by the fish weight and dividing by the nominal dose provided to the fish. Tissue concentrations of individual PRCs were expressed on a lipid equivalent basis (Clip(eq); Daley et al. 2012) according to:

$$C_{lip(eq)} = \frac{C_{wet}}{f_{lip} + 0.05f_{LDP}}$$

Equation 3.1
where $C_{\text{wet}}$ is the wet chemical concentration (ng/g) in the sample, flip and $f_{LDP}$ is the fraction of lipid and lean dry protein, respectively. The LDP in the sample is calculated by subtracting the lipid weight from the dry weight and expressing as a fraction to the wet sample weight.

To correct for individual variation in size and dose received, I took two approaches to evaluate the experimental data. First, I presented the ratio of PRC lipid equivalent concentrations in dorsal muscle (DM)/whole body carcass (WB). The DM/WB ratio was determined for each individual fish and averaged across fish for a given time point. Since unassimilated PRCs in the IP injection oil would be found in carcass samples, but not in dorsal muscle, an observation of similar lipid equivalent concentrations in dorsal muscle and carcass samples would indicate inter-tissue equilibration (Drouillard et al. 2004). Thus, under an equilibrium partitioning paradigm, it was expected that the DM/WB ratio would be less than 1 at early time points and approach a value of one as the IP dose assimilation is completed.

Second, I used a native PCB compound as an internal benchmark to correct for individualized differences in the tissue distribution of native PCBs apart from equilibrium partitioning. In this case, all study fish were exposed to PCBs in the Detroit River by natural means. Regardless of whether the fish achieved steady state with their food or environment when they were captured, it can be assumed that native PCBs would have achieved steady state within
the tissues of individual fish at the time of sampling and over the extended laboratory acclimation period. PCB153 was selected as the benchmark because of its ubiquitous distribution throughout the environment and well understood environmental fate and bioaccumulation behaviour. For benchmarking, the DM/WB ratio for a given PRC is divided by the DM/WB ratio of PCB 153 determined in an individual fish. Thus, it was expected that the benchmarked ratio would approach a value of 1 when the PRC tissue distribution approaches the same tissue distribution of PCB 153. For brevity, target dose fractions, DM/WB ratios and benchmarked DM/WB ratios are presented for selected chemicals: PRC (IUPAC #'s) 21, 89, and 205. The selected PCB congeners have log Kow values reflective of the range present in the PRC mixture.

Lipid equivalent concentrations of PRCs 21, 89, 205 and native PCB 153 within and between time intervals were compared using analysis of variance (ANOVA) and Tukey's post-hoc tests. ANOVA was also used to test for changes in native PCB 153 concentrations in control fish sacrificed after 6 h and 8 d. Significant differences were represented by a p-value < 0.05. A one sample t-test was used to test for differences in the benchmarked DM:WB concentration ratios for PRCs 21, 89, 205 from the expected ratio of one at each time interval.

Results

There were no mortalities of fish following IP dosing and completion of the experiment. The concentration of PCB 153 in control fish sacrificed at the beginning and end of the study was not significantly different (p<0.05; ANOVA) from one another. There was also no evidence of PRC-PCBs in control fish
sacrificed over the study duration. The target dose fraction (expressed as a percentage) and the coefficient of variation (CV) for PRC congeners 21, 89, 205 and native PCB 153 across the experimental period is summarized in Table 3.1. The target dose fraction varied by chemical and averaged 35.29±8.74% (mean ± SE), 84.98±14.4% and 51.02±6.47% for PCBs 21, 89 and 205, respectively. Although the assimilated fraction of PCBs 21 and 205 was less than 100%, PRC 89 was within 1 standard deviation of 100% dose assimilation. There were no significant differences between the concentrations of PRCs 21, 89, 205 and PCB 153 between different sampling time points (ANOVA, p < 0.05) post injection. The range in CVs across sampling days for native PCB 153 was 38.62-80.79 (Table 3.1). This range is similar to those measured for PRCs 21, 89 and 205 (24.13-100.75; Table 3.1) suggesting that IP dosing was able to achieve a similar precision in individual dose as was achieved by life time feeding exposures of round gobies in the field.

Figure 3.1 summarizes the DM/WB ratio for native PCBs 153 and 180. For PCB 153 and 180, the average ± SD DM/WB ratio was 1.16±0.77 and 1.36±0.83 across time. The ratios were generally within a factor of 2 of the theoretical value of 1 except for the time 0 point where ratios were 2.33 and 2.75 for PCBs 153 and 180, respectively. Figure 3.1 also summarizes DM/WB ratios for PRCs 21, 89 and 205. Within 6 h following dosing, the DM/WB ratio was 1.24±1.03, 1.19±0.73 and 1.24±0.65 for the three congeners and were within one standard deviation of the theoretical value of 1. Across time points, the average DM/WB ratio was 1.02±0.87, 1.03±0.84 and 1.54±1.22 for the three congeners,
respectively. Only time point 4 showed deviation from the expected ratio, with a higher than expected ratio evident for PRC 205 (2.75±2.48) that was consistent in magnitude to the time 0 point ratio measured for PCB 180 (Figure 3.1). Figure 3.2 summarizes the benchmarked DM/WB ratios for PRCs 21, 89 and 205 over time. For any given time point and PRC congener, the benchmarked DM/WB ratio was not significantly different from the expected value of 1 (one sample t-test, p < 0.05).

**Discussion**

By convention, acclimation periods of several days to a week are typically used following IP dosing to allow chemical assimilation and inter-tissue distribution to take place before experimental treatment groups are analyzed (Paterson et al. 2010). While the release of PCB congeners from orally administered oil carriers appears to be slower than from naturally ingested diet items (Sijm et al. 1993), the present study challenges the belief that this claim also holds true for IP injections of PCBs dissolved in oil (Andersson et al. 2001), i.e. unassimilated oil in the peritoneal cavity does not act as a long term reservoir of injected chemical in fish. The results of this study challenge previous research on the Zebrafish which attributed a 90% loss in PCB dosage to leakage out of the IP injection puncture wound (Andersson et al. 2001). In my study, losses of dosed chemical due to leakage of oil from the wound would likely affect all PRCs equally and as such did not account for the near complete assimilation of PCB 89. While oil leakage through a puncture wound may be realized in each dosing situation, it was most likely negligible in the current study. The lack of significant
differences in PCB concentrations with time indicates negligible elimination of chemical following dosage which is expected for highly hydrophobic chemicals such as PCBs over the short time frame of the study (Paterson et al. 2010). This suggests that chemical-to-chemical differences in the target dose fraction were due to either error in the concentration assigned to standards and nominal dose or potentially influenced by removal of small amounts of tissue (liver, egg and brain) from samples prior to processing carcass. Based on Figures 3.1 and 3.2, PRC-PCBs were assimilated into muscle within 6 hours of IP dosing. The average percentage of target dose assimilated in dorsal muscle 6-hours post injection was 27.52 ± 11.85% for PCB 21, 68.24± 26.56% for PCB 89 and 62.48 ± 19.32% for PCB 205. Thus the need for several days of acclimation post dosing would appear unnecessary.

The difference in tissue resistance when inserting the needle into the body cavity compared to muscle makes detection of injection mistakes simple and easy to avoid. For small fish such as round goby, IP injection volumes need to be kept small in order to avoid inundation of blood with oil during assimilation of injected material. Error propagated by manual injection of small oil volumes (as small as 1.5 μL in one 3 g fish in the present study) undoubtedly contributed to individual variation in the received dose. Despite this limitation, the coefficient of variation following IP dose was similar to the error observed for native PCBs in individual fish exposed in the field. Greater precision of dosage would be expected when dosing larger fish since larger injection volumes could be used and measured with greater confidence. The rationale for comparing dorsal
muscle to whole body carcass in the present study was that IP injections do not introduce oil directly into muscle. Individual variation of benchmarked ratios was lower than the non-benchmarked ratios, particularly for time point 4, suggesting that factors other than lipid and lean dry protein contribute to tissue partitioning of PCBs outside of random analytical error. Both lipid equivalent tissue distribution ratios and benchmarked ratios yielded a similar interpretation of rapid PRC assimilation by fish. However, muscle represents a highly perfused tissue and therefore would be expected to receive the assimilated dose rapidly following chemical entry into blood. Further research to investigate the kinetics of chemical distribution to slowly perfused tissues such as skin and fat would be useful to determine time lags related to between tissue distribution post assimilation.

The results from the present study validate the use of IP injections as an acceptable method for administering relatively precise PCB doses to small fish such as the round goby. The IP dose is rapidly assimilated into highly perfused tissues within hours of dosing. To the best of my knowledge, the rapid uptake and distribution of PCBs to muscle has not previously been described in a small fish species over such short time scales. Overall, IP dosing appears to be an effective and precise exposure method for conducting toxicokinetics studies such as whole body elimination rates following a short acclimation period of < 1 d post-dosing.

References

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Fisk, AT, Norstrom, RJ, Cymbalisty, CD, Muir, DCG. (1998). Dietary Accumulation and Depuration of Hydrophobic Organochlorines:


Perch (*Perca flavescens*). *Canadian Journal of Fisheries and Aquatic Sciences*. 64: 1222-1233.


Table 3.1: Average percentage of the nominal dose measured in individuals for selected PRC-PCBs 21, 89 and 205 at 6 hours, 2 days, 4 days and 8 days post-injection. Target dose fraction represents the percent of dose detected in the whole body carcass across sampling time points for select PRC-PCBs. The average coefficient of variation is presented for selected PRCs and native PCB 153 across days.

<table>
<thead>
<tr>
<th>Day</th>
<th>PRC 21</th>
<th>PRC 89</th>
<th>PRC 205</th>
<th>PCB 153</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average mass of nominal dose injected (ng)</td>
<td>149.375</td>
<td>119.500</td>
<td>119.500</td>
<td></td>
</tr>
<tr>
<td>Target Dose Fraction (%)</td>
<td>Day 0.25</td>
<td>31.529</td>
<td>61.449</td>
<td>40.833</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>40.417</td>
<td>85.822</td>
<td>47.725</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>55.535</td>
<td>125.471</td>
<td>69.941</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>16.688</td>
<td>67.189</td>
<td>45.591</td>
</tr>
<tr>
<td>Average CV</td>
<td>Day 0.25</td>
<td>27.005</td>
<td>24.126</td>
<td>39.334</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>50.701</td>
<td>54.405</td>
<td>38.950</td>
</tr>
<tr>
<td></td>
<td>Day 4</td>
<td>42.024</td>
<td>39.058</td>
<td>46.618</td>
</tr>
<tr>
<td></td>
<td>Day 8</td>
<td>100.749</td>
<td>65.341</td>
<td>96.149</td>
</tr>
</tbody>
</table>
**Figure 3.1:** Dorsal muscle/whole body concentration ratios for native PCBs 153 and 180 (A) and non-environmental IP dosed PCBs 21, 89, and 205 (B). Error bars represent ± 1SE. Horizontal dashed lines represent the expected dorsal muscle/whole body concentration ratio for native and non-environmental PCBs upon complete tissue assimilation.
Figure 3.2: Dorsal muscle/whole body concentration ratios for non-environmental IP dosed PCBs 21, 89 and 205 benchmarked to PCB 153 to account for individual variation. Error bars represent ± 1SE. Horizontal dashed lines represent the expected dorsal muscle/whole body concentration ratio for native and non-environmental PCBs upon complete tissue assimilation. IP dosed congeners approach a ratio of 1.6 hours post-injection.
CHAPTER 4

DETERMINATION OF FIELD METABOLIC RATES IN ROUND GOBIES (NEOGOBIUS MELANOSTOMUS) FROM THREE INVADED GREAT LAKES

Introduction

Since the early 1800’s, human-mediated transport has resulted in the introduction of over 180 alien species to the Laurentian Great Lakes (Holeck et al. 2004, Mills et al. 1993). Ricciardi and MacIsaac (2000) discovered a pattern of invasion unique to the Great Lakes ecosystem: approximately 70% of the alien species discovered since 1985 originated from the Black, Caspian and Azov Seas (Ponto-Caspian region). This pattern of invasion derives not only from the climatically matched environments found in the Great Lakes and the Ponto-Caspian regions, but also from the tolerance of Ponto-Caspian biota to habitats of variable water levels and salinities (Ricciardi and MacIsaac, 2000; Kocovsky et al. 2011). In 1990, the round goby (Neogobius melanostomus) was first discovered in the St. Clair River (Jude et al. 1992). Within the first 5 years of their reported invasion, round gobies spread throughout all of the Great Lakes (Marsden et al. 1997). The impacts round gobies have had on the Great Lakes biological communities includes the disruption of food webs (Ng et al. 2008), alteration of energy pathways (Johnson et al. 2005), modification of pollutant transfer (Morrison et al. 2000) and reduction in biodiversity (French and Jude, 2001). It has been suggested that the metabolic capacity of round gobies exceeds that of native species in regions of establishment, thereby making it an effective competitor (Cross and Rawding, 2009); however, few case studies have
quantified the energy requirements of round goby populations or examined how physiological tolerance, related to factors such as metabolic efficiency across temperatures (Lee and Johnson, 2005) varies between Great Lakes populations. Evidence of significant genetic diversity between round goby populations is provided by Brown and Stepień (2009), where they reported genetically distinct populations in Lake Ontario and Lake Huron. An examination of metabolic efficiency, i.e. metabolic rate of fish in response to changing environmental conditions, in round gobies from genetically distinct Great Lakes populations would examine whether genetic divergence parallels differences in physiological tolerance to temperature change in a novel environment.

The most common method for analyzing the standard metabolic rate (SMR) of a species uses respirometry to directly measure an organism's rate of oxygen usage under resting and fasted conditions. In 2005, Lee and Johnson measured the temperature and size dependence of SMR for round gobies and developed the first bioenergetic model for this species. However, the use of respirometry has limitations because it only provides a short-term measure of SMR under artificial conditions and fails to incorporate acclimation capacity and metabolic efficiency over seasonal temperature cycles (Lee and Johnson, 2005; Paterson et al. 2007). Drouillard et al. (2009) provided a method for the estimation of an organism's field metabolic rate (FMR) through the use of a chemical tracer depuration approach. In this method, animals are dosed via intraperitoneal (IP) injection with non-environmental polychlorinated-biphenyl (PCB) performance reference compounds (PRCs) (Raeside et al. 2009) and the
elimination of each PRC is tracked through time under free-living conditions. The main route of chemical elimination in fish occurs via loss across their gill surfaces during respiration according to Equation 4.1 (Drouillard et al. 2009; Paterson et al. 2010):

\[ Q_V = \frac{FMR}{D_{O_2} \cdot \bar{C}_{O_2} \cdot E_{O_2}} \]  

(Equation 4.1)

where \( FMR \) is the organism’s field metabolic rate (kJ/g body weight/d); \( D_{O_2} \) is the oxycalorific coefficient for converting oxygen respired to energy of fish (14.30 kJ/g O\(_2\)) (Norstrom et al. 1976); \( \bar{C}_{O_2} \) is the weighted average concentration of dissolved oxygen in water over time (g O\(_2\)/mL water); and \( E_{O_2} \) is the oxygen exchange efficiency across the gills (0.6; unitless). Thus, for hydrophobic organic chemicals chemical elimination (\( k_{total} \)) can be modelled according to the following equation:

\[ k_{total} = \frac{E_W Q_V}{BCF} \]  

(Equation 4.2)

where \( Q_V \) is the gill ventilation rate (mL/g/d), \( E_W \) is the chemical exchange efficiency across the gills (0.54, unitless), and \( BCF \) is the biota/water equilibrium partition coefficient; \( BCF \sim \) fraction of lipid (\( X_{lipid} \))· octanol water partition coefficient of the chemical (\( K_{OW} \)) (Drouillard et al. 2009). Finally, combining Equation 1.2 and Equation 1.3 allows for the determination of an organism’s FMR:

\[ FMR = \frac{k_2 \cdot D_{O_2} \cdot \bar{C}_{O_2} \cdot E_{O_2} \cdot X_{lipid} \cdot K_{OW}}{E_W} \]  

(Equation 4.3)
In this study I tracked the elimination rates \( (k_{\text{total}}) \) of 14 PRC congeners in round goby populations as a surrogate measure of field metabolic rate during a period of rapid temperature change. Round goby populations tested included fish collected from Lake St. Clair, Lake Huron and Lake Ontario. Among these populations, Lake Huron round gobies have the greatest differences in genetic structure compared to Lake St. Clair and Lake Ontario populations (Brown and Stepie, 2009). Specifically, I tested the hypothesis that field metabolic rates and temperature acclimation capacities varies between round goby populations, such that Lake St. Clair and Lake Ontario fish will show greater similarities in FMR response compared to Lake Huron populations. This hypothesis was generated under the assumption that genetic characteristics of round goby sub-populations are matched with environmental temperature characteristics in their respective invasive ranges.

**Materials and Methods**

*Sample collection and fish husbandry*

IP injections were used to calculate the FMRs of round gobies from three Great Lakes populations. Individuals were sampled by angling, beach seine and minnow traps from 2 locations in Lake St. Clair (42°20'14.8452"N, 82°54'58.1466"W and 42°18'25.0776"N, 83°4'31.1376"W), 2 locations in Lake Huron (43°18'47.0844"N, -81°45'56.0844"W and 44°33'52.4448"N, -80°26'55.158"W) and 2 locations in Lake Ontario (44°2'29.9652"N, -77°3'28.584"W and 44°2'53.0478"N, -77°3'3.0018"W). Prior to the initiation of the experiment, individuals were held in 550 liter polyethylene flow-through aquaria
under ambient environmental temperature and photoperiod in the aquatic facility at the Great Lakes Institute for Environmental Research (University of Windsor, Windsor, ON, Canada). Throughout the course of the experiment, fish were fed *ad libitum* Premium Soft Krill Pellet from Angels Plus (Olean, NY, USA). Water temperatures were measured four times daily by digital temperature loggers submersed within each tank (HOBOware Pro Version 3.4.0, Onset Computer Corporation). Water quality was monitored weekly by measuring pH, dissolved oxygen, temperature, conductivity and oxidation/reduction potential (Hydrolab, Campbell Scientific Corp., Edmonton, AB, Canada) (See Table 4.1). This research was conducted with approval from the University of Windsor's Animal Care Committee.

*IP injection with PRC-PCB congener mixture and sacrifice schedule*

Environmentally rare PCBs, henceforth designated as performance reference compounds (PRCs), were selected as dosing chemicals such that they could be analytically distinguished from native (environmentally accumulated) PCBs (Raeside et al. 2009; O'Neil et al. 2013). The dosing mixture contained the following PCBs (IUPAC #s): 13, 21, 23, 43, 62, 89, 57, 68, 112, 125, 166, 204 and 205 derived from individual standards of neat chemical (AccuStandard, New Haven, CT, USA). This mixture was initially dissolved in hexane and then diluted in sunflower oil to a nominal dose of 40ng/μL for PCBs 13, 23, 43, 62, 89, 68, 112, 125, 166, 204, 205; 50ng/mL for PCB 21; and 200ng/mL for PCB 57. The log Kow values for the PRC compounds ranged between 5.06 and 8.0 (Hawker and Connell, 1988).
After acclimation to experimental conditions for a minimum of 4 weeks, round gobies of masses (mean ± SE) 11.76 ± 0.92g from Lake St. Clair, 8.52 ± 0.43g from Lake Huron and 6.41 ± 0.38g from Lake Ontario were dosed on Day 0 of the experiment (September 24, 2012) using a 10µL or 25µL Hamilton MICROLITER® Syringe, depending on total volume injected. Fish were randomly assigned as control or treatment individuals for each lake population. Prior to dosing, each fish was netted from its holding tank, lightly anaesthetized with clove oil (40mg/L) and weighed. Treatment fish were injected with a mass dependent volume of the PRC mixture to achieve the target nominal dose. Target tissue concentrations for individual PRCs in fish tissue were 20ng/g for PCBs 6, 13, 23, 43, 62, 89, 68, 112, 125, 166, 204, 205; 25 ng/g for PCB 21 and 100ng/g for PCB 57. Control fish were sham dosed with a mass dependent volume of sunflower oil containing no PRCs. After injection, fish were returned to their population-specific holding tanks; control and treatment individuals were separated by a mesh divider. The total numbers of fish within each population at the initiation of the experiment were as follows: Lake St. Clair \(N = 88\) treatment, \(N = 50\) control; Lake Huron \(N = 85\) treatment, \(N = 47\) control; and Lake Ontario \(N = 88\) treatment, \(N = 50\) control.

I used annual water temperature data recorded for Lake St Clair in 2011 to identify specific thermal conditions that would dictate when fish would be sacrificed. Sample dates were selected to encompass points within Lake St. Clair’s cooling period as described in Figure 4.1. Four experimental and two control individuals from each lake population were sacrificed on days 0.25, 2, 25,
60, 87, 95 and 97 of the study. On each sacrificed day fish were euthanized using a clove oil/ethanol solution (1:9) then weighed, sexed, and measured for standard length (mm). After processing, each individual was ground into a whole body homogenate using a stainless steel blender and placed in labeled, hexane rinsed aluminum tins. Samples were stored in a freezer at -23ºC until chemical analysis.

Procedure for tissue analysis of PCB content

The moisture, lipid and PCB content of each sample was analyzed as per Daley et al. (2009). For each set of 6 samples extracted, a reference homogenate (Detroit River Carp), method blank, PCB 34 recovery standard, external PCB standard (Quebec Ministry of Environment Congener mix; AccuStandard, NewHaven, CT, USA) and PRC standard (AccuStandard, New Haven, CT, USA) were analyzed. To summarize, activated sodium sulfate(15g) was used to grind approximately 0.5g of tissue using a mortar and pestle and then packed into glass columns containing 25mL of 50:50(v/v) dichloromethane:hexane. Each sample was spiked with 50µL of PCB 34 as an internal recovery standard and left for 1 hour. As the columns dripped, an additional 15mL of 50:50 dichloromethane:hexane was added to the columns and eluted. Once elution was complete, the samples were concentrated to ~ 2mL using vacuum evaporation. Lipid determination involved bringing the extracts to a total volume of 10mL. One mL of the extract was removed to determine neutral lipid content of the sample by placing it on an aluminum weigh boat and allowing the solvent to evaporate (Drouillard et al. 2004). The remaining extract was
subsequently concentrated to ~2mL using vacuum evaporation for Florisil cleanup (Lazar et al. 1992). Collection of the first fraction of extract used 50mL of 50:50 (v/v) dichloromethane:hexane and collection of the second fraction of extract used 50mL of 15:85 (v/v) dichloromethane:hexane. Each fraction of each sample was collected in separate receiving flasks, concentrated to ~0.5mL using vacuum evaporation, brought to a final volume of 1mL with isoctane and stored in 2mL glass capping vials. Each fraction was analyzed for PCBs by gas chromatography electron capture detection (GC-ECD) (Lazar et al. 1992).

Data Analysis

The average percent recovery of internal PCB 34 standard was 93.81 ± 11.21% (mean ± SD ) and ranged between 70.01-118.82%. The concentration of native PCBs in reference homogenates were within 2 standard deviations of the analytical laboratory database values. No corrections were made for PCB recoveries where concentrations are reported. Analysis of variance (ANOVA), analysis of covariance (ANCOVA), linear regression and principal component analysis (PCA) were used for statistical analyses and completed using SYSTAT software (Systat Software Inc. 2008). Significance levels of α < 0.05 were used as a criteria for significant differences for ANOVA, ANCOVA and linear regressions. Analysis of variance (ANOVA) was used to test for significant differences in the fraction of whole body lipids and body weights for individuals from each population as a function of time. Round goby FMRs (i.e. chemical elimination rates, $k_{\text{total}}$) were calculated from linear regressions of the natural logarithm of whole body lipid-normalized PRC PCB concentrations as a function
of time. In the equation $y = mx + b$, $m$ represents $k_{\text{total}}$, the elimination rate of the chemical. ANOVAs were used to test the significance of $k_{\text{total}}$ for each congener within each lake population. By testing for differences in the $k_{\text{total}}$ of congeners between lake populations, I was able to infer differences in their FMRs. To evaluate whether there were significant differences in the $k_{\text{total}}$ values for PRC-PCB congeners between lake populations, principal component analysis (PCA) was performed. Subsequent ANOVA was used to test for the significance of the population x time interaction term for PCA scores for each PCA axis.

Furthermore, a post-hoc analysis separated individuals from all three lake populations into small ($\leq 6.9$ cm standard length (SL)), medium ($7.0$-$8.4$ cm SL) and large ($\geq 8.5$ cm SL) size classes as per Ray and Corkum (1997). After testing for the study day x population interaction using ANOVA, study day was used as a covariate in ANCOVA to evaluate whether there were significant differences in the $k_{\text{total}}$ values for PRC-PCB 21 between size classes.

**Results**

Water temperatures showed large changes over the 97 day study duration commencing at $18.54^\circ$C and ending at $2.76^\circ$C. The average rate of temperature change experienced was $0.42^\circ$C·d$^{-1}$. Throughout the course of the study the observed mortality for Lake St. Clair, Lake Huron and Lake Ontario were 7.4%, 1.5% and 1.0% respectively. Figure 4.3 provides the mean fraction of lipid for individuals from each population as a function of time. There were significant differences in the fraction of lipid for Lake St. Clair and Lake Ontario populations ($p < 0.05$) over time, but no significant differences were found in the fraction of
lipid for the Lake Huron population (p > 0.05). The average whole body lipid fractions ± SE for Lake St. Clair, Lake Huron and Lake Ontario were 0.036 ± 0.002, 0.029 ± 0.002 and 0.034 ± 0.004, respectively for the entire study period. The increase in % lipid over the experiment was 77.1 % for Lake St. Clair, 9.3% for Lake Huron, and 33.9% for Lake Ontario. Thus, while body weight changes appeared to be negligible, there was some evidence for a change in the proximate composition of tissues, favoring higher lipid contents at the end of the study. In addition, there was a significant negative correlation between fish body lipid and temperature yielding the following equations:

\[
\%\text{Lipid} = -0.0011 \times \text{Temperature(°C)} + 0.0449; R^2 = 0.257 \text{ (Lake St. Clair)}; \\
\text{Equation 4.1}
\]

\[
\%\text{Lipid} = -0.001 \times \text{Temperature(°C)} + 0.0461; R^2 = 0.3416 \text{ (Lake Huron)}; \\
\text{Equation 4.2}
\]

\[
\%\text{Lipid} = -0.003 \times \text{Temperature(°C)} + 0.0317; R^2 = 0.215 \text{ (Lake Ontario)}; \\
\text{Equation 4.3}
\]

Control fish, collected in conjunction with treatment fish were analyzed for the presence of PRC-PCBs. No PRC-PCBs were detected in controls indicating no recycling of PRC-PCBs in tanks following their elimination from fish. Most injected PRC-PCBs demonstrated significant elimination over the course of the study. The exceptions were PCB 6 and 13 which had a number of non-detections in dosed fish which precluded accurate determination of \(k_{\text{total}}\) for these compounds. PCB 205, the most hydrophobic congener, exhibited significant
(ANOVA, p < 0.05) elimination in only 1 goby population (Lake Huron) (Table 4.2). PCB 125 was significantly (ANOVA, p < 0.05) eliminated by two goby populations (Lake St. Clair and Lake Huron) and non-significantly (ANOVA, p < 0.05) eliminated by the Lake Ontario population (Table 4.2). Figure 4.5 illustrates temporal trends of the lipid normalized PRC-PCB concentrations ± SE for representative low, medium and high octanol water partition coefficients (K\text{ow}) congeners (PCB 21, K\text{ow} = 5.5; PCB 57, K\text{ow} = 6.17; PCB 204, K\text{ow} = 7.30) over time. As demonstrated in Figure 4.5 PRC elimination was generally K\text{OW} dependent, proceeding most rapidly for PCB 21 and decreasing for more hydrophobic congeners.

Linear regressions of the natural logarithm of PRC-PCB concentrations in fish lipids through time for each of the PCB congeners and goby populations are summarized in Table 4.2. A plot of the chemical elimination rate coefficient (k\text{total}) versus chemical K\text{OW} is provided by Figure 4.6. There was a strong negative relationship between chemical elimination rate coefficient and chemical K\text{OW} up to log K\text{OW} values of 6.5, after which the decreasing trend in PRC-PCB elimination fails to decrease with further increases in chemical hydrophobicity. The lack of K\text{OW} dependent chemical elimination for these highly hydrophobic chemicals are likely a result of pseudo-elimination occurring as a consequence of lipid dilution rather than true chemical elimination occurring in the study. Indeed, the population specific trends in elimination for the most hydrophobic congeners tend to follow the same trends reported for lipid content of fish tissues.

The principal component analysis generated a total of 5 axes, however
only axis 1 and axis 2 had significant loadings (> 0.7). Furthermore, axis 1 and axis 2 explained 75.4% and 10.6% of total model variation respectively. PRC-PCBs 43, 68, 57, 89, 112, 125, 166, 204 and 205 were strongly associated with axis 1 and PRC-PCB 21 was strongly associated with axis 2. PRC-PCB 62 was not strongly associated with any axis. ANOVAs were ran for PCA scores generated for PCA axes 1 and 2. For axis 1, the population x time interaction term was not significant (ANOVA, p > 0.05), which indicated no significant differences in the \( k_{\text{total}} \) values between round goby populations for PRC-PCBs 43, 68, 57, 89, 112, 125, 166, 204 and 205. For axis 2, the population x time interaction term was also not significant (ANOVA, p > 0.05), indicating no difference in the \( k_{\text{total}} \) values between round goby populations for PRC-PCB 21. To explore this relationship further, I classified individuals from all lake populations into small (\( \leq 6.9 \) cm standard length (SL)), medium (7.0-8.4 cm SL) and large (\( \geq 8.5 \) cm SL) three size classes as per Ray and Corkum (1997). Post-hoc ANOVA on \( k_{\text{total}} \) values for PRC-PCB 21 found a non-significant (p > 0.05) interaction between study day and fish size. After removing the interaction term and using study as a covariate, there was a significant difference (p < 0.05) between the \( k_{\text{total}} \) values for PRC-PCB 21 between size classes.

**Discussion**

In the present research, elimination of PRC-PCBs were used as a surrogate measure of field metabolic rate (FMR) for round gobies. Previous studies have demonstrated that PCB elimination by fish is dominated by elimination across the gills (Paterson et al. 2010; Drouillard et al. 2009), such that
the rate of chemical elimination is regulated by gill ventilation rate (mL·g⁻¹ fish·d⁻¹) and chemical-specific properties, such as the degree of chlorine substitution, which are related to diffusive resistances across the gills. Drouillard et al. (2009) further showed that gill ventilation rate can be related to oxygen consumption rates (i.e. metabolic rate of fish) following correction for oxygen concentration in water and the oxygen extraction efficiency of fish gills (See Equations 4.1 and 4.2). Furthermore, they determined that the daily loss of PCBs by yellow perch were strongly dependent on water temperature, a chief determinant of fish metabolic rate (Drouillard et al. 2009). Evidence for the temperature-dependence of metabolic rate in fish has been documented in studies on a variety of other fish species, including European sea bass (*Dicentrarchus labrax*) (Claireaux and Lagardère, 1999), spined loach (*Cobitis taenia*) (Maciak and Konarzewski, 2010), mulloway (*Argyrosomus japonicus*: Sciaenidae), and yellowtail kingfish (*Seriola lalandi*: Carangiidae) (Pirozzi and Booth, 2009). Research directly related to the metabolic rates of round gobies is presented by Lee and Johnson (2005), where respirometry was used to measure the routine metabolic rates of round gobies of various sizes. In their study, Lee and Johnson (2005) showed that round gobies exhibited a five-fold increase in oxygen consumption between experimental temperatures of 3 and 27°C and that oxygen consumption decreased 2-fold between fish of 2 and 30g. Their results suggested that the reduced metabolic rates (i.e. increased metabolic efficiency) of larger round gobies supersedes that of smaller individuals.

While most PRC-PCBs exhibited significant elimination by round goby
populations, an unexpected observation from this research was the lack of $K_{ow}$ dependent elimination for PRC-PCBs having log $K_{ow}$s greater than 6.5. PCBs 112, 125, 166, 204 and 205 all had similar $k_{tot}$ values (ranging from 0.005 to 0.012 d$^{-1}$) despite a two order magnitude difference in chemical hydrophobicity. The lack of trends observed between chemical hydrophobicity and $k_{tot}$ measured for high $K_{ow}$ PCBs in the present study suggests that other factors (e.g. changes in proximate tissue composition, pseudo-elimination, and lipid dilution) may have contributed to the observed elimination patterns. Although elimination across the gills contributes significantly to the overall elimination of PCBs in fish (Drouillard et al. 2009), for congeners with $K_{ow}$ values higher than $10^5$ this relationship breaks down; respiratory elimination becomes less significant compared to dietary elimination for super hydrophobic chemicals (Kelly et al. 2004). Other pathways that contribute to chemical elimination in fish include fecal egestion and metabolic transformation (Gobas et al. 1988; Arnot and Gobas, 2004; Kelly et al. 2004). Furthermore, negative relationships between chemical hydrophobicity and elimination rates for PCBs have been commonly reported for fish (Paterson et al. 2010; Paterson et al. 2007; Fisk et al. 1998; Sijm et al. 1983; Niimi and Oliver, 1983), although some exceptions have been noted such as an increase in chemical elimination rates for extremely hydrophobic chemicals (Fisk et al. 1998; Buckman et al. 2006). Fisk et. al (1998) present the idea that a PCB congener's inverse relationship between the hydrophobicity and time to steady state ultimately influences its availability for elimination. That is, congeners of higher $K_{ow}$ achieve steady state within an
organism slower than congeners with lower $K_{ow}$, and are more readily available for elimination in comparison. Previous studies have shown that the half-lives, or the time to eliminate half of the chemical burden present within an organism, are shortest in small fish (Niimi and Oliver, 1983), thereby owing to their increased rates of chemical elimination (Fisk et al. 1998). In my post-hoc analysis, I found significant differences (ANCOVA, $p < 0.05$) in $k_{total}$ for PRC-PCB 21 between the size classes of fish from all lakes (Figure 4.7). The small size class of fish from all of the lakes had the fastest $k_{total}$, which fits with the findings of previous outlined above. It is important to take note that the Lake St. Clair population is skewed, with no individuals falling under the small size classification and most individuals falling under the large size classification. Furthermore, the observed increases in lipid content of gobies from Lake St. Clair and Lake Ontario suggest that pseudo-elimination may have taken place for highly hydrophobic chemicals like PRC-PCB 205. In other words, the decrease in lipid normalized PCB concentration occurred due to lipid dilution as opposed to mass elimination of chemical from the body. These effects would be expected to affect all chemicals equally, whereas diffusive based elimination would most likely only occur for less hydrophobic chemicals ($3 < K_{ow} < 6$). Validation of this hypothesis would require performing mass balance calculations which unfortunately could not be done with the current data set. I speculate that although fish were consuming minimal amounts of artificial feed throughout the course of this experiment and experienced non-significant ($p > 0.05$, ANOVA) growth, these fish were accumulating lipids without growth (See Figure 4.3). Although each goby was
initially injected with a PRC-PCB dose to achieve the same whole body concentration, the given size range of gobies at day zero (1.8-25.7g) involved considerable differences in the mass of chemical injected into individuals. Since individual gobies were not uniquely tagged, individual growth corrections and mass balance calculations could not be performed.

In this study, non-environmental PCB congeners were dosed to fish in order to track, on a relative basis, differences in field metabolic rates of different Great Lakes round goby populations. The null hypothesis for the study was that different fish populations would show similar elimination rates of chemicals following dosing and exposure to similar environmental conditions. Based on results from PCA analysis, I accept the null hypothesis after finding non-significant (p > 0.05) differences in the elimination rates of PRC-PCB congeners 21, 43, 62, 68, 57, 89, 112, 125, 166, 204, and 205 between lake populations. Combining the results of the PCA analysis and the potential for pseudo-elimination of higher $K_{OW}$ chemicals as described previously, the weight of evidence suggests no major differences in PCB elimination between populations and hence similar field metabolic rates for the three populations.

While it has been shown that round goby populations within Lake Huron and Lake Ontario are genetically distinct from other Great Lakes populations (Brown and Stepien, 2009; Dillon and Stepien, 2001), traits governing the successful range distribution of this species remain conserved across Great Lakes populations. Round gobies are characterized by a wide range of physiological tolerance to environmental conditions (Corkum et al 1998) including
variable water levels, salinities (Ellis and MacIsaac, 2009) and temperatures (Lee and Johnson, 2005) which facilitated their successful transport to the Great Lakes via transoceanic shipping vessels (Holeck et al. 2004; Ricciardi and MacIsaac, 2000; Ellis and MacIsaac, 2009). Neilson and Stepien (2009) presented the European range distribution of various gobiid species in the native Ponto-Caspian region, and showed that round goby populations are distributed over the greatest latitudes at both the northern and southern range (50ºN and 40ºN latitude) compared to all other species. This suggests that round gobies have the greatest physiological tolerance to temperature of all gobiid species within their native ranges (Neilson and Stepien, 2009) and in turn may have been the most tolerant to Great Lakes conditions.

In the present research, round gobies were subject to substantial temperature shifts over the course of the study necessitating that individuals undergo warm-to-cold water acclimation, a process that typically entails substantial protein turnover in order to express temperature appropriate enzymes necessary for homeostasis (Schmidt-Nielsen, 1990). Given the genetic differences previously reported among the three study populations, it was expected that such differences would manifest themselves in the acclimation responses of different goby populations to rapid changes in temperature. This hypothesis is consistent with population genetic theory which proposes that the potential of a species to adapt to its local environment is influenced by its genetic diversity (Lee, 2002). That is, populations with increased genetic diversity are expected to display a broader range of phenotypes that are differentially suited to
their introduced environment. Overall, I found no consistent differences in the FMRs of genetically distinct round goby populations from Lake St. Clair, Lake Huron and Lake Ontario when subjected to a rapid change in environmental temperatures. While genetic differences between Great Lakes populations of round gobies have been well documented (Dillon and Stepien, 2001; Stepien and Tumeo, 2006; Brown and Stepien 2009), results from my study show that physiological tolerance, as measured by temperature dependent FMR appears to be conserved across populations. Future research should aim to investigate relationships between the genetic diversity of round goby populations within the Great Lakes and other measures of physiological tolerance such as tolerance to dissolved oxygen, turbidity or other stressors, as well as biotic interactions including attributes of intra- and interspecific competition. A greater understanding of how genetic diversity contributes to phenotype expression in round gobies would provide insight into this invasive species' potential for continued population growth and range expansion throughout the Great Lakes.

References


Raeside, AA, O'Rourke, SM, Drouillard, KG. (2009). Determination of in situ Polychlorinated Biphenyl Elimination Rate Coefficients in the Freshwater Mussel Biomonitor *Elliptio Complanata* Deployed in the Huron-Erie
Corridor, Southeast Michigan, USA, and Southwest Ontario, Canada.


Table 4.1: Weekly water quality parameters measured in flow-through holding tanks used to evaluate field metabolic rates of round gobies between October 2, 2013 to December 30, 2012. Parameters include water temperature (°C), specific conductivity (SPC) (mS/cm), dissolved oxygen (mg/L), pH and oxidation/reduction potential (mV).

<table>
<thead>
<tr>
<th>Date (MM-DD-YY)</th>
<th>Temperature (°C)</th>
<th>SPC (mS/cm)</th>
<th>Dissolved Oxygen (mg/L)</th>
<th>pH</th>
<th>Oxidation/Reduction Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-2-12</td>
<td>16.5</td>
<td>0.239</td>
<td>10.46</td>
<td>7.48</td>
<td>292</td>
</tr>
<tr>
<td>10-9-12</td>
<td>14.8</td>
<td>0.215</td>
<td>10.99</td>
<td>7.99</td>
<td>281</td>
</tr>
<tr>
<td>10-16-12</td>
<td>12.4</td>
<td>0.229</td>
<td>9.98</td>
<td>7.82</td>
<td>327</td>
</tr>
<tr>
<td>10-23-12</td>
<td>13.65</td>
<td>0.311</td>
<td>11.94</td>
<td>7.94</td>
<td>320</td>
</tr>
<tr>
<td>10-30-12</td>
<td>10.11</td>
<td>0.210</td>
<td>12.55</td>
<td>7.66</td>
<td>309</td>
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<tr>
<td>11-9-12</td>
<td>6.98</td>
<td>0.256</td>
<td>12.98</td>
<td>7.56</td>
<td>287</td>
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<tr>
<td>11-15-12</td>
<td>7.02</td>
<td>0.223</td>
<td>12.45</td>
<td>7.82</td>
<td>326</td>
</tr>
<tr>
<td>11-21-12</td>
<td>8.51</td>
<td>0.241</td>
<td>13.02</td>
<td>7.54</td>
<td>315</td>
</tr>
<tr>
<td>11-30-12</td>
<td>5.78</td>
<td>0.250</td>
<td>12.57</td>
<td>7.62</td>
<td>328</td>
</tr>
<tr>
<td>12-08-12</td>
<td>6.22</td>
<td>0.234</td>
<td>12.22</td>
<td>7.85</td>
<td>316</td>
</tr>
<tr>
<td>12-13-12</td>
<td>5.00</td>
<td>0.236</td>
<td>14.71</td>
<td>6.46</td>
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</tr>
<tr>
<td>12-17-12</td>
<td>5.75</td>
<td>0.233</td>
<td>11.59</td>
<td>7.10</td>
<td>287</td>
</tr>
<tr>
<td>12-21-12</td>
<td>5.93</td>
<td>0.241</td>
<td>13.52</td>
<td>6.72</td>
<td>354</td>
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<tr>
<td>26-12-12</td>
<td>2.69</td>
<td>0.259</td>
<td>12.80</td>
<td>7.71</td>
<td>359</td>
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<td>30-12-12</td>
<td>2.42</td>
<td>0.282</td>
<td>12.91</td>
<td>7.67</td>
<td>373</td>
</tr>
</tbody>
</table>
Table 4.2: Lipid normalized chemical elimination rates ($k_{total}$) for each PRC-PCB congener in round gobies from Lakes St. Clair Huron and Ontario. PRC $K_{OW}$ values are from Hawker and Connell (1988). $N$ is the number of individuals fish used to estimate $k_{total}$, $p$ is the p-value from the analysis of variance (ANOVA), and $r^2$ is the strength of the correlation. Non-significant $k_{total}$ values are indicated by (*).

<table>
<thead>
<tr>
<th>PCB (IUPAC #)</th>
<th>$K_{OW}$</th>
<th>Lake St. Clair $k_{total}$ ± SE ($N$, $p$, $r^2$)</th>
<th>Lake Huron $k_{total}$ ± SE ($N$, $p$, $r^2$)</th>
<th>Lake Ontario $k_{total}$ ± SE ($N$, $p$, $r^2$)</th>
</tr>
</thead>
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<tr>
<td>21</td>
<td>5.51</td>
<td>-0.027 ± 0.005 (27, 0.000, 0.566)</td>
<td>-0.040 ± 0.004 (27, 0.000, 0.752)</td>
<td>-0.032 ± 0.005 (20, 0.000, 0.637)</td>
</tr>
<tr>
<td>43</td>
<td>5.75</td>
<td>-0.011 ± 0.004 (28, 0.008, 0.213)</td>
<td>-0.013 ± 0.002 (28, 0.000, 0.591)</td>
<td>-0.011 ± 0.003 (26, 0.002, 0.313)</td>
</tr>
<tr>
<td>62</td>
<td>5.89</td>
<td>-0.016 ± 0.004 (28, 0.001, 0.312)</td>
<td>-0.026 ± 0.003 (28, 0.000, 0.689)</td>
<td>-0.013 ± 0.003 (26, 0.001, 0.344)</td>
</tr>
<tr>
<td>68</td>
<td>6.26</td>
<td>-0.012 ± 0.004 (28, 0.007, 0.220)</td>
<td>-0.009 ± 0.002 (28, 0.001, 0.340)</td>
<td>-0.008 ± 0.003 (26, 0.029, 0.149)</td>
</tr>
<tr>
<td>57</td>
<td>6.17</td>
<td>-0.016 ± 0.005 (28, 0.002, 0.298)</td>
<td>-0.014 ± 0.005 (28, 0.006, 0.225)</td>
<td>-0.016 ± 0.004 (26, 0.001, 0.334)</td>
</tr>
<tr>
<td>89</td>
<td>6.07</td>
<td>-0.013 ± 0.004 (28, 0.002, 0.299)</td>
<td>-0.010 ± 0.002 (28, 0.000, 0.406)</td>
<td>-0.008 ± 0.003 (26, 0.030, 0.148)</td>
</tr>
<tr>
<td>112</td>
<td>6.45</td>
<td>-0.009 ± 0.004 (28, 0.016, 0.174)</td>
<td>-0.006 ± 0.003 (28, 0.023, 0.152)</td>
<td>-0.009 ± 0.003 (26, 0.017, 0.184)</td>
</tr>
<tr>
<td>125</td>
<td>6.51</td>
<td>-0.010 ± 0.004 (28, 0.035, 0.127)</td>
<td>-0.008 ± 0.002 (28, 0.003, 0.269)</td>
<td>-0.005 ± 0.004 (26, 0.156, 0.044) *</td>
</tr>
<tr>
<td>166</td>
<td>6.93</td>
<td>-0.011 ± 0.004 (28, 0.008, 0.212)</td>
<td>-0.006 ± 0.003 (28, 0.018, 0.167)</td>
<td>-0.008 ± 0.004 (26, 0.040, 0.130)</td>
</tr>
<tr>
<td>204</td>
<td>7.30</td>
<td>-0.012 ± 0.005 (28, 0.022, 0.154)</td>
<td>-0.006 ± 0.003 (28, 0.030, 0.137)</td>
<td>-0.009 ± 0.003 (26, 0.020, 0.172)</td>
</tr>
<tr>
<td>205</td>
<td>8.00</td>
<td>-0.007 ± 0.004 (28, 0.112, 0.059) *</td>
<td>-0.006 ± 0.003 (28, 0.041, 0.118)</td>
<td>-0.006 ± 0.004 (26, 0.108, 0.067) *</td>
</tr>
</tbody>
</table>
Table 4.3: Component loadings for select PRC-PCB congeners from principal component analysis (PCA). Component loadings of 0.7 were considered significant and are indicated by (*). PCA axes 1 and 2 explained 75.4% and 10.6% of model variation, respectively. Across PRC-PCBs, most chemicals loaded on PCA axis 1; only PRC-PCB 21 loaded on PCA axis 2. PRC-PCB 62 did not load on either axis.

<table>
<thead>
<tr>
<th>PRC congener</th>
<th>Axis 1 Component Loadings</th>
<th>Axis 2 Component Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>0.491</td>
<td>-0.747*</td>
</tr>
<tr>
<td>43</td>
<td>0.957*</td>
<td>-0.195</td>
</tr>
<tr>
<td>62</td>
<td>0.623</td>
<td>-0.628</td>
</tr>
<tr>
<td>68</td>
<td>0.976*</td>
<td>0.072</td>
</tr>
<tr>
<td>57</td>
<td>0.765*</td>
<td>0.081</td>
</tr>
<tr>
<td>89</td>
<td>0.968*</td>
<td>-0.001</td>
</tr>
<tr>
<td>112</td>
<td>0.957*</td>
<td>0.152</td>
</tr>
<tr>
<td>125</td>
<td>0.925*</td>
<td>0.204</td>
</tr>
<tr>
<td>166</td>
<td>0.961*</td>
<td>0.114</td>
</tr>
<tr>
<td>204</td>
<td>0.895*</td>
<td>0.202</td>
</tr>
<tr>
<td>205</td>
<td>0.879*</td>
<td>0.214</td>
</tr>
</tbody>
</table>
Figure 4.1: Experimental temperatures observed during the evaluation of field metabolic rates of round gobies between Day 0 (September 24, 2012) to Day 97 (December 30, 2013). Arrows indicate sampling dates.
Figure 4.2: Mean body weight ± S.E. as a function of time for round gobies originating from Lake St. Clair (■), Lake Huron (△), and Lake Ontario (●). A total of 6 round gobies (4 treatment and 2 control) were sampled from each population on each sacrifice day (N = 42 for each population). Lake Ontario's population showed significant (p < 0.05, ANOVA) growth between the beginning and end of the study.
Figure 4.3: Mean fraction of lipid ± S.E (% lipid / 100), as a function of time for round gobies originated from Lake St. Clair (■), Lake Huron (Δ), and Lake Ontario (●). A total of 6 round gobies (4 treatment and 2 control) were sampled from each lake population on each sacrifice day (N = 42 for each population). Lake Ontario’s population showed significant (p < 0.05, ANOVA) growth between the beginning and end of the study. Lake Ontario’s population showed significant (p < 0.05, ANOVA) growth between the beginning and end of the study.
Figure 4.4: Mean fraction of lipid ± S.E (% lipid / 100) as a function of temperature for round gobies originating from Lake St. Clair (■), Lake Huron (△), and Lake Ontario (●). A total of 4 treatment individuals were sampled from each lake population on each sacrifice day (N = 28 for each population). Lake Ontario’s population showed significant (p < 0.05, ANOVA) growth between the beginning and end of the study. Solid line represents Lake St. Clair linear regression, $y_{LSC} = -0.0011x + 0.0449$, $R^2 = 0.26$. Dotted line represents Lake Huron linear regression, $y_{LH} = -0.001x + 0.0461$, $R^2 = 0.34$ and dashed line represents Lake Ontario linear regression, $y_{LO} = -0.0003x + 0.0317$, $R^2 = 0.22$. 
Figure 4.5: Mean lipid normalized concentrations ± SE (ng·g⁻¹) of PCB 21, 57 and 204 over time for round gobies originating from Lake St. Clair (■, solid line), Lake Huron (Δ, dotted line), and Lake Ontario (●, dashed line). A total of 4 treatment individuals were sampled from each lake population on each sacrifice day (N = 28 for each population). Slope of the line represents the chemical elimination rate (k_{total}) (See Table 4.2).
Figure 4.6: Relationship between $K_{OW}$ and average $k_{total} \pm S.E.$ (elimination rate) for all PRC-PCB congeners for round gobies originating from Lake St. Clair (■), Lake Huron (△), and Lake Ontario (●). $K_{OW}$ values for PRC-PCB congeners are from Hawker and Connell (1988).
Figure 4.7: Elimination rate ($k_{\text{total}}$) of PRC-PCB 21 for small (●, top graphic, ≤ 6.9 cm standard length (SL)), medium (■, middle graphic, 7.0-8.4 cm SL) and large (▲, bottom graphic, ≥ 8.5 cm SL) size classes of round gobies originating from Lake St. Clair, Lake Huron and Lake Ontario as a function of time. There were no small size class round gobies from Lake St. Clair. The slopes of the linear regressions represent $k_{\text{total}}$ for PRC-PCB 21. $R^2$ is the correlation coefficient. The dotted line represents the small size class linear regression, $y_S = -0.0128x + 8.1198$, $R^2 = 0.570$; the dashed line represents the medium size class linear regression, $y_M = -0.0094x + 7.5574$, $R^2 = 0.040$ and the solid line represents the large size class linear regression, $y_L = -0.01x + 7.0755$, $R^2 = 0.004$. 

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CHAPTER 5

GENERAL DISCUSSION

General Discussion

The research presented in this thesis has improved our understanding of the influence of temperature on physiological processes in two closely related invasive fish species; round and tubenose gobies. In chapter 2, I presented a comparison of standard metabolic rates (SMRs) between round and tubenose gobies using intermittent flow of respirometry. To the best of my knowledge, this was the first study to present respirometry measurements on tubenose goby for comparison with the more well studied round goby. Overall the results from chapter 2 were consistent with other studies examining the temperature dependence of SMRs in fish (Cech et al. 1994; Lee and Johnson, 2005; Clarke and Johnston; 1999; Mesa et al. 2013). The findings of this study showed that in general, tubenose gobies had higher $\text{SMR}_{\text{min}}$ than round gobies at most temperatures except the 30°C and suggested that tubenose gobies reached their metabolic optima at a lower temperature. When round and tubenose goby $\text{SMR}_{\text{total}}$ were compared, tubenose gobies demonstrated higher average $\text{SMR}_{\text{total}}$ across all temperature treatments. At higher temperatures, tubenose goby $\text{SMR}_{\text{total}}$ increased over that of round goby due to elevated activity associated with a heat stress response. This suggests that acute stress occurred in the tubenose goby compared to a lower stress response in the round goby, even though both species were outside their temperature optima. Such trends are expected between species with different temperature optima and temperature
tolerance breadth (Ford et al. 2004). The metabolic efficiency of a species, as described by $\text{SMR}_{\text{min}}$ and $\text{SMR}_{\text{total}}$, over a range of temperatures may provide a useful metric to compare and contrast the acute stress response and temperature tolerance of closely related species pairs. Due to the gap in literature concerning the tubenose goby, continued research is imperative to gain an improved understanding of this species' physiological tolerance to environmental stressors such as turbidity and low dissolved oxygen, life history traits such as reproductive strategy and fecundity, and feeding ecology as described by diet preferences.

Chapter 3 addressed a gap in literature regarding the time required for fish tissues to assimilate hydrophobic chemicals following IP injection and investigated whether or not the tissue distribution of a chemical following IP dosing is equivalent to dietary exposure methods. Andersson et al. (2001) investigated the latter and demonstrated that an IP injection of 20 PCB congeners in Zebrafish (*Danio rerio*) resulted in an equivalent chemical distribution to tissues after 7 days when compared to fish dosed by food. This chapter examined the assimilation of non environmental polychlorinated biphenyl (PCB) reference compounds (PRCs) in round gobies after IP injection and compared tissue distribution of the dose to the tissue distribution of PCBs bioaccumulated by the same fish through natural exposure ("native" PCBs). Since round gobies are a benthic dwelling fish, they are in close contact with contaminated sediments and are characterized by a high bioaccumulation of PCBs (Vanderploeg *et al.* 2002). Using native PCBs as an internal benchmark, I
determined the shortest sampling time required for IP injected PRC-PCBs and native PCBs to achieve similar tissue distributions and evaluated the time required to complete chemical assimilation of the IP dose. After expressing the ratio of PRC-PCB lipid equivalent concentrations in dorsal muscle (DM)/whole body carcass (WB) and benchmarking with native PCB 153, tissue distributions of PRC-PCBs approached tissue distributions of PCB 153 as indicated by the DM/WB ratio 6 hours post-injection. To the best of my knowledge, the rapid uptake and distribution of PCBs to muscle has not been previously described in a small fish species over such short time scales. Overall, my results suggested that IP injection was an effective exposure method to use when conducting toxicokinetics studies, such as the rate of chemical elimination, following a short acclimation period of < 1 day post injection.

Chapter 4 used the elimination rates ($k_{total}$) of 14 PRC-PCB congeners in round goby populations from Lake St. Clair, Lake Huron and Lake Ontario as a surrogate measure of field metabolic rate (FMR) during a period of rapid temperature change. Previous research has found that round gobies from Lake Huron populations are the most genetically distinct, and least genetically diverse compared to other populations within the Great Lakes (Brown and Stepien, 2009). However, it is important to note that the analysis by Brown and Stepien (2009) looked only at the allelic structure of round goby populations, not specific genes coding for thermal tolerance. Round gobies were dosed with 14 PRC-PCB congeners via IP injection and chemical elimination rates were evaluated for 97 days to test the hypothesis that Lake St. Clair and Lake Ontario populations
would show greater similarities in FMR response compared to Lake Huron populations (less genetically diverse). My study found that most PRC-PCB congeners (43, 68, 89, 112, 166, 204 and 205) did not show population specific differences in the rate of chemical elimination ($k_{\text{total}}$). However, PRC-PCB congeners 21, 62, 57 and 125 showed significantly different chemical elimination between populations, although this trend was not consistent across chemicals. In other words, no single population showed consistently higher or lower elimination rates across multiple PRC-PCB congeners. In conclusion, there were no significant differences in the FMRs of genetically distinct round goby populations from Lake St. Clair, Lake Huron and Lake Ontario when subjected to a rapid change in environmental temperatures. While genetic differences between Great Lakes populations of round gobies have been well documented (Dillon and Stepien, 2001; Stepien and Tumeo, 2006; Brown and Stepien 2009), results from my study indicate that physiological tolerance, as measured by temperature dependent FMR appears to be conserved across populations. Future research should aim to investigate relationships between the genetic diversity of round goby populations within the Great Lakes and other measures of physiological tolerance. Improving our understanding and how genetic diversity contributes to phenotype expression in round gobies would provide insight into this invasive species' potential for continued population growth and range expansion throughout the Great Lakes.

References
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