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Comparison of physiological performance characteristics of two Great
Lakes invasive fish species: Round Goby (*Neogobius melanostomus*) and
Tubenose Goby (*Proterorhinus semilunaris*)

By

Xin Sun

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

2016

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Comparison of physiological performance characteristics of two Great Lakes invasive
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(*Proterorhinus semilunaris*)

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March 15th, 2016

DECLARATION OF CO-AUTHORSHIP/PREVIOUS PUBLICATION

I. Co-Authorship Declaration

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). In all chapters, the primary contributions, main ideas, field work, experimental procedures and data analysis were performed by the X. Sun. The contribution of co-authors was to provide input on the experimental design and provide editorial revisions on drafts.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-authors to include the above materials in my thesis.

I certify that, with the above qualification, this thesis and the research to which it refers, is the product of my own work.

II. Declaration of Previous Publication

This thesis includes one original paper that has been previously submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication Title	Publication Status
<i>Chapter 3</i>	<i>Determination of PCB elimination in round goby (Neogobius melanostomus) and tubenose goby (Proterorhinus semilunaris)</i>	<i>In press</i>

Chapter 3 of this thesis “Determination of PCB elimination in round goby (*Neogobius melanostomus*) and tubenose goby (*Proterorhinus semilunaris*)” has been submitted to *Bulletin of Environmental Contamination and Toxicology*. This article was co-authored by Drouillard K and Johnson T.

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ABSTRACT

This thesis compared physiological tolerance and bioenergetic requirements in a pair of Great Lakes invasive fish from the family Gobiidae: the round goby (*Neogobius melanostomus*) and the tubenose goby (*Proterorhinus semilunaris*). Physiological tolerance studies compared the response of the two species to acute thermal stress. The 12 h LC50 (lethal temperature contributing to 50% mortality) for round goby (33.4°C) was significantly higher than the tubenose goby (31.9°C). The LT50 (lethal time contributing to 50% mortality) for round goby was also longer compared to tubenose gobies. Routine metabolic rates of the two goby species were estimated using a depuration tracer modelling approach based on PCB elimination measured in the two species under 21.4°C. There were no significant differences in the rate of PCB elimination or estimates of RMR between round and tubenose goby. This implies that the two species have a similar bioenergetic requirements at their preferred temperature.

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CHAPTER I - GENERAL INTRODUCTION

1.1 Introduction

Invasive species are taxa that are introduced outside of their natural range either intentionally or unintentionally by human activity (IUCN 2000). Alien invasive species (AIS) are species which have negative ecosystem impacts or economic consequences associated with their establishment (Mills et al. 1993). Alien invasive species are a leading cause of species extinctions and habitat destruction in the world (Clavero and Garcia-Berthou, 2005; Jelks et al. 2008). The negative effects of AIS include altering nutrient cycling (Bunnell et al., 2015), disrupting food webs (Ng et al., 2008), transferring pollutant (Morrison et al., 2000) and reducing the biodiversity of native populations through predatory, competitive, parasitic interactions and ecosystem/habitat modification (French and Jude, 2001; Vanderploeg et al., 2002). The Laurentian Great Lakes basin, as a commercially active and shipping intensive freshwater ecosystem, has been suffering large scale invasions since the opening of the Welland Canal in 1959. By 2010, human-mediated transportation resulted in more than 180 AIS (Kornis and Vander Zanden, 2010). Among the large number of non-indigenous species, AIS originating from the Black, Caspian and Azov Seas (Ponto-Caspian region) constitute 70% of Great Lakes AIS entering the system since 1985 (Ricciardi and MacIsaac, 2000).

In the early 1990's, Gobiidae, native to the Ponto-Caspian, were first reported in

the Huron-Erie corridor (St. Clair River) of the Laurentian Great Lakes basin and are thought to have been introduced by ship ballast water (Jude et al., 1992). The presence of tubenose goby (*Proterorhinus semilunaris*) was first reported in Lake St. Clair in April, 1990. Its discovery was soon followed by reports of round goby (*Neogobius melanostomus*) in same water body in June, 1990 (Jude et al., 1992). Round gobies subsequently exhibited very rapid spread throughout the Great Lakes basin and were widely distributed within 5 years of their first identification (Marsden et al., 1996). In contrast, tubenose goby have remained more restricted in their Great Lakes distribution. Until 2001, the tubenose goby range 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 (Fuller et. al., 2013). If rate of range expansion is used as a metric of invasion success, round goby clearly ranks as the more successful AIS compared to the tubenose goby in the Laurentian Great Lakes. Given the high morphological similarity of round and tubenose goby, similar time and position of entry and differences in invasive spread, these two AIS are considered an appropriate pair for the comparison of physiological performance metrics that contribute to invasive species success.

A suite of characteristics of successful invasive species was proposed by Ehrlich (1989) 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) physically larger than most related species, (7) fertilized females are able to colonize

alone and (8) the ability to function in a wide variety of physical conditions (physiological tolerance). The list of characteristic invasive species traits was expanded by Lodge (1993) to include: early succession, climatically matched to invaded environment, low native species diversity and absence of predators. Undoubtedly, all aquatic invasive species possess at least one of the traits outlined by Ehrlich (1989) and Lodge (1993).

Thermal tolerance is often considered a key factor limiting species distributions (Braby and Somero, 2006). This is especially true for aquatic organisms where thermal constraints on metabolic rate coupled with dissolved oxygen availability at elevated water temperatures contribute to a combination of heat and hypoxia stress (Cossins et al., 1987). Many studies have found that successful invasive species display higher physiological tolerance to environmental stressors compared to native species (Braby and Somero, 2006). For example, Sorte et al. (2010) conducted research on hull fouling communities in Bodega Harbor, California and found that introduced species were more tolerant than native species to higher temperatures. Research by Braby and Somero (2006) reported that the invasive mussel, *Mytilus galloprovincialis* was also more tolerant to high water temperatures than the co-existing native species (*Mytilus trossulus*). Thus, understanding the thermal tolerance of a species, and the mechanistic response to thermal stress, enables predicting the response of a species to shifts in their temperature exposure that occur during range expansion, natural or human mediated alien invasions, or in response to climate change.

Several studies on invasive species attempt to assess a species' capacity for range expansion by examining its range distribution and estimating the physiological tolerance of the species based on the extremes of environmental attributes at the edges of its natural range (Braby and Somero, 2006). However, the short-term physiological tolerance that may be experienced during transport such as in ship ballast, or in habitats that experience high daily temperature fluctuation such as small tributaries or ditches that may be used during range expansion, are often ignored. Species whose acute tolerances are sufficient to survive extreme conditions experienced during transport / dispersal are more likely to survive not just the initial dispersal conditions, but more likely to take advantage of multiple transport pathways, enhancing their overall rate of spread post invasion as well. Generation of more direct empirical measurements of physiological tolerance, such as temperature based LC50's (lethal temperature contributing to 50% mortality in an exposed population) to directly compare physiological tolerance in sets of Great Lakes invaders would therefore be useful to evaluate why some invaders such as round goby demonstrate much faster rates of post-invasion spread compared to similar species, e.g. tubenose goby.

Additionally, physiological performance such as metabolic rate or annual energy budget is also considered a potential factor that contributes either to invasion success or ecosystem impact of an invader (McMahon, 2002). Determination of metabolic rate is particularly useful because it not only mirrors physiological demands for oxygen (Cross and Rawding, 2009), but also simultaneously reflects diminished muscular performance when it is likely most important for fish. It has been suggested

that the metabolic rate of round gobies exceeds that of native species in regions of establishment, thus contributing to a higher ecological footprint related to prey items consumed relative to biomass produced (Cross and Rawding, 2009). Alternatively, a higher metabolic rate in an AIS can be a disadvantage under resource limiting conditions. Although some research has been performed to measure metabolic rate of round goby (Lee and Johnson 2005) and estimate annual energy budgets of this species (Ng et al., 2009), few case studies provide direct comparisons of the energy budget of round goby with other invaders such as tubenose goby. O'Neil (2013; MSc thesis) measured standard metabolic rates (SMR) of both fish species and observed similar SMRs at low temperature conditions, but potentially elevated metabolic rates of tubenose goby at high water temperature (i.e. 28°C and 30°C). However, standard metabolic rate represents only one of several components contributing to the total energy budget of a fish and methods to compare the metabolic rate of free living fish would provide additional information, especially when applied on a comparative species basis.

Bioenergetic tracer approaches have been developed using mercury (Stern, et al., 2001), isotopes of cesium, and polychlorinated biphenyls (PCBs) to estimate energetic costs of free living fish (Macdonald et al., 2000; Madenjian et al., 2000; Paterson et al., 2007; McLeod et al. 2015). Commonly, these techniques infer food consumption rates of fish based on measured rates of chemical bioaccumulation in fish populations over a set time interval (Metcalf, 1986; Andersson et al. 2001). However, bioaccumulative approaches to bioenergetics require multiple assumptions necessary to account for the

eliminated fraction of the chemical from fish during the time interval of study. The approaches must also address and account for variation in chemical uptake/assimilation resulting from incorporation of multiple prey items in the diet of field collected fish. Drouillard et al. (2009) generated a bioenergetic/toxicokinetic model for PCBs used to describe elimination of PCBs from yellow perch. This model provides an opportunity to apply PCBs as a depuration tracer and deviates from the classic bioaccumulation approach. By studying PCB loss over time, several assumptions of the more complex bioaccumulation/bioenergetic tracer models are circumvented. Under this approach, fish are pre-dosed with non-environmental tracers, released to a system of study and then recaptured to determine the fraction of chemical eliminated over time. The eliminated fraction is then related to gill ventilation rate offering an alternative method of estimating the oxygen consumption rate of fish. This model has potential application to measure bioenergetics of free living fish under experimental conditions and in small systems where recapture success of dosed individuals has a high likelihood. However, prior to implementing the method as a quantitative approach to fish bioenergetics, PCB toxicokinetics in the fish species to be characterized first need to be calibrated and critical assumptions about the importance of gill verses fecal elimination need to be validated.

1.2 Thesis Objectives

The objective of this thesis is to compare selected physiological performance metrics between invasive populations of round and tubenose gobies.

Chapter 2 contrasted acute thermal stress in each species by measuring the temperature LC50 in field collected round and tubenose goby. This research was developed to test the null hypothesis that round and tubenose goby exhibit similar mortality responses under acute temperature stress.

Chapter 3 characterized the elimination rates of PCBs in round and tubenose gobies under constant temperature conditions consistent with the optimal range of each species. The whole body elimination rate coefficient (k_{tot} ; d^{-1}) is a key toxicokinetic parameter necessary to implement either a bioaccumulation or depuration-tracer approach to fish bioenergetics estimates. This study tested the following hypotheses:

- i) Elimination of non-environmental PCBs from round and tubenose goby decreases as a function of chemical hydrophobicity, and
- ii) Elimination rates of PCBs for round and tubenose goby are similar to one another when animals are housed under common environmental conditions.

Chapter 4 provided the first case study to apply PCB depuration-tracer models to estimate routine metabolic rates (RMR) of fish. The study compared RMR of individual chemical tracers dosed into fish to contrast two types of models; one that considers only depuration of chemical across the gills and a second model that considers both gill and fecal depuration. Upon evaluation of model performance, and selection of the appropriate model framework, the approach was then used to estimate RMR of round and tubenose goby living in a common environment at their optimal

temperature. The null hypotheses tested in Chapter 4 included:

- i) Model I, which assumes gill elimination is the dominant elimination pathway for PCBs by fish, provides a similar estimate of RMR in a given fish species for all tracer chemicals being studied.
- ii) Model II, which assumes both gill and fecal elimination of PCBs by fish, will generate lower RMR estimates for more hydrophobic PCBs relative to less hydrophobic chemicals.
- iii) The RMR of round goby is equivalent to the RMR of tubenose goby under tank held conditions at their optimal temperature.

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CHAPTER II - COMPARISON OF THE ACUTE UPPER THERMAL TOLERANCE OF ROUND GOBY (*NEOGOBIUS MELANSTOMUS*) AND TUBENOSE GOBY (*PROTERORHINUS SEMILUNARIS*)

2.1 Introduction

Species distributions are ultimately limited by their physiological tolerance which places constraints on an organism's ability to survive environmental stress induced by the abiotic environment they inhabit (Lexer et al., 2003; Hampe and Petit, 2005; Sexton et al., 2009). Among several parameters that influence a species' viability to extreme environmental stress, thermal tolerance is often considered a key factor limiting species distributions (Braby and Somero, 2006). This is the case for aquatic organisms where temperature/metabolic rate associations coupled with reduced dissolved oxygen availability at elevated water temperatures contribute to a combination of hypoxia and heat stress (Cossins et al., 1987). Thus, understanding the thermal tolerance of a species, and the mechanistic response to thermal stress, enables predicting the response of a species to shifts in their temperature exposure that occur during range expansion, natural or human mediated alien invasions, or in response to climate change.

Thermal tolerance is important in understanding alien invasive species' (AIS) range expansion (Huey et al., 2012). The ability of a species to tolerate temperature fluctuations may enable it to survive various filters, or barriers, presented throughout the invasion process that include: i) temperature stress during human mediated

transfer (Leidenberger, 2015), ii) thermal conditions of the receiving environment (Sorte et al., 2010), iii) temperature dependent competitive interactions (i.e. biotic interactions that constrain realized niche) between the invader and other species in the receiving environment (Braby and Somero 2006), iv) thermal conditions operating in connecting corridors linking the receiving environment with surrounding colonizable habitats, and/or v) thermal variation during subsequent human mediated transfers at the local and regional scale post-invasion enhancing species spread.

Braby and Somero (2006) reported that the temperature tolerance of the invasive blue mussel (*Mytilus galloprovincialis*) was a major factor influencing its range distribution and could be used to predict the future invasion success of this species in the North Pacific and other areas. In addition, research by Sorte et al. (2010) on hull fouling communities in Bodega Harbor, California, found that introduced species were often more tolerant to high temperature than native species. The above case studies imply that successful introduced species are more thermally tolerant than co-existing to native species. Base on the above, potential AIS may be ranked such that successful invasive species are likely to have greater temperature tolerance compared to less successfully introduced species or that temperature tolerance is likely to play an important role in AIS range expansions post introduction, especially when animals are subject to highly variable environmental conditions.

In the early 1990's, the family Gobiidae were first reported in the Huron-Erie corridor of the Laurentian Great Lakes and are thought to have been introduced by ship ballast water (Jude et al., 1992). The presence of tubenose goby (*Proterorhinus*

semilunaris) was first reported in Lake St. Clair in April, 1990. Its discovery was soon followed by reports of round goby (*Neogobius melanostomus*) in same water body (Jude et al., 1992). Round gobies subsequently exhibited very rapid spread throughout the Great Lakes Basin and were widely distributed within 5 years of their first identification (Marsden et al., 1996). In contrast, tubenose goby have remained more restricted in their Great Lakes distribution. Until 2001, the tubenose goby range 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 (Fuller et. al., 2013). Thus, if range expansion is used as a metric of invasion success, round goby clearly ranks as the more successful AIS compared to the tubenose goby in the Laurentian Great Lakes.

The objective of the present study was to compare the acute thermal tolerance of invasive populations of round and tubenose gobies. Given the wider distribution and rapid range expansion of round goby compared to tubenose goby, it is hypothesized that round goby will exhibit higher thermal tolerance compared to tubenose goby.

2.2 Methods

Past approaches used to determine thermal tolerance have used either static or dynamic temperature adjustment methods. The static hold lethal temperature (SHLT) method quantifies mortality of replicated groups of organisms after transfer from an acclimatized temperature directly to a set of treatment temperatures over a fixed

period of time. The LC50, representative of the upper incipient lethal temperature, is then extrapolated using linear regression analysis on transformed mortality data against treatment temperature. The dynamic approach exposes fish as individuals or in groups to a linear temperature ramp and monitors time to mortality of each individual in the test population. The CTMax is subsequently calculated as the mean temperature at which the population of individuals tested succumbs to mortality. Both methods produce different estimates of lethal temperature (Bennett and Judd 1992). The SHLT is normally lower than the CTMax for a given species (Bennett and Beitinger 1997). This is because thermal shock and handling stress contributed during initial transfer of animals from the acclimatized to treatment temperature often contributes the largest stress (Bennett and Judd 1992). Dynamic approaches are less susceptible to the above problem and more consistent with how fish are exposed to thermal fluctuations in the environment (Bennett and Judd 1992). However, the dynamic approach is sensitive to the temperature ramping rate used (Hutchison 1976). Another issue with the dynamic approach is that lags between the onset of critical stress leading to mortality and the actual observed time of mortality can mask the true lethal temperature of the species due to the continuous temperature ramp being applied. The present study adopted a hybrid design between the SHLT and dynamic approach. Fish were acclimatized at their optimal temperature of 22°C (Lee and Johnson 2005) prior to initiating trials. During each trial, groups of fish were subjected to a slow stepped temperature ramp of 2°C·hr⁻¹, beginning at the optimal temperature until the trial target temperature was reached. Pilot studies indicated that no fish mortality occurred during the ramping to

highest treatment temperature given the $2^{\circ}\text{C}\cdot\text{hr}^{-1}$ ramping rate. Once the target temperature was achieved, the temperature was held constant for a period of 12 h and standard LC50 approaches were applied.

Experimental

Round and tubenose gobies were collected from the Detroit River ($42^{\circ} 20'14.8452''\text{N}$, $82^{\circ} 54'58.1466''\text{W}$) between July to October, 2014. Animals were collected by seine and identified on site. After capture, the fish were transported to the laboratory and maintained communally in a 550 liter flow through plastic tank. The housing tank was sectioned using plastic mesh and round and tubenose gobies were kept in each section separately. The housing tank received and drained water into a separate reservoir containing a heater/chiller unit and biofiltration system. The water temperature was maintained at $22\pm0.5^{\circ}\text{C}$ and monitored using digital temperature loggers (Onset Computer Corporation, Bourne, MA, USA). Fish were fed live blood worms three times per week until satiation. Water quality parameters, dissolved oxygen, pH, and ammonia were tested on a weekly basis. Animals were maintained in the holding tanks at constant temperature for 2 months prior to use in trials.

Experimental trials involved placing groups of 10 fish per species into a 50L observation aquarium containing water from the holding reservoir at 22°C . The observation aquarium was divided in half by plastic mesh with each species kept on a separate side. Water temperatures were increased at a rate of $2^{\circ}\text{C}\cdot\text{hr}^{-1}$ until the target temperature was reached. Temperatures in the observation tank were monitored

manually every 10 minutes using a thermometer immersed in the tank. Once the target temperature was reached, the water temperature was maintained at the target for 12 h. During each trial, a separate control aquaria was set up identical to the observation aquaria. The control tank contained 5 fish of each species separated from one another by plastic mesh. The control tank was maintained at a constant temperature of 22°C over the trial duration and monitored in similar fashion as the treatment. Target temperatures used in the study were 31, 32, 33, 34 and 35°C. Triplicate trials were performed for each species and temperature.

After achieving the target temperature, the response of fish was constantly monitored for 12 h. A metric of impending death was applied in place of actual mortality. Pilot study data indicated that the onset of muscle spasms was highly indicative of pending death within approximately 30 minutes. Thus, during trials, individuals exhibiting muscle spasms were immediately removed from the tank and euthanized by overdose of an anesthetic agent (MS222). The time at which each fish succumbed to muscle spasms was recorded. At the end of the 12 h trial the cumulative %mortality of each species was determined.

The above studies were performed following ethical approval of experimental protocols as reviewed by the University of Windsor's local animal care committee.

2.3 Data analysis

Percent mortality data generated for each trial was transformed using probit tables.

Linear regression was performed on probit transformed mortality data against \log_{10} temperature. The LC₅₀ was determined based on the above linear regression to extrapolate the temperature at which 50% mortality occurred. Analysis of variance (ANOVA) was used to test if the slope of the regression was significantly different from zero. In this analysis, the unit of replication was 1 tank triplicated for each temperature and study species. A general linear model (GLM) was applied to test the species x temperature interaction term for probit transformed mortalities. An observation of a significant interaction term indicated significant differences in the LC₅₀ between the species.

Time to mortality data were combined across individual trials of each species and temperature treatment. Data were \log_{10} transformed and linear regression was performed between log time to death versus probit transformed percent mortality. The above regression equation was used to extrapolate the time to 50% mortality at each temperature treatment for each species designated as LT₅₀. Analysis of variance was used to test for differences in the meantime to mortality between species. All statistical tests were performed using OriginPro 9.1.

2.4 Results

There was no evidence of major stress to fish in the holding tank before the experiment commenced although 6.25% mortality rate occurred during the 2 month holding period prior to initiating the study. Water quality measurements were within

experimental norms and fish feeding behaviour was considered normal. For all experimental trials conducted, control fish mortality was 0%. There were also no incidences of fish mortality occurring during the temperature ramp period for any of the individual trials. However, given the high mortalities of tubenose goby experienced at the 34°C temperature trials and relatively short time to death experienced at this temperature, only round gobies were tested at the highest 35°C temperature trial.

The standard length and weight of round and tubenose gobies used across trials was $4.90 \pm 0.26\text{cm}$ (mean \pm S.E.), $2.27 \pm 0.46\text{g}$ and $4.57\pm 0.26\text{cm}$, $1.92 \pm 0.42\text{g}$, respectively. There were no significant differences in mean body size between treatments ($p=0.514$). There were also no significant differences between the mean body size of a given species across different temperature treatments.

In general, tubenose gobies were found to exhibit higher mortalities compared to round gobies during individual temperature trials (Figure 2.1) The general linear model indicated a significant species x temperature interaction ($p<0.05$) indicating significant

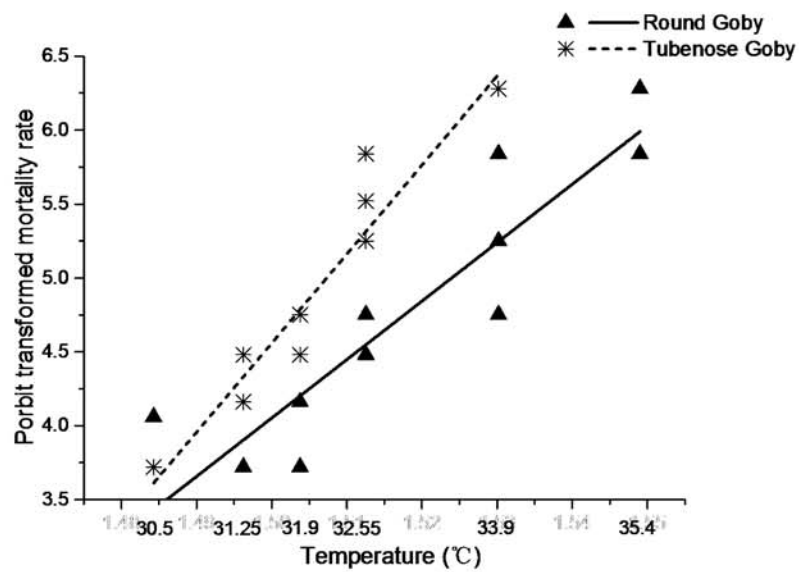


Figure 2.1 The relationship between temperature and mortality in round and tubenose goby. The two lines represent linear regression to calculation LC50 of each species.

differences in the LC50 between species. The extrapolated LC50 values were 33.4°C for round goby and 31.9°C for the tubenose goby, respectively.

Time to death for tubenose gobies was more rapid than for round gobies at a given temperature (Figure 2.2). LT50 for tubenose gobies ranged from 127.7 to 1,209.2 min over the 32.9 to 33.9°C temperature range. For round gobies, LT50 ranged from 527.6 min to 2,496.1 min over the same range. In both cases, fish mortalities at the 31°C temperature were too low to compute LT50's. For each temperature treatment LT50 was significantly shorter ($p < 0.05$; ANOVA) for tubenose gobies compared to round gobies. LT50 values for both species exhibited a decreasing trend with increasing trial temperature (Figure 2.3). Although the number of temperature treatments are limited for tubenose gobies, significant relationships between LT50 and temperature existed for both species:

$$\text{Round Goby: } \log(1/\text{LT50}) = 1.42 \times \text{Temperature} - 45.13; R^2 = 0.8570 \quad (1)$$

$$\text{Tubenose Goby: } \log(1/\text{LT50}) = 3.40 \times \text{Temperature} - 107.13; R^2 = 0.9554 \quad (2)$$

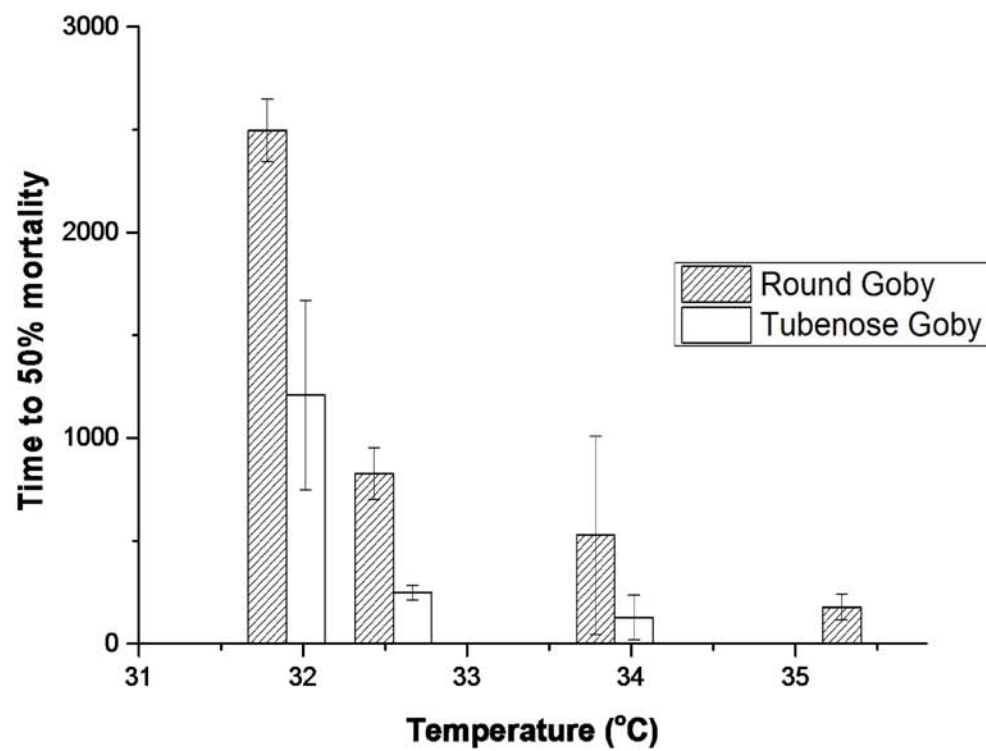


Figure 2.2 Time to 50% mortality (LT50) for Round and Tubenose Goby at 31.9, 32.4, 33.9, and 35.4°C.

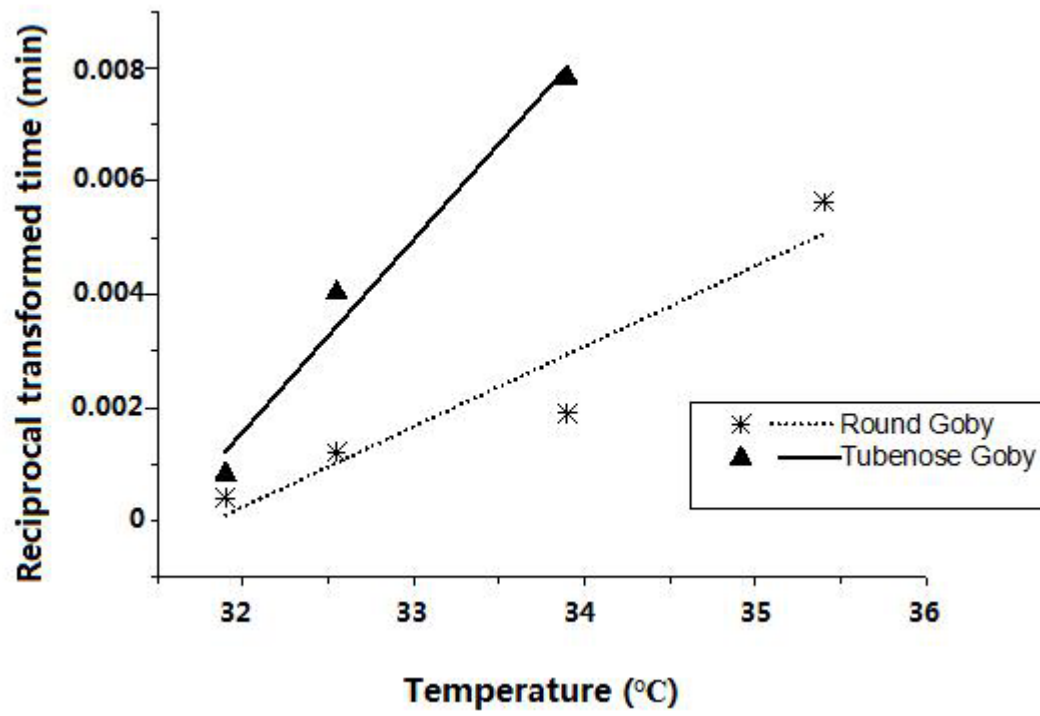


Figure 2.3 Relationship between time to 50% mortality (LT50) and temperature for Round and Tubenose Goby.

2.5 Discussion

Many studies have found that thermal tolerance is a limiting factor influencing the distribution of ectotherms (Lexer et al., 2003; Hampe and Petit, 2005). Braby and Somero (2006) reported that the invasive Mediterranean mussel (*Mytilus galloprovincialis*) was more tolerant to high water temperatures than the native blue mussels species. Sorte et al. (2010) found that in a marine fouling community, introduced species were more tolerant than native species to high temperatures. In general, they put forward an argument that successful invaders have higher thermal tolerance when compared to co-existing native species. In this study, two sets of established invasive species that occupy a similar ecological niche but differ in the rate of spread post-establishment were contrasted with respect to thermal tolerance. Similar to the observations of Sorte et al. (2010), round gobies, which demonstrated higher rates of range expansion relative to tubenose goby, were also found to have higher thermal tolerance. Round gobies had both higher LC50 and LT50 values under acute thermal stress.

Laboratory studies of thermal tolerance of Gobiidae remain somewhat limited in the literature. Matern (2001) measured the critical thermal maximum (CTMax) at eight temperature and salinity combinations in the marine Shimofuri goby (*Tridentiger bifasciatus*), an invader of the San Francisco estuary, California. For this species, mean CTMax in 10 and 20°C acclimated gobies was 31-37°C and 37 °C respectively, depending upon salinity conditions that ranged from 5 ‰ to 20 ‰. Unfortunately no information was available on critical thermal maximum of round or

tubenose goby for comparison with the above species.

The thermal tolerance for invasive species is more commonly inferred based on their geographic distribution in their native range and considering the thermal maxima encountered within their native range. However, this process can combine realized niche with fundamental niche and will likely underestimate the true thermal tolerance of a species. Environmental temperatures in the native and invasive distribution of round goby (*Neogobius melanostomus*) are reported to range from -1 to 31°C (Ng and Gray, 2011). These data are consistent with the results presented here, the 12 hour LC50 of round goby was 33.4°C and an LT50 at 31°C was not detectable for either species. Thus, mortalities experienced by round goby under acute thermal stress commence after 32°C and they can survive these conditions for longer periods of time compared to tubenose goby.

Water temperatures in the lethal range of either species are not likely to be encountered in the open lake or riverine environment of the Huron-Erie corridor or other Great Lakes habitats within the range invaded by round or tubenose goby. However, these temperature extremes may occur on an intermittent basis in shallow/turbid ditches and small creeks which provide connectivity between adjacent aquatic habitats. Based on differences observed in thermal tolerance of the two species, we hypothesize that round gobies would have a better capacity to take advantage of small aquatic corridors subject to rapid diurnal heating, allowing more rapid infiltration and spread to adjacent water bodies post invasion. Alternatively, the number of days whereby such systems represent a thermal barrier for tubenose goby

but not round goby are likely limited and therefore thermal tolerance in itself is unlikely to provide a full explanation for the very large differences in invasive range expansion experienced by these two species. Additional research to quantify fundamental niche across other stressor axes such as low dissolved oxygen, turbidity, pH and other water quality characteristics would be useful to provide a better picture of multi-dimensional fundamental niche differences experienced by these two species.

2.6 References

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CHAPTER III - DETERMINATION OF PCB ELIMINATION IN ROUND ROUND GOBY (*NEOGOBIUS MELANSTOMUS*) AND TUBENOSE GOBY (*PROTERORHINUS SEMILUNARIS*)

3.1 Introduction

Two invasive fish species, the round goby (*Neogobius melanostomus*) and the tubenose goby (*Proterorhinus semilunaris*), entered the Laurentian Great Lakes by ballast water in 1990 (Jude et al. 1992). Impacts of these invasive gobies on the Great Lakes biological communities includes the disruption of food webs (Ng et al. 2008), alteration of energy pathways (Johnson et al. 2005), predation on native fish eggs and larvae (French and Jude, 2001) and modification of pollutant transfer (Morrison et al. 2000).

Persistent organic pollutants (POPs), as exemplified by organochlorines such as polychlorinated biphenyls (PCBs), exhibit high bioaccumulation potentials in organisms and food webs. Bioconcentration, which describes chemical exposures through respiratory surfaces (Neely et al., 1974; Leblanc, 1995) and biomagnification, reflective of chemical exposure through food (Connolly and Pedersen, 1988; Gobas et al., 1993), are major exposure mechanisms for POP bioaccumulation in fish. PCBs are highly hydrophobic and accumulate in organic materials in aquatic systems, with sediments becoming the ultimate repository of these contaminants (Davis et al., 2007). Given bans in PCB production and use within North America since the 1970's, sediments have reverted from being sinks for POPs to major sources for chemical

entry into water and the food web (Morrison et al. 2000). Indeed, chemical fugacity of PCBs in sediments of aquatic and marine systems typically exceed the chemical fugacity of surface and overlying water at the sediment/water interface (Gobas and MacLean, 2003) increasing the importance of sediment associated contaminants as pollutant sources to aquatic food webs (McLeod et al., 2015).

Gobies are benthic fish species which lack a swim bladder. They have high dietary plasticity and consume a variety of benthic organisms including crustaceans, insect larvae, molluscs, small fishes and fish eggs (Djuricich and Janssen 2001; Polacik et al., 2009; Stevove, 2013). Of the two study species, round gobies are also known to consume dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*) which reflect another set of Caspian derived AIS that have established large populations throughout the Great Lakes and have contributed to enhanced benthic production of these systems at the expense of pelagic food web production (Ghedotti et al., 1995; Diggins et al., 2002; Hecky et al. 2004). Owing to their high abundance post invasion, gobies are routinely incorporated into the diets of larger fish including important commercial and recreational sport fish species such as smallmouth bass (*Micropterus dolomieu*), walleye (*Stizostedion vitreum*) and lake trout (*Salvelinus namaycush*) (Thomas et al., 2002). Thus, gobies have become an important link in coupling benthic and pelagic food webs that enhance the movement of sediment associated POPs into the food web and potentially increases the frequency of fish consumption advisories issued to the public (Morrison et al., 2000; Kwon et al., 2006).

Although bioaccumulation kinetics of PCBs in fish have been documented in many species (Fisk et al., 1998; Fisk et al., 2001; Cappelletti, 2015) including bioaccumulation models developed for round goby (Ng and Gray 2011), there are no published studies measuring PCB elimination kinetics in round or tubenose gobies. The whole body elimination rate coefficient (k_{tot}) is a key toxicokinetic parameter used to estimate chemical half life and time required to reach steady state by fish and a necessary component of biokinetic bioaccumulation models. The k_{tot} value is also required for fish bioenergetic tracer models used to estimate fish feeding rates based on bioaccumulation rates of tracer chemicals such as PCBs (McLeod et al. 2015).

In this present study, k_{tot} values for 14 non-environmental PCBs were determined in round goby and tubenose goby. The k_{tot} values were measured in comparable sized fish near the optimal temperature of each species in order to contrast chemical kinetics in these two Great Lakes invaders.

3.2 Methods

Twenty-four round gobies and twenty-four tubenose gobies were collected from the Detroit River (42° 20'14.8452"N, 82° 54'58.1466"W) by seine net between July 1 and Sept 15, 2013. At the time of collection, the more abundant round gobies were size graded to match similar sizes of tubenose gobies being captured in the field. During collection, fish were placed in a cooler containing water from the site location and transported back to Aquatic Research Facility of the Great Lakes Institute for

Environmental Research (University of Windsor, Windsor, ON, Canada) within 3 h of collection.

Individuals of each species were held in a 550 litre polyethylene tank divided into four parts using plastic mesh glued to the sides of the tank. Two larger partitions held 20 round gobies and 20 tubenose gobies and two smaller partitions held 4 round gobies and 4 tubenose gobies later designated as internal controls. The tank was set up as a recirculating system in which water was pumped in and drained to a larger reservoir system under constant aeration and containing a heating/chiller unit. A biofiltration unit was added to the reservoir to maintain water quality. The photoperiod was kept at ambient environmental conditions. Fish were fed blood worms (*Glycera dibranchiata*) to satiation every other day throughout the acclimation and experimental duration. Water temperatures were measured four times daily by digital temperature loggers submersed in each tank (Onset Computer Corporation, Bourne, MA, USA). Water quality was monitored weekly by measuring pH, dissolved oxygen, temperature, conductivity and oxidation/reduction potential (Hydrolab, Ott Corp., Loveland, CO, USA; Appendix 1). All experimental research was conducted with approval from the University of Windsor Animal Care Committee.

A set of 14 environmentally rare PCBs were used to dose fish so as not to interfere with PCBs previously bioaccumulated by the fish from the Detroit River. The dosing mixture contained the following PCBs (IUPAC #s): 6, 13, 21, 23, 43, 62, 89, 57, 68, 112, 125, 166, 204 and 205 derived from individual standards of each 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 6, 13, 23, 43, 62, 89, 68, 112, 125, 166, 204, 205; 50ng/mL for PCB 21; and 200ng/mL for PCB 57. The log K_{ow} values for the PRC compounds ranged between 5.06 and 8.0 (Hawker and Connell, 1988).

Just prior to dosing, experimental fish were weighed, sexed and measured for total length following light anesthesia by immersion in a solution of MS222 at 140 mg/L until the fish lost swimming equilibrium. Each fish was administered an intraperitoneal injection (IP) using a 10μL Hamilton MICROLITER® Syringe. The volume of injection was 0.5 uL/g body weight to achieve nominal target doses of 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. Control fish were sham dosed with an equivalent volume of sunflower oil. Following injection, fish were allowed to recover and placed back into the experimental tank. Fish were held 2 d before sampling to allow tissue re-distribution of the injected PCBs (O’Neil et al. 2013). Four fish of each species were destructively sampled on day 0 (2 days following IP injection), 15, 30, 60 and 90. All sacrificed fish were weighed, measured for total length and sexed. Control fish were sampled on Day 0 and Day 90 of the study. Additionally, ages of each individual was estimated by the following equation (Pauly, 1987)) to distinguish the developmental stages of each experimental fish.

$$L_t = L_{\infty} (1 - e^{-K(t-t_0)})$$

where L_t is length of fish at T age, K is growth parameter, t_0 is size of fertilized

egg, L_{∞} asymptotic length.

The neutral lipid and PCB content of each sample was analyzed by solid/liquid chromatography as described in Daley et al. (2009). Prior to extraction, each sample was spiked with PCB 34 as an internal recovery standard. Neutral lipid content was determined gravimetrically using dichloromethane/hexane (1:1 v/v) extracts by removing 10% of the extract volume and weighing the dried residues (Drouillard et al. 2004). The remaining extract was cleaned up by activated florisil chromatography as described by Lazar et al. (1992). Modifications to the Lazar procedure involved use of 50 mL of hexane to collect the first fraction followed by a second fraction consisting of 50 mL 15:85(v/v) dichloromethane:hexane. Each fraction was collected in separate receiving flasks and concentrated to a final volume of 1mL in isooctane. Analytical determination of PCBs was performed by gas chromatography-electron capture detection (GC-ECD) as described by Lazar et al. (1992). For each set of 4 samples extracted, a method blank, PCB 34 recovery standard, external PCB standard (Quebec Ministry of Environment Congener mix; AccuStandard, New Haven, CT, USA) and non-environmental PCB standard (AccuStandard, New Haven, CT, USA) was analyzed. No PCB peaks were detected in any of the analytical blanks run with sample extractions.

Analytical precision was checked by comparing native PCBs in reference homogenates with laboratory control charts and were found to be within 2 standard deviations of the database values for each batch. Mean internal standard recoveries for PCB 34 were $64.47 \pm 16.68\%$ and ranged of 31.69 - 107.74%. Owing to the low

recoveries present in a few samples, all data were PCB 34 recovery corrected prior to analysis.

After testing normality using probability plots, variables related to fish body weight and fish lipid mass were ln transformed to conform to normality assumptions of ANOVA. A general ANOVA model was used to evaluate differences in body weight or lipid mass between the two species and through time and to test for significant differences in the species x time interaction term which tests for differences in growth rate between the species. ANOVA's were also performed without time as a variable to test for differences in the average body mass or lipid mass between the species.

Special attention was paid to PCB 205 which is not expected to be eliminated to any appreciable extent over the 90 d depuration study on account of its highly hydrophobic characteristics. PCB 205 is the most hydrophobic PCB present in the PRC dosing solution ($\log K_{ow} = 7.55$) and expected to be eliminated at the slowest rate compared to other congeners. If PCB 205 is shown to undergo no significant elimination from fish, it could subsequently be used to correct concentrations of other PCB congeners present within the dosing mixture. This additional correction would therefore account for variation in the received IP dose of individual fish while also correcting for growth dilution experienced by individual fish. In effect, PCB 205 is used in the same manner as the analytical recovery standard applied at the experimental scale. A general ANOVA was performed on ln transformed concentration data (lipid normalized concentration as well as total chemical mass in fish) to determine if PCB 205 was significantly eliminated with time. Following

verification that PCB 205 was not eliminated in either species of fish, all other non-environmental PCBs in each individual were corrected for PCB 205 lipid normalized concentration. The correction procedure was as follows:

$$C_{PCB(x,c)} = C_{PCB(x)} \cdot [C_{PCB(m205)}/C_{PCB(205)}] \quad (3.1)$$

where $C_{PCB(x,c)}$ is the PCB 205 corrected, lipid normalized concentration (ng/g lipid) in the sample, $C_{PCB(x)}$ is the uncorrected lipid normalized concentration of the PCB in the sample, $C_{PCB(m205)}$ is the mean lipid normalized concentration of PCB 205 measured in all fish of the same species over the study, $C_{PCB(205)}$ is the lipid normalized concentration of PCB 205 measured in the sample.

Whole body elimination rate coefficients (k_{tot}) values were determined by linear regression of a plot of $\ln C_{PCB(x,c)}$ versus time and establishing k_{tot} as the slope from the above relationship. k_{tot} values were only reported for PCB congeners whose slopes were negative and significantly different than zero.

3.3 Results

Water temperatures were constant at $21.40 \pm 0.48^{\circ}\text{C}$ and showed no changes with time (Appendix 1). No fish mortalities occurred throughout the course of the study. The mean whole body weight and length (mean \pm S.E.) for round and tubenose gobies were $2.88 \pm 0.059\text{g}$, $5.29 \pm 0.033\text{cm}$ and $2.85 \pm 0.047\text{g}$, $5.21 \pm 0.028\text{cm}$ and were not significantly different from one another ($p = 0.502$). Individual from the two species

were at relatively early life stages relative to the maximum lengths. The mean whole body lipid content of round and tubenose gobies was $4.07 \pm 0.35\%$ and $3.93 \pm 0.31\%$, respectively, and was not significantly different from each other ($p = 0.082$). Figure 3.1 demonstrate trends of body weight and mass of lipid for fish from each species as a function of time. Neither whole body lipid weight nor body weight showed significant changes ($p > 0.05$) with time in either species.

Environmental background correction calculated for control fish housed within the same tanks as experimental fish and sampled on day 0 and 90 was $2.98 \pm 0.50\%$ in round goby and $7.10 \pm 0.93\%$ in tubenose goby, respectively of the experimental fish. Thus, any effect of non-environmental background PCBs measured in treatment fish was removed.

Figure 3.2 demonstrates changes in whole body \ln transformed PCB 205 mass in individual fish through time for each species. PCB 205 exhibited a non-significant positive slope with time in round gobies (ANOVA, $p = 0.121$) and non-significant negative slope with time in tubenose gobies (ANOVA, $p = 0.371$). Figure 3.4 presents changes in lipid normalized concentrations of \ln transformed PCB 205 in each fish species. As in the case for the mass balance, lipid normalized concentrations of PCB 205 did not exhibit a significant change with time in either species (ANOVA, $p = 0.065$ for round goby, $p = 0.163$ for tubenose goby). Given the lack of elimination of PCB 205, all other PCB concentrations were PCB-205 corrected as described by Eq. 3.1.

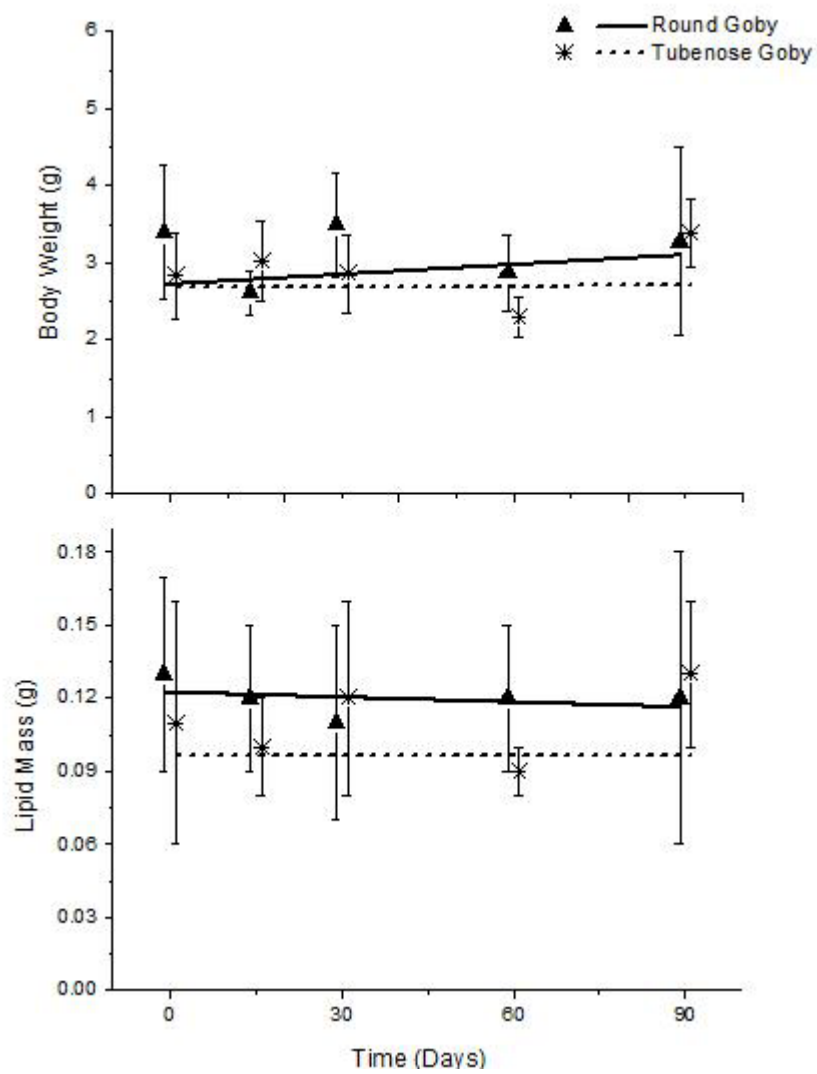


Figure 3.1 Body weight and lipid changes with time (mean \pm S.E.) for round and tubenose goby.

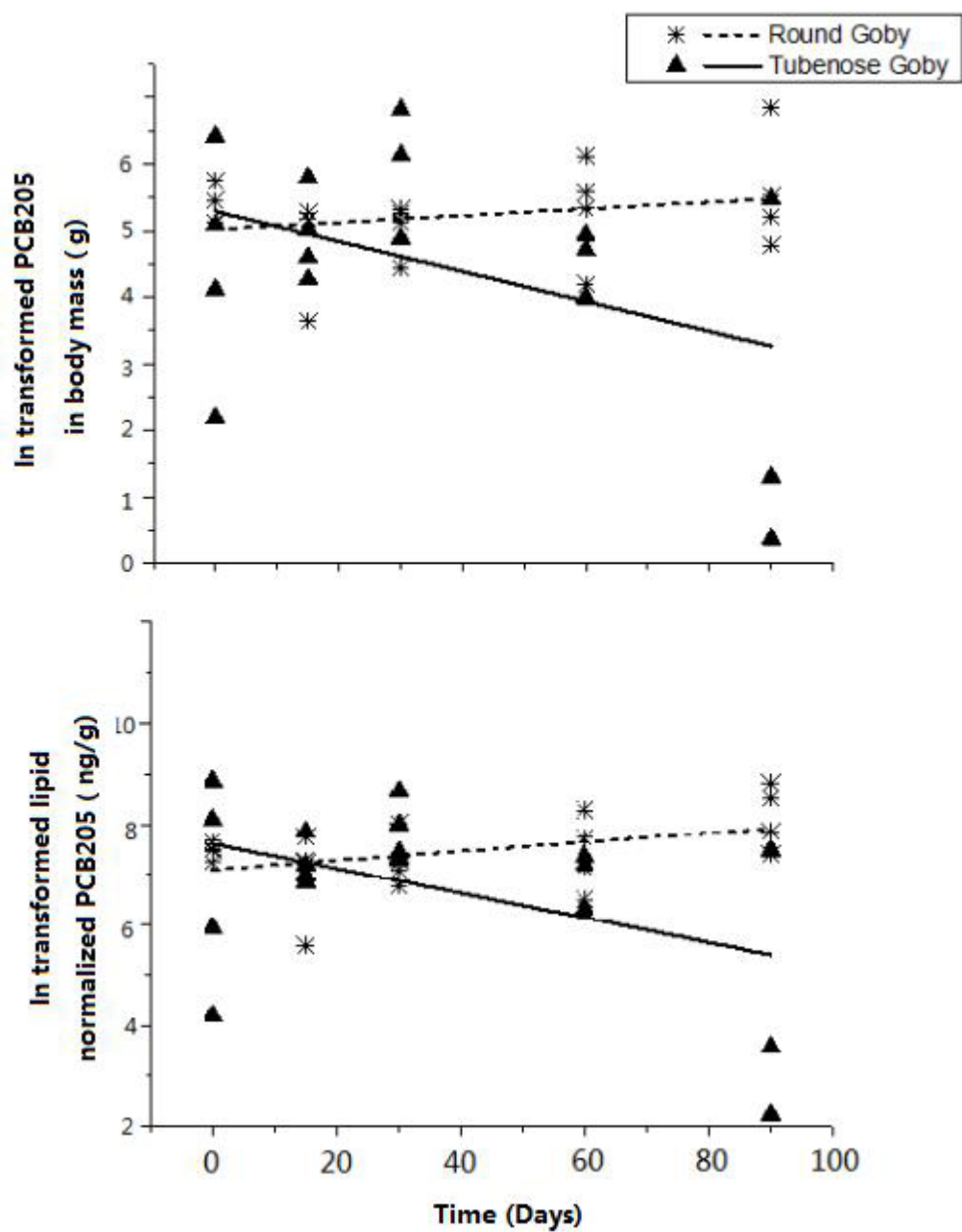


Figure 3.2 ln transformed mass balance and ln transformed lipid normalized PCB 205 dosed to fish with time in round and tubenose goby.

Less hydrophobic PRC-PCBs were observed to undergo significant elimination over the study. Valid k_{tot} values could not be generated for PCB 6 and 13 owing to the large number of non-detected concentrations in dosed fish, likely because of very rapid elimination of these compounds from fish. There was an overlap interference of PCB 23 with environmental PCB congener (PCB # 49) when each peak of PCB came out from GC-ECD, precluded its determination of k_{tot} . Significant elimination of PCBs 21, 57, 62, 68, and 89 was observed for round goby (Table 3.1). For tubenose goby, significant elimination was observed for PCBs 21, 57, 62, 68, 125 and 166 (Table 3.1). Figures 3.3 present elimination trends for 4 selected PCBs (PCBs 21, 57, 62 and 68) which underwent significant elimination in round goby and tubenose goby.

A principle component analysis was performed on the data matrix comprised of PCBs 21, 57, 62 and 68 which demonstrated significant elimination with time for both species. Only the first principle component had an eigenvalue greater than 1 (3.38) and was found to explain 84.5% of the variation of the data. All four PCB congeners exhibited strong loadings (correlation coefficients ranging from 0.86 to 0.96 on PCA 1). A general linear model was then performed to test for differences in PCA scores by species, differences with time and the species x time interaction term. Time was highly significant ($p < 0.001$; ANOVA) as expected given that only significantly eliminated PCB congeners were included within the model. Both the species ($p > 0.2$; ANOVA) and species x time interaction term ($p > 0.7$; ANOVA) were non-significant indicating that the dose received by each species and the rate of chemical elimination by each species was similar to one another.

		Round Goby			Tubenose Goby		
Chemical	Log K _{ow} ¹	k _{tot} ±SE (d ⁻¹)	N	R ² (p)	k _{tot} ±SE(d ⁻¹)	N	R ² (p)
PCB 21	5.41	0.031±0.005	20	0.70 (<0.001)	0.027±0.008	19	0.33(<0.01)
PCB 62	5.73	0.036±0.007	20	0.57(<0.001)	0.016±0.007	19	0.22(<0.05)
PCB 57	5.97	0.028±0.006	20	0.53(<0.001)	0.023±0.005	19	0.50(<0.001)
PCB 68	6.06	0.015±0.006	20	0.20(<0.02)	0.013±0.006	19	0.18(<0.05)
PCB 89	6.06	0.028±0.007	20	0.39(<0.05)	NS		
PCB 125	6.27	NS			0.017±0.004	19	0.52(<0.001)
PCB 166	6.63	NS			0.017±0.003	19	0.70 (<0.001)

Table 3.1 Whole body elimination coefficients for PCBs demonstrating significant elimination in round and tubenose goby. log K_{ow} values from Hansen et al. (1999). NS indicates no significant relationship existed between the PCB congener and k_{tot}.

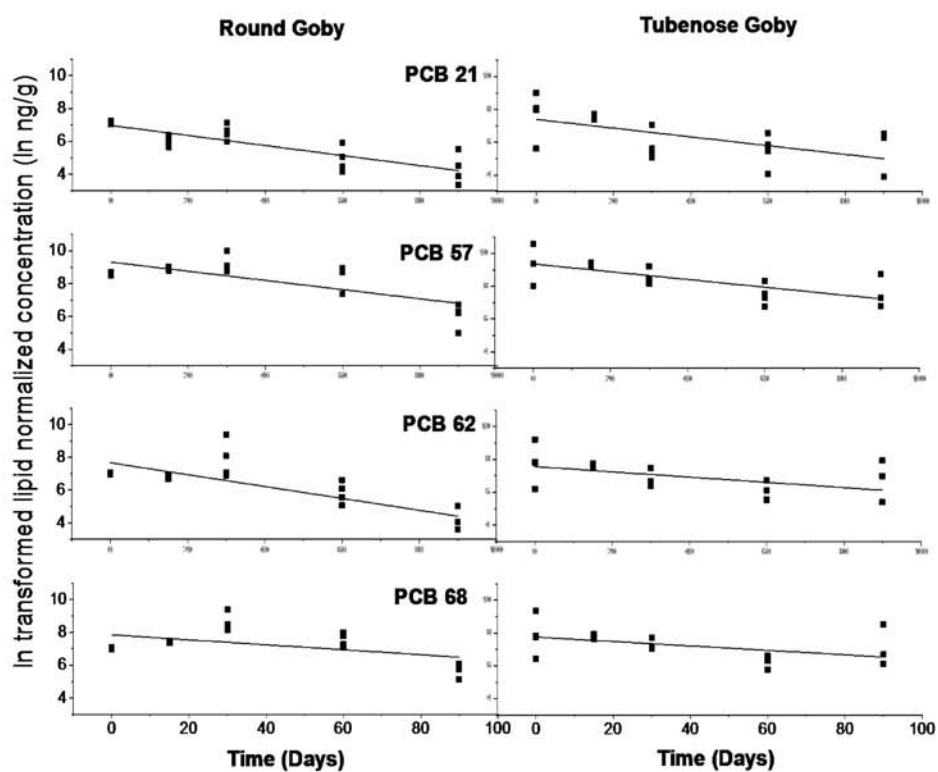


Figure 3.3 Trends in elimination of PCB 21, 57, 62, 68 in round and tubenose goby.

Figure 3.4 presents trends in k_{tot} as a function of chemical $\log K_{\text{OW}}$. There was an overall pattern of higher elimination for the least hydrophobic chemical (PCB 21). However, linear regression between k_{tot} and K_{OW} revealed no significant relationship ($p>0.05$; ANOVA) for either species or for data for the combined species ($p>0.05$; ANOVA).

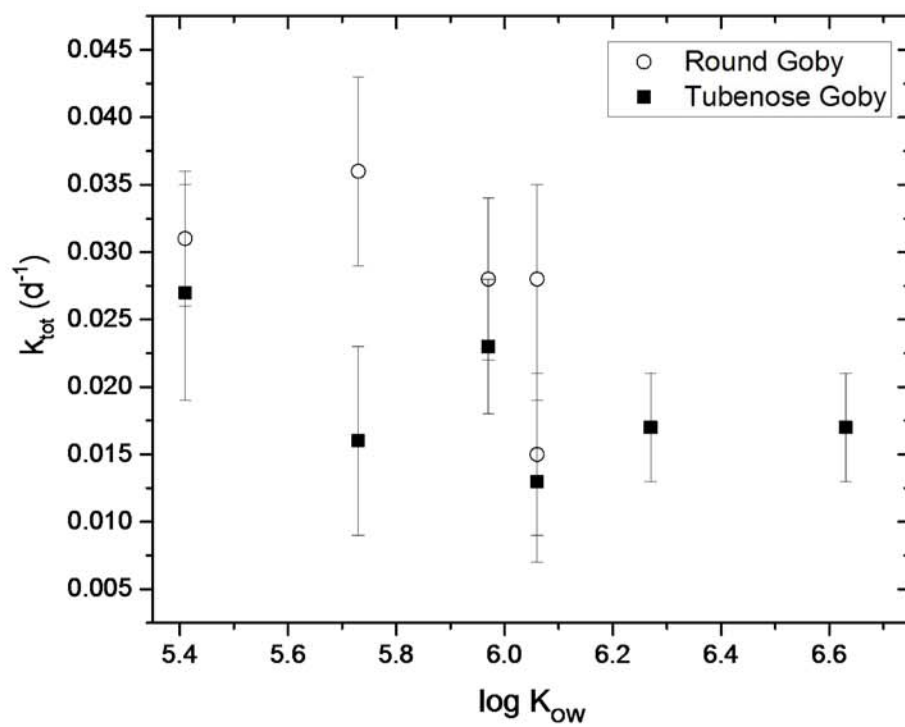


Figure 3.4. K_{tot} as a function of $\log K_{ow}$ in round and tubenose goby.

3.4 Discussion

Although chemical whole body elimination rates of each PCB generally have a negative relation with increased K_{ow} , the trend of this relation is not a constant. An unexpected observation from this research was the negligible elimination for PCBs with $\log K_{ow}$ greater than 6.4. The lack of trend observed between chemical hydrophobicity and k_{tot} measured for high K_{ow} PCBs in the present study suggests that other factors may have contributed to the observed elimination patterns. However, despite using small fish these experiments, the results of this study contrast with the conclusions of Paterson et al. (2007) who reported significant elimination of high K_{ow} ($\log K_{ow} > 6.5$) chemicals for yellow perch $< 10g$ while negligible elimination for yellow perch $> 50g$. In fact, small perch in Peterson et al.'s (2007) research demonstrated high growth rates over the duration of the experiment, while medium and large perch exhibited minimal or no growth, respectively. Sijm and Van der Linde (1995) predicted that an order of magnitude increase in body size would result in a substantial decrease in chemical elimination rates. In this case, growth dilution in small individuals might be a major contribution to whole body concentration reductions. However, no significant growth was observed in either of our two goby species. Therefore we conclude that high K_{ow} PCBs have negligible elimination even for the small fish $< 3g$.

In the present study, elimination of less hydrophobic PCBs (PCB 21, 57, 62, 68) was similar between round and tubenose goby. The PCB elimination rates observed in round and tubenose goby during constant temperature were similar to those observed

for small perch in Paterson et al.'s (2007) study during the summer period at their preferred temperature (22°C). Similarly, the half-life range of 22-25 days we estimated for round and tubenose goby for PCB21 were very similar to that reported for juvenile rainbow trout (PCB18 $t_{1/2}$ =24) reared at their preferred temperature (12°C) and fed a diet contaminated with PCBs (Fisk et al., 1998). Rainbow trout are considered a cold water species typically occupying 12 - 20 °C waters with a preferred temperature 11 °C (Cocherell et al., 2014). Round and tubenose goby, however, are eurythermal species inhabiting -1 to 30°C water with a metabolic optimum temperature of 23 °C (Lee and Johnson, 2005). Although positive relationships between chemical hydrophobicity and their respective half-lives have been established for a variety of organisms spanning a range of body sizes (Niimi and Oliver, 1983), these studies illustrate that four species of small fish (round goby, tubenose goby, yellow perch, rainbow trout) exhibited similar elimination rates in less hydrophobic PCBs when temperature reached their metabolic optimum. These results may indicate that the elimination of chemical might proceed through similar mechanisms, and elimination kinetics determined for similar-sized individuals reared at their metabolic optimum temperatures can eliminate PCBs at similar rates.

PCB elimination has been consistently shown to follow first order kinetics (Sijm et al., 1993; Paterson et al., 2010). Although there is a slight negative relationship between k_{tot} and K_{ow} , the elimination rates of individual PCBs for the two goby species used in this study showed no significant difference as a function of $\log K_{ow}$ (ANOVA, $p>0.05$). These results are in contrast with Li et al. (2015) who found a

strong negative relationship between PCB whole body elimination and $\log K_{ow}$ in goldfish (*C. auratus*) (linear regression, $p < 0.01$), even though a similar body size of 2.32g and a water temperature of 21-23°C were used. This might be because an insufficient number of PCBs were used in our study. Li et al. (2015) used 24 different PCB elimination rates while we used only 5 (Round Goby) or 6 (Tubenose Goby) which may not have been adequate to generate a significant relationship. However, our k_{tot} results are very close to Hattula and Karlog (1973) who reported the half-life for the sum PCB at 21 days in goldfish (1.8g) held at 21-23°C, which corresponds to an elimination rate of 0.03 d^{-1} . Future research should explore the relationship between K_{ow} and k_{tot} with larger number of individual PCBs.

Overall, we found similar PCB elimination kinetics for similar-sized round and tubenose goby when subjected to metabolic optimum temperatures. Future research should compare the chemical elimination rates for the two species at other temperatures spanning the range experienced during the seasonal thermal cycle. Such work would improve our understanding of metabolic efficiency and acclimation capacity of these two Great Lakes invaders.

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CHAPTER IV - USE OF NOVEL TRACER DEPURATION APPROACH TO COMPARE ROUTINE METABOLIC RATES (RMR) OF ROUND GOBY (*NEOGOBIOUS MELANSTOMUS*) AND TUBENOSE GOBY (*PROTERORHINUS SEMILUNARIS*)

4.1 Introduction

Metabolic rate is a physiological measure of the rate at which organisms utilize energy from assimilated food resources to satisfy energy requirements for organismal functioning. Understanding metabolic rate is critical for investigating ecological processes at multiple levels of organization (Brown et al., 2004). Chemical tracers such as mercury (Hg), cesium isotopes (^{137}Cs) and polychlorinated biphenyls (PCBs) have been used to provide estimates of fish routine metabolic rate (RMR) and in situ fish activity costs (Zheng et al., 2015; Madenjian et al., 2000; Paterson et al., 2007; McLeod et al. 2015). In all of the above cases, chemical tracers were interpreted within a bioaccumulation model framework where in situ fish feeding rates were solved based on the mass balance of tracer accumulated in fish tissues over time. The tracer mass balance necessitates the use of a tracer-specific toxicokinetic model that considers chemical exposure of consumed food items while accounting for time consuming in tracer assimilation during food digestion and tracer loss to various elimination processes (Trudel and Rasmussen 2001; McLeod et al. 2015).

Despite different examples of their application in aquatic systems, there are a number of challenges to bioaccumulation-tracer approaches of estimating RMR.

Variation in the composition of prey items consumed by fish and differences in tracer concentrations across different diet items can bias feeding rate estimates if this is not correctly accounted for by the model or if individual differences in diet composition occur among sampled fish (McLeod et al. 2015). For PCBs, chemical assimilation efficiency is also variable across different diet types which contributes additional uncertainty in RMR estimates when fish incorporate multiple prey (Liu et al. 2010; Selck et al. 2012). For Hg, little is known about the actual mechanisms of elimination of this chemical by fish. Trudel and Rasmussen (1997) generated empirical models of Hg elimination that considered temperature and body size. However, critics argued that Hg and ^{137}Cs elimination are themselves governed by fish metabolic rate and therefore are co-variates of fish feeding rate, the main state variable for which the model is being solved for (Rennie et al. 2005). This causes the model solution to become circular. Madenjian et al. (2014) argued that Hg elimination differs between fish sexes and is potentially under hormonal control, providing another complication to modeling elimination when Hg is used as the chemical tracer.

For PCBs, elimination is known to occur mainly by diffusive processes that include loss of chemical to water via gills and to feces as a result of organism/fecal chemical exchange (Gobas et al. 1988; Drouillard et al. 2009). Metabolic biotransformation of PCBs by fish is also known to occur, but considered negligible in magnitude compared to diffusive losses (Paterson et al. 2010). Drouillard et al. (2009) described a bioenergetic model used to predict congener specific PCB elimination by yellow perch. After model calibration, the authors concluded that most

PCB elimination by perch occurred via gills. This interpretation differed from previous models which assumed a transition in importance from gill to fecal dominated losses of PCBs with increasing chemical hydrophobicity (Gobas et al. 1988). Paterson et al. (2010) tested this by simultaneously measuring fecal elimination and whole body elimination of PCBs in goldfish. They observed that fecal elimination was less than 10% of whole body PCB losses, even for the most hydrophobic congeners, and attributed the remaining chemical elimination to gill losses.

Under a condition where gill elimination dominates whole body PCB elimination, the Drouillard et al. (2009) model could be re-arranged to solve for gill ventilation rates based on measurements of PCB elimination. Gill ventilation rate can then be used to calculate average oxygen consumption rates of fish offering an alternative chemical tracer method for estimating RMR. Under a tracer depuration approach, fish are dosed with non-environmental chemical tracers, released to the study system and recaptured at later time intervals to determine tracer loss. Given that a tracer depuration model has fewer parameters compared to a bioaccumulation model, its use could generate more precise estimates of RMR. Furthermore, if variation in the received dose by individuals is minimized through use of precise dosing methods, e.g. intraperitoneal (IP) injections (O'Neil et al. 2014), many of the issues related to multi-diet exposures and associated uncertainties inherent in tracer-bioaccumulation models would be avoided. Last, given that each PCB congener differs in its elimination rate as regulated by its chemical hydrophobicity (Gobas et al. 1988;

Drouillard et al. 2009; Paterson et al. 2010), multiple PCB tracer compounds could be used to track different time scales of animal release/recapture intervals or be used to cross validate one another in RMR estimates.

Alternatively, failure of the assumption that tracer elimination occurs only by elimination across of gills will cause overestimates of RMR for a given PCB congener. Furthermore, if the relative contribution of fecal to whole body elimination varies with chemical hydrophobicity as hypothesized by Gobas et al. (1988), this will result in congener specific differences in RMR that would positively correlate with chemical hydrophobicity. Under this condition, the tracer depuration model would have to be expanded to incorporate a fecal elimination sub-model and solved accordingly.

In this study, two tracer-depuration models are applied to fish depuration data in order to estimate of RMR of round and tubenose gobies. Model I uses a depuration tracer model to estimate RMR assuming all PCB elimination occurs across fish gills. Model II incorporates chemical loss to water and feces in order to estimate RMR. In order to assess accuracy of tracer depuration RMR estimates, the model generated RMRs were compared against standard metabolic rates (SMR) calculated for fish based on established allometric models generated for the species (Lee and Johnson 2006).

4.2 Methods

General Model

Details of the PCB toxicokinetic model are found in Drouillard et al. (2009). The basic tracer depuration model expanded into biogenergetic terms is given as follows:

$$dC_{org}/dt = - (G_v \cdot AE_w \cdot C_{org})/K_{b,w} - [(1-AE_d) \cdot G_{feed} \cdot AE_{chem} \cdot C_{org}]/K_{b,ex} - k_g \cdot C_{org} \quad (1)$$

where C_{org} is the PCB concentration in the animal (ng/g body weight), G_v and G_{feed} are gill ventilation (mL/g body weight/d) and feeding rate (g food/g body weight/d); AE_w , AE_d and AE_{chem} are efficiency terms (unitless) that describe chemical exchange efficiency between the animal and water via gills, diet digestability (g dry feces produced/g dry food consumed) and dietary chemical assimilation efficiency from ingested food; $K_{b,w}$ and $K_{b,ex}$ are partition coefficients for the chemical between animal and water (mL/g body weight) and between animal and its feces (g feces/g body weight); and k_g is the specific growth rate of tissues (/d). Gill ventilation rate, feeding rate and growth are further defined as bioenergetic terms as follows:

$$G_v = RMR / (D_{O_2} \cdot C_{O_2} \cdot E_{O_2}) \quad (2)$$

where RMR is in units of kJ/g body weight/d; D_{O_2} is the oxycalorific coefficient (14.30 kJ/g O_2); C_{O_2} is the concentration of oxygen in water (g O_2 /mL water) and E_{O_2} is the oxygen exchange efficiency across the gills (0.6; unitless).

$$G_{feed} = RMR/ED_{food} + G/ED_{food} \quad (3)$$

where ED_{food} is the energy density of food (kJ/g food) and G is the energy associated with tissue growth (kJ / g body weight/d).

$$k_g = G/ED_{org} \quad (4)$$

where ED_{org} is the energy density of the animal (kJ/g body weight).

Finally, the whole body elimination rate coefficient (k_{tot}) is set equal to $(dC_{org}/dt)/C_{org}$.

Substituting Eqs. 2-4 into Eq. 1 and solving for RMR yields:

$$RMR = \frac{k_{tot} \cdot K_{B,EX} - k_g \cdot \left(\frac{AE_{chem} \cdot ED_{org}}{ED_{food}} - \frac{AE_d \cdot AE_{chem} \cdot ED_{org}}{ED_{food}} + K_{b,ex} \right)}{\left(\frac{AE_w \cdot K_{B,EX}}{K_{b,w} \cdot D_{O2} \cdot C_{O2} \cdot AE_{O2}} + \frac{AE_{chem}}{ED_{food}} - \frac{AE_d \cdot AE_{chem}}{ED_{food}} \right)} \quad (5)$$

Equation 5 can be simplified under different sets of experimental conditions. Under a condition of no growth (i.e. $k_g = 0$) and no elimination to feces, Eq. 5 is simplified to Model I and has the following solution:

$$RMR = \frac{k_{tot} \cdot K_{b,w} \cdot D_{O2} \cdot C_{O2} \cdot AE_{O2}}{AE_w} \quad (6)$$

Under a condition of no growth but elimination occurring to both water and feces, Eq 5 is reduced to Model II and is solved as:

$$RMR = \frac{k_{tot} \cdot K_{b,ex}}{\left(\frac{AE_w \cdot K_{b,ex}}{K_{b,w} \cdot D_{O2} \cdot C_{O2} \cdot AE_{O2}} + \frac{AE_{chem}}{ED_{food}} + \frac{AE_{diet} \cdot AE_{chem}}{ED_{food}} \right)} \quad (7)$$

The partition coefficients ($K_{b,w}$ and $K_{b,ex}$) are calculated based on proximate composition of tissues or feces as defined by Arnot and Gobas (2004) and referenced against the *n*-octanol/water partition coefficient (K_{OW}) of the PCB congener such that:

$$K_{b,w} = (X_{lipid(org)} + 0.05 \cdot X_{LDP(org)}) \cdot K_{OW} \quad (8)$$

where $X_{lipid(org)}$ and $X_{LDP(org)}$ are the mass fraction of neutral lipids and lean dry protein in the animal. The constant 0.05 specifies the partition capacity of lean dry protein

relative to lipids in the system (Debruyne and Gobas, 2007). X_{LDP} is estimated as the dry tissue weight minus lipid weight divided by the wet whole body weight of the animal. The animal to feces partition coefficient is given by:

$$K_{b,ex} = (X_{lipid(org)} + 0.05 \cdot X_{LDP(org)}) / (X_{lipid(ex)} + 0.05 \cdot X_{LDP(ex)}) \quad (9)$$

where $X_{lipid(ex)}$ and $X_{LDP(ex)}$ are the mass fraction of neutral lipids and lean dry protein in the feces.

4.3 Data interpretation

PCB k_{tot} values were obtained from Chapter 3. k_{tot} values were used in conjunction with estimates of model parameters generated by Model I and Model II to estimate RMR of fish. Table 4.1 and 4.2 summarizes mean and standard deviations applied for each of the model parameters employed in RMR calculations for each model in both species. Monte Carlo simulations, using Crystal Ball software, were used to calculate uncertainty of RMR estimates for each species using Model I and Model II calculation methods. The mean and standard deviation of each measured parameter was applied in Model I and II. For all other model parameters where assumed values were used, estimates of standard deviation were obtained from the literature or as described below. For parameters assigned as continuous variables, a log normal distribution was assumed. For parameters which were efficiency terms (e.g. AE_{diet} , AE_w , AE_{Chem} , ect), a triangle distribution was used setting the minimum and maximum values equal to 1 standard deviation from the mean. Each Monte Carlo

Round Goby	PRC 21	PRC 62	PRC 68	PRC 57	PRC 89	Source
$\log K_{ow}$	5.41±0.030	5.73±0.040	6.06±0.070	5.97±0.060	6.06±0.070	Measured
AE_w	0.54±0.025	0.54±0.025	0.54±0.025	0.54±0.025	0.54±0.025	Quoted
$D_{O_2} (kJ \cdot g^{-1})$	14.3±2.86	14.3±2.86	14.3±2.86	14.3±2.86	14.3±2.86	Quoted
E_{O_2}	0.6±0.25	0.6±0.25	0.6±0.25	0.6±0.25	0.6±0.25	Quoted
K_{tot}	0.031±0.01	0.036±0.015	0.015±0.013	0.028±0.012	0.028±0.016	Measured
$C_{O_2} (10^{-6})$	8.83±1.97	8.83±1.97	8.83±1.97	8.83±1.97	8.83±1.97	Measured
X_{lipid}	0.041±0.007	0.041±0.007	0.041±0.007	0.041±0.007	0.041±0.007	Measured
X_{ldp}	0.159±0.007	0.159±0.007	0.159±0.007	0.159±0.007	0.159±0.007	Measured
X_{fldp}	0.802±0.163	0.802±0.163	0.802±0.163	0.802±0.163	0.802±0.163	Measured
X_{fl}	0.041±0.005	0.041±0.005	0.041±0.005	0.041±0.005	0.041±0.005	Measured
AE_{chem}	0.931±0.03	0.931±0.03	0.931±0.03	0.931±0.03	0.931±0.03	Quoted
ED_{food}	3.907±0.079	3.907±0.079	3.907±0.079	3.907±0.079	3.907±0.079	Measured
AE_{diet}	0.685±0.106	0.685±0.106	0.685±0.106	0.685±0.106	0.685±0.106	Quoted

Table 4.1 Values of each parameter used in Model I to calculate routine metabolic rates in round goby. Where AE_w is chemical exchange coefficient (unitless) (Drouillard et al., 2009), D_{O_2} is oxycalorific coefficient ($kJ \cdot g^{-1}$), E_{O_2} is the oxygen exchange efficiency across gills (unitless) (Drouillard et al., 2009), k_{tot} is chemical elimination rates ($mg \cdot g^{-1} \cdot day^{-1}$), C_{O_2} is dissolved oxygen concentration ($mg \cdot mL^{-1}$). X_{lipid} is fraction of lipid in fish. X_{ldp} is fraction of lean dry weight in fish. X_{fldp} fraction of lean dry weight in food, X_{fl} is fraction of lipid in food. AE_{chem} is chemical assimilation coefficient (unitless) (Liu et al., 2010), ED_{food} is food density ($kJ \cdot g^{-1}$) and AE_{diet} is dietary assimilation coefficient (unitless).

Tubenose Goby	PRC 21	PRC 62	PRC 68	PRC 57	PRC 125	PRC 166	Source
logK_{ow}	5.41±0.030	5.73±0.040	6.06±0.070	5.97±0.060	6.27±0.09	6.63±0.15	Measured
AE_w	0.54±0.025	0.54±0.025	0.54±0.025	0.54±0.025	0.54±0.025	0.54±0.025	Quoted
D_{O2} (kJ·g⁻¹)	14.3±2.86	14.3±2.86	14.3±2.86	14.3±2.86	14.3±2.86	14.3±2.86	Quoted
E_{O2}	0.6±0.25	0.6±0.25	0.6±0.25	0.6±0.25	0.6±0.25	0.6±0.25	Quoted
K_{tot}	0.027±0.017	0.016±0.014	0.013±0.012	0.024±0.011	0.017±0.007	0.017±0.005	Measured
C_{O2} (10⁻⁶)	8.83±1.97	8.83±1.97	8.83±1.97	8.83±1.97	8.83±1.97	8.83±1.97	Measured
X_{lipid}	0.039±0.006	0.039±0.006	0.039±0.006	0.039±0.006	0.039±0.006	0.039±0.006	Measured
X_{ldp}	0.161±0.008	0.161±0.008	0.161±0.008	0.161±0.008	0.161±0.008	0.161±0.008	Measured
X_{fldp}	0.802±0.163	0.802±0.163	0.802±0.163	0.802±0.163	0.802±0.163	0.802±0.163	Measured
X_{fl}	0.041±0.005	0.041±0.005	0.041±0.005	0.041±0.005	0.041±0.005	0.041±0.005	Measured
AE_{chem}	0.931±0.03	0.931±0.03	0.931±0.03	0.931±0.03	0.931±0.03	0.931±0.03	Quoted
ED_{food}	3.907±0.079	3.907±0.079	3.907±0.079	3.907±0.079	3.907±0.079	3.907±0.079	Measured
AE_{diet}	0.685±0.106	0.685±0.106	0.685±0.106	0.685±0.106	0.685±0.106	0.685±0.106	Quoted

Table 4.2 Values of each parameter used in Model II to calculate routine metabolic rates in round goby. Where AE_w is chemical exchange coefficient (unitless) (Drouillard et al., 2009), D_{O2} is oxycalorific coefficient (kJ·g⁻¹), E_{O2} is the oxygen exchange efficiency across gills (unitless) (Drouillard et al., 2009), k_{tot} is chemical elimination rates (mg·g⁻¹·day⁻¹), C_{O2} is dissolved oxygen concentration (mg·mL⁻¹). X_{lipid} is fraction of lipid in fish. X_{ldp} is fraction of lean dry weight in fish. X_{fldp} fraction of lean dry weight in food, X_{fl} is fraction of lipid in food. AE_{chem} is chemical assimilation coefficient (unitless) (Liu et al., 2010), ED_{food} is food density (kJ·g⁻¹) and AE_{diet} is dietary assimilation coefficient (unitless).

simulation was run for 10,000 iterations and outputs of RMR from each trial were saved. A tornado plot was used to evaluate the sensitivity of each model to different parameters included in the model. RMR trial values were then randomly taken from the crystal ball simulation outputs for a randomly selected PCB congener that was commonly eliminated by both species. The Monte Carlo simulated data were treated as independent RMR estimates and used in an ANOVA to evaluate the likelihood that significant differences in RMR between the two species occurred. These evaluations were performed with 5, 10, 20, 100 and 1000 random trial values as a means of assessing the relative power of the test result and to inform on the likelihood of differences in RMR between species.

4.4 Results

Routine metabolic rates were calculated by Model I and Model II from k_{tot} data. The mean \pm standard error RMR generated across significantly eliminated PCB congeners were 0.19 ± 0.09 kJ/g body weight/d and 0.23 ± 0.23 kJ/g body weight/d for round and tubenose gobies, respectively based on model I (Figure 4.1; Table 4.3). Model II estimates of RMR were lower at 0.070 ± 0.002 kJ/g body weight/d and 0.055 ± 0.001 kJ/g body weight/d for round and tubenose gobies, respectively (Figure 4.1; Table 4.3). RMR estimates generated using Model I, differed across PCB congeners and exhibited a significant increasing trend with chemical hydrophobicity. Differences in RMR estimates across chemicals were not apparent when estimated by Model II.

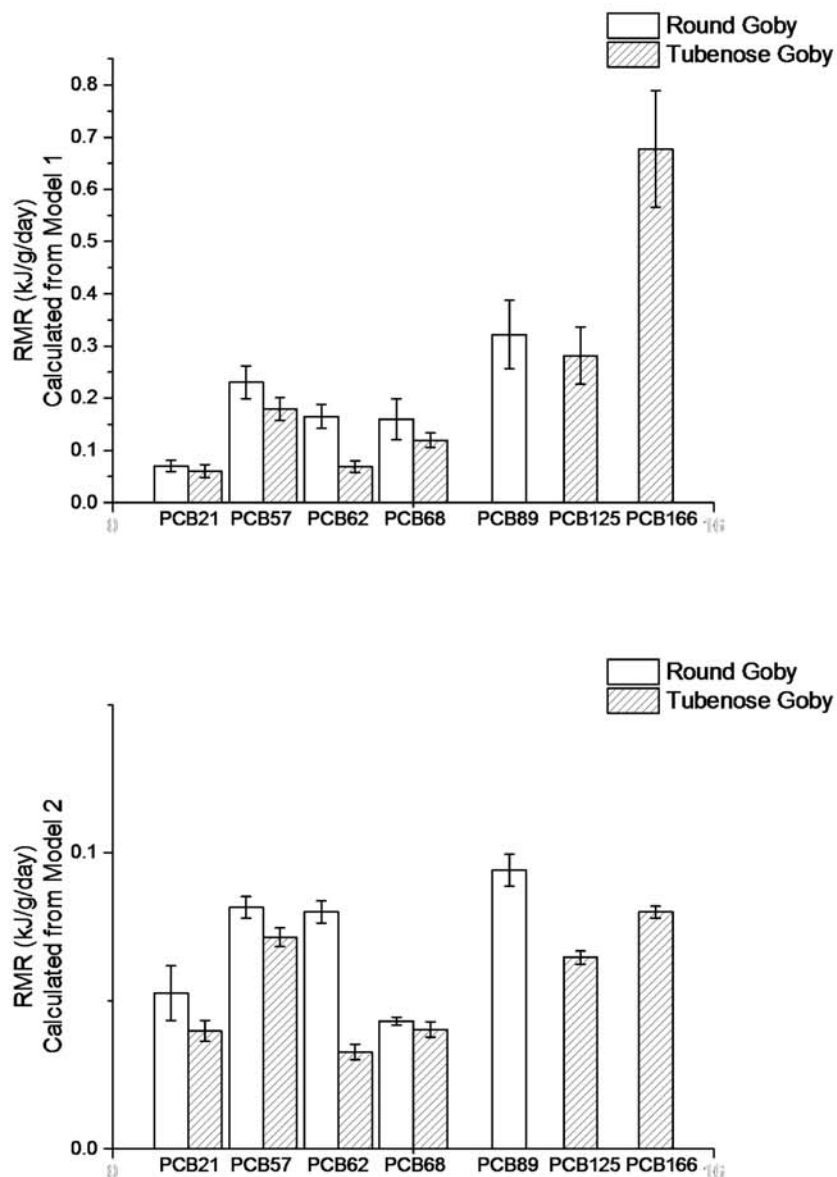


Figure 4.1 Routine metabolic rate (kJ/g/day) for Round and Tubenose Goby calculated by Model I (top graphic) and Model II (bottom graphic).

Model I

Round Goby	lg Kow	RMR (kJ/g/day)	RMR/SMR	Average ratio
PRC 21	5.51	0.05743	1.531	4.069±0.491
PRC 57	5.89	0.23708	6.319	
PRC 62	6.26	0.15997	4.264	
PRC 68	6.17	0.15626	4.165	
Tubenose Goby	lg Kow	RMR (kJ/g/day)	RMR/SMR	Average ratio
PRC 21	5.51	0.06021	1.462	2.599±0.333
PRC 57	5.89	0.11931	2.898	
PRC 62	6.26	0.17934	4.356	
PRC 68	6.17	0.06921	1.681	

Model II

Round Goby	lg Kow	RMR (kJ/g/day)	RMR/SMR	Average ratio
PRC 21	5.51	0.05261	1.40227	1.716±0.129
PRC 57	5.89	0.08164	2.17595	
PRC 62	6.26	0.08005	2.13353	
PRC 68	6.17	0.04317	1.1507	
Tubenose Goby	lg Kow	RMR (kJ/g/day)	RMR/SMR	Average ratio
PRC 21	5.51	0.03981	0.96711	1.120±0.105
PRC 57	5.89	0.07155	1.73801	
PRC 62	6.26	0.0327	0.79422	
PRC 68	6.17	0.04041	0.98172	

Table 4.3 Estimated routine metabolic rate (RMR, kJ/g/day) and field metabolic rate/standard metabolic rate ratios (RMR/SMR) for different chemical tracers in round goby and tubenose goby using Model I (upper) and Model II (lower)

The ratio of RMR/SMR was generated as a further evaluation of the accuracy of tracer-generated RMR estimates. Standard metabolic rates were estimated for each species based on experimental temperature and fish body size using the SMR model reported by in O'Neil (2013) from respirometry studies. Given that fish were maintained in an aquaculture facility, RMRs were expected to be within 50% of SMRs owing to additional metabolic costs of specific dynamic action, waste excretion and small amounts of activity within the confined space of the experimental tanks (Brett, 1964; Claireaux et al., 2006). Based on Model I, the RMR/SMR ratio for round and tubenose gobies were 4.069 ± 0.491 and 2.599 ± 0.333 , respectively. For model II, the ratios of RMR/SMR in the two species were 1.716 ± 0.129 and 1.120 ± 0.105 , for round and tubenose gobies (Table 4.3). These results imply that Model II yielded metabolic rate estimates that more closely approached the expected metabolic rate in both species.

Monte Carlo simulation trial values generated using Model II estimates of RMR were subsequently used to evaluate the likelihood of species differences in RMR. Four PCB congeners (PCB 21, 57, 62, 58) exhibited significant elimination by both species over the study duration. A randomization procedure was used to select one of the 4 congeners (PCB 21) for evaluation. ANOVA's were subsequently performed to detect species differences in RMR using Monte Carlo generated trial values as independent replicates. The first ANOVA was run using 5 randomly selected Monte-Carlo generated trial values for each species. Subsequent ANOVAs increased the theoretical power of the test by using 10, 50, 100 and 1000 trial values.

In the case of 5 trial values, no significant differences (ANOVA, $p = 0.875$) were observed in the RMR estimates between species. There were also no significant species differences when replicate trial values per species were increased to 10, 50 and 100 trials. Differences by species ($p < 0.01$) occurred when 1000 replicated trial values were applied. These data indicate that the likelihood of species differences in RMR are very low given the uncertainty present in both measured and model parameters outlined in Table 4.3.

A sensitivity analysis was performed for RMRs estimated by Model II for PCB 21 and 166 in the tubenose goby. The above congeners were chosen to be representative of model performance generated using a low and high K_{OW} congener to determine whether model sensitivity varies according to chemical hydrophobicity. Figure 4.2. presents a Tornado Plot generated from the sensitivity analysis completed for each chemical. The Tornado plot specifies the minimum and maximum RMR values generated over the range of values chosen for a given parameter across Monte Carlo iterations. For both the low and high K_{OW} chemicals, the RMR model was found to be most sensitive to uncertainty in the k_{tot} estimate. For PCB 21, individual estimates of RMR across Monte Carlo trials varied by as much as $\pm 63\%$ from the mean estimate as a result of uncertainty in k_{tot} . For PCB 166, RMR values across trials varied by $\pm 29\%$ owing to uncertainty in k_{tot} estimates. These differences in model sensitivity between chemicals are attributed to differences in the precision of k_{tot} measurements given that the relative standard deviation was lower for the high K_{OW} congener. The

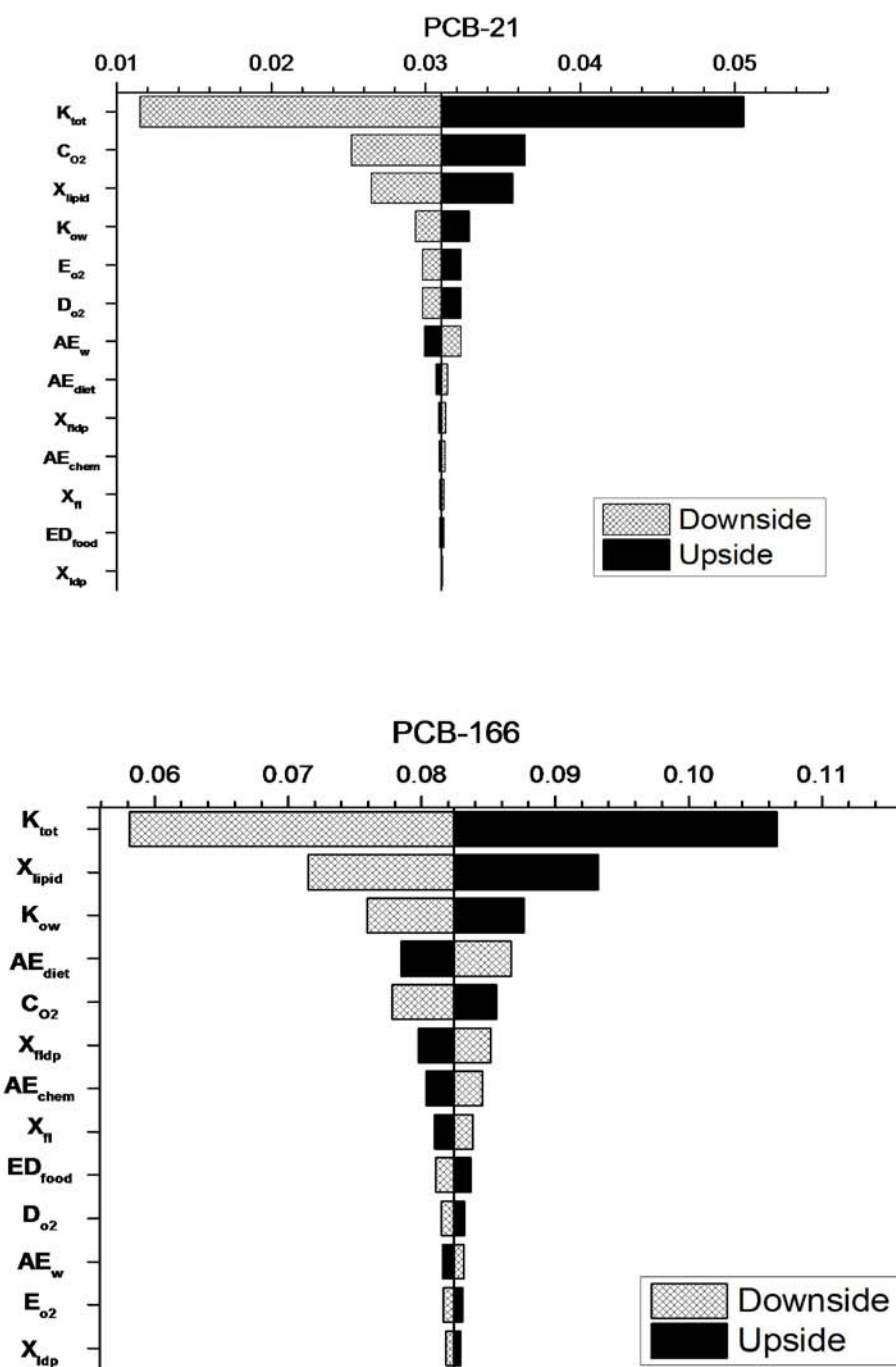


Figure 4.2 Tornado plot demonstrating the contribution of each parameter to model uncertainty in RMR estimates for low Kow (PCB 21) and high Kow (PCB 166) congeners in tubenose goby.

sensitivity analysis subsequently showed differences in the rank order of parameters contributing to additional model uncertainty. For PCB 21, variation in dissolved oxygen concentrations, fraction of lipid in the animal and K_{ow} were the next largest contributors to model uncertainty resulting in $\pm 19\%$, $\pm 15\%$ and $\pm 6\%$ differences in RMR estimates from the mean value across iterations. For PCB 166, fraction of lipid in the animal, chemical assimilation efficiency from diet and K_{ow} resulted in $\pm 13\%$, $\pm 8\%$ and $\pm 5\%$ model uncertainty in RMR estimates. Differences in rank order parameter contributions to model uncertainty between the congeners are due to the different chemicals behaving differently within the model. Essentially, Model II collapses into Model I for less hydrophobic chemicals and thus variation in parameters present in both model I and model II will have a greater contribution to model uncertainty. For the more hydrophobic chemicals, parameters associated with fecal egestion (inversely related to AE_{diet}) become more important and stronger contributes to uncertainty when parameter error is included.

4.5 Discussion

Drouillard et al. (2009) provided model solutions enabling use of PCBs as chemical depuration tracers that could be used to infer fish ventilation rates and subsequently estimate oxygen consumption rates. However, this inference is largely based on Model I which assumes that loss of PCBs to feces represents a negligible elimination route by fish. Paterson et al. (2007) reported that only 10% of PCBs are

lost via egestion in yellow perch. Similarly, Paterson et al. (2010) found that only 5% of the PCB ($\log K_{ow}$ from 5.7 to 7.8) whole body elimination measured in Japanese koi could be explained by fecal egestion following determination of fecal egestion rate coefficients. The above observations support application of Model I over Model II to estimate RMR of fish. Alternatively, Gobas et al. (1988) generated a model which predicted that fecal egestion of PCBs becomes the predominate mechanism of chemical elimination for chemicals with $\log K_{ow}$ values exceeding 6. This would imply Model I could only be used for less hydrophobic congeners (i.e. $< \log K_{ow}$ 5.5) and that Model II would be more accurate for chemicals exhibiting higher hydrophobicity.

The present research generated two lines of evidence in order to distinguish the applicability of Model I versus Model II used to estimate RMRs of gobies. The first was to test whether Model I or Model II generated similar estimates of RMR across different chemicals that varied in their hydrophobicity. The present results showed that Model I generated different values of RMRs across chemicals and that RMR was positively correlated with chemical K_{ow} . In contrast, Model II estimates of RMR were more consistent across different PCB congeners and were not correlated with chemical hydrophobicity.

The second line of evidence was based on interpretation of the magnitude of RMR/SMR ratios generated by the two models as compared to expectations from Wisconsin fish bioenergetics model predictions. Under a Wisconsin bioenergetic model, the RMR is predicted according to:

$$\text{RMR} = \text{SMR} \cdot \text{A} + \text{SDA} + \text{U}$$

Where SMR is the standard metabolic rate (kJ/g/d), A is an Activity Multiplier (unitless), SDA is specific dynamic action (kJ/g/d) and U refers to energy losses to excretion (kJ/g/d). Calculation algorithms for SDA and U are generally derived from SMR. For example, SDA is typically estimated to be 17.2% of the product of SMR·A. Excretion is commonly modelled as a temperature dependent function but typically constitutes 10-12% of the product of SMR·A near the fish's optimal temperature (Drouillard et al. 2009). Fish activity represents the largest unknown and greatest possible modifier of RMR. Under standard model applications, the Wisconsin model applies an activity multiplier of 2 for natural populations of fish experiencing normal foraging costs (Facey et al., 1990). Activity multipliers of 3 – 4 are considered extreme and approach the scope for activity for many fish species as elucidated under forced swimming trials (Garcia et al., 2012). Alternatively, when fish are held under confined conditions and have low foraging costs, as is the case of aquaculture held fish, activity multipliers between 1 and 1.5 are typically recommended (Klinger et al., 2015).

The fish from experimental studies used to calibrate the chemical tracer model in the present research were held in a tank near their optimal temperature and fed a maintenance ration that contributed to negligible growth. Thus an activity multiplier between 1 and 1.5 would be considered appropriate to this population and the expected RMR/SMR ratio would therefore range from 1.28 to 2.01 when considering the above estimates of SDA and U.

Table 4.3 demonstrates that the RMR/SMR ratio generated under model I averaged 2.6 ± 0.3 and 4.0 ± 0.5 for tubenose and round goby and were higher than the expected RMR/SMR ratio. In particular, congener specific estimates of RMR using model I as high as 6 are highly unlikely except for vigorously exercised fish which is not likely given the husbandry conditions of fish under study. In contrast, the RMR/SMR predictions generated for round goby using Model II were consistent with the Wisconsin model predictions. For tubenose goby, RMR/SMR ratios generated using Model II were close to 1 and somewhat less than expected but generally closer to expectations than equivalent estimates generated from Model I. Taken together, Model II appears to be the most appropriate method for estimating fish ventilation rate and oxygen consumption using PCBs as bioenergetics tracers.

There was no clear evidence for species differences in the RMR's of round and tubenose gobies based on computed values of RMR from Model II and consideration of the uncertainty associated with these model estimates. Direct testing of differences in RMR between species is difficult because of the large number of parameters incorporated into the model and propagation of error across parameters. Monte Carlo trial iterations were instead used to infer statistical power and establish the likelihood of observing species differences in RMR given model uncertainty. These trials indicated that very large replicate sizes approaching 1000 observations would be needed to detect species differences in RMR and suggest that species differences in true RMR values are unlikely. This is consistent with the interpretation generated in

Chapter 3 which showed that k_{tot} values were not statistically different between the two species.

Model sensitivity evaluation further indicates that measurement error in k_{tot} was the single highest contributor to model uncertainty. Thus, generating more precise estimates of k_{tot} would lead to an improvement in the ability of the RMR model to discriminate between treatments (i.e. species effect or other experimental manipulations). Strategies towards improving the precision of k_{tot} estimates vary depending on the chemical tracer employed. For the least hydrophobic chemicals such as PCB 21, increasing the sampling resolution during early time steps would yield more precise estimates of k_{tot} given that some fish had not retained any of the initial dose by the end of the study duration. Whereas for more hydrophobic chemicals, extending the duration of the depuration trial over longer time periods would be more beneficial. Thus, the time frame over which integrated estimates of RMR in relation to study goals should be considered when attempting to optimize a sampling design in order to generate more precise k_{tot} and RMR estimates.

Overall, the study results indicate that PCBs can be used as chemical tracers of fish bioenergetics as applied under depuration conditions. However, the data suggest that fecal losses of PCBs needs to be accounted for in order to estimate fish ventilation rate and oxygen consumption rates. In the present study, fecal egestion of PCBs was estimated according to a general model using literature based estimates of diet digestability (AE_{diet}), proximate composition of feces (X_{lipid} and X_{LDP}) and animal/fecal exchange efficiency (assumed to be equal to AE_{chem}). More accurate

RMR estimates could be generated by empirically measuring parameters used within the fecal elimination sub-model and by validating assumptions, i.e. that the organism/feces exchange efficiency is equal to the dietary assimilation efficiency of the chemical (Drouillard et al. 2012). There is a general paucity of information about fecal elimination rate coefficients of PCBs from fish with measured estimates limited to a single study (Paterson et al. 2010). Further research to generate more accurate fecal-elimination sub-models would be useful to improve the accuracy the bioenergetics tracer model particularly when it is applied to highly hydrophobic tracer compounds. Alternatively, given that less hydrophobic chemicals behave more like Model I compounds, utilization of a larger number of lower chlorinated PCB tracer chemicals would negate the need for fecal loss corrections.

Overall, there were no differences in the RMR of round and tubenose goby which suggests that at the size range and temperature tested, both species have similar energy requirements. Thus, neither round or tubenose goby would be expected to have a stronger impact on food resource provisioning nor would either have a competitive advantage over one another under resource limited conditions. However, this study was limited to the preferred temperature range of the two species and additional investigations should be performed in order to test differences in RMR between the species across different temperatures. For example, Chapter 2 of this thesis indicated that tubenose goby suffer higher thermal stress at elevated temperatures compared to round goby and therefore differences in RMR between the species might be expected at temperatures above 30°C. Lastly, care was taken to ensure that both species were of

similar size to provide a direct comparison of RMRs. However, in the wild, round goby typically achieves much larger sizes compared to tubenose gobies. Thus, measurement of RMR over the full size range of each species would be necessary to fully compare their ecological footprints within their invasive range.

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CHAPTER V - GENERAL DISCUSSION

5.1 General Discussion

The present thesis provides a useful set of case studies to estimate the potential invasive risks for a round and tubenose goby and improves our understanding of the metabolic requirements in two closely related Great Lakes invasive fish species using novel chemical tracer approaches. Round and tubenose goby were introduced to the Laurentian Great Lakes via Lake St. Clair at approximately the same time (early 1990's) and by the same hypothesized transport vector (ship ballast waters). Both species bear many similarities in their morphological characteristics, benthic habitat and benthic invertebrate feeding strategies. Despite these similarities, round gobies exhibited very rapid expansion post-establishment with their range encompassing the entire Great Lakes basin within 5 years of their introduction. In contrast tubenose goby remained largely restricted in its distribution over the same time frame.

This thesis compared selected attributes related to physiological tolerance and metabolic performance of the two species to determine whether differences in fundamental niche or realized niche could explain differences in invader distribution success. Fundamental niche is explicitly associated with physiological tolerance of a species in question. Chapter 2 of this thesis compared the temperature tolerance of round and tubenose goby to determine whether or not species differences in survivorship under acute thermal stress occurred. Realized niche is constrained

relative to fundamental niche as it considers both physiological tolerance to environmental conditions coupled with additional constraints generated from biological interactions (e.g. pathogens, competitors and predators) in a given environment. In chapter 4, routine metabolic rates were compared between round and tubenose goby as a first step in evaluating potential differences in realized niche. Routine metabolic rate provides a measure of the energy requirement of a given species which relates to its trophic impact on the environment (i.e. the number of prey items consumed per unit body mass and time) and its competitiveness under food resource limitation (i.e. animals with higher food requirements are more likely to become extirpated relative to competitors under resource limited conditions). By measuring and comparing routine metabolic rates of round and tubenose goby, inferences about the trophic impact footprint or likelihood of establishing in ecosystem with limited food resources could be assessed.

Chapter 2 tested thermal tolerance of the two species by determination of 12 h lethal water temperatures causing 50% mortality (LC50) and time to lethality of 50% of fish under acute temperature conditions (LT50). Exposure to high water temperatures, and the ability to survive such exposures, is hypothesized to be important to the spread and post-establishment distribution of an invader. For example, small water bodies (e.g. ditches and creeks) are commonly subject to rapid daily thermal fluctuations. These same water bodies can also act as transport conduits for established invaders to extend their range into adjacent habitats. Thus species that have high physiological tolerance to acute temperature stress can potentially access

and utilize a larger number of connecting water bodies and more rapidly extend their range compared to those restricted by lower temperature tolerance. The results of chapter 2 demonstrated round goby to have a higher physiological tolerance to acute temperature stress relative to tubenose goby. The 12 h LC50 for round goby was 33.4°C and was significantly higher than tubenose goby which had an LC50 of 31.9°C. Thus the null hypothesis that round goby and tubenose goby have a similar temperature tolerance is rejected. Furthermore, based on the relationships presented for time to 50% lethality with temperature; 50% of round gobies would be capable of surviving water temperatures of 32°C for up to 28 h whereas 50% mortality of tubenose gobies would be expected after approximately 4 h under similar environmental conditions. It is plausible that diurnal fluctuations in small water conduits such as ditches and creeks achieve high temperatures of 32°C for several hours within the Great Lakes region during the day whereas such conditions are unlikely to extend over period exceeding 12 h or longer due to temperature decreases at night. As such, physiological tolerance differences measured between round and tubenose goby may place realistic constraints on their ability to take advantage of different types of connecting corridors that could facilitate their invasive spread.

Alternatively, thermal tolerance represents only one dimension of physiological tolerance differences between the two species. Future research in this area should consider extending these results to characterize temperature tolerance under cold conditions to assess whether the lower acute temperature tolerance of tubnose goby under high temperatures represents a trade-off to tolerance of low temperature

conditions. Additionally, physiological tolerance differences across other types of stressors likely to affect invasive species spread and fitness would be useful to measure. For example, differences in the two species' tolerance to low oxygen conditions, turbidity, contaminants, exposure to algal toxins etc. would be useful to understand.

Chapter 3 of the thesis was concerned with calibrating and comparing the elimination of hydrophobic chemicals from round and tubenose goby. Non-environmental polychlorinated biphenyls (PCBs) have the potential to be utilized as bioenergetics tracer chemicals but require calibration of chemical toxicokinetics in the species of interest prior to their use as tracer compounds. Elimination rate constants of 14 PCBs from fish were evaluated under constant temperature conditions (21.4 °C) over a period of 90 days. Growth of fish was negligible over the depuration period owing to reduced diet provisioning. For round goby, significant elimination was measured for 5 PCB congeners (PCB 21, 62, 68, 57 and 89) with k_{tot} values ranging from 0.015 – 0.031 d⁻¹ and corresponding half lives of 22.4 to 46.2 d. For tubenose goby, elimination rate coefficients were measured for 6 PCBs (PCBs 21, 62, 68, 57, 125 and 166) ranging from 0.013 – 0.027 d⁻¹ and half lives in the range of 25.7 to 53.3 d. No significant elimination was observed for chemicals having log K_{OW} values exceeding 6.6. There was a general, but non-significant, trend of decreasing k_{tot} value with increasing chemical hydrophobicity. The lack of K_{OW} trend on k_{tot} was not consistent with our hypothesis of K_{OW} dependent elimination (Chapter 1). However, this result was likely due to the small K_{OW} range over which measurable k_{tot} values

were generated. Both longer depuration times and more precise dosing methodologies would be necessary to generate robust enough data sets to further test the hypothesis of K_{ow} dependent PCB elimination in these species. A second and central hypothesis tested in Chapter 3 was whether or not PCB elimination rates were similar between the two fish species. Multivariate data reduction approaches (PCA) indicated similar behaviour of the four commonly eliminated PCB congeners from the two species (PCB 21, 62, 68 and 57) such that a single PCA axis was capable of explaining 84.5% of the variation in the data. A general linear model performed on PCA 1 scores indicated that there were no species differences in PCB elimination for the commonly eliminated PCB congeners. As such, the hypothesis formulated in Chapter 1, that there are no differences in the elimination of PCBs in round and tubenose goby was accepted. Future research in this area should be performed to quantify PCB elimination rate coefficients over a larger K_{ow} range. To accomplish this, depuration trials extended over 120 to 180 d should be considered. In addition, larger number of fish replicates per sample time point would increase the statistical power to detect differences in species elimination rates. One potential source of error in this study was the precision of dosing fish. Intraperitoneal dosing offers a degree of precision for hydrophobic chemicals. However, the small size of fish used in the present study necessitated dosing fish with very small volumes of dosing oil (5-15 μ L) which presents technical difficulties in achieving dosing precision. These limitations and their contribution to error in k_{tot} measurements are unlikely to be resolved using alternative dosing strategies (e.g. oral administration of PCBs via food). Therefore

more robust sampling strategies coupled with longer depuration trials are required to more effectively quantify k_{tot} differences between species and extend the range of congeners for which significant k_{tot} estimates are generated.

Chapter 4 of this thesis applied a novel depuration tracer modelling approach to determine routine metabolic rates (RMR) of the two fish species using PCB elimination rate coefficients determined in Chapter 3. RMRs were computed using two different model solutions that differed with respect to assumptions about the role of gill and fecal elimination to whole body elimination of PCBs from fish. Model I assumed that PCBs loss via gills is the dominate route of chemical elimination from fish and that whole body elimination rates can be used as proxy measurements of fish gill ventilation rates. Model II considers PCB elimination to be a function of gill ventilation and fecal elimination with the proportion of fecal elimination increasing as a function of increasing chemical K_{OW} . Model II applies a correction factor for fecal elimination of PCBs prior to estimating gill ventilation rates of fish. A comparison of model performance indicated that Model II was the most appropriate method for estimating RMR's using PCBs as depuration tracers in gobies. Model II estimates of RMR remained constant for different PCBs, regardless of chemical hydrophobicity, whereas Model I generated RMR estimates were positively related to tracer chemical hydrophobicity. Second, Model II yielded RMR estimates that were generally between 20 to 77% higher than standard metabolic rate (SMR) estimates of these fish based on previously described temperature and allometric scaling models. These estimates were in-line with Wisconsin bioenergetics models based predictions which

indicate that aquaculture/tank housed fish would have RMRs on the order of 28 to 201% higher than SMRs depending on the magnitude of activity multiplier used in such calculations.

Chapter 4 represents the first attempt to calibrate and apply a depuration tracer model to estimate fish routine metabolic rate. Overall, the data indicate that PCB depuration tracer models can provide a promising tool for estimating RMR of fish. However, the results of Chapter 4 imply that correction of PCB elimination due to fecal egestion losses is necessary to properly estimate fish RMRs. In the present work, I used a generic model for estimating fecal elimination of PCBs based on previously generated sub-models present in generic fish bioaccumulation models. However, more accurate estimates of RMRs could be generated if PCB fecal losses were measured in conjunction with k_{tot} values in depuration studies or if more accurate species specific k_{eg} sub-models were generated. Measuring PCB fecal losses would necessitate collection of feces and determination of the feces/carcass PCB distribution coefficient coupled with measurements of fecal egestion rate. Both of these critical parameters are potentially food and temperature dependent. To date, only one study (goldfish, Li et al. 2015) has been completed to empirically measure fecal loss of PCBs in fish. Thus, future research to generate better sub-models for prediction of fecal elimination of PCBs is needed to advance the PCB-RMR technique. Specifically, empirical measurements of fecal egestion efficiency (expressed as the ratio of chemical fugacity in feces compared to carcass during depuration), temperature dependence of fecal egestion efficiency, food specific dependence of fecal egestion efficiency and

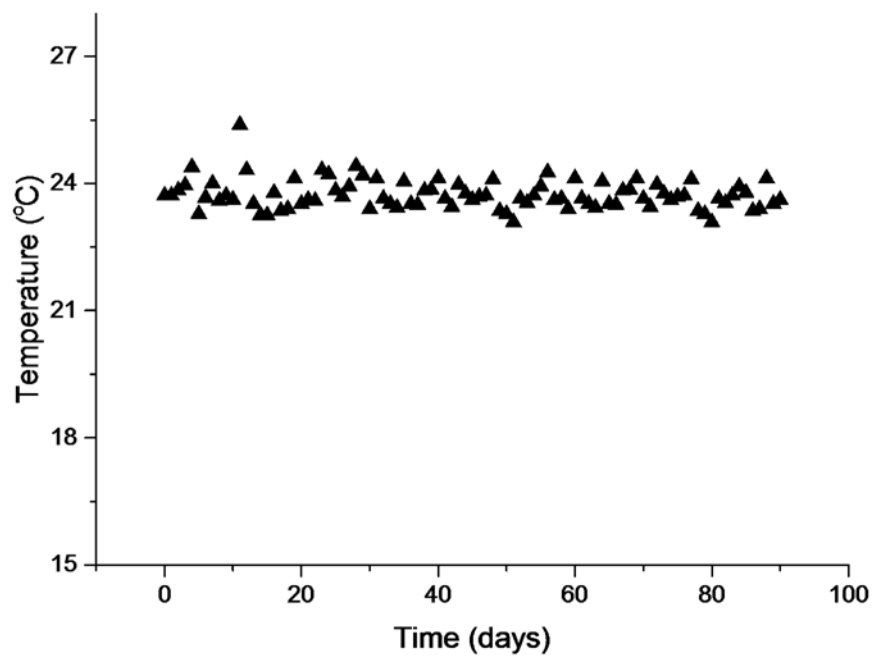
temperature/food dependence of fecal egestion rates would be useful to advance toxicokinetic submodels of PCB fecal losses by fish.

Coupled with data generated from Chapter 3 (lack of species differences in PCB elimination by fish), data from Chapter 4 imply that there were no differences in the RMR of round and tubenose goby under the husbandry conditions of the study. Chapter 4 substantiated this conclusion by performing sensitivity and model uncertainty studies. Greater statistical power could be achieved by reducing variation in empirical measurements of k_{tot} in the same manner as recommended in the discussion pertaining to Chapter 3. With respect to realized niche, research from this thesis implies that similar sized tubenose and round gobies have a similar ecological footprint in the Great Lakes given that bioenergetics needs are generally comparable between the species. Furthermore, the lack of difference in RMR between the two species would imply that neither species is at a competitive disadvantage under low food conditions. However, such conclusions are based on a very limited comparison of RMR at a single temperature and in limited size range of both species. Evaluation of the full scope of realized niche differences between the species would necessitate a much more expansive study of differences in feeding ecology, disease pathology resistance, predation susceptibility and competitive differences between the two gobies with one another and relative to native Great Lakes competitors within their respective established invasive ranges.

APPENDICES

Date	Temperature (°C)	pH	DO (mg/L)	Conductivity (s/m)	ORP (mV)
2014/4/4	20.02	8.38	11.99	0.474	310
2014/4/11	20.92	8.3	11.47	0.464	308
2014/4/18	21.42	8.51	9.59	0.538	344
2014/4/25	21.47	8.6	9.42	0.517	327
2014/5/2	21.07	8.45	12.9	0.508	315
2014/5/9	21.49	8.62	9.42	0.517	327
2014/5/16	21.18	8.69	6.77	0.633	342
2014/5/23	21.17	8.84	6.41	0.635	339
2014/5/30	21.54	8.72	6.95	0.637	327
2014/6/6	21.8	8.66	7.48	0.641	321
2014/6/13	21.74	8.53	7.54	0.639	319
2014/6/20	21.69	8.77	7.62	0.654	321
2014/6/27	21.77	8.76	8.05	0.646	319
2014/7/4	21.84	8.64	8.43	0.637	324
2014/7/11	21.81	8.63	8.41	0.629	326

Appendices 1. Water quality parameters measured during the experimental period.



Appendices 2. Temperature in water during the experimental duration.

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