SEX, CONTAMINATION AND MOVEMENT IN AN INVASIVE FISH

SEX, CONTAMINATION AND MOVEMENT IN AN INVASIVE FISH

BY

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ABSTRACT

Animal movement has had a long history of study in the fields of behavioural ecology and toxicology, but rarely is the ecological context of behaviour in toxicology directly addressed. To explore how movement might be influenced by both sex differences and habitat contamination, I conducted studies on the round goby, an invasive fish, in a highly polluted part of Lake Ontario. In the first half of my dissertation I examined the reproductive biology of this species, finding evidence of multiple male reproductive tactics, and extended this to predict sex differences in goby movement. I showed that male fish were more exploratory in the laboratory, and over multiple years moved further in the field than females. This difference may predict variation in sex ratio along a round goby invasion front. Second, I accumulated multiple lines of evidence for contaminant exposure in these fish, validating their utility as a contaminant sentinel species in the field. With the same battery of behavioural tests, I revealed that round goby collected from cleaner sites were more exploratory than fish from highly contaminated sites in the laboratory, but moved similar distances in the field. Although changes in activity level are the most frequently used behavioural measure of contaminant exposure, the ecological relevance of change was not apparent in this study. These results challenge the utility of movement as an integrated biomarker of contaminant exposure beyond the laboratory.

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the other has been indispensable and I still haven't a clue how she does it. Another tremendous thank-you must go to my fellow students, past and present lab members Viktoria Mileva, Natalie Sopinka, Matthew Taves, Marian Wong, Karen Cogliati, John Fitzpatrick, Cody Dey and Adam Reddon for mutual support, collaboration and a lot of fun in the lab, the field, and the World Outside. I have had many wonderful student assistants and collaborators over the years: Luke Bowley, Michal Galus, Holly Hynes, Krista Gooderham, Alix Stosic, Stephanie Tong, Grace Wang, Kyle Empringham, Claire Schiller and Laura King. They have been indispensible in the lab and the field.

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DECLARATION OF ACADEMIC ACHIEVEMENT

This dissertation is organized in a sandwich format as approved by McMaster University. It consists of six chapters and two appendices **Chapter 1** provides a broad introduction to the influence of sex and contaminant exposure on movement, the study species and the study site used in my research. **Chapters 2, 3** and **4** are manuscripts that have been published. **Chapter 5** is currently under review. **Chapter 6** provides a synthetic discussion of the results of **Chapters 2-5**, places them in the context of existing literature and describes avenues of future research. **Appendix A** reviews papers that examine sex differences in fish movement, and **Appendix B** reviews papers that examine contaminant effects on fish movement.

CHAPTER 1 – General Introduction

Author: Julie R. Marentette

CHAPTER 2 – Multiple male reproductive morphs in the invasive round goby (*Apollonia melanostoma*)

Authors: Julie R. Marentette, John L. Fitzpatrick, Robert G. Berger, Sigal Balshine

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Comments: This manuscript was conceived by JRM and SB. JRM collected the samples, analysed the data, and wrote the manuscript under the supervision of SB. JLF performed the video analyses of sperm velocity. RGB performed the hormone assays with the help of JRM and JLF. Reprinted with permission.

CHAPTER 3 – Laboratory and field evidence of sex-biased movement in the invasive round goby

Authors: Julie R. Marentette, Grace Wang, Stephanie Tong, Natalie M. Sopinka, Matthew D. Taves, Marten A. Koops, Sigal Balshine

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Chapter 4 – Signatures of contamination in invasive round gobies (*Neogobius melanostomus*): a double strike for ecosystem health?

Authors: Julie R. Marentette, Krista L. Gooderham, Mark E. McMaster, Tania Ng, Joanne L. Parrott, Joanna Y. Wilson, Chris M. Wood, Sigal Balshine

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Chapter 5 – Behaviour as biomarker? Round goby (*Neogobius melanostomus*) from highly contaminated areas show decreased movement in the laboratory but not in the field

Authors: Julie R. Marentette, Stephanie Tong, Grace Wang, Natalie M. Sopinka, Matthew D. Taves, Marten A. Koops, Sigal Balshine

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Chapter 6 – *General Discussion*

Author: Julie R. Marentette

Appendix A

Author: Julie R. Marentette

Appendix B

Author: Julie R. Marentette

Chapter 1

GENERAL INTRODUCTION

1.1 MOVEMENT IN BEHAVIOURAL ECOLOGY

Locomotion, the displacement of an individual from one point in space to another, is an integral component of most behaviour. Searching for food or shelter, escaping from predation, competing with conspecifics, migration and the pursuit of mates all require movement. Alterations to movement on a spatial or temporal scale will influence these behaviours and in turn impact fitness (Dingle and Holyoak 2001). Movements generated by an individual are a product of the interactions between that animal's internal state (motivation and readiness to move), navigation capacity (cognitive and sensory abilities), motion capacity (morphology and associated physiological traits), and external environmental factors (e.g., patch quality, or predator density; Nathan et al. 2008, **Figure 1.1a**). In behavioural and evolutionary ecology, studies of movement can address relationships between any pair or grouping of these factors, although a focus on effects of external factors and motion capacity has dominated the literature (Holyoak et al. 2008).

The term *movement* is often ill-defined and used to mean many different behaviours (Holyoak et al. 2008). For the sake of clarity, in this thesis I use *movement* to encompass a family of three related behaviours that vary in magnitude on temporal and spatial scales: the rate of locomotion on a scale of minutes or hours, the size of area over which activity occurs (i.e., home ranges) or over which

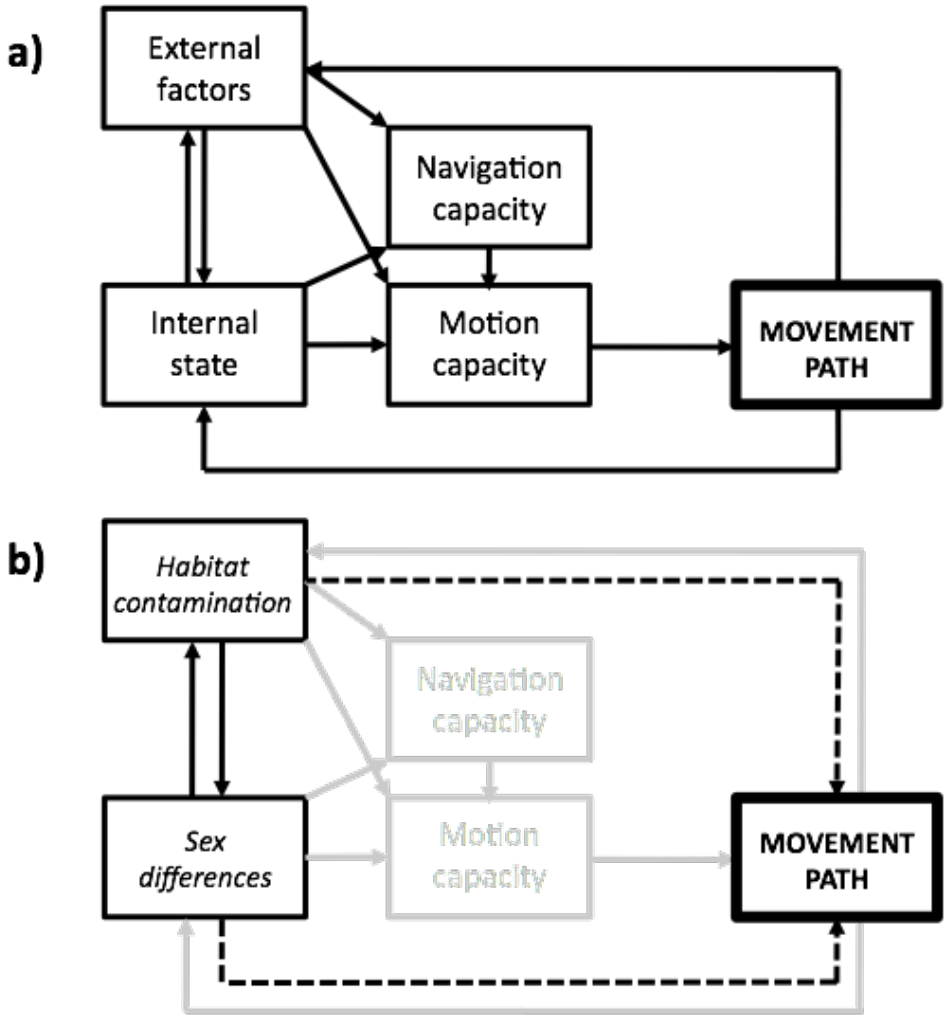

Figure 1.1. **a)** A schematic representation of the conceptual framework for the study of movement ecology, adapted from Nathan et al. 2008. Arrows indicate the direction of effects of factors on the individual's movement path. *External factors*: abiotic or biotic factors affecting the movement of an individual. *Internal state*: the physical state of the individual determining why it moves. *Motion capacity*: the morphological traits that determine how an individual moves. *Navigation capacity*: the physical traits that determine where an individual moves. *Movement path*: where an individual goes. **b)** The aim of this dissertation in understanding round goby movement paths, phrased in terms of the framework proposed by Nathan and others (2008). Dashed lines indicate the direction of indirect effects (i.e., bypassing navigation and motion capacity, which are in gray to signify they are not directly examined in this thesis).

individuals contest access (i.e., territories) on a scale of weeks and months, and the distance of dispersal from natal areas or previous breeding areas on a scale of years. These three categories correspond to movement steps, movement phases, and lifetime tracks, respectively (Nathan et al. 2008). Where appropriate, I use more specific terminology.

Sex differences in movement have been best studied in terms of the dispersal of birds and mammals at both the natal and adult stage (Pusey 1987, Greenwood 1980; see **Appendix Section A.1** for a more limited list of such studies in fishes). Movement relating to dispersal or home range size can confer benefits (permitting access to better food, territory or mates, reducing competition with conspecifics or inbreeding risks), but at some risk of reduced survival and therefore fitness (heightened exposure to predators, energetic expenditures; Perrin and Goudet 2001, Dingle and Holyoak 2001). The tradeoffs (costs and benefits) associated with movement often differ between the sexes (Pusey 1987). In many species, male potential reproductive rate exceeds that of females: males are limited by access to mates and females are limited by access to resources such as patch quality and food supply (Bateman 1948). Sex-biased dispersal can be produced depending on which factor is higher, local mate competition (promoting male movement) or local resource competition (promoting female movement; Perrin and Mazalov 2000). Mating systems reflect species-specific levels of sexual selection and thus the intensity of competition for resources and mates. Mating systems often covary with sex differences in movement (Greenwood 1980). For example, male-

biased dispersal is often observed in polygynous mammals where males defend females, while female-biased dispersal is most common in socially monogamous, male-territory-holding birds (Pusey 1987, Perrin and Goudet 2001). Similar sex differences are also observed in shorter-distance movements such as juvenile exploration, daily foraging activities and home range size. The cognitive and physiological machinery for dealing with spatial tasks will also, therefore, differ between the sexes (Gaulin 1992). This can be seen where members of one sex may excel at simple spatial tasks in comparison to the other, in parallel to sex differences in movement patterns on larger scales in the field (e.g. meadow voles, *Microtus pennsylvanicus*, Gaulin and Fitzgerald 1986; reviewed in Jones et al. 2003).

1.1.1 Why do individuals vary in movement?

A wide variety of internal state changes and external factors (in the sense of Nathan et al. 2008; **Figure 1.1a**) influence the navigation and motor capacities of animals for movement. Movement may vary with ontogeny; as an animal ages, it may increase or decrease its general activity patterns, the area over which it roams on a daily basis, or its propensity to disperse (Bowler and Benton 2005, Börger et al. 2006). Movement also varies with health or body condition; individuals may adjust their movements and other behaviours accordingly to motion capacity and their need to either acquire resources or conserve energy (e.g., condition- or phenotype-dependent dispersal; Ims and Hjermann 2001, Clobert et al. 2009). Animals with greater metabolic needs, due to larger body size or faster growth rate, may range

more widely to support those needs (McNab 1963, Gittleman and Harvey 1982). Seasonal, climatic or habitat-related changes can produce dramatic differences in movement within a single individual as well (Börger et al. 2006).

Variation in movement can be produced by more fixed differences in internal states such as endocrine function and neural development. These proximate causes for movement can differ between the sexes (Jones et al. 2003, Börger et al. 2006) or among alternative reproductive morphs or life history tactics (e.g., foraging polymorphisms) within a single sex (Sinervo et al. 2000, McCairns and Fox 2004; see **Appendix Section A.2** for a list of studies in fishes with male alternative reproductive tactics). More recently, consistent between-individual variation in behavioural syndromes has come under scrutiny (Sih et al. 2004a, Sih et al. 2004b). Variation across individuals in personality traits such as propensity towards boldness, gregariousness, and aggression sometimes all covary with different patterns of movement across temporal and spatial scales, and particularly influence dispersal (reviewed by Cote et al. 2010).

1.2 MOVEMENT IN TOXICOLOGY

There are two roles for behaviour in toxicology: 1) behaviour as a simple biomarker of exposure, and 2) behaviour as a link between individual, population and ecosystem health (Lipp 2002). A *biomarker* can be defined as any measurable biological response (on a molecular, cellular, individual, population, community or ecosystem scale) to a chemical substance indicating either exposure or toxic effect

(Peakall 1994). *Per se*, biomarkers are monitoring and assessment tools (Peakall et al. 2002). The utility of behaviour as a biomarker has not been without controversy. In a positive vein, behavioural biomarkers have been promoted because they can be readily and non-invasively measured, and also because they can be more sensitive than physiological or structural biomarkers, as behaviour can integrate multiple effects at the genetic, developmental, endocrine, neural and metabolic levels (Peakall et al. 2002, Gerhardt 2007). Conversely, it has been argued that not all behaviours are easy to quantify, that invasive biomarkers still need to be taken regardless of behavioural observations, and that behaviour is not universally more sensitive than other biomarkers (Peakall 1996, Gerhardt 2007). Another obstacle is that, despite numerous proposals, standardization of even simple behavioural tests has never occurred (e.g., Drummond and Russom 1990, Kane et al. 2005, Gerhardt 2007), not even for one of the simplest behaviours to observe, spontaneous locomotion (Little and Finger 1990). In general, the inherent variability in behaviour across species, sexes and phenotypes, the lack of standardization, and other problems have impeded the more widespread use of behaviour in risk assessments (Atchison et al. 1987, Little 1990, Grue et al. 2002, Gerhardt 2007).

Behaviour may also be used to understand toxicant-induced changes in populations, communities and ecosystems. The functioning of these higher levels of biological organization can be considered as the summed result of the health and behaviour of individuals in afflicted areas (Amiard-Triquet 2009). Changes to behaviour often can produce changes in population structure and growth rates,

community composition and ecosystem integrity by affecting individual survival and reproduction. Behavioural changes often lack specificity – multiple contaminants can produce similar alterations to behaviour (e.g., hyperreactivity, hypoactivity, etc.; Barron 2002). While this is a challenge for the use of behaviour as a biomarker, where specificity is a prized attribute, when it comes to ecological indicators, a generalized response may in fact be an asset (Peakall 1994, Peakall et al. 2002, Gerhardt 2007).

The most frequently-studied behaviour in toxicology is a change in activity, usually the rate of locomotion or its characteristics (Little and Finger 1990, Bayley 2002, Sloman and Wilson 2006; see **Appendix Section B.1** for a table of such studies on fishes). Almost everything an organism does involves moving, and if the capacity to move is impaired, then everything downstream can also become impaired.

1.2.1 Toxic mechanisms of behavioural change

Toxicants can affect animal movement through a variety of proximate mechanisms (see Barron 2002 and Sloman and Wilson 2006 for reviews). As toxic substances can simultaneously influence an animal's internal physiological state, neurology and morphology (thus, motion capacity and internal readiness to move), as well as cause indirect effects on external factors such as habitat quality, there is the potential for contaminants to affect movement through all four components of the movement ecology paradigm (**Figure 1.1a**, Nathan et al. 2008). For example,

toxicants such as waterborne copper and cadmium can directly enter and impair sensory systems such as olfaction, impeding the ability of aquatic organisms to detect stimuli and respond appropriately to them, which in turn may affect movement decisions (Scholz et al. 2000, Scott et al. 2003). Many contaminants such as organophosphate (malathion, diazinon, chlorpyrifos, etc.) and carbamate (IPBC, carbofuran, etc.) pesticides and methylmercury are neurotoxic; they can act through a change in the levels of neurotransmitters such as dopamine and norepinephrine (Fingerman and Russell 1980, Smith et al. 1995, Bretaud et al. 2002) or associated enzymes (such as acetylcholinesterase, AChE; Brewer et al. 2001, Rao et al. 2005), either acutely or over the course of development. Toxicant exposure can increase an individual's metabolic load (Campbell et al. 2002). When energy reserves are diverted to increased enzymatic activity of the liver (to break down accumulating toxicants, or to repairing contaminant-induced cell damage, such as that associated with aluminum exposure; Allin and Wilson 1999), there will be less energy available for other activities. Also, direct physical damage by chemical means, such as injury to fish gills, may cause animals to seek out areas of relatively higher dissolved oxygen (Schmidt et al. 2005) or reduce their activity altogether (Allin and Wilson 1999, Allin and Wilson 2000) in order to cope with less efficient respiration. Endocrine-disrupting compounds (EDCs) can impair proper development of behaviours, causing sex-atypical movement patterns or alter motivation to move and feed under predation risk (Bell 2004). Toxicants can also induce an overall

physiological stress response in animals, which in turn can modify behavior (Barton 2002).

1.3 MOTIVATION FOR THESIS: WHY STUDY MOVEMENT?

Behaviour of individuals is modulated by lower levels of biological organization (i.e., molecular biology, physiology), and directly affects higher levels of organization (i.e., population and community structure) through the survival and reproduction of individuals (Dingle and Holyoak 2001, Amiard-Triquet 2009). Natural dispersal patterns of individuals along with natality and mortality rates combine to affect the composition and distribution of populations over time (Anholt 1997). This becomes particularly important in understanding the progress of events such as invading species or range expansions (Kokko and López-Sepulcre 2006), populations subject to exploitation, and/or populations in decline due to anthropogenic impacts (Macdonald and Johnson 2001). These special instances reflect the intersection of behavioural ecology with conservation biology (Reed 2002). Furthermore, movement behaviour in the long term (i.e., dispersal) can often be correlated or studied in proxy by simple behaviours on more laboratory-appropriate spatiotemporal scales (e.g., Cote et al. 2010).

In toxicology, the need to understand whether contaminant-induced changes in behaviour manifest as effects at the population level has been recognized for decades (Eisler 1979, Heinz 1989, Sprague 1971). However, these ecological implications remain poorly addressed (Grue et al. 2002), in part because of many

logistical challenges in establishing causal links between multiple levels of biological organization (Anholt 1997). In 1985, Peakall reviewed avian behavioural toxicology and concluded that there was little evidence of behavioural changes manifesting as deleterious impacts on wild populations. The situation has remained largely the same 25 years later. To date there is little real-world support for the often-mentioned belief that toxicants, via altered behaviour, can have negative influence on wild population health ("pious hopes;" Heinz 1989, Grue 1994, Peakall 1996, Grue et al. 2002). One step in addressing the validity of these pious hopes might be to establish whether behavioural abnormalities observed in short-term experiments or laboratory assays parallel patterns of wild behaviour and even population-level consequences in the field, something rarely attempted (but see Weis et al. 2000, Weis et al. 2001 for notable exceptions).

1.4 STUDY SPECIES: ROUND GOBY

In this thesis I have focused my research linking behaviour and contaminants on one species, the round goby (*Neogobius melanostomus*, briefly *Apollonia melanostoma*). This is a small benthic fish and member of the speciose Gobiidae family (Nelson 2006). It is native to Ponto-Caspian region of Europe, which invaded North America and western Europe in the past several decades through accidental transfer in the ballast water of ships (Jude et al. 1992, Corkum et al. 2004). This animal is euryhaline, invading fresh and brackish waters alike; it is tolerant of a wide range of temperatures, and consumes a diet of benthos, emphasizing mollusks

(Pinchuk et al. 2003). Reproduction in round goby is seasonal, with a prolonged period of spawning lasting from April through September in North America (Corkum et al. 1998, Young et al. 2010). Young receive parental care from males who build nests in territories established in crevices or under rocks. Nest-guarding males turn black and develop distinctive enlarged round heads, using a combination of sounds, visual displays and pheromones to attract females (Corkum et al. 1998, Pinchuk et al. 2003, Corkum et al. 2006, Rollo et al. 2007).

Following their invasion, round goby have become an important dietary component for many predators (Somers et al. 2003, Reyjol et al. 2010). Their abundance poses a challenge for native competitors such as Johnny darter, logperch, and mottled sculpins (Janssen and Jude 2001, Lauer et al. 2004, Balshine et al. 2005), and round goby have complex interactions with sportfishes; goby eat fish eggs, but also serve as prey for adults (Steinhart et al. 2004, Reyjol et al. 2010). Round goby are pollution-tolerant (Pinchuk et al. 2003) and also thought to serve as a contaminant vector in Great Lakes foodwebs, due to their ability to eat contaminant-bioconcentrating dreissenid mussels in large quantities – prey few other Great Lakes fishes can consume (an idea first suggested by Jude et al. 1995).

A more recent idea is that round goby may also be a suitable sentinel species for habitat contamination (Yule et al. 2006, Bowley et al. 2010). A *sentinel species* is one whose populations are used to monitor habitat contamination and thus evaluate environmental health and risks posed to humans. Useful characteristics that sentinel species may possess include: 1) a benthic diet, and thus exposure to sediment-

contaminated prey, 2) high exposure to sediments, 3) restricted mobility ensuring long-term exposure to site conditions, being 4) abundant and 5) easy to capture, 6) playing an integral role within the foodweb as both predator and prey, such that effects on the sentinel species may indicate impacts on other species as well, 7) high fecundity, high growth rate and an early age to maturation, which facilitate a rapid response to environmental change and 8) an absence of fishing pressure, reducing confounding impacts on populations (Munkittrick 1992, Gibbons 1997). These are all qualities that round goby possess (MacInnis and Corkum 2000a, MacInnis and Corkum 2000b, Ray and Corkum 2001, Pinchuk et al. 2003, Johnson et al. 2005, Young et al. 2010).

1.5 STUDY SITE: HAMILTON HARBOUR, LAKE ONTARIO

My research on the round goby has been exclusively within Hamilton Harbour, a 2150 ha embayment on the western tip of Lake Ontario. More than two centuries of intense urban and industrial (particularly steel mill) development have permanently altered the shoreline structure of the harbor (Hamilton Harbour Remedial Action Plan 1992) and affected contaminant loadings in both the sediments and water column; primary or "A list" contaminants include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), iron, arsenic, cadmium, lead, zinc and mercury (Hamilton Harbour Remedial Action Plan 1992 and 2003, Zeman 2009). Hamilton Harbour has been designated as an Area of Concern by the International Joint Commission (IJC 1999). The first round goby was

collected in Hamilton Harbour in 1999, with an inferred first colonization event some years prior; it has become an abundant inhabitant of the harbor nearshore in the years since (Young et al. 2010, Vélez-Espino et al. 2010).

1.6 AIMS OF THESIS

The aims of this thesis are threefold. 1) To increase understanding of the reproductive biology of an invasive fish. 2) To investigate this species as a sentinel of habitation contamination in a well-characterized Area of Concern. 3) To evaluate the movement patterns of this animal in light of my discoveries about round goby reproductive habits and patterns of contaminant exposure, in both the laboratory and the field (**Figure 1.1b**).

1.7 STRUCTURE OF THESIS

In this thesis I examine first round goby reproductive biology and second, this fish's utility as a contaminant sentinel. I use a consideration of movement as an overarching theme uniting both components. **Chapter 2** evaluates evidence for the existence of two alternative reproductive tactics in round goby males. **Chapter 3** examines the implications of round goby male tactics, and the species' mating system, in looking for patterns of sex differences in movement. **Chapter 4** examines variation in contaminant biomarker patterns in round goby taken from various areas in Hamilton Harbour. Many of these biomarker patterns take into account potential differences due to sex and male tactic. **Chapter 5** takes a look at another

perspective on round goby movement – differences with respect to habitat contamination. **Chapter 6** summarizes the results of my thesis, highlights the major contributions to research and notes possible extensions of my work in new directions.

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Chapter 2

MULTIPLE MALE REPRODUCTIVE MORPHS IN THE INVASIVE ROUND GOBY (*APOLLONIA MELANOSTOMA*)

2.1 ABSTRACT

Alternative male reproductive tactics are taxonomically widespread. In such species, parental, or conventional, males express secondary sexual characteristics, court females and guard offspring, while smaller parasitic or sneaker males avoid the costs of courtship and parental care by performing sneak fertilizations. Theory predicts that sneakers will invest more in testes mass and produce more competitive ejaculates than parentals because sneakers always experience sperm competition while parental males experience sperm competition only when a sneaker is present. Here we present convergent lines of evidence supporting the existence of alternative male reproductive tactics in round gobies (*Apollonia melanostoma,* formerly *Neogobius melanostomus*), a recent invader in the Great Lakes. Dark morph males exhibited secondary sexual characteristics, were larger and had higher plasma 11-ketotestosterone concentrations than light morphs, while light morph males invested more in ejaculates (both testes mass and sperm density). Both male morphs had enlarged urogenital papillae, but papillae were relatively longer in light morph males. Sperm tail length did not differ between morphs, and sperm from dark morphs swam faster than sperm from light morphs. Our data strongly argue for the presence of alternative tactics in round gobies,

support some predictions from sperm competition theory and align with empirical observations in other taxa. For species of concern like the invasive round goby, it is critical to consider such evidence of alternative male mating tactics when constructing population growth models and assessment of invasion success and impacts.

2.2 INTRODUCTION

Male alternative reproductive tactics (MARTs) emerge when competition for mating opportunities is fierce and the potential exists for some males to reduce fitness costs by exploiting the reproductive investment of other males (Oliveira et al. 2008). Among fishes, MARTs are particularly common and are taxonomically widespread, because external fertilization is prevalent (allowing simultaneous sperm release from several males), somatic growth is indeterminate (creating large variance in body size and resource sequestering abilities among males), and paternal care is common (providing strong fitness pay-offs to male sneakers that avoid the costs of courtship and parental care; Oliveira 2006, Taborsky 1998). The aim of this study was to comprehensively examine the possibility of MARTs in the round goby *(Apollonia melanostoma,* formerly *Neogobius melanostomus*), a recent prolific Ponto-Caspian invader of the Laurentian Great Lakes and aquatic biotas in northeastern Europe (Corkum et al. 2004).

Although the round goby poses a serious threat to native fauna ecology and conservation (e.g., Jude et al. 1995), its reproductive habits are still not fully

understood. Breeding fish are difficult to observe in the wild (Wickett and Corkum 1998) and spawning is rarely achieved under laboratory conditions (L.D. Corkum, University of Windsor, personal communication; J.R. Marentette, personal observation), complicating efforts to study reproduction directly. However, understanding round goby reproduction is critically important in terms of predicting the ecological impacts of this invasive species. Round gobies belong to the speciose teleost family Gobiidae that contains several species with male alternative reproductive tactics, including the common goby, *Pomatoschistus microps* (Magnhagen 1992), black goby, *Gobius niger* (Mazzoldi and Rasotto 2002), and the sand goby, *Pomatoschistus minutus* (Svensson 2004). Sneaking behaviour has been observed in the round goby (C. Murphy, University of Alberta, personal communication) and has been reported to occur in a number of publications (MacInnis 1997, Corkum et al. 1998, Marentette and Corkum 2008). However, these reports are based on limited laboratory behavioural observations and a few morphological observations.

To investigate the claim of MARTs in round gobies, we examined the external morphology, internal anatomy, endocrinology and sperm characteristics of male round gobies from Lake Ontario in light of sperm competition theory and current knowledge of MARTs in vertebrates. In general, male tactics can be divided into two categories. Parental males, sometimes called conventional, type I or bourgeois males, are large, invest more in growth than in reproduction, defend territories, court females, exhibit secondary sexual characteristics, and have elevated androgen

concentrations (Oliveira et al. 2008). In contrast, sneaker, type II or parasitic males are smaller, invest in reproduction at the cost of growth, and lack secondary sexual characteristics. Rather than court females, these sneaker males add their ejaculate surreptitiously to spawnings in progress by stealth, speed, or by imitating females (Oliveira et al. 2008). Males will be subject to asymmetric risks of sperm competition, the competition between sperm from rival males to fertilize a female's ova (Parker 1970), depending on the reproductive tactic employed. Because parental males are sometimes able to sequester mates and drive off competitors including sneakers, they experience a relatively lower risk of sperm competition. In contrast, sneaker males experience sperm competition during every mating, as by definition they only release sperm in the presence of a parental male. Thus, in order to overcome their disadvantage, sneaker males are expected to invest more in sperm number than parental males (Parker and Ball 2005). Sperm competition is also thought to lead to sperm with longer flagella that swim faster (Ball and Parker 1996). Sperm tail length is associated with sperm swimming speed (Fitzpatrick et al. 2009) and, in external fertilizers, sperm swimming speed predicts fertilization success in competitive matings (Gage et al. 2004).

We predicted that if two alternative reproductive tactics exist in round gobies, one morph (presumably the parental male morph) would be larger than the other morph (the sneaker male morph). Since secondary male sexual traits are associated with high levels of androgens, we predicted that plasma concentrations of 11-ketotestosterone (11-KT), the primary fish androgen, would be higher in

parental males than in sneaker males (Oliveira et al. 2008). We also predicted that parental males would invest comparatively less in testes mass than sneaker males, and that sneaker males, which encounter higher levels of sperm competition, will produce more sperm that have longer flagella and swim faster than the sperm of parental males (Ball and Parker 1996; Parker and Ball 2005).

2.3 METHODS

Round gobies (n = 1295) were collected in minnow traps or by electrofishing from Hamilton Harbour between June 26 to August 23 2006, and May 16 to August 29 2007, and from nearby Jordan Harbour in Lake Ontario on July 21 2006. Traps baited with 25 g frozen corn were set in < 1 m of water every two weeks, and were collected after 24 hr. Fish were then transported to McMaster University and maintained in aerated laboratory aquaria before processing.

The sexes were differentiated by the shape of the urogenital papilla, which is broad and square in females, but narrow and pointed in males (Miller 1984). Fish that did not possess identifiable papillae were classified as juveniles, and all males with small flat papillae were classified as non-reproductive (non-spawning males). Dissections revealed the size and maturity of testes and confirmed this male classification scheme. All males with erect papillae were shown by dissection to have mature testes (see measurements below). Males with erect papillae that had dark to black bodies were classified as dark morph males and we considered these to be putative parental males, since nest-holding round goby males have been

reported to have black nuptial coloration (Corkum et al. 1998). Males with erect urogenital papillae that had light, mottled juvenile or female-shaped bodies (see below) were then classified as light morph males and putative sneakers. Using this classification scheme, of the 752 adult males captured, 144 were classified as dark morph and 151 as light morph males, with the remainder classed as non-reproductive males.

Fish were all measured for total length (TL), head width (taken across the posterior orbital edge), and urogenital papilla length (anterior insertion to posterior tip), all to 0.1 mm (**Figure 2.1**). Total body mass and the mass of the gonads were measured to 0.001 g. The gonads of male round gobies have two sets of paired organs: 1) testes and 2) accessory structures also called seminal vesicles or sperm-duct glands (Miller 1984). The function of these accessory glands has been linked to sperm storage, production of mucus for laying sperm trails (lines of sperm embedded in mucus, laid on nest surfaces), and pheromone production (Mazzoldi et al. 2005; Jasra et al. 2007; **Figure 2.1**). Hence the mass of testes and accessory glands were recorded separately. Based on these measures we calculated a somatic mass as the total body mass – total gonad mass (testes and accessory glands combined). We also calculated a papilla index or PI (papilla length / TL x 100%), testicular somatic index or TSI (testes mass / somatic mass x 100%), and accessory gland somatic index or AGSI (accessory gland mass / somatic mass x 100%).

Blood samples were collected in heparinized 10 μL micro-capillary tubes from a representative sample of 14 dark morph and 14 light morph males by caudal

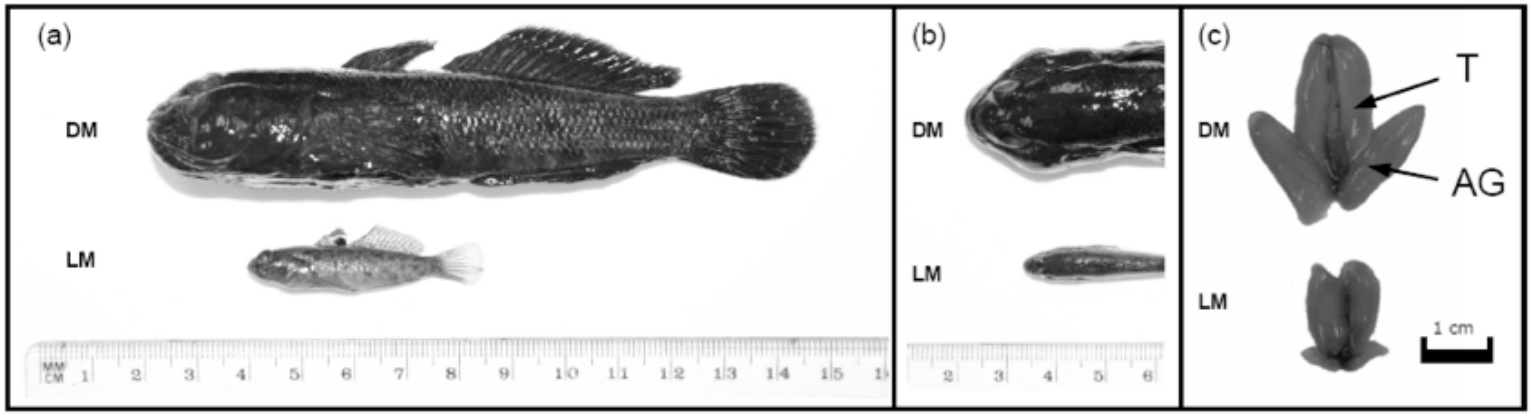

Figure 2.1. a) External morphology (lateral view) of a dark morph male (DM) and a light morph male (LM) showing differences in colour and body size. **b)** Anterior dorsal view of a dark morph male (DM) and a light morph male (LM) showing differences in head width. **c)** Dissected testes of dark morph males (range 96.9 – 131.3 mm TL, top row) and light morph males (range 45.0 – 70.6 mm TL, bottom row). This photo shows the relative investment in lobes of the testes (T) versus accessory glands (AG). A scale appears below (in cm).

severance 0-2 d after capture, and spun at 14500 RPM for 10 minutes. Collection day was random, did not vary between the two morphs and had no correlation with 11-KT concentrations for either morph (Spearman rho, $p > 0.2$ for both dark and light morphs). The plasma was then removed and frozen at -80°C. Steroids were extracted from the samples by shaking 5 µL of plasma with 5 ml of diethyl ether. After freezing the aqueous phase in an acetone/dry ice bath, the organic phase was decanted and dried. The dry extracts were resolubilized in 500 µL EIA buffer and frozen at -20ºC to be assayed for 11-KT at a later date. On the day of the assay procedure, the parent solution was thawed and further diluted with EIA buffer to generate a 1:200 working solution. 11-KT levels were assessed using a commercially available Enzyme Immunosorbant Assay kit (Cayman Chemical Inc.). Standards and samples were run in duplicate on a single plate. Plates were read using a microplate reader with a single filter at 405 nm (Bio-Tek Instruments Inc., model Elx 808). The percent bound for each sample was calculated using the maximum bound value as reference, and were excluded from analysis if their values fell outside 20-80% bound.

Milt was collected from the dabbed dry papillae of a representative sample of 14 dark morph and 21 light morph males in 2007 following submersion in ice water. This process typically induced ejaculation, and further milt was produced by gentle abdominal massaging. Urine was expelled by abdominal massage before milt samples were taken to avoid contamination or activation of sperm. We also measured the spermatocrit, or the proportion of solid packed material in semen,

after centrifugation. Spermatocrit values are used as indicators of spermatozoa density and correspond well to density measures obtained from manual haemocytometer-based sperm counts (Tvedt et al. 2001). Spermatocrit was measured by filling a microhematocrit capillary tube with milt (sperm and seminal plasma) and centrifuging this tube for 10 minutes in a microhematocrit centrifuge. This procedure causes sperm cells to pack down in the capillary tube, forming an opaque layer below a clear layer of seminal fluid. The spermatocrit was recorded as the percent of total volume formed by the sperm cells (Liljedal et al. 1999). Sperm smears (5 μL of milt or testicular fluid diluted in 200 μL distilled water) were collected from 21 dark morph and 11 light morph males in 2006 and 2007. Ten clearly visible spermatozoa were photographed at 400x magnification under phase contrast from each male (Prosilica EC-650 camera, software Astro IIDC v3.02.01), and the length (μm) of each flagellum measured, tip to midpoint of head, with ImageJ 1.37v (Wayne Rasband, National Institutes of Health, U.S.A., available at http://rsb.info.nih.gov/ij) (Fitzpatrick *et al*. 2009). A mean sperm length was then computed for each male.

Video recordings of sperm from 10 dark and 11 light morph males caught in 2007 were captured at 200x magnification, under phase contrast, at 240 frames per second using a Prosilica EC-650 camera. Sperm were collected from either milt or dissected testes and activated by 200 μL of filtered water from Hamilton Harbour. Sperm velocity for each male was measured in one second intervals as the VAP (smoothed path velocity, μm/sec), using a CEROS video analysis program

(Hamilton-Thorne Research, Beverly, Maine, U.S.A.) at 20, 30, 45, 60, 90, 120, 180, 240, 360, 480, and 600 seconds. Only spermatozoa whose forward movement was traced for at least 0.33 sec and samples with at least five swimming sperm per time period were analyzed.

2.3.1 Statistical analyses

All analyses were performed using JMP 5.0.1a (SAS Institute, Inc., 2002). Despite their common use to measure reproductive investment, ratio indices such as GSI, TSI or AGSI have recently come under scrutiny (Tomkins and Simmons 2002) because such measures assume isometry of body proportions in animals of varying size. As the assumption of isometry is rarely met, using these indices may lead to incorrect conclusions about the differences between groups. Hence we used ANCOVA models as proposed by Tomkins and Simmons (2002) to account for allometry among individuals and accurately detect differences in allometry between groups. However, as the use of the ratio indices is extremely common in studies of fish reproduction, and the interpretation of ANCOVA models can be complicated by significant covariate-main effect interaction terms, we employed both methods (after Neff et al. 2003), to examine differences between parental and sneaker males and facilitate comparisons with published studies.

Body measures were log-transformed and testes mass, accessory gland mass, papilla length and head width data were fitted to an ANCOVA model, with male morph as a factor and either the logarithm of somatic mass or TL as the covariate.

Where covariate-morph interaction terms were not significant ($p > 0.05$), they were removed from the model (Engqvist 2005) and only the subsequent model F values were reported. Indices (TSI, AGSI, PI), concentrations of 11-KT, sperm tail length, and spermatocrit values were analyzed by Student's t-test (arcsine- or log-transformed if necessary) or by the normal approximation to the Wilcoxon rank sum nonparametric test where normalization could not be achieved. Sperm velocity was examined using a repeated-measures ANOVA as the log-transformed median VAP with time and male morph as fixed factors. Means, standard errors, medians and ranges for all measurements are provided in **Table 2.1**.

2.4 RESULTS

2.4.1 External body morphology

Dark morph males were larger ($t_{293} = 19.8$, $p < 0.0001$) and heavier ($t_{293} = 19.3$, $p < 0.0001$, **Table 2.1**, **Figure 2.1a**) than light morph males. Dark morph males had wider heads compared with light morphs (ANCOVA, whole model $F_{2,292} = 1865$, $p < 0.0001$; male morph $F_{1,292} = 54.3$, $p < 0.0001$**; Figure 2.1b**).

2.4.2 Gonads and urogenital papillae

Light morph males invested nearly three times as much as dark morphs in testes (2.8 times more massive; $Z = -10.62$, $p < 0.0001$, **Figure 2.2a**), while dark morph males invested twice as much as light morphs in accessory glands (their

Table 2.1. Summary statistics for body measurements of dark and light morph males. TSI = testicular somatic index. AGSI = accessory gland somatic index. PI = papilla index. 11-KT = 11-ketotestosterone. Indices are expressed as the percentage of somatic mass (TSI, AGSI), total length (PI) or ejaculate volume (spermatocrit). [a] n = 144 parental, 151 sneaker males. [b] n = 14 dark, 14 light morph males. [c] n = 14 dark, 21 light morph males. [d] n = 21 dark, 11 light males.

	Dark Morph Males		**Light Morph Males**	
	Mean (SE)	**Median (Range)**	**Mean (SE)**	**Median (Range)**
Total Length (mm)[a]	98.9 (1.6)	96.3 (61.5 – 161.0)	65.5 (1.2)	65.5 (43.5 – 90.3)
Total Mass (g)[a]	14.23 (0.78)	11.26 (2.97 – 56.02)	3.94 (0.16)	3.67 (1.07-10.14)
TSI (%)[a]	1.60 (0.06)	1.46 (0.26 – 5.64)	4.22 (0.18)	4.22 (0.60 – 9.59)
AGSI (%)[a]	0.70 (0.03)	0.64 (0.00 – 2.07)	0.39 (0.03)	0.34 (0.00 – 1.20)
PI (%)[a]	5.17 (0.07)	5.09 (3.39 – 7.96)	6.71 (0.12)	6.59 (3.66 – 11.26)
11-KT (ng/mL)[b]	3.29 (0.60)	1.81 (0.79 – 10.65)	0.87 (0.60)	0.56 (0.22 – 2.31)
Spermatocrit (%)[c]	56.4 (5.2)	48.3 (38.5 – 98.8)	88.2 (2.2)	92.3 (62.5 – 100)
Sperm Tail Length (µm) [d]	30.28 (0.38)	30.35 (27.82 – 33.17)	30.15 (0.52)	30.16 (26.85 – 33.22)

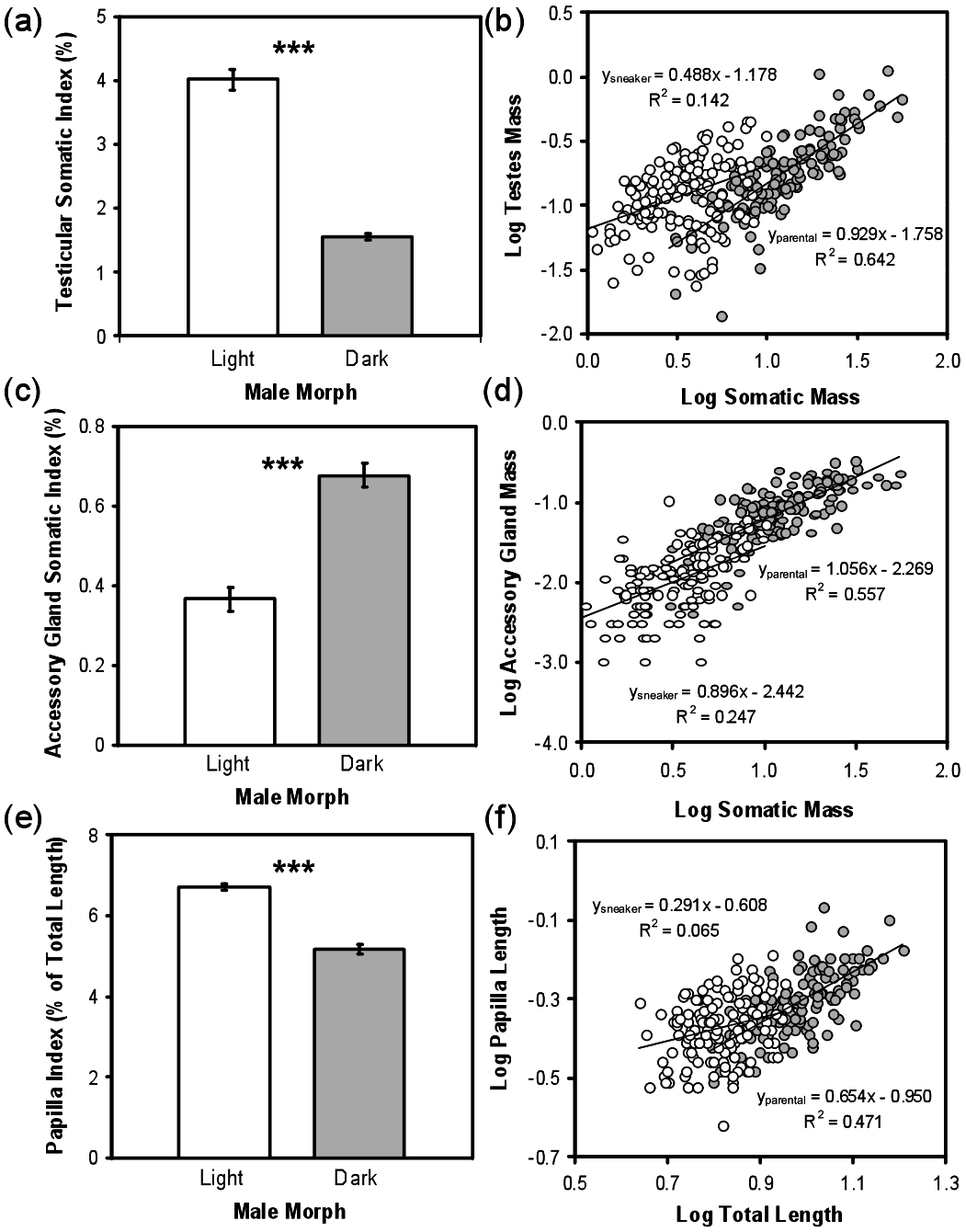

Figure 2.2. Measures of reproductive investment in male round goby. Gray bars or circles = dark morph males. White bars or circles = light morph males. Asterisks (***) indicate significant differences between morphs at $p < 0.0001$. **a)** Mean ± SE of the testicular somatic index. **b)** Plot of log testes mass versus log of somatic mass. **c)** Mean ± SE of the accessory gland somatic index. **d)** Plot of log accessory gland mass versus log of somatic mass. **e)** Mean ± SE of the papilla index. **f)** Plot of log papilla length versus log of total length.

median score was 2.0 times more massive; Z = 8.36, p < 0.0001, **Figure 2.1c** and **2.2c**). Light morph males had relatively longer urogenital papillae (t_{268} = 11.1, p < 0.0001, **Figure 2.2e**). ANCOVA models confirmed that light males had relatively larger testes (whole model $F_{3,\ 289}$ = 79.1; male morph $F_{1,\ 289}$ = 33.4, p < 0.0001, **Figure 2.2b**) and that dark morph males invested significantly more than light morphs in accessory gland tissue (whole model $F_{2,274}$ = 361.7; male morph $F_{1,274}$ = 28.3, p < 0.0001, **Figure 2.2d**). No morph difference in papilla length was observed when an ANCOVA model was applied (whole model $F_{3,266}$ = 53.6; male morph $F_{1,266}$ = 1.5, p = 0.23, **Figure 2.2f**) but the significant interaction between the covariate (log somatic mass or log TL) and the main effect of male morph made it difficult to interpret main effects (covariate-morph interaction terms were $F_{1,289}$ = 15.6, p < 0.0001 for testes mass and $F_{1,266}$ = 10.8, p = 0.0012 for papilla length). Adding and subtracting 1 SD from the covariate of one male group (a method suggested by Tomkins and Simmons 2002 to overcome significant covariate interaction terms) did not yield the same male morph effect.

2.4.3 Hormones and sperm

Dark morph males had significantly higher concentrations of plasma 11-KT than light morph males (t_{26} = 4.2, p = 0.0003, **Figure 2.3**). Spearman rho correlations showed that 11-KT levels did not vary within a male morph with the number of days spent in the laboratory prior to testing (R_s = 0.35 and -0.06, p > 0.2 for both dark and light morphs respectively). Sperm tail length did not vary between

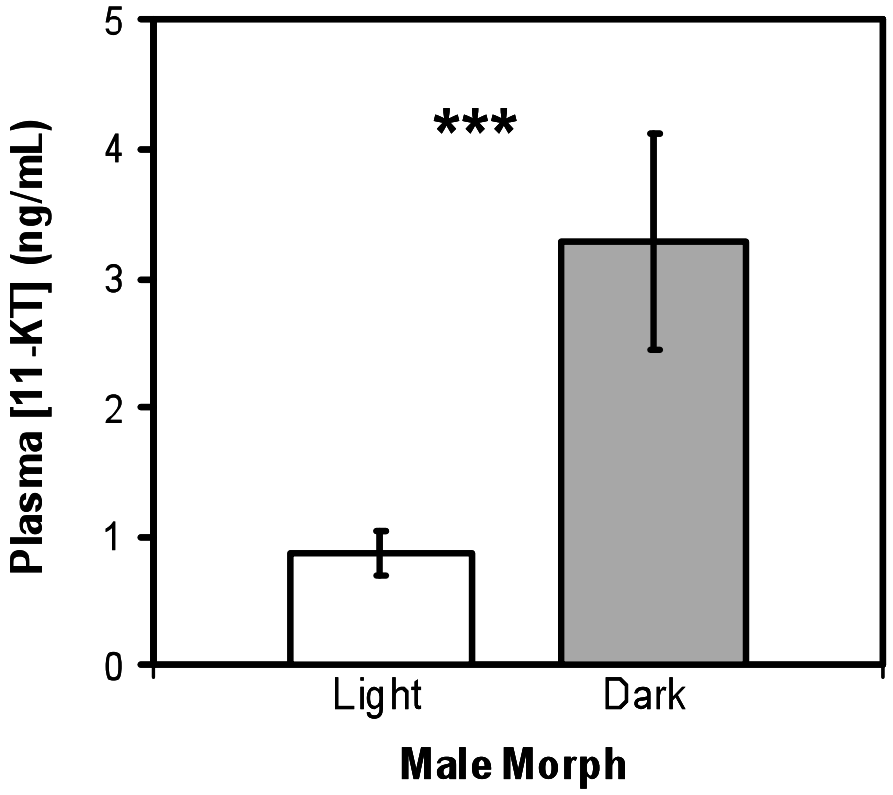

Figure 2.3. Differences in mean ± SE plasma concentrations (ng/mL) of the primary fish androgen 11-ketotestosterone for dark morph (gray bar) and light morph (white bar) males.

dark and light morph males (t_{30} = 0.2, p = 0.84). However, light morph males had a higher density of sperm in their ejaculates than did dark morphs (median score was 1.89 times greater; Z = -3.9, p < 0.0001, **Figure 2.4a**). Sperm velocity decreased with time ($F_{10,89}$ = 14.5, p < 0.0001). However, a significant interaction between time and male morph indicated that the rate of decline in sperm velocity was dependent on male morph ($F_{10,89}$ = 2.3, p = 0.02). Sperm velocity did not differ significantly between morphs alone ($F_{1,19}$ = 0.2, p = 0.67; **Figure 2.4b**). Dark morph sperm swam faster than light morph sperm only at 180 and 240 seconds post-activation (Tukey, p < 0.05, **Figure 2.4b**).

2.5 DISCUSSION

The multiple lines of evidence pursued in this study, including data on external morphology, reproductive investment, endocrinology and sperm characteristics, converge to corroborate the hypothesis that the round goby possesses two morphologically and physiologically distinct male morphs that we predict correspond to alternative male reproductive tactics. The dark morph has traits corresponding to a parental male tactic, and the light morph has traits corresponding to a sneaker male tactic. One morph, the dark morph, was physically larger, invested more in accessory glands and had higher concentrations of plasma 11-KT. These traits are seen in parental males. The other morph, the light morph, invested more in testes mass and had a greater volume of sperm in their ejaculate

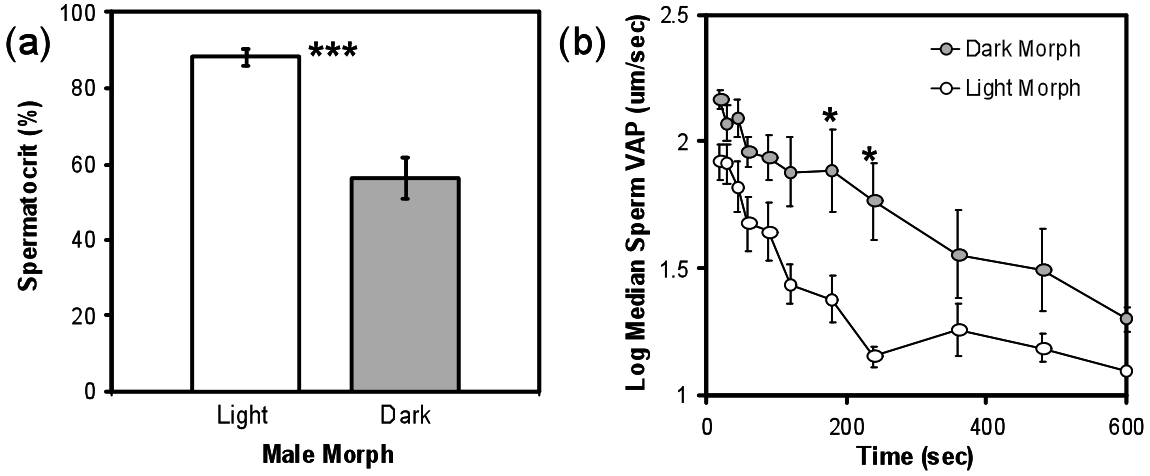

Figure 2.4. a) Differences in spermatocrit for dark morph (gray bar) and light morph (white bar) males. **b)** Changes in median VAP (smoothed path sperm velocity) with time in dark morph (black circles) and light morph (white circles) males. Asterisks (*) indicate differences between morphs at $p < 0.05$.

than did dark morphs. These traits are seen in sneaker males. These results are consistent with the predictions of sperm competition theory (Parker 1970) and empirical observations in many species (Oliveira et al. 2008, Montgomerie and Fitzpatrick 2009). Although a number of different fish studies have confirmed that parental males will have higher levels of 11-KT (Oliveira 2006), to our knowledge this is the first demonstration of this pattern in Gobiidae, one of the most speciose of teleost fish families (at least 1875 species, in more than 200 genera; Nelson 2006).

Our results based on ratio indices (assuming isometry), and those based on ANCOVA models (assuming allometry), both support the finding of greater testicular investment in sneakers and greater accessory gland investment in parentals. A relatively longer urogenital papilla in sneaker males compared to parental males has not, to our knowledge, been previously reported in gobiids or for any other fish species. In the Azorean rock-pool blenny *Parablennius sanguinolentus parvicornis*, it is the bourgeois or parental males that exhibit longer and wider papillae than the satellite morph (Oliveira et al. 2001). It is possible that a relatively longer papilla allows sneaker males to fertilize eggs more efficiently or from a greater distance than if they had a papilla proportionately equal in length to parental males. There may be a threshold papilla length required for fertilization success, but the non-significant effect of male morph in the ANCOVA model, complicated by a significant morph-covariate interaction, suggests that papilla differences between tactics needs to be studied further and interpreted carefully.

Among fishes especially, sperm competition is expected to have selected for longer sperm and/or faster swimming speed because the first sperm that swims down the micropyle on the egg fertilizes that egg (Yanagimachi et al. 1992). Yet, at most time periods we found no differences in sperm length or sperm swimming speed between morphs. While Burness et al. (2004) reported that sneaker males had longer sperm, in accordance with theoretical predictions, in fact the majority of studies that have examined sperm morphology of males exhibiting alternative reproductive tactics have failed to find differences in sperm length within species (Gage et al. 1995; Leach and Montgomerie 2000; Fitzpatrick et al. 2007). Thus the selective forces acting on sperm morphology and function in species with alternative reproductive tactics remain unresolved. An exploration of differences in sperm energetics between tactics may help resolve these issues (Burness et al. 2005; Fitzpatrick et al. 2007, Fitzpatrick et al. 2009).

While the predicted pattern of faster sperm in light morph (putative sneaker) males was not found in our study, there remains the distinct possibility that in the earlier time periods (i.e., from 0 to 20 seconds post-activation) not measured in this work, round goby light morph sperm does indeed swim at greater velocities. Burness et al. (2004) showed that sperm from bluegill sneakers swam faster than parental males but that it did so *only* at time periods < 20 seconds. In fact, sperm from bluegill parental males swam faster than sneaker males at later time periods, consistent with our results. The initially faster sperm in bluegill sneakers followed by a rapid decline in swimming speed was interpreted by Burness et al. (2004) as a

trade-off between speed and longevity in ejaculates. A similar trade-off may be present in round gobies. Interestingly, in the black goby, sneaker sperm was faster than that of parental males both at 0 and 30 minutes post activation but in the grass goby sperm velocity did not differ between tactics (Locatello et al. 2007). Similarly, round goby light morphs may invest solely in elevated sperm production – a possibility supported by the higher density of sperm per unit ejaculate obtained from light morphs. This would suggest that in competitive spawnings, round goby light morph males might rely on sperm number rather than sperm quality to secure fertilizations. Alternatively, parental male sperm laid down in sperm trails (known to be produced by gobiids; Miller 1984) well in advance of fertilization may be selected for prolonged swimming capacity compared to sperm of sneaker males, which may release ejaculates only in the presence of a spawning female. Further examination of sperm and ejaculate characteristics in round gobies is needed, with particular emphasis on early phases of activation, to answer these questions.

In this study we provide evidence for the presence of multiple male morphs in round gobies and argue that these morphs represent alternative male tactics. Yet, key pieces of evidence remain to be resolved before alternative male reproductive tactics can be conclusively identified in round gobies. The most urgent of these is rigorous behavioural confirmation of tactics and establishing that light morphs engage in parasitic spawnings. If light males are indeed sneakers then it would be valuable to know if they rely on stealth and speed, or act as a female mimic to gain reproductive success. Further, although not definitive, evidence of multiple

paternity from egg clutches collected from wild nests could indicate the presence of any cuckoldry.

The round goby and its reproductive habits are of interest not only because of the known impacts of this species on native fauna, but also because there has been a call to stem further invasions through exploitation of chemical (Corkum 2004) or auditory (D. Higgs, University of Windsor, personal communication) reproductive signaling. The presence of alternative reproductive tactics in round gobies is likely to have profound implications for population growth via annual recruitment of juveniles. This recruitment will be influenced by the number and quality of males providing care – which in turn will depend on the availability of nesting sites and the degree of male-male competition for these nests.

The frequency of light to dark morph males in our study was approximately 1:1. We predict that the frequency of light to dark morph males will reflect the various stages of an invasion. For example, when a new habitat is being initially colonized, male – male competition should be low as all males will be able to access nest sites. Hence, alternative male reproductive tactics will not be favoured. However, as the population grows and becomes more dense, nest sites may become limited and male-male competition is expected to increase, favouring the evolution of alternative male tactics, and sneakers (as light morphs) would be expected to be more common. Iguchi et al. (2004) reported evidence for an escalation of male-male competition, in invasive versus native populations of smallmouth bass (*Micropterus dolomieu*), possibly due to heightened competition for nest sites in the new habitats.

However, when Jones et al. (2001) examined sneaking rates in two sand goby populations, one with high and one with low nest densities, they found no difference in rates of sneaking. The degree of sexual selection itself rather than nest density per se (as one contributor to sexual selection) may be the factor that will select for increased alternative male reproductive behaviour. In addition, invasive populations of round gobies in North America reach sexual maturity faster, have shorter lifespans, and are smaller than round gobies from native populations (MacInnis and Corkum 2000). Life history trait modulation may have facilitated round goby invasion speed and range expansion as invasive populations apparently cycle more quickly. Having a relatively plastic life history, which may include the presence of alternative male reproductive tactics, may arm a species with the ability to adaptively respond to sudden changes in environmental conditions or to novel environments and hence this flexibility may predispose round gobies to successful invasions (Balázová-L'avrincíková and Kovác 2007).

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Chapter 3

LABORATORY AND FIELD EVIDENCE OF SEX-BIASED MOVEMENT IN THE INVASIVE ROUND GOBY

3.1 ABSTRACT

Activity levels are modulated by trade-offs between reducing predation risk and the need to move in order to find food or mates. Because these trade-offs affect males and females differently, many species show sex-specific movement, dispersal patterns and spatial navigation capacities, with the sex that gains the most from territory ownership often dispersing less. Unlike mammals and birds, sex differences in movement among fishes remain poorly studied, and the connections between tests of movement propensity in the laboratory and in the field are rarely made. Here we examine the differences in movement between male and female round goby (*Neogobius melanostomus*) in both laboratory and field settings. This fish species is invasive in North America and currently undergoing further range expansions. In the laboratory, round goby males were more active and explored a novel environment more readily than did females. A large-scale mark-recapture study in Lake Ontario over two years revealed that males moved more than females between years, but there were no within-year sex differences. Thus, round goby display male-biased movement patterns, providing a comparison point to dispersal patterns in other taxa. Understanding sex-specific movement of round goby in the field will also help predict dispersal and population dynamics, both in areas where

round goby have already become established, and where they are continuing to invade.

3.2 INTRODUCTION

Greater levels of activity, exploration and dispersal can increase foraging or mating opportunities, but may also increase predation (Werner and Anholt 1993; Smith and Blumstein 2008). The influence of this trade-off on reproductive success differs for males versus females, and as a result, sexes frequently differ in movement patterns and spatial abilities (Jones et al. 2003). Natal and breeding dispersal, two major types of movement, are typically greater for the sex that has more to gain in terms of increasing mate encounter rates or reducing inbreeding and kin competition. The benefits of philopatry also influence dispersal patterns, with the more philopatric sex often having more to gain from a well-known territory or home range with access to familiar shelter and food (Greenwood 1980; Clobert et al. 2001; Dingle and Holyoak 2001; Bowler and Benton 2005). Sex differences in movement are not limited to large-scale dispersal events. Home range or territory size, general activity levels and spatial navigation capacity are often greater in one sex, usually the dispersing sex (Gaulin 1992, Jones et al. 2003).

Sex-specific movement patterns are frequently generalized by taxon and mating system. Male-biased dispersal predominates in polygynous mammals, while dispersal is often female-biased in socially monogamous birds (Greenwood 1980). Sex-specific movement patterns among fish taxa, however, are much less frequently

studied. In a wide variety of polygamous species where males do not care for young, males appear to disperse, or range, further (African lions, *Panthera leo*, Pusy and Packer 1987; brook trout, *Salvelinus fontinalis,* Hutchings and Gerber 2002; túngara frogs, *Physalaemus pustulosus,* Lampert et al. 2003; mosquitofish, *Gambusia affinis*, Cote et al. 2010b). In monogamous species or those with sex role reversal (male parental care and a female-biased operational sex ratio), the opposite pattern is found, with greater movement by females (Florida scrub-jays, *Aphelocoma coeruslescens*, Woolfenden and Fitzpatrick 1984; red-necked phalaropes, *Phalaropus lobatus*, Reynolds and Cooke 1988; cardinalfish, *Apogon niger*, Okuda 1999). Fishes provide an opportunity to decouple parental care from mating behaviour, as many species exhibit both male-only parental care and typical sex roles (with male-biased operational sex ratios, where males are the more competitive sex). In such systems, males may still range over greater areas than females, particularly outside of the breeding season, and simply reduce their movements and territory range during breeding (fluvial sculpins, *Cottus pollux*, Natsumeda 2001 and Natsumeda 2007; gobiid fish *Rhinogobius* spp., Osugi et al. 1998), when female movements may exceed males (blenniid fish, *Blennius sanguinolentus*, Santos and Almada 1988; smallmouth bass, *Micropterus dolomieu*; Savitz et al. 1993).

Measurements of sex-specific or individual spatial ability or activity in the laboratory can be used as a tool for understanding and predicting movements in the field (Jones et al. 2003, Cote et al. 2010). To date, however, only a few studies have done this (Cote et al. 2010). Time to navigate a maze (meadow voles, *Microtus*

pennsylvanicus, Gaulin and Fitzgerald 1986), laboratory dispersal (mosquitofish, *Gambusia* spp.; Rehage and Sih 2004), exploration (female great tits, *Parus major,* Dingemanse et al. 2003; bullhead fish, *Cottus perifretum*, Kobler et al. 2009), asociality (common lizard, *Lacerta vivipara*, Cote and Clobert 2007) and boldness (killifish, *Rivulus hartii*, Fraser et al. 2001) have all been linked to greater home ranges and/or dispersal for individuals, sexes or species in the field.

The aims of our study were twofold. 1) To explore sex differences in movement in an understudied group of vertebrates, the fishes–and thus build a picture of how fishes fit in the theoretical framework for dispersal. 2) To link behaviour in the laboratory with that observed in the field. We also wished to consider how sex differences in movement may impact patterns of invasion. We used the round goby (Gobiidae: *Neogobius melanostomus*) to address all three issues. The round goby is a benthic, euryhaline fish found in Ponto-Caspian Europe and invaded regions of both western Europe and the North American Great Lakes basin (Jude et al. 1992; Corkum et al. 2004). Males exhibit alternative reproductive tactics (**Chapter 2**), which may affect movement. Nest-guarding males are dark in colour and exhibit both territoriality and parental care (hereafter, guarding males), and a light female-like morph that may parasitize, or sneak, spawnings and exploit the paternal efforts of guarding males (hereafter, sneaker males; **Chapter 2**). Competition for nest sites and females is believed to favour larger guarding males (Charlebois et al. 1997, Corkum et al. 1998). Round goby undergo seasonal migrations from deeper water to shallow nearshore rocky areas to spawn during

the spring and summer (Pinchuk et al. 2003). During the breeding season the site fidelity of adults is thought to be high (e.g., Ray and Corkum 2001) though long-distance movement (> 1 km) of adults has also been observed (Wolfe and Marsden 1998; Balshine et al. unpublished data). Round goby continue to invade new habitats (Poos et al. 2009), and if sex differences in exploration, ranging and dispersal exist in the round goby, then differences in sex composition may distinguish an invasion front from more established populations.

In this study, we explored activity differences between male and female round goby in a small-scale laboratory context, and a large-scale field context, where natural movements were evaluated through a two-year mark-recapture study. In the two-year period of the field study we evaluated movement within and outside the breeding season, as well as across years. We had three major predictions. First, we predicted that males would move more than females in most circumstances: over the long term and outside of the breeding season. This prediction was based on the fact that round goby are polygamous and sexually dimorphic, with males growing larger (Young et al. 2010) and faster (MacInnis and Corkum 2000a) than females. Larger, faster-growing individuals may move more than smaller ones because they have greater metabolic needs (McNab 1963; Gittleman and Harvey 1982). Moving over a greater home range may mean greater access to food and faster growth, which may be particularly important for males because females prefer large males (Corkum et al. 1998) and larger males fare better in competition for resources such as nest sites (Gaulin 1992, Bowler and Benton 2005, Clobert et al.

2009). Larger individuals are also better able to escape predation by gape-limited predators (Persson et al. 1996), reducing the costs of movement. Second, we predicted that during the breeding season only, guarding males would move less than females as they would be limited by the need to defend small territories and nests (Natsumeda 2001, Sunobe and Nakazono 1999, Taru and Sunobe 2002). Females, on the other hand, continue to forage through the breeding season, are likely to visit multiple nests over a large area to spawn up to six times over the breeding season (Charlebois et al. 1997, Natsumeda 2001). Third, we predicted that among males, sneakers might move more than guarding males during the breeding season, as sneakers need not be constrained to a single nest territory and may access and parasitize the spawning efforts of more than one guarding male. Outside of the breeding season, however, larger guarding males were predicted to move more than the smaller sneaker males.

3.3 METHODS

3.3.1 Collection of fish

Round goby were collected from three locations in Hamilton Harbour, Lake Ontario, Canada (43°17′ N 79°50′ W; **Figure 3.1**), where they have been present since the mid 1990's (Vélez-Espino et al. 2010), and breed from May to early August (Young et al. 2010). The nearshore littoral population of round goby is male-biased (Young et al. 2010). Collection locations were approximately 4 km apart, had mixed

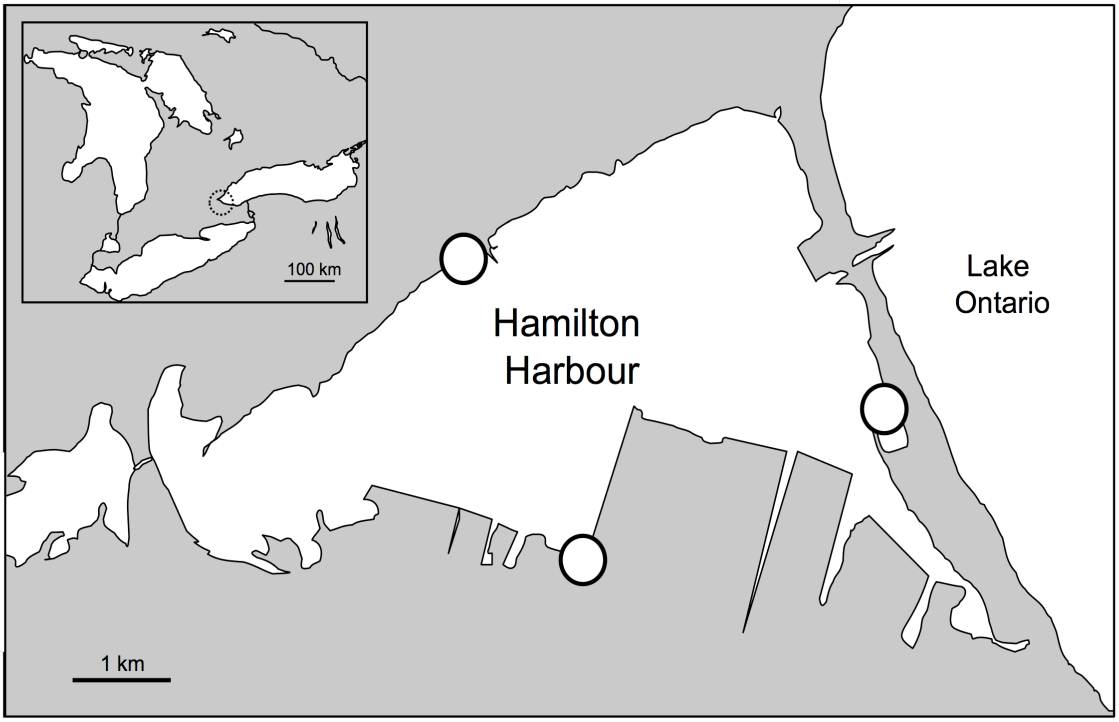

Figure 3.1. A map of round goby collection sites in Hamilton Harbour, Ontario, Canada, including the three locations where round goby for laboratory experiments were obtained and the mark-recapture study was run (white circles). Inset: the lower Laurentian Great Lakes: from the left, Huron, Erie and Ontario, with the location of Hamilton Harbour circled. The scale in km is indicated.

sand, cobble and boulder substrates, and were similar in water parameters such as turbidity, oxygen concentration and temperature (**Chapter 4**). Fish were collected in commercial minnow traps baited with 30 g frozen corn, set at a depth of 1 m or less, for 24 hours. Round goby were transported back to the laboratory and maintained in groups of 3-6 fish separated by sex. Fish were housed in 60 L aquaria equipped with AquaClear 50 external box filters and two airstones at 21 $\pm$ 1 ºC, with a gravel substrate and several 15-cm long, 5-cm diameter PVC tubes for shelter. Male and female round goby are readily distinguished by an examination of the external urogenital papilla, which is pointed in males and blunt in females. Males were assigned one of three reproductive states based on external characteristics: guarding male (erect urogenital papilla, black nuptial coloration and swollen cheeks), sneaker male (erect urogenital papilla but no secondary sex characteristics), and non-reproducing male (a small, flat papilla; **Chapter 2**; Young et al. 2010). Male reproductive status was further confirmed after experiments based on dissection and the presence or absence of well-developed testes and accessory glands during the breeding season (a gonadosomatic index or GSI, gonad (testes) weight (g) / somatic weight (g) x 100, of > 1% = reproducing males; Young et al. 2010). Female reproductive status was assigned as gravid or non-gravid after dissection based on a GSI of > 8 % for gravid females (Young et al. 2010). Fish were allowed to acclimate to the laboratory for at least two and no more than seven days prior to testing and were fed once daily ad libitum with Nutrafin Basix fish flakes, except on the day of testing.

3.3.2 Movement in the lab

This experiment was conducted between 16 May and 25 July 2008. Round goby (*N* = 198) were maintained under a reversed 16L:8D light schedule to facilitate behavioural observations under nocturnal breeding conditions. This species is more active at night (Dubs and Corkum 1996; Diana et al. 2006). Fish were tested in a five-chambered arena under red light (total dimensions 2.5 m long, 75 cm wide, 15 cm deep; each segment 50 cm long and 75 cm wide; **Figure 3.2a**). The arena had sand substrate to 1 cm depth. Each chamber was equipped with one AquaClear Mini filter, and three clear half-tube acrylic shelters. Chambers were separated by transparent dividers; each with a 25 cm x 15 cm central gap in the center as an entrance to the next chamber. The water within the arena was changed once daily to reduce the influence of fish odours between trials and fish were started from alternating ends of the arena in subsequent tests.

In each trial, a group of three sex-matched fish were tested together. Groups rather than single fish were used because round goby naturally exist in high densities (Chotkowski and Marsden 1999) and pilot studies indicated that fish in triads were more active than when observed individually (a mean increase of 2.0 $\pm$ 0.6 movements/min, *N* = 81, 95% confidence interval of 0.8—3.1 movements/min). The three individuals in each group were not matched in size, to facilitate individual identification. The observer was blind to the sex of the fish during testing.

Each group of three was placed in one of the end chambers, with access to the rest of the arena temporarily blocked by barriers, to acclimate for 30 min.

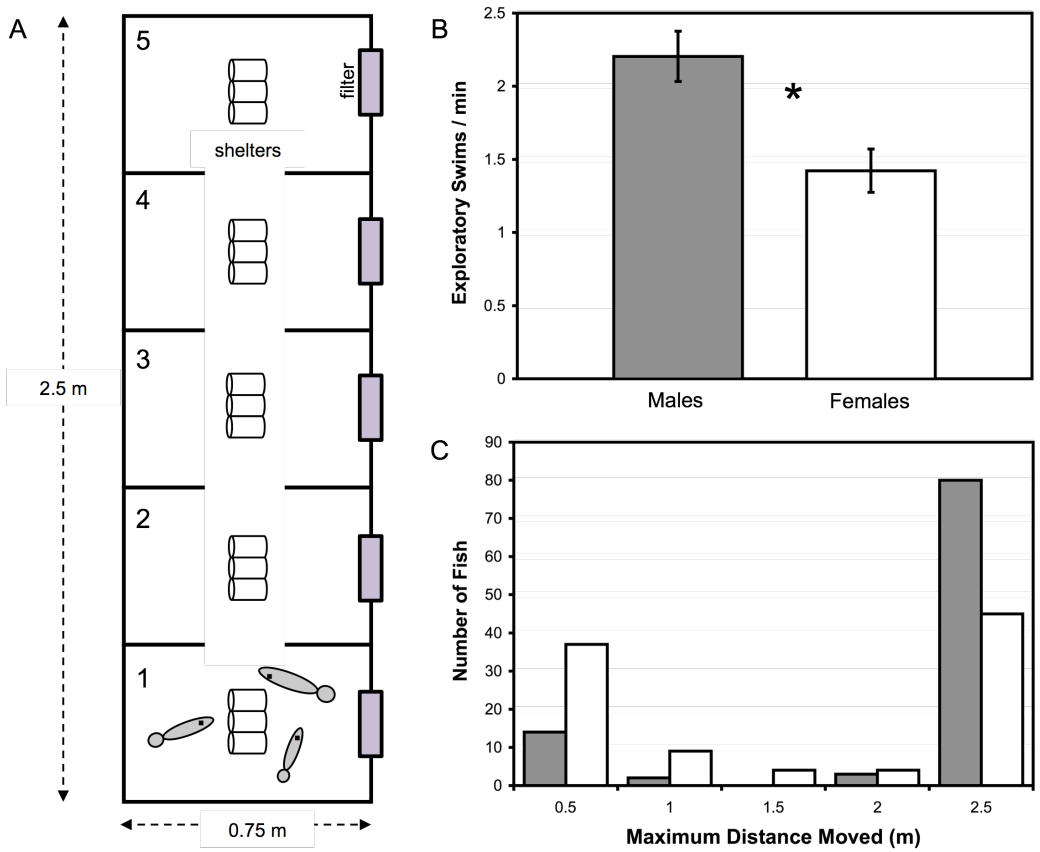

Figure 3.2. **A)** Schematic representation of the 2.5 m long testing arena with five chambers of length 0.5 m each. Fish were placed in sex-matched triads in one chamber (1) and were able to enter other chambers (2-5) after acclimation. Fish in image are not to scale. **B)** Differences in mean ± SE exploratory swims/min for males and females. **C)** The number of male (N = 99) and female (N = 99) round goby to move from 1 to 5 chambers during the experimental test period of 30 min, representing distances of 0.5 to 2.5 m. An asterisk (*) denotes significant differences between the sexes. Males = gray bars, females = white bars.

During the last 15 min of the acclimation, each individual was observed (in randomized order) for 5 min and all behaviour exhibited was recorded. Behaviour (obtained for N = 180 fish) was classed as social interactions, horizontal locomotion, exploration and sheltering (see **Table 3.1** for details). In a pilot study of 20 individual fish, each introduced to a 90 L testing aquarium and observed for 1-min periods at 15-min intervals over a 1 h trial, exploration occurred at high rates after entry into a novel environment, and then declined over the hour (RM-ANOVA, $F_{4,76}$ = 6.7, P < 0.0001), but horizontal locomotion did not ($F_{4,76}$ = 0.4, P = 0.80). At the end of the acclimation period, the temporary barrier blocking the opening to the second chamber was removed, allowing all three fish to freely disperse within the arena for 30 min. The experimenter noted the time at which each fish first left the starting chamber (time to begin dispersal, in sec), the furthest chamber each fish reached in the test period (maximum dispersal), and the total number of chamber switches made. At the end of each trial, the fish were removed, euthanized and dissected to confirm both sex and reproductive status.

3.3.3 Movement in the field

Between May 5 and November 6 2009, and May 5 to November 3 2010, we conducted a large-scale mark-recapture study of round goby in Hamilton Harbour. We selected a mark-recapture technique to study round goby movement in the field because this methodology has been used by many other studies and offered many logistic advantages (Hutchings and Gerber 2002, Croft et al. 2003; see below). Traps

Table 3.1. Behavioural categories for round goby experiments.

Category	Behaviour	Description
Horizontal Locomotion	Hop	Fish movement of ≤ 1 body length
	Swim	Sustained horizontal movements in water column of > 1 body length
	Dart	Rapid swim of > 1 body length
Exploration	Swim	Sustained, repeated, frequently vertical movements in water column with mouth oriented at perimeter of aquarium
Sheltering	Dig	Fish inserts snout into substrate, takes mouthful of substrate and ejects it
	Self-burial	Fish rapidly shimmies to bury body in substrate
Social interactions	Bite	Following a rapid approach, one individual opens and closes its mouth on another; the bitten fish darts away
	Chase	One individual rapidly approaches another without contact; the approached fish darts away

were set at each of three sites along a 30-m stretch of shoreline (six traps, each six m apart) and all fish caught were tagged as above with a unique identifying VIE four-mark tag (Visible Implant Elastomer, Northwest Marine Technologies, Inc.) in four of 12 possible locations on the body. Fish were tagged in five cohorts between May 5 and August 21 2009; recaptures continued to be monitored weekly (2009) or bimonthly (2010) until the end of the study. In total 881 fish were tagged (N = 539 males, 328 females, 14 juveniles). Each fish was sexed and its total mass, total length, and reproductive condition (based solely on the external appearance of the urogenital papilla and secondary sexual characteristics; **Chapter 2**) were noted. Recaptured individuals were identified, re-weighed and measured, their recapture location recorded, and then they were released at that same recapture location. Traps for other round goby studies were also occasionally set in the same general area. Any tagged round goby opportunistically collected in these traps that were not part of the mark-recapture study were re-weighed and measured as usual, and the distance along the shore to the nearest mark-recapture trap was calculated in determining the total distance moved by the fish. All recaptured fish were assigned a maximum distance traveled, across all capture events (in m), a maximum number of elapsed days between first and last captures as measures of residence time and site fidelity, and a traveling rate (maximum distance traveled (m)/week, with weekly units defined as maximum days elapsed/7 days).

For the first year of the study, recaptured fish could be assigned to one of three mutually exclusive seasonal categories based on the dates at which they were

first and last seen. These categories were i) breeding season residents, ii) post-breeding season residents, or iii) year-long residents for fish that were first observed during the breeding season and last seen after the breeding season. A fourth category encompassed all fish seen in the second year of the study, iv) returning residents. Previous research has indicated that the number of gravid females in the study area declines dramatically by August (Young et al. 2010), and male sperm is reduced in number and speed in this month (J. R. Marentette, personal observation, Sopinka 2010). Based on these findings August 15 was selected to demarcate the end of breeding season.

Two additional supporting studies were also conducted. To evaluate whether tags influenced mortality or would be lost over time, a group of 10 VIE-tagged fish were maintained and monitored in the laboratory from June 2009 to April 2010 when the last round goby in the laboratory died. No tag losses were observed in this period, and no mortalities occurred in the first two months of the study. To examine potential sex differences in trap response, a laboratory study was run between July 10 and August 27 2010. Groups of four fish (two male, two female) were given two 16-h overnight trials spaced two days apart. In a trial, each group of four was placed in a 90 L aquarium equipped with an AquaClear 50 filter and a sand substrate to a depth of 2 cm. Groups were placed either inside or outside a minnow trap baited with 30 g frozen corn. Both entrances to the minnow trap were open, and the order of trial presentation (inside or outside the trap) was randomized. After 16 h, the fish that successfully entered or escaped the trap were identified. Of 52 fish, 38% of

females (N = 10) and 58% males (N = 15) entered the trap, but only one individual (a male) was observed to escape the trap. There were no sex differences in rates of laboratory trap entry (χ^2 = 1.9, P = 0.17) or escape (χ^2 = 1.0, P = 0.31).

3.3.4 Statistical analyses

All data analysis was performed using the program JMP 5.0.1a for MacIntosh (SAS Institute, Inc., 2002). Behavioural data (rates/min) and morphological data (e.g., fish total length) from the laboratory experiment were log or arcsine square-root transformed to normalize where possible. As individual fish were observed as part of a group of three, behavioural data were examined with linear mixed models (residual maximum likelihood method) incorporating sex and collection site as fixed main effects and Group ID as a random effect, nested within Sex to account for the fact that all members within a group were of the same sex. Differences in laboratory behaviour between fish of different reproductive states was examined in linear mixed models separately within each sex, using Status as fixed main effect and Group ID as a random effect. Fish were given a binary score for some measurements, such as reaching the furthest point of the laboratory apparatus (i.e., "yes" or "no") and these data were analyzed with a logistic regression model followed by post-hoc Wald chi-square tests to determine the effect of sex. Male round goby are larger than females (Charlebois et al. 1997; Young et al. 2010) and so a covariate of log total length was used in models, but removed where it was not significant. The number of days spent in the laboratory, which varied between 2 and

7, was never a significant covariate in models of fish behaviour and was therefore removed. Non-significant interaction terms were also removed from models. Post-hoc differences between sexes or reproductive states were identified, where necessary, using Tukey HSD tests or a non-parametric equivalent (Zar 1999). Where data could not be normalized through transformation, and in the case of field movement data, non-parametric statistics such as the Kruskal-Wallis test, normal approximation to the Wilcoxon rank-sum test, or Spearman rho correlations, were used. Comparisons of numbers recaptured (male versus female) were achieved using chi-square tests.

3.3.5 Ethical note

Animal handling protocols for these studies were approved by the McMaster University Animal Research Board (AUP # 06-10-61) in accordance with the Canadian Council for Animal Care guidelines.

3.4 RESULTS

3.4.1 Movement in the lab

Male fish explored more than females in the acclimation phase ($F_{\text{sex } 1,56} = 6.7$, $P = 0.01$, **Figure 3.2b**), although the sexes did not differ in horizontal locomotion ($F_{\text{sex } 1,56} = 1.3$, $p = 0.27$) or in sheltering behaviour ($F_{\text{sex } 1,56} = 1.3$, $P = 0.12$). Males showed greater dispersal through the arena than females in the test phase: they

made more chamber switches ($F_{\text{sex } 1,62}$ = 16.3, P = 0.0001) and those that dispersed began dispersal sooner than females ($F_{\text{sex } 1,57}$ = 8.0, P = 0.007). More males than females reached the fifth and furthest chamber of the testing arena (logistic regression, Wald χ^2_{sex} = 25.2, P < 0.0001; **Figure 3.2c**).

Reproductive status had little impact on round goby behaviour in this experiment. Gravid and non-gravid females did not differ from each other on any measure, nor did female GSI correlate with any behavioural data (ANCOVAs, effect of status P's > 0.10; Spearman rho, P's > 0.30). Sneaker males showed the most horizontal locomotion among males in the acclimation phase ($F_{\text{status } 2,58}$ = 7.1, P = 0.002), but did not disperse differently from other males in the test phase. Guarding males made more chamber switches than non-reproductive males ($F_{\text{status } 2,63}$ = 4.5, P = 0.02). Male GSI also did not correlate with any behavioural data (Spearman rho, P's > 0.10).

Behaviour during the acclimation phase and the testing phase was correlated. Fish that exhibited more exploration in the acclimation phase began dispersal sooner (r_s = -0.22, N = 132, P = 0.01), dispersed farther (r_s = 0.51, N = 180, P < 0.0001) and made a greater number of chamber switches (r_s = 0.45, N = 180, P < 0.0001) in the test phase.

Body size did not correlate with round goby movement. Fish total length did not relate to exploration behaviour, the time to begin dispersal, the distance dispersed or the number of chamber switches (Spearman rho, all P's > 0.10). Aggression was size-based and generally directed by larger individuals toward

smaller individuals, with the smallest of three fish receiving the most aggressive acts in a group (r_s = 0.40, p < 0.0001). However, the amount of aggression received did not affect how soon fish dispersed, how far they dispersed, or the number of chamber switches made (Spearman rho, all *P*'s > 0.10).

3.4.2 Movement in the field

Of the 881 fish tagged, 19.0% (*N* = 167) were recaptured the same year (2009). About one third (*N* = 54) were recaptured more than once, with three individuals being caught six times after tagging (two females, one male). Male (*N* = 106, 19.7%) and female (*N* = 61, 18.6%) return rates were similar (χ^2 = 0.2, *P* = 0.70). Among the reproductive males, more guarding males (*N* = 34, 23%) than sneaker males (*N* = 20, 11.6%) were ultimately recaptured (χ^2 = 7.2, *P* = 0.007). No juveniles were recovered.

In 2010, the second year of the study, 1.5% (*N* = 13, nine male and four female) of the original 881 fish were recaptured. All fish possessed a complete set of four VIE tags. Seven fish had not previously been recaptured, increasing the total recapture rate for the study to 20.0% (*N* = 176). Of the four fish that had been recaptured in 2009 as well as 2010, three had been year-long residents, and one was a post-breeding season resident.

Across the study, known residence times varied from 1-168 days in 2009 and up to 386 days in 2010, and absolute distances moved ranged from 0-18 m (**Table 3.2**). To control for the amount of time elapsed between sightings, we used

Table 3.2. Median (and range) values for the absolute distances moved, in meters (m), and the number of days elapsed between first and last captures, categorized by round goby sex, reproductive tactic, and whether the fish was a resident i) during the breeding season, ii) after the breeding season, iii) year-long in 2009, or iv) a returning resident in 2010. Days elapsed between captures for returning residents were calculated as the difference between the last capture in 2009 and the first capture in 2010. GM = guarding males. SM = sneaker males. NRM = non-reproductive males.

Time of Residence	Measure	All Males	All Females	GM	SM	NRM
Breeding Season	Distance (m)	0 (0-18)	0 (0-18)	0 (0-9)	0 (0-18)	0 (0-12)
	Days	17 (1-71)	20 (1-85)	14 (1-58)	19.5 (1-71)	29.5 (7-70)
	N	29	21	9	10	10
Post-breeding Season	Distance (m)	0 (0-12)	0 (0-6)	0 (0-12)	0 (0-3)	0 (0-12)
	Days	22.5 (1-64)	14 (1-64)	36.5 (1-63)	13 (3-50)	20 (1-64)
	N	58	21	20	6	32
Year-long	Distance (m)	0 (0-9)	3 (0-15)	6 (3-9)	0 (0)	0 (0-3)
	Days	57 (25-134)	79 (28-168)	89 (57-134)	59 (25-93)	47.5 (27-91)
	N	19	19	5	4	10
Returning	Distance (m)	6 (0-12)	1.5 (0-6)	12 (6-12)	n/a	6 (0-12)
	Days	290 (233-386)	322 (282-386)	282 (233-386)	n/a	321 (271-386)
	N	9	4	5	0	4

travelling rate in m/week to compare movement distances between sexes or among male reproductive tactics in round goby residents.

3.4.2.1 Breeding season residents

Males and females did not differ in their rate of travel (Z = 0.06, P = 0.95). The travelling rate of guarding, sneaker and non-reproductive males were similar (H_3 = 0.24, P = 0.89), and guarding males and females moved at similar rates (Z = 10.45, P = 0.66; **Figure 3.3a**).

3.4.2.2 Post-breeding season residents

Males traveled faster than females, but not significantly so (Z = 1.69, P = 0.09), after the breeding season ended. Among male tactics, there were no differences in rates of travel (H_3 = 2.37, P = 0.31). Post-breeding season guarding males traveled faster than did females (Z = 2.16, P = 0.03; **Figure 3.3b**).

3.4.2.3 Year-long residents

In fish present over the entire 2009 sampling period, males and females moved at similar rates (Z = 1.02, P = 0.31). Guarding males moved faster than either sneaker males or non-reproductive males (H_3 = 10.43, P = 0.005). Guarding males, however, did not move differently than females (Z = 2.03, P = 0.15; **Figure 3.3c**). In no measure did year-long residents differ from fish found only in either the breeding or post-breeding season. Year-long residents were similar in total length to other

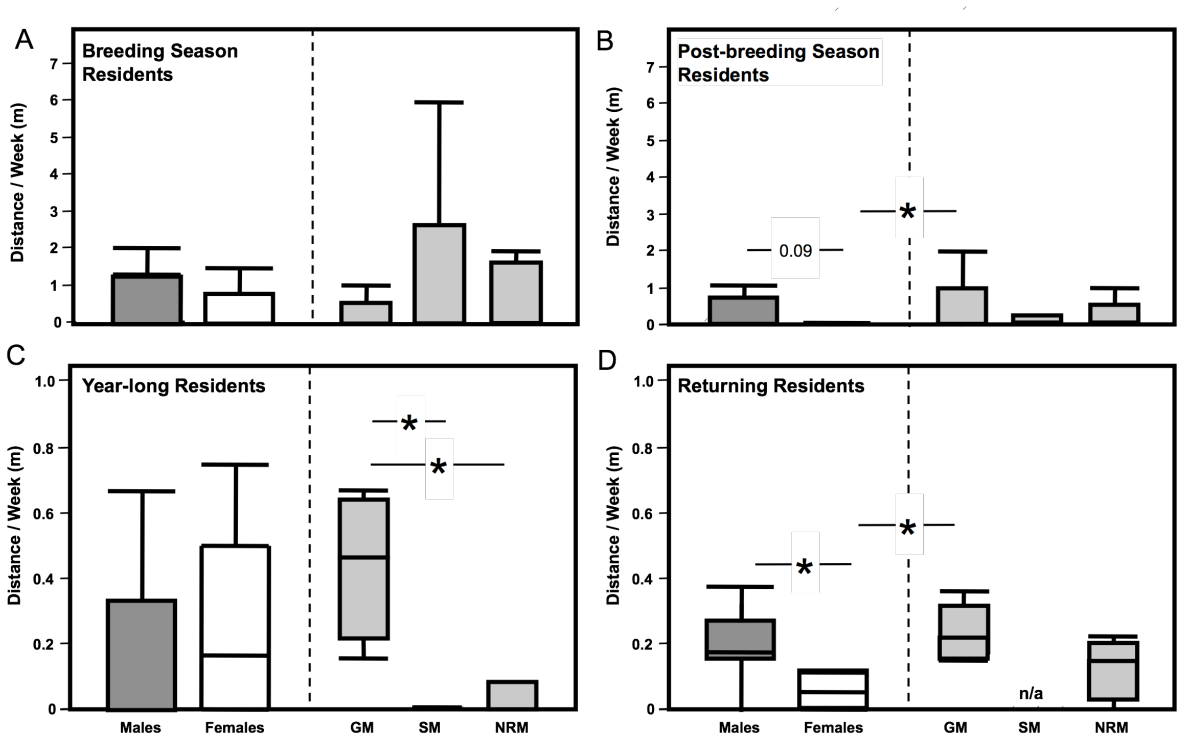

Figure 3.3. Median, quartile and 95% percentile boxplot distributions of rate of travel (in m/week) across sexes (left; males dark gray, females white) and male reproductive states (right; light gray) in **A)** the breeding season (May-August); **B)** the post-breeding season (August-November), **C)** year-long residents who spanned the breeding and post-breeding seasons, and **D)** returning residents recaptured in the second year of the study. GM = guarding males, SM = sneaker males, NRM = non-reproductive males. Lines between pairs with an asterisk (*) indicate significant differences ($P < 0.05$) between pairs (based on Wilcoxon rank-sum tests), and one non-significant result is indicated as $P = 0.09$.

residents, controlling for sex ($F_{2,163\ \text{resident}} = 1.87$, $P = 0.16$), nor did they differ from breeding or post-breeding season fish in the rate of travel per week, within each sex or reproductive status (Kruskal-Wallis tests, P's > 0.10).

3.4.2.4 Returning residents

Males moved farther and faster than females ($Z = 2.25$, $P = 0.024$; **Figure 3.3d**). Larger fish in general traveled faster, but not significantly so (Spearman rho, $r_s = 0.48$, $N = 13$, $P = 0.09$). Three of the males were identified as guarding males in both years, four as non-reproducing in both years, and two males were identified as non-reproducing in 2009 and as guarding males in 2010. Males that presented as non-reproducing in both years grew more than males that presented as guarding males by the second year (measured as a percent change in total length; $Z = 2.3$, $P = 0.02$). The sex difference in movement between years was driven by guarding males, which moved more than females, and not by non-reproductive males ($H_3 = 7.0$, $P = 0.03$; **Figure 3.3d**).

3.5 DISCUSSION

In the laboratory, round goby males exhibited more exploratory behaviour, dispersed farther and dispersed sooner in a novel laboratory environment than did females. In the field, males also moved greater distances than females. Guarding males did not move more than sneaker males in the laboratory, but did so over a year in the field. Although larger round goby are thought to aggressively displace

smaller round goby to suboptimal habitat in the field (Ray and Corkum 2001), neither body size nor the amount of aggression received appeared to modulate round goby movements in our laboratory studies.

In the laboratory, where males were not given the opportunity to reproduce or guard territories, our predictions were supported—males moved more than females. In the field, our predictions of greater male movement were supported for post-breeding season movements, and movement between years. Our predictions of greater female movement during the breeding season were not supported. Why did we not see less movement in guarding males relative to females? First, there may be no difference in home ranges or territorial behavior between guarding males and females. This may be particularly true for guarding males that at the time of capture did not currently have egg clutches in their nest, due to clutch loss, not having yet spawned, or having reared a previous clutch to the juvenile stage. Males with eggs are believed to reduce feeding (Charlebois et al. 1997; Corkum et al. 1998) and we felt these were unlikely to be trapped. A reduction in movement around a territory may only be apparent for that window of time when guarding males are actively parenting.

The differences in movement rates or space use between males adopting alternative reproductive tactics have rarely been examined or quantified (see Gladstone 1987, Petersen 1987, Mboko and Kohda 1999, Sunobe and Nakazono 1999, Manabe et al. 2009). In our study, guarding and sneaker male round goby were equally explorative in the laboratory. In the field, guarding males moved more than

sneaker males, as predicted, but the pattern was only obvious over the course of an entire year. It may be the case that sneaker males associate closely with one or a few nests only, thus also showing relatively restricted home ranges (as has been observed with ruff, *Philomachus pugnax*, Van Rhijn 1973; blennies, *Blennius sanguinolentus,* Santos and Almada 1988; and cichlids, *Telmatochromis vitattus*, Ota and Kohda 2006).

Mark-recapture was preferred as a low-impact methodology for our study. Surgical introduction of ultrasonic or PIT tags is more invasive than VIE tag injection and thus can affect fish health and ability to move. Accurate individual fish identification based on in-person watches have been used successfully with cottid fish in shallow rivers (e.g., Natsumeda 2001) but would be difficult to achieve in deeper lakes for this rock-dwelling, cryptic and primarily nocturnal fish species. The distances between traps (6 m) used in our study were smaller than the reported mean 48-h distance moved by round goby in a semi-natural enclosure (7.3 m; Cookingham and Ruetz III 2008). However, a finer-scale assessment of movement may have been necessary to detect subtle but real differences in movements between sexes or across male tactics within a single year or season. Although the close physical monitoring required for in-person PIT tag detection is known to disturb round goby and could disrupt the maintenance of normal behaviour like ranging (e.g., Cookingham and Ruetz III 2008), the use of stationary arrays to detect PIT tags could be useful in this species.

We recaptured 20% of tagged round goby. Tag loss or handling-related mortality seems unlikely to have significantly influenced our recapture rate estimates, as control fish tagged and maintained in the laboratory showed no tag loss even after several months. Also, all fish recaptured in the second year of this study had a complete set of tags. The 80% of fish not recaptured may have remained in the area, but simply avoided or escaped traps. Our laboratory control studies indicated that trap escape is a possibility but may happen only at low rates (<2%), and also that not all fish enter a baited trap even when it is within close quarters. Some other fish probably dispersed out of the mark-recapture study area. In a 2003 mark-recapture study in the same study location of Hamilton Harbour (Balshine et al. unpublished data), three long-distance dispersers were opportunistically captured (one female travelling 50 m, and two males, 4 and 8 km) from a population of 231 tagged fish. Long-distance dispersers may mediate round goby invasion fronts, estimated to move at a rate of 1.0 km/year (Bergstrom et al. 2008).

How do sex differences in movement in this species compare with other taxa? Greater male movement in round goby exhibited in the laboratory and in the field matches up with predictions based on the round goby's polygamous mating system. Like most other polygamous species (mammals, fishes) where males compete more vigorously for access to mates (Greenwood 1980, Dobson 1982), male goby move more than females, both in a short-term spatial task and over the long term (across years) in a natural environment. Sex biases in movement may represent dispersal away from kin toward new mating opportunities, or an attempt to access better

resources. Passive dispersal of round goby larvae over many km is considered to be a major contributor to genetic exchange among populations (Hensler and Jude 2007; Hayden and Miner 2009), where not driven by human transport (LaRue et al. 2011), so adult movement away from kin may be unnecessary and unlikely for round goby. The effects of 6-12 m movement differences on reproductive success of males versus females reproductive success are not known. Movement between years may represent the establishment of newer, higher quality home ranges and nesting territories in rocky nearshore regions where our study was conducted. It is worth noting that the 0—18 m linear nearshore movements revealed in this study are within the same spatial scale as home ranges identified for other benthic fish species of similar body sizes; slimy sculpins (*Cottus cognatus*, Cunjak et al. 2005), fluvial sculpin (*Cottus pollux*, Natsumeda 2001, 2007), and other gobiids (Osugi et al. 1998; Sunobe and Nakazono 1999).

To our knowledge, this study represents the largest-scale and longest-lasting study of adult round goby movement in the field, where much is not yet known, and the only to take into account fish sex and reproductive tactic. A mark-recapture study of round goby in Lake Michigan yielded only 6.2% recaptures (Wolfe and Marsden 1998); one individual was recovered 2 km away after 213 days. Cookingham and Ruetz III (2008) observed that 85% of stocked round goby dispersed out of a 20 x 20 m area in Muskegon Lake after two weeks. A third study recovered 58% of fish in the Detroit River over a five-week period, and calculated the mean diurnal home range of these fish to be 5 m^2 over 1 hr (Ray and Corkum 2001). Round goby may move

more widely at night, however (Natsumeda 1998). Our study indicated moderate site fidelity on the basis of recapture rates, home ranges with a linear axis of 6 m or less, and very high site fidelity in terms of actual distances moved within and between years. Assuming a winter migration to deeper waters, our study suggests that the round goby has strong annual homing abilities, or may simply be undergoing far shorter winter migrations in North America than in its native range (Pinchuk et al. 2003).

Movement at all life stages is key to understanding the dynamics of established as well as invading populations of many species. Upriver invasion fronts of round goby (e.g., Poos et al. 2009) may be mediated primarily through natural dispersal of adults or older juveniles (range expansion) as well as human-assisted transfers, because these occur in areas where passive dispersal of larvae cannot occur. The sex of the first round goby to be recorded in newly invaded areas is not often reported; however, Ojaveer (2006) describes the first three fish collected from the northeast Baltic Sea to be male. Our experimental behavioural data and field records of long-distance dispersers both suggest that movement of males may precede that of females at the invasion front, like that of male coho salmon (*Oncorhynchus kisutch,* Anderson and Quinn 2007) or male western bluebirds (*Sialia mexicana*, Duckworth and Badyaev 2007), both species undergoing range expansions into non-native habitats. This predicted greater male bias has been found in round goby invasion fronts (Gutowsky and Fox 2011). It is possible that other factors independent of sex, such as asociality (Cote et al. 2010b) or increased aggression

(Duckworth and Badyaev 2007) play important roles in determining which round goby first enter new habitats. Invasion fronts thus offer a novel context in which to examine whether field predictions of sex- or individual-specific differences in movement are borne out.

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Chapter 4

SIGNATURES OF CONTAMINATION IN INVASIVE ROUND GOBIES (*NEOGOBIUS MELANOSTOMUS*): A DOUBLE STRIKE FOR ECOSYSTEM HEALTH?

4.1 ABSTRACT

The invasive round goby has a recognized role in transferring contaminants through foodwebs, but little work has been done on contaminant impacts on round gobies themselves. Here we present the first case study of contaminant biomarkers and subpopulation structure variation in round gobies, in relation to habitat contamination, within a Canadian Area of Concern, Hamilton Harbour. Copper and cadmium were elevated in livers of fish from contaminated habitats. Although catch abundances were similar across sites, fish were smaller, a greater proportion of fish were female and more males were in reproductive condition in contaminated sites. Fish from contaminated areas showed more fin loss. Males from contaminated sites showed intersex gonads and genitalia. Ethoxyresorufin-*o*-deethylase (EROD) activity was higher in fish collected near polycyclic aromatic hydrocarbon (PAH)-rich sediments. The results indicate that contaminants impact the characteristics of round goby populations, which could affect ecosystems beyond toxicant biomagnification. This study also confirms that round gobies can be abundant in polluted habitats, which may draw predators – facilitating mobilization of contaminants in foodwebs.

4.2 INTRODUCTION

The aquatic biotas of the Laurentian Great Lakes have been shaped by a succession of invading species, one of which is the round goby (*Neogobius melanostomus*). This small benthic fish invaded all five Great Lakes by 1998 (Mills et al. 2003), possibly aided by multiple introduction events in ship ballast water (Jude 1997). The rapid range expansion of the round goby and its explosive population growth were facilitated by its ability to specialize on dreissenid mussels (*Dreissena polymorpha* and *D. rostriformis bugensis*), to outcompete native species for food and shelter, and to spawn several times each breeding season (Corkum et al. 2004). The round goby is now a dominant part of the biomass in many areas of the lower Great Lakes (Johnson et al. 2005) and its influence continues to expand as round gobies invade more inland waterways (e.g., Poos et al. 2010). These fish are now key players in Great Lakes aquatic ecology, both as an integral and important part of the diets of piscivorous fishes, reptiles and waterbirds (Mills et al. 2003; Somers et al. 2003; King et al. 2006), and as a competitor and potential causal agent in the decline of native benthic fishes such as darters, logperch and sculpins (Jude et al. 1995; Dubs and Corkum 1996; Janssen and Jude 2001; Lauer et al. 2004; Balshine et al. 2005).

The benthivorous diet of round gobies with its emphasis on dreissenids, and the role of gobies as prey for many species, has additional implications. As first suggested by Jude and others (1995), the round goby is one of the few fish in the Great Lakes capable of eating the abundant dreissenid mussels, which are

invertebrates known to bioconcentrate contaminants in their tissues while filter–feeding. This means that persistent contaminants may have found a new route for bioaccumulation in higher trophic levels of the Great Lakes (Morrison et al. 2000). Kwon and colleagues (2006) found evidence for biomagnification of PCBs (polychlorinated biphenyls) in simplified foodwebs across several sites in Lake Erie, although this hasn't been found in all studies (Hanari et al. 2004). Round gobies may be contributing to the biomagnification of other organic contaminants like perfluoroctanesulfonate (PFOS; Kannan et al. 2005), and metallic toxicants as well. A diet of round gobies, for example, might be responsible for the sustained mercury loads in smallmouth bass in Lake Erie even as sediment mercury contamination declines (Hogan et al. 2007).

Round gobies have the potential to impact local ecosystems due to contaminant transfer, but what effects do contaminants have on round gobies? As a pollution-tolerant species (Pinchuk et al. 2003), round gobies may be able to substantially colonize heavily polluted areas of the Great Lakes in greater numbers than native fishes, and possibly attract or support predator populations in these regions. One such area is Hamilton Harbour in western Lake Ontario, Canada. This 2150 hectare embayment is a Canadian International Joint Commission (IJC) Area of Concern (International Joint Commission 1999) with a long history of contamination and habitat modification, stemming from both local steel mills and local urban settlements that have discharged sewage, agricultural, road and other effluents (Hamilton Harbour RAP 1992). The Harbour is the location of Randle Reef,

a polycyclic aromatic hydrocarbon (PAH)-rich coal tar deposit ranked as the second most contaminated aquatic location in Canada (Hamilton Harbour RAP 1992). Remedial efforts have made progress in recent years toward cleaning the Harbour (Hall et al. 2006), but many contaminants continue to persist at problematic levels. Pollutants of primary concern ("A list" contaminants) include organic contaminants of industrial origin (PCBs and PAHs), and metals such as arsenic, cadmium, lead, iron, and zinc (Hamilton Harbour RAP 2003). Nonetheless, Hamilton Harbour continues to support a speciose ecosystem (fishes, waterfowl and terrestrial life) fed by a local wetland serving as an important fish and avian breeding ground (Cootes Paradise Marsh; Hamilton Harbour RAP 1992). It continues to serve as a resource for human waterfront activities, including sportfishing and boating, although consumption of Harbour fish is regulated (Hamilton Harbour RAP 2008).

Round gobies were first discovered in Hamilton Harbour in 1999 (Hamilton Harbour RAP 2003), making this one of the last areas of the Great Lakes to be invaded. Within two years of the initial report, round gobies were well established in the embayment (Young et al. 2010). Since their invasion round gobies have become an important prey item for Harbour predators, including the abundant avian piscivore, the double-crested cormorant *Phalacrocorax auritus* (Somers et al. 2003). If contaminant exposure affects round goby populations in highly impacted areas of Hamilton Harbour (through bioconcentration and bioaccumulation, or through changes in abundance or vulnerability), then it follows that predators consuming round gobies in those areas should also be affected.

The aim of this study was to examine the consequences of living in contaminated areas using the round goby population of Hamilton Harbour as a model system. This fish's limited adult mobility (Ray and Corkum 2001) suggests that known field conditions at fish collection sites should be reflected in biomarkers of contaminant exposure. We contrasted several toxicant biomarkers (7-ethoxyresorufin-*o*-deethylase, or EROD, activity, incidence of intersex, and morphological abnormalities), and direct measurements of contaminants (concentrations of copper, nickel and cadmium in body tissues) of round gobies collected from two heavily contaminated (PCB-, metal- and PAH-rich), and two relatively less contaminated areas of Hamilton Harbour. We also compared subpopulation characteristics (body size, age, abundance as catch per unit effort or CPUE, and sex ratio) between the two highly contaminated sites and the two cleaner sites.

4.3 METHODS

4.3.1 Site selection and collection of fish

Round gobies were collected during the breeding season (MacInnis and Corkum 2000a) from four nearshore sites in Hamilton Harbour (**Figure 4.1**) between May 3 and October 26 in 2006 (n = 846), April 19 and October 23 in 2007 (n = 695) and April 23 and October 24 in 2008 (n = 643). In the summer months, the

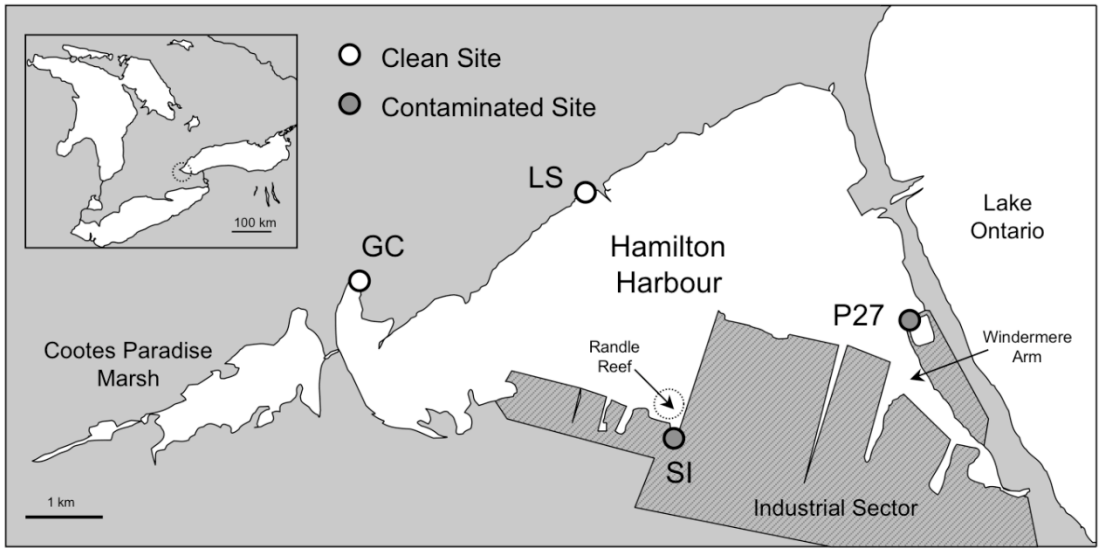

Figure 4.1. Collection sites within Hamilton Harbour, Canada (43°17' N, 79°49' W) identified as a low impact or clean site (open circles): Grindstone Creek (GC) and LaSalle Park Marina (LS); or a high impact, contaminated site (gray circles): Pier 27 (P27) and Sherman Inlet (SI). Hamilton Harbour is shown in relation to the Laurentian Great Lakes (inset). Scales of maps are indicated.

nearshore or littoral zone (< 7 m depth) represents the oxygenated portion of the Harbour (Hamilton Harbour RAP, 2003). Shallow warm waters in rocky habitats (rich in crevices) are where male round gobies typically establish nesting territories from which they court females and guard eggs (Charlebois et al. 1997). Sites were selected from known distributions of PAHs, metals and PCBs in harbour sediments (Hamilton Harbour RAP 1992, 2003; Zeman 2009), as these are the dominant contaminants of concern in this water body. Two cleaner sites were located along the relatively less impacted northern shore of the Harbour, at the mouth of Grindstone Creek (GC; 43°17'21'' N, 79°53'13'' W) and at LaSalle Park Marina (LS; 43°18'1'' N, 79°50'47'' W), and two highly contaminated sites were located along the southern and eastern shores, near a region of heavy steel industry discharges and historical coal tar dumping known as Randle Reef (Sherman Inlet, or SI; 43° 17' 3'' N, 79° 47' 33'' W; Murphy 2000) and a confined, dredged-sediment disposal facility next to a high-use shipping channel known as Windermere Arm (Pier 27, or P27; 43°17'3'' N, 79°47'33'' W). Based on sediment contaminant databases generated between 1975 and 1989, sites near both SI (467 ng/g) and P27 (638 ng/g) have higher concentrations of PCBs than either GC or LS (30 and 124 ng/g), and both SI (1400-1470 ug/g) and P27 (20-67 ng/g) have higher concentrations of PAHs than GC and LS (< 17 ng/g; Zeman 2009). PAH and PCB concentrations in the Harbour have remained stable from 1990–2004 (Zeman 2009). Three sites had boulder and cobble cover extending to approximately 3 m from shore to a sand/silt substrate bottom, while one site (GC) had a mud substrate with few hard structures.

All sites were similar in water clarity with typical Secchi depths > 1 m except for GC where the waters were consistently turbid (mean ± SE Secchi depth 20 ± 1.3 cm; Chi square test, χ^2_3 = 61.3, p < 0.0001). Sites were also similar in water temperature ($F_{7,151}$ = 0.6, p = 0.6) and in dissolved oxygen within years ($F_{7,143}$ = 7.9, p < 0.0001, site $F_{3,143}$ = 2.7, p = 0.05; Tukey HSD, p > 0.05 for all).

Round gobies were collected with minnow traps baited with 30 g frozen corn, and set for 24 hours in < 1 m of water. Fish were either processed on-site or transported back to the laboratory for processing within two days of capture. Round gobies processed for rapid collection of blood and tissue samples were placed in an ice-water bath followed by cervical severance; otherwise, fish were euthanized in a solution of benzocaine.

4.3.2 Site catch characteristics & morphological measurements

Sex was determined by an examination of the urogenital papilla (Miller, 1984) and confirmed by dissection. Fish could be assigned one of five reproductive states, as follows: gravid or reproductive female (RF, females with gonadosomatic index (GSI; see below) ≥ 8 %); adult but non-reproductive female (NRF, females with GSI < 8%); parental male (PM; males with a swollen urogenital papilla, black body colouration, enlarged head, developed testes and accessory glands); sneaker male (SM, males with a swollen urogenital papilla, a female-typical brown body colouration and narrow head, and developed testes); and adult but non-reproductive male (NRM, males with a small flat urogenital papilla and undeveloped

testes and accessory glands, GSI << 1%) (Young et al. 2010; **Chapter 2**). The standard length (SL), total length (TL), width of the head (across the posterior orbital edge; a wider head representing one of the secondary sex characteristics of reproductive parental males; **Chapter 2**) and length of the urogenital papilla (tip to anterior insertion at the posterior edge of the anus) were measured with calipers to 0.1 mm. Fish total mass, as well as the mass of the liver and gonads (accessory glands in males recorded separately) were recorded to 0.001 g. Fulton's condition factor K was calculated as total mass : TL^3 x 100. The gonadosomatic index (GSI) was calculated as gonad mass : somatic mass x 100%, where somatic mass = total mass – gonad mass. Similarly, the hepatosomatic index (HSI) was calculated as liver mass : somatic mass x 100%, where somatic mass = total mass – liver mass. In males, the accessory gland somatic index (AGSI) was calculated as the mass of the organ : somatic mass x 100%. Indices such as HSI and GSI are recognized to inadequately account for allometric differences across fish of varying size (Tomkins and Simmons, 2002), but are very commonly used and thus reported to facilitate comparisons with other studies.

In 2007, digital photographs were taken of the ventral surface of 197 round gobies, representing approximately 10 fish per reproductive category from all four Hamilton Harbour sites. The length and width (at the midpoint of the length) of the urogenital papilla as well as the length and area of the pelvic suction disc (fused pelvic fins characteristic of gobiid fishes) were determined in mm or mm^2 using the program ImageJ (Wayne Rasband, National Institutes of Health, U.S.A., available at

http://rsb.info.nih.gov/ij). The width:length ratio of the urogenital papilla has been used in other gobiids to track male-to-female sex change (Carlisle et al. 2000).

4.3.3 Body burdens of copper, nickel and cadmium

Eight size-matched males from three sites (LS, P27 and SI; n = 24) were obtained on September 12 and 26, 2008 and processed to quantify copper (Cu), nickel (Ni) and cadmium (Cd). Too few females and too few fish from GC were collected to form part of this study. Fish were euthanized in benzocaine and the liver, gills, and gut removed (gut cut longitudinally to remove contents), rinsed in 0.9% NaCl solution (analytical grade, Sigma-Aldrich, prepared in NANOpure II water, Sybron/Barnstead, Massachusetts, U.S.A.) to remove surface bound metals, and placed on ice. Tissue was weighed and digested in 3-5 volumes of 1 or 2 N trace-metal grade HNO_3 (Fisher Scientific) at 65°C for two days. Samples were shaken on the first day of digestion. The tissue digest was diluted to 1% HNO_3 for Cd, Cu and Ni analyses by a Graphite Furnace Atomic Absorption Spectrometer (Varian Spectra AA-20 with graphite tube atomizer [GTA-110], Mulgrave, Australia). Standards from Fisher Scientific (Toronto, Canada) were used for calibration in every 40 samples. Certified reference material, TM15 (National Water Research Institute, Burlington, Canada) was analyzed for validation in each run. Recovery of metals was within 20%. A maximum of 5% difference in metal concentrations between duplicates of samples was accepted. Tissue metal concentrations were expressed in $\mu g \cdot g^{-1}$ wet weight. To determine the importance of waterborne (ratios < 1) versus dietary

(ratios > 1) routes of metal exposure, the gut:gill ratio was calculated in each fish. LS fish had larger livers than fish from P27 or SI (effect of site $F_{2,18} = 7.9$, $p = 0.003$).

4.3.4 EROD activity

Livers were collected from freshly euthanized animals up to two days after capture between May 22 and September 11 in 2007 (n = 179), and on the day of capture between June 16 and July 14 in 2008 (n = 35). The samples were individually wrapped in aluminium foil and stored at –80°C until processing. Each liver was then thawed, bisected, and one half of the organ was homogenized in buffer (50 mM Tris HCl, 0.15 M KCl; 4 $mL \cdot g^{-1}$ tissue) at 4°C. Liver homogenates were then centrifuged for 10 minutes at 750 g and 10 minutes at 12 000 g, at 4°C (Sorvall Legend RT centrifuge). The supernatant S9 fraction was drawn off and the protein content of the S9 fraction (in $\mu g \cdot mL^{-1}$) was determined with 0.5 µL of sample, taken in duplicate, for a bicinchoninic acid protein assay adapted for a 96-well plate (Pierce Kit 23225, Thermo Scientific; Smith et al. 1985). The remainder was stored at –80°C until assayed for EROD activity (Kennedy et al. 1993) with Gen5 data analysis software and a Synergy microplate reader (BioTek Instruments, Inc.). EROD activity was calculated as pmol resorufin $\cdot$ min^{-1} $\cdot$ mg^{-1} of liver protein. In 2007, fish held in the laboratory for one to two days had lower EROD activity than fish processed on the day of collection ($F_{2,176} = 7.7$, p = 0.0006). Hence only fish processed on day 0 were used in further analyses. In this study, SI males were

smaller than males of other sites, but females did not differ in TL (site x sex $F_{3,128}$ = 4.3, p = 0.007).

4.3.5 Gonad histology

The gonads of a subset of 6-31 reproductive males (PM and SM tactics) and 7-18 females per site were collected from the four sites in 2007. Gonad samples were preserved in 10% neutral buffered formalin for 24 hours immediately after dissection from freshly euthanized fish, then placed in 70% ethanol for long-term storage (approximately nine months elapsed before processing). A randomly selected gonad half (either the left or right ovary in females, or the left or right lobes of the testes and accessory glands in males) was embedded in paraffin (Fisher Scientific) and sectioned at either 4 μm (males) or 6 μm (females) using Leica RM2155 and RM2235 microtomes. Embedded ovary samples were first soaked in glycerin and warm water, followed by immersion in ice, before sectioning to prevent loss of sample. Sections from the middle of the gonad sample were stained with hematoxylin and eosin (Leica Autostainer XL). Longitudinal sections from three areas of the testis were obtained per male, spaced at least 30 μm apart. Sections of all three areas per male were scanned using a light microscope at 100 X power to identify oocytes, indicating the presence of intersex. One section of each female ovary was digitally photographed at low magnification (2.5 X or 5 X) on a Leica DMR microscope with a Qimaging Qcam (Fast 1394) and the images stitched together to create a composite image of the entire section (Northern Eclipse 7.0, Empix Imaging,

Inc.). The single stitched image was analyzed using ImageJ to determine a) the number of nucleated oocytes of one of three main categories (primary oocytes, early vitellogenic ova, late vitellogenic ova), and b) the area of at least 10 nucleated cells in each category (mm^2).

4.3.6 Statistical analyses

Data were log- or arcsine-square-root-transformed where necessary to meet parametric test requirements. Data from GC were excluded from analyses when too few fish were collected. Morphological data were examined as one-, two- or three-factor ANOVAs or ANCOVAs with a combination of sex, reproductive status, site and/or year as independent variables, and where applicable, log TL or log somatic mass (total mass – mass of organ of interest) as the covariate. Non-significant factor-covariate interaction terms were removed from ANCOVA models. Significant differences between groups were determined with *post-hoc* Tukey Honestly Significant Difference (HSD) tests. In cases where transformation was not successful in satisfying parametric test requirements, non-parametric Kruskal Wallis (KW) tests were used, and differences among groups identified using non-parametric post-hoc tests (Zar 1999). Statistics for which non-parametric tests were performed were reported as medians and ranges. Non-parametric correlations were examined with the Spearman rho (r_s) test. Ratio data (for sexes, number of reproductive to non-reproductive individuals, and for parental and sneaker male tactics) were analyzed for departure from a 1:1 ratio with chi square (χ^2) tests, and were

examined for differences among sites and/or years with heterogeneity chi square tests. Site or year differences among sets of ratios were identified by sequentially removing the largest or smallest site or year value in a set, and then recalculating the heterogeneity chi square on the remaining ratios until no pairwise differences are achieved (a technique recommended by Zar, 1999).

4.3.7 Ethical note

All animal handling methods met criteria for McMaster University Animal Research Ethics Board (AREB) (AUPs # 03-09-54 and 06-10-61) according to standards of the Canadian Council on Animal Care.

4.4 RESULTS

4.4.1 Evidence of contaminant burdens (Ni, Cu, Cd)

The livers of fish from one of the contaminated sites (P27) contained higher levels of Cd than those found in fish from the cleaner site (LS, $F_{\text{liver } 2,21} = 5.2$, $p = 0.02$; **Figure 4.2a**); gill and gut concentrations of Cd did not vary among sites ($F_{\text{gill } 2,21} = 2.2$, $p = 0.13$, $F_{\text{gut } 2,21} = 1.1$, $p = 0.4$). The livers but not the gills or the gut of fish from the other contaminated site (SI) had higher levels of Cu compared to fish from the clean site ($F_{\text{liver } 2,21} = 4.2$, $p = 0.03$, $F_{\text{gill } 2,21} = 3.6$, $p = 0.04$, $F_{\text{gut } 2,21} = 1.4$, $p = 0.3$; **Figure 4.2b**). Ni concentrations in fish tissues did not vary across sites ($F_{\text{liver } 2,21} = 2.8$, $p = 0.08$, $F_{\text{gill } 2,21} = 0.2$, $p = 0.8$, $F_{\text{gut } 2,21} = 2.7$, $p = 0.09$; **Figure 4.2c**). The gut:gill ratio of Ni

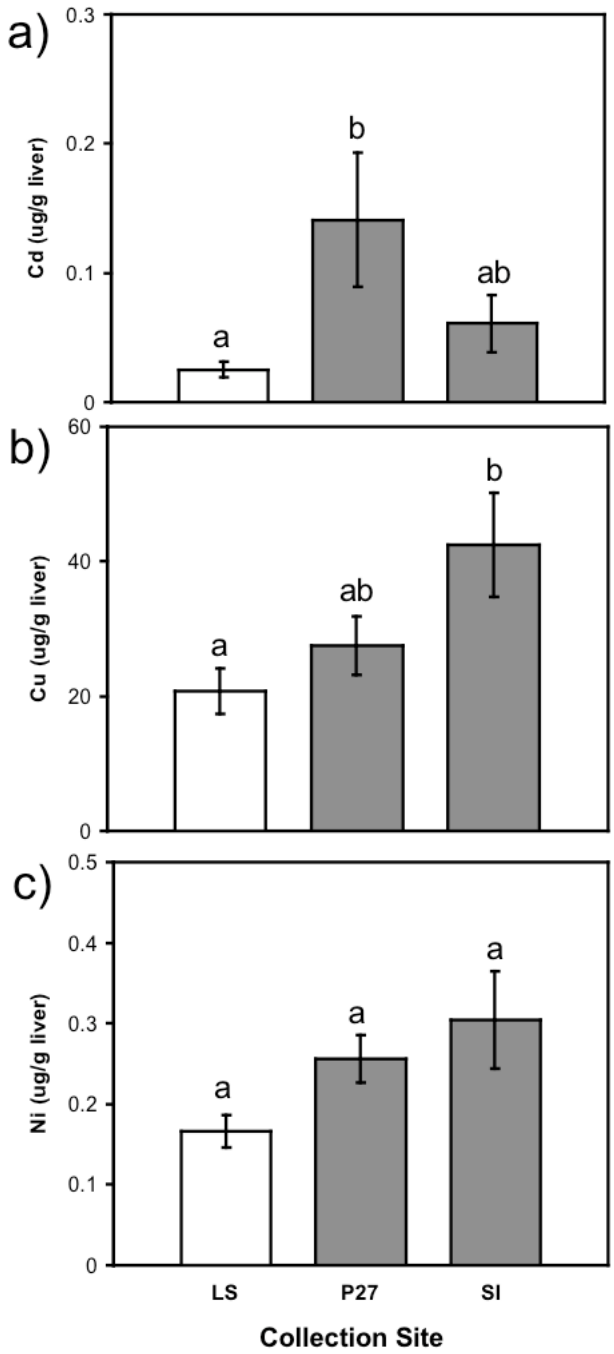

Figure 4.2. Body burdens of contaminants in round gobies from clean and contaminated sites. White bars = clean site. Gray bars = contaminated site. **a)** Mean ± SE liver Cd burdens for male round gobies (n=8). **b)** Mean ± SE liver Cu burdens for male round gobies (n=8). **c)** Mean ± SE liver Ni burdens for male round gobies (n=8). Letters represent differences among sites (Tukey HSD, $p < 0.05$).

and Cd were similar across sites ($p > 0.1$; pooled mean ± SE of 1.17 ± 0.12 for Ni and 12.75 ± 2.19 for Cd); for Cu, the gut:gill ratio was higher for P27 males (0.78 ± 0.14) than for SI males (0.37 ± 0.07, $F_{2,21} = 3.6$, $p = 0.043$), but LS males did not differ from either (0.58 ± 0.11). Confidence intervals (95%) generated for the gut:gill ratios indicated that Cd was acquired primarily through the gut in all sites, Cu was acquired primarily through the gills, but only in fish from two of three sites (LS and SI), and neither route of entry predominated for Ni acquisition in fish tissues.

4.4.2 Subpopulation variation related to site contamination

Catch rates at the four sites were not related to the degree of site contamination and varied across years for which data were available (**Table 4.1**). Fish from the clean sites, GC and LS, were longer and heavier than fish from the contaminated sites, P27 or SI, and male round gobies were both longer and heavier than same-site females (effect of sex*site $F_{3,2047} = 8.2$ and $F_{3,2174} = 8.2$, p's < 0.0001; **Figure 4.3a** and **Table 4.2**). The digital photographs revealed that the fused pelvic fins of males and females from one of the contaminated sites, SI, were typically damaged, covering a smaller area of the ventral surface than in fish from any other site (effect of site $F_{3,190} = 3.8$, $p = 0.01$, **Figure 4.3b**). Round goby investment in liver tissue did not consistently vary with site, only with reproductive status (effect of status $F_{4,2101} = 48.7$, $p < 0.0001$; NRFs > NRMs > PMs > SMs and RFs; **Tables 4.2** and **4.3**). There was also no clear effect of site on reproductive investment (**Tables 4.2** and **4.3**). Only SM fish showed a decrease in gonad size in contaminated areas, in

Table 4.1. Median and range of catch rates per unit effort (round gobies per minnow trap) across years at clean (GC, LS) and contaminated (P27, SI) sites. Letters indicate significant differences among sites within a year (KW tests, $p < 0.05$; post-hoc non-parametric tests, $p < 0.05$).

SITE	2007	2008	Total
GC	0 (0 – 7)[a] n = 62	0 (0 – 6)[a] n =16	0 (0 – 7) [a] n = 78
LS	4 (0 – 16)[b] n = 126	4 (0 – 35)[b] n = 78	4 (0 – 35) [b] n = 204
P27	4 (0 – 19)[b] n = 77	3 (0 – 28)[c] n = 72	3 (0 – 28) [c] n = 149
SI	2 (0 – 25)[c] n = 81	5 (0 – 19)[b] n = 47	3 (0 – 25) [c] n = 128

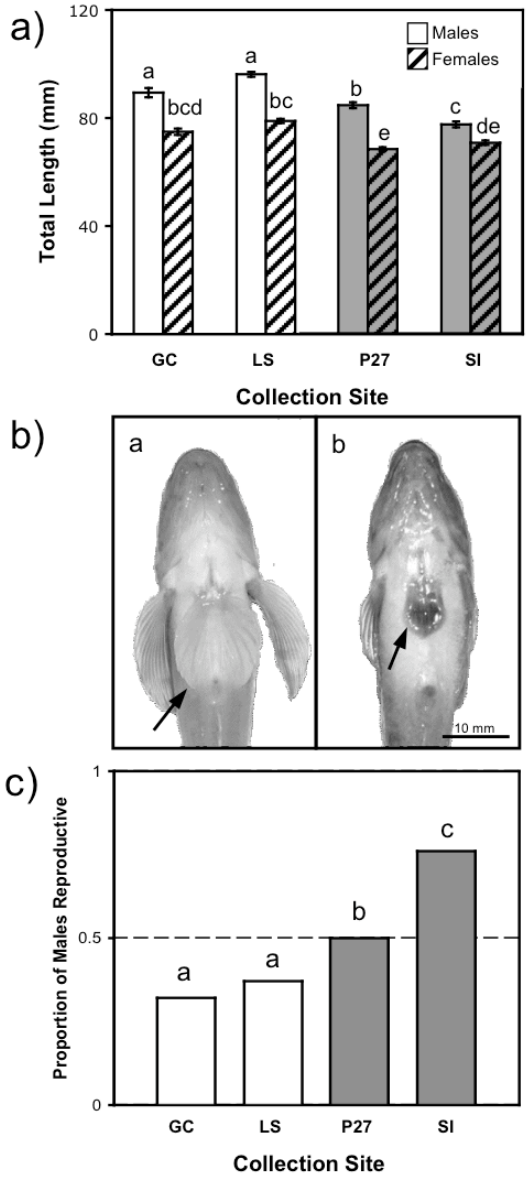

Figure 4.3. Characteristics of round goby catches at different sampling sites. White bars = clean site. Gray bars = contaminated site. Open bars = male data and hatched bars = female data where indicated. **a)** Differences in mean ± SE total length (cm) for male and female round gobies across sites. Letters represent significant differences among sites and sexes (Tukey HSD, $p < 0.05$). **b)** Photographs of the ventral anterior aspect of a round goby with a normal pelvic disc (panel a) and an eroded disc characteristic of many fish from contaminated areas (panel b). Arrows indicate the disc, and the scale of the photographs is given in mm. **c)** The proportion of all adult males found to be in reproductive condition (parental or sneaker male) across sites, pooled across years. Letters indicate significant differences between sites (Chi square, $p < 0.05$).

Table 4.2. Morphological measures of round gobies at clean (GC, LS) and contaminated (P27, SI) sites, by sex and reproductive status. Reported values are means (SE; TL and mass) or medians and ranges (indices). Letters indicate significant differences among group means (log-transformed data, Tukey HSD, $p < 0.05$) or medians (non-parametric post-hoc comparisons, $p < 0.05$). TL = total length. K = mass : TL^3 x 100. HSI = hepatosomatic index. GSI = gonadosomatic index. AGSI = accessory gland somatic index (male only). M = all males. F = all females. PM = parental males, SM = sneaker males, NRM = non-reproductive males, RF = reproductive females, NRF = non-reproductive females.

	Sites	n	TL (mm)	Mass (g)	Fulton's K	HSI (%)	GSI (%)	AGSI (%)
M	GC	126	89.4 (1.7)[a]	10.3 (0.5)[a]	11.5 (0.3)[a]	2.4 (0-10.6)[a]	0.2 (0-10.1)[a]	0.1 (0-1.5)[a]
	LS	613	96.2 (1.1)[b]	14.4 (0.4)[b]	13.5 (0.2)[b]	2.5 (0.1-8.4)[a]	0.1 (0 -14.3)[a]	0.0 (0-6.4)
	P27	342	84.7 (1.1)[a]	10.0 (0.4)[a]	11.5 (0.2)[a]	2.2 (0-7.0)[b]	0.2 (0-8.0)[b]	[ab]
	SI	278	77.7 (1.1)[c]	7.6 (0.4)[c]	10.4 (0.2)[c]	2.0 (0-5.2)[c]	0.3 (0-7.8)[c]	0.0 (0-6.9)[b]
								0.2 (0-2.0)[c]
F	GC	73	74.9 (1.2)[a]	5.8 (0.3)[ab]	9.5 (0.2)[a]	2.2 (0.1-10.9)[a]	4.5 (0.3-19.7)[a]	
	LS	288	78.9 (0.8)[a]	7.0 (0.2)[a]	10.8 (0.1)[b]	2.5 (0-20.7)[a]	1.9 (0.1-23.2)[b]	
	P27	218	68.5 (0.7)[b]	4.7 (0.2)[c]	9.1 (0.1)[a]	2.1 (0-6.1)[b]	3.9 (0-23.3)[a]	
	SI	246	71.0 (0.8)[b]	5.2 (0.2)[bc]	9.4 (0.1)[a]	1.8 (0-6.2)[b]	4.3 (0-28.8)[a]	
PM	GC	24	96.4 (2.5)[ab]	12.8 (1.0)	13.3 (0.3)[abc]	2.4 (0.2-6.0)	1.1 (0.2-2.1)	0.6 (0-1.5)
	LS	149	111.9 (1.7)	[ab]	15.9 (0.4)[a]	2.7 (0.5-6.9)	1.2 (0-7.5)	0.4 (0-2.3)
	P27	119	[c]	22.9 (1.0)[c]	14.0 (0.3)[b]	2.6 (0.3-5.2)	1.2 (0-3.8)	0.5 (0-2.9)
	SI	83	99.4 (1.6)[a]	15.4 (0.9)[a]	12.8 (0.4)[c]	2.4 (0-5.1)	1.3 (0.1-4.7)	0.4 (0-1.2)
			92.3 (2.0)[b]	12.4 (1.0)[b]				
SM	GC	15	77.6 (4.6)[a]	5.5 (1.0)	9.4 (0.7)	1.8 (0-10.6)	5.2 (0-10.1)[a]	0.4 (0-1.3)[a]
	LS	88	68.6 (1.4)[ab]	4.7 (0.3)	9.2 (0.2)	1.1 (0.2-6.1)	4.7 (0.1-14.4)	0.3 (0-6.4)[a]
	P27	63	64.5 (1.8)[b]	4.3 (0.5)	8.8 (0.3)	1.2 (0-6.6)	[a]	0.3 (0-6.9)[b]
	SI	134	67.2 (1.0)[ab]	4.4 (0.2)	8.8 (0.2)	1.4 (0-5.2)	4.1 (0.1-13.6)[b]	0.2 (0-2.0)[c]

	Sites	n	TL (mm)	Mass (g)	Fulton's K	HSI (%)	GSI (%)	AGSI (%)
							3.3 (0.1-7.8) [c]	
NRM	GC	87	89.6 (2.0) [a]	10.4 (0.6) [a]	11.4 (0.3)[a]	2.6 (0.3-8.0) [a]	0.1 (0-0.7) [a]	0.0 (0-0.3) [a]
	LS	376	96.4 (0.9) [b]	13.7 (0.4) [b]	13.5 (0.2)[b]	2.7 (0.1-8.4) [a]	0.1 (0-0.7) [a]	0.0 (0-0.8) [b]
	P27	160	81.8 (1.3) [c]	8.2 (0.4) [c]	10.7 (0.2)[a]	2.4 (0.1-7.0) [b]	0.1 (0-0.9) [a]	0.0 (0-0.7) [b]
	SI	61	81.0 (2.0) [c]	8.0 (0.6) [ac]	10.8 (0.4)[a]	2.7 (0.2-5) [a]	0.1 (0-1.0) [b]	0.0 (0-0.3) [a]
RF	GC	23	73.7 (2.3) [ab]	5.6 (0.5) [ab]	9.7 (0.4)[ab]	1.3 (0.1-10.9)	12.4 (8.5-19.7) [a]	
	LS	54	80.4 (2.0) [a]	7.3 (0.5) [a]	11.3 (0.3)[a]	1.6 (0-5.0)	12.8 (8.1-23.2) [a]	
	P27	60	68.9 (1.3) [b]	5.0 (0.3) [b]	9.7 (0.3)[b]	1.4 (0-5.2)	17.3 (8.1-23.3) [b]	
	SI	74	70.6 (1.4) [b]	5.2 (0.4) [b]	9.4 (0.2)[b]	0.9 (0-5.0)	12.6 (8.1-28.8) [a]	
NRF	GC	50	75.4 (1.4) [ab]	5.8 (0.3) [ab]	9.4 (0.2)[a]	2.7 (0.5-5.8) [a]	1.9 (0.3-7.8)[ab]	
	LS	234	78.6 (0.8) [a]	7.0 (0.2) [a]	10.7 (0.2)[b]	2.6 (0-20.8) [a]	1.2 (0.1-7.9)[a]	
	P27	158	68.3 (0.9) [c]	4.5 (0.2) [c]	8.9 (0.6)[a]	2.3 (0-6.1) [b]	2.1 (0-7.9)[ab]	
	SI	172	71.1 (0.9) [bc]	5.3 (0.2) [bc]	9.4 (0.2)[a]	2.2 (0-6.2) [b]	2.5 (0-8.0)[b]	

Table 4.3. Results of ANCOVA models (effect of site) on liver and gonad investment in round gobies of different reproductive states. Models were run on log-transformed data with log somatic mass as covariate; *post-hoc* differences were identified with Tukey HSD tests (letters). PM = parental males, SM = sneaker males, NRM = non-reproductive males, RF = reproductive females, NRF = non-reproductive females. Clean sites: GC, LS. Contaminated sites: P27, SI.

	PM	**SM**	**NRM**	**RF**	**NRF**
Liver	$F_{3,369} = 1.4$ $p = 0.25$	$F_{3,293} = 1.5$ $p = 0.21$	$F_{3,678} = 0.63$ $p = 0.60$	$F_{3,197} = 0.74$ $p = 0.53$	$F_{3,608} = 0.50$ $p = 0.68$
Gonad	$F_{3,369} = 1.2$ $p = 0.32$	$F_{3,294} = 9.0$ $p < 0.0001$[a]	$F_{3,673} = 9.6$ $p < 0.0001$[b]	$F_{3,206} = 4.6$ $p = 0.004$[c]	$F_{3,605} = 1.7$ $p = 0.17$
Accessory Gland	$F_{3,369} = 1.8$ $p = 0.14$	$F_{3,294} = 3.7$ $p = 0.01$[d]	$F_{3,673} = 5.5$ $p = 0.0009$[e]		

[a]LS > P27,SI. [b]SI > P27,LS; GC > LS. [c]P27 > SI. [d]LS > SI. [e]All > LS.

both ANCOVA models of testes investment and GSI values; SMs from both contaminated sites of SI and P27 had smaller testes than SMs from LS, and accessory glands of SMs from SI were also smaller than those of LS. Three of the sites showed a male-biased catch (χ^2 tests, all $p < 0.001$), while catches from one of the contaminated sites, SI were not significantly different from a 1:1 male:female sex ratio (across years $\chi^2 = 1.96$, $p = 0.16$). This site, SI, also had the highest ratio of reproductive to non-reproductive (R:NR) males, followed by the other contaminated site, P27, and then the two cleaner sites GC and LS both had the lowest ratio of R:NR males across years (pairwise χ^2 tests, $p < 0.0001$; **Figure 4.3c**). Ratios of parental to sneaker males (PM:SM) varied among sites. GC exhibited no bias in the ratio ($\chi^2 = 2.1$, $p = 0.15$) while LS and P27 both exhibited strongly PM-biased ratios ($\chi^2 = 15.9$ and 17.5, $p < 0.0001$). SI had a strikingly strong SM-biased ratio in 2006-2007 ($\chi^2 = 12.8$ and 11.2, $p < 0.001$) but an unbiased ratio in 2008 ($\chi^2 = 0.01$, $p = 0.91$).

4.4.3 EROD activity

The effect of fish collection site on EROD activity varied marginally with reproductive status ($F_{15,120} = 8.3$, $p < 0.0001$; site x status $F_{9,120} = 1.8$, $p = 0.07$; **Table 4.4**). Within each status, site effects were only apparent for NRF fish (Tukey HSD, $p < 0.05$) where NRFs from SI had higher activity than NRFs from any other site. Within each site, NRM fish consistently had the highest EROD activity, and RF fish the lowest (NRMs > RFs, Tukey HSD, $p < 0.05$). PM and SM males had

Table 4.4. Mean ± SE EROD activity (pmol · min^{-1} · mg^{-1}) for round gobies of different sexes and reproductive states. Fish from four Hamilton Harbour sites were collected in 2007 and 2008. RM = reproductive male (parental and sneaker males), RF = reproductive female, NRM = non-reproductive male, NRF = non-reproductive female). Letters indicate significant differences among groups (Tukey HSD, $p < 0.05$).

Site	RM	RF	NRM	NRF
GC	664.7 (354.7) n = 3	50.5 (12.8) n = 2	1821.7 (390.9) n = 6	176.7 (54.9) [a] n = 4
LS	166.8 (42.9) n = 10	79.8 (25.6) n = 3	3053.3 (624.8) n = 8	742.1 (409.0) [a] n=17
P27	404.3 (170.2) n = 10	139.9 (61.1) n = 6	2223.1 (788.9) n = 13	777.0 (307.1) [a] n = 11
SI	611.1 (244.9) n = 13	384.2 (97.2) n = 6	4933.8 (1438.8) n = 9	2605.2 (738.7) [b] n = 15

equivalent EROD activities and were pooled into one category, RM (reproductive males; $t_{33} = 0.2$, $p = 0.8$). When considering reproductive status alone, NRM fish had the greatest EROD activity, and NRF fish had higher activity than RFs ($F_{3,131} = 20.9$, $p < 0.0001$).

4.4.4 Reproductive impacts & indicators of endocrine disruption

Male and female round gobies had different width:length ratios of the urogenital papilla, with males having longer, narrower papillae and thus smaller ratios than females ($F_{1,183} = 89.7$, $p < 0.0001$; **Figure 4.4a**). The width:length ratio did not vary with reproductive status or site in females ($F_{4,71} = 1.3$, $p = 0.27$). Among reproductive males (PM and SM fish together), LS fish had smaller, more male-typical length:width papilla ratios than fish from either contaminated site (P27 or SI), but all reproductive males regardless of site still had smaller ratios than females ($F_{3,148} = 45.9$, $p < 0.0001$, **Figure 4.4a**). There was no correlation of GSI with the degree of male papilla feminization (papilla ratio) in either clean ($r_s = 0.3$, $p = 0.9$) or contaminated-site ($r_s = 0.16$, $p = 0.23$) reproductive males.

No intersex was detected in males collected from the two clean sites GC (n = 6), LS (n = 22), or from fish from one of the contaminated sites, P27 (n = 26). Four of 31 males (12.9% of males) from SI showed the presence of oocytes. Of these, two still showed production of spermatozoa; two did not show development of seminiferous lobules. One male possessed a few developed vitellogenic ova both

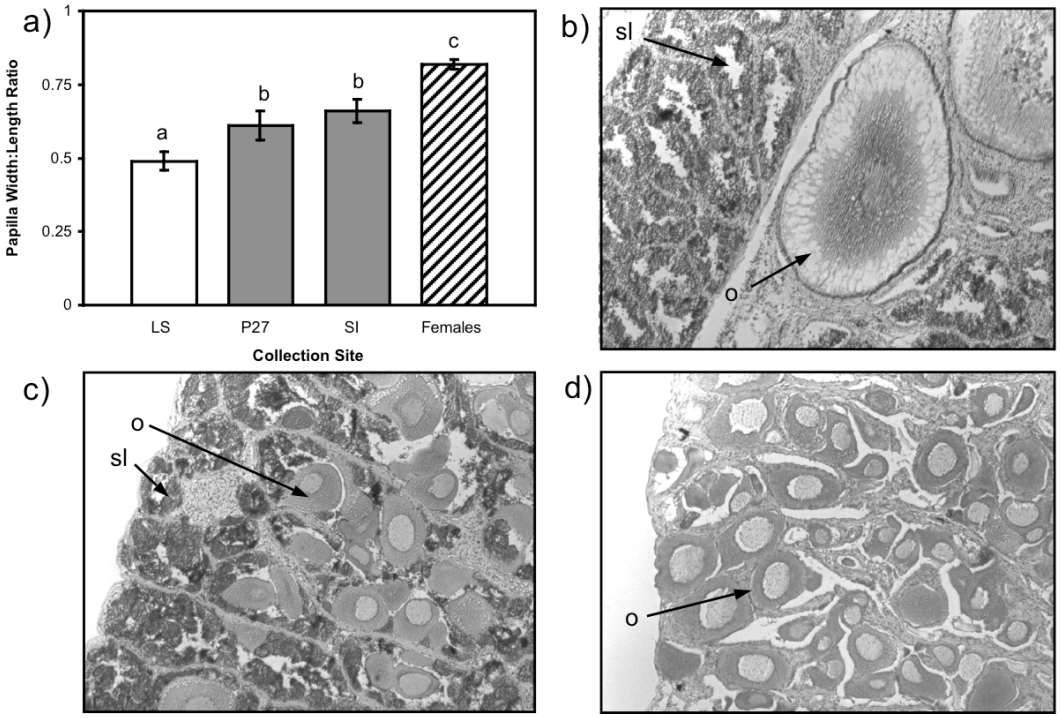

Figure 4.4. Intersex in male round gobies. White bars = clean site. Gray bars = contaminated site. Open bars = male data and hatched bars = female data where indicated. Microphotographs were taken at 100x magnification. **a)** Mean ± SE ratios of the urogenital papilla width:length in reproducing males from three sites compared to a pooled sample of females from all sites. Letters represent significant differences among sites (Tukey HSD, $p < 0.05$). Papillae of males from contaminated sites were more female-like in their ratios than males of a clean site. **b)** Male round goby testis from SI showing vitellogenic oocytes (o) and seminiferous lobules (sl). **c)** Male round goby testis from SI showing the presence of both primary oocytes (o) and seminiferous lobules (sl). **d)** Male round goby testis from SI showing presence of primary oocytes (o) but no developed seminiferous lobules.

distally and medially placed within the testis (**Figure 4.4b**); three males had primary oocytes scattered throughout the testis sections, with or without spermatogenesis evident (**Figures 4.4c** and **4.4d**).

Female gamete size did not differ across sites for any of the three stages of oocyte development: primary oocyte (effect of site $F_{3,49}$ = 2.4, p = 0.08); early vitellogenic ova (effect of site $F_{3,48}$ = 0.6, p = 0.62); or late vitellogenic ova (effect of site $F_{3,34}$ = 0.9, p = 0.45), nor did gonad investment vary across sites in these fish (effect of site $F_{3,47}$ = 2.1, p = 0.11). Females from one of the contaminated sites (SI) had more primary oocytes than females from one of the clean sites, LS (KW, H = 8.1, p = 0.02) but no differences were observed across females from different sites in the numbers of early vitellogenic (KW, H = 0.6, p = 0.73) or late vitellogenic ova (KW, H = 4.4, p = 0.11).

4.5 DISCUSSION

4.5.1 Subpopulation variation related to site contamination

The smaller mean fish sizes observed in contaminated areas relative to clean areas could be attributed to either a) an inhibition of growth and/or b) higher mortality and thus fewer older fish in contaminated sites (Newman and Clements 2008). A previous study elsewhere in the Great Lakes has reported a maximum age of two years for males and three years for females (MacInnis and Corkum 2000b). Establishing the ages of individuals and their growth rates from otolith annuli will

be a crucial next step to understanding the shift in body size in gobies of Hamilton Harbour's contaminated regions.

A male-biased catch is commonly reported for round gobies in nearshore areas (Corkum et al. 2004; Shemonaev and Kirilenko 2009; Young et al. 2010; but see Kovtun 1980; Bergstrom et al. 2008). This is despite the fact that males are thought to experience high mortality after each breeding season (Charlebois et al. 1997; MacInnis and Corkum 2000b; Pinchuk et al. 2003). Interestingly, a female-biased sex ratio in round gobies has been reported in the Detroit River, another Area of Concern (MacInnis and Corkum 2000b). The absence of male bias in the nearshore of most highly contaminated region in Hamilton Harbour (Sherman Inlet) could be due to higher mortality rates for male versus female fish. Endocrine disruption of larval or juvenile sexual development could also yield a higher than normal percentage of female hatched fry by feminization of males in a critical window (van Aerle et al. 2002). However, round goby larvae are expected to disperse over very great areas (Hayden and Miner 2009), so it is possible but unlikely that the lack of sex bias is due to an increased production of female offspring in Sherman Inlet, since larvae from all over the Harbour may settle in the area. In addition, little is known about the range of values expected for parental to sneaker ratios in round goby populations. The increased prevalence of sneaker males relative to parental males in the highly polluted site of Sherman Inlet could be due to differential migration or mortality of the two male tactics (perhaps, an increased attraction of sneaker males to areas with many females), or an increased

tendency of reproductively maturing males in contaminated areas to take on the sneaker tactic. A potential role for endocrine disruption during this sexual maturation phase and tactic adoption phase would be an interesting avenue of future research.

Reproductive investment in round gobies was not compromised in habitats with contamination. Lower gonadal investment has been reported in many species collected from contaminated areas, but the opposite can also occur (Schlenk et al. 2008). For example, elevated reproductive investment has been observed in populations of mummichog (*Fundulus heteroclitus*) that demonstrate tolerance to habitat contamination (Weis 2002). The lack of a change in gonad size with contaminant exposure, and a higher proportion of reproductive individuals in contaminated areas may mean that round gobies are relatively unimpaired by exposure to contaminants in terms of reproductive investment. Alternatively, the long breeding season and repetitive spawning habits of the round goby may mean that individuals are not at the same point of the reproductive cycle when sampled, and this asynchrony, or antagonistic effects by different contaminants (Schlenk et al. 2008), masks small but otherwise real changes in gonad investment and reproductive capacity.

4.5.2 Body burdens of contaminants

Metal distributions in round goby tissues varied with fish collection site and related to sediment metal contamination data (Zeman 2009). In these reports,

cadmium levels were higher in sediments sampled near Pier 27 (17.1 ug/g) than in Sherman Inlet (4.3 ug/g), but sites near LaSalle were not measured. Copper levels were higher in sediments near Sherman Inlet (in the range of 110—197 ug/g) than near Pier 27 (102 ug/g) but were similar to LaSalle (113 ug/g). Nickel sediment deposits were distributed across the Harbour in the range of 16-75 ug/g, above the lowest effect level but below the severe effect level according to provincial sediment quality guidelines; interestingly, nickel concentrations at LaSalle (53 ug/g) exceeded those at Pier 27 (41 ug/g) and Sherman Inlet (32 ug/g; Zeman 2009). In round gobies, cadmium levels were highest in livers of Pier 27 males, and copper levels were highest in livers of Sherman Inlet males. However, liver burdens of nickel did not vary significantly among sites. The gut:gill ratios indicate a predominantly dietary route of entry for cadmium throughout the Harbour, and predominately waterborne route of entry for copper. Cadmium is one of the "A list" contaminants for Hamilton Harbour (i.e., a contaminant of primary concern for remediation) and these results point to a potential role for round gobies in contaminated areas mobilizing cadmium to piscivores in the region, including sportfishes that may be consumed by humans.

4.5.3 EROD activity as indicator of PAH exposure

Increases in EROD activity are expected with exposure to contaminants that bind the aryl hydrocarbon receptors (AhR) in the liver, which commonly include PAHs, coplanar PCBs and other planar halogenated aromatic hydrocarbons

(PHAHs). The magnitude of EROD activity for round gobies in Hamilton Harbour is greater than that seen for brown bullheads from this same region (Arcand-Hoy and Metcalfe 1999) or centrarchids from contaminated streams in the southern USA (Brammell et al. 2004). This may reflect not only species-specific differences but also the limited mobility of the round goby leading to a chronic high level of exposure to PAHs and PHAHs. PAHs are likely to be rapidly metabolized and cleared by the body (Arcand-Hoy and Metcalfe 1999) and so measurements of PAHs or their metabolites may not adequately reflect PAH exposure. Although we did not measure the presence of PAH metabolites in our samples, the loss of EROD activity after even one night in clean laboratory water would point to a very quick clearance of the inducing substance, again supporting PAH exposure as the likely causal agent of patterns of EROD induction in our study.

EROD patterns are known to be modulated by 17β-estradiol or E_2 (Navas and Segner 2001; Elskus 2004), and so it is not surprising that male round gobies had higher EROD activity levels than did females, nor that non-reproductive individuals of both sexes had higher activities than reproductive individuals. Further studies exploring differences in underlying endocrine patterns are likely to parallel EROD patterns.

4.5.4 Endocrine disruption?

Low rates of intersex, present only at one contaminated site (SI), do indicate that some degree of early exposure to endocrine disrupting compounds (EDCs)

may be occurring (van Aerle et al. 2002). Naturally occurring rates of spontaneous, non-contaminant-related intersex are not known for this species. The very rare presence of vitellogenic follicles also indicates that male round gobies in contaminated sites are likely to be producing vitellogenin, another common biomarker of endocrine disruption that was not examined in this study. The intersex rates described in this study appear to be much lower than those reported for other species in Hamilton Harbour, such as white perch (*Morone americana*) where levels of intersex reach over 50% (Kavanaugh et al. 2004). This may be because larval round gobies hatch in different locations from the larvae of other species (many of which spawn in the nearby wetland of Cootes Paradise Marsh), and thus are exposed to different EDCs, or because round gobies have a different thresholds for EDC-determined intersex development.

More female-like urogenital papillae occurred in reproducing males of both contaminated sites, similar to what has been found in sand gobies (*Pomatoschistus minutus*) exposed to EDCs (Kirby et al. 2003). The papilla width:length ratio appears to be a more sensitive indicator of round goby endocrine disruption than histologic scoring of oocytes in testes. The simplicity of this measure means that papilla shape would be an efficient metric to track in other studies where endocrine disruption of round gobies is of interest or suspected.

4.5.5 Summary

Round gobies appear to be site-faithful enough to reliably differ in their indicators of contaminant exposure, reflecting the conditions of their site of capture. For this reason, round gobies may be a potentially useful bioindicator species throughout their global range, in the Great Lakes and beyond. Understanding round goby movements within and between clean and contaminated areas will be critical to understand the gradients formed by clean and contaminated fish in terms of home range, and thus the gradient of their possible impacts.

The contaminant body burdens in particular have worrisome implications for the remediation of Hamilton Harbour. As more piscivores like double-crested cormorants switch their diets to include an increasing proportion of round gobies (Somers et al. 2003), the risk of accumulating contaminants increases. This will impact the ability to deregulate sportfish consumption guidelines for species that are known to consume round gobies (e.g., largemouth bass, yellow perch, walleye). As PCBs are a primary contaminant of concern in Hamilton Harbour (Hamilton Harbour RAP 2008), quantifying both PCB body burdens and biomagnification of PCBs in round gobies and their predators in this region is an important area of future research. PAHs are readily metabolized and excreted (Arcand-Hoy and Metcalfe 1999) and are not expected to bioaccumulate in organisms, including round goby predators. Nonetheless, predators consuming round gobies with high levels of PAHs will experience pulses of exposure to this carcinogenic class of

contaminants (Balch et al. 1995), and if predators are drawn to areas with PAHs as a result of round goby prey, the predators themselves may become exposed to coal tar deposits and suffer the effects of these direct exposures in addition to consequences of consuming contaminated prey.

Contaminant-modulated shifts in round goby populations (e.g. altered sex ratios, size and age structure, dispersal and reproductive rates) could also alter round goby vulnerability to specific predators, as well as their dietary composition. Foraging preferences of round gobies, such as rates of consumption of dreissenid mussels, chironomids, ostracods and other fishes vary with round goby size (Jude et al. 1995; Walsh et al. 2007). Body size is also likely to affect which piscivorous species prefer them as prey, or which size classes of predators may eat round gobies (e.g. Dietrich et al. 2006). Contaminant exposure can also make fish more vulnerable behaviourally to predation (reviewed in Sloman and Wilson 2006). Any or all of these interacting factors could modify the structure of foodwebs, and thus the flow of energy and contaminants throughout such foodwebs, on a local scale. This potential modification warrants future studies.

Round gobies are known to be tolerant of pollution (Pinchuk et al. 2003). Have the heavily contaminated seas of Europe pre-armed this resilient species with adaptations to cope with such environments? Isolated populations living in contaminated areas can and do adapt to contaminant levels that are fatal to conspecifics from other regions (Nacci et al. 2002). One characteristic taken to indicate such adaptation is the loss or reduction of a cytochrome P450 1A (CYP1A;

EROD) response, since CYP1A or AhR-mediated interactions are often key to the toxicity of many compounds (Nacci et al. 1999; Brammell et al. 2004; Van Veld and Nacci 2008). Our data indicate that round gobies in Hamilton Harbour can mount a CYP1A response, but it is not possible to determine, at this stage, whether adaption to Hamilton's PAH-laden sediments has occurred.

The presence of abundant round goby populations in highly contaminated areas is likely to attract predators, or at least support higher numbers of predators than could exist in those regions otherwise. This may facilitate, or possibly accelerate, contaminant transfer in a way that operates in tandem with the role round gobies have in contaminant transfer because of their diet of dreissenid mussels. Round gobies are found throughout the Great Lakes and more inland waters (Poos et al. 2010), so similar situations may arise in other IJC Areas of Concern where contaminant bioaccumulation is a remedial priority. For these reasons, the possibility that pollution-tolerant round gobies can draw or sustain more predators in contaminated areas is a question, with many implications for conservation and remediation, which is worthy of future exploration.

4.6 CONCLUSIONS

Round gobies from different areas of Hamilton Harbour, a Canadian Area of Concern, exhibited signs of contaminant exposure that are similar to known sediment distributions of PAHs, metals and PCBs in the embayment. Fish in the heavily PAH- and metal-contaminated area of Sherman Inlet (SI), near Randle Reef,

showed elevated copper burdens and higher EROD activity, consistent with PAH exposure, while fish from Pier 27 (P27), near the heavily PCB- and metal-contaminated area of Windermere Arm, show elevated cadmium body burdens. Fish in both contaminated sites were smaller, had higher metal body burdens and signs of endocrine disruption not observed in fish from cleaner areas of the Harbour. The multiple lines of evidence indicate that there is likely a causal link between contaminant exposure, and shifts in the morphological and physiological characteristics of the fish inhabiting these locations. The impacts that such population-level changes will have on round goby vulnerability to predation, and thus the mobilization of persistent contaminants from round gobies to piscivorous predators, are important future directions for population-level studies in this system.

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Chapter 5

BEHAVIOUR AS BIOMARKER? ROUND GOBY (*NEOGOBIUS MELANOSTOMUS*) FROM HIGHLY CONTAMINATED AREAS SHOW DECREASED MOVEMENT IN THE LABORATORY BUT NOT IN THE FIELD

5.1 ABSTRACT

Changes in animal movement (frequency or speed of locomotion) following exposure to a toxicant are frequently considered a biomarker of contaminant exposure and is one of the most widely reported behavioral results in toxicological literature. However, the ecological consequences of such behavioural changes, such as effects on toxicant transfer in foodwebs, are far less well understood, complicated in part by the short-term nature of laboratory experiments and the lack of complementary field studies where the nature of toxicant exposure is more complex. Here we examine whether naturally-exposed individuals of the round goby, a benthic, site-loyal fish, move in a manner similar to conspecifics from less contaminated habitats. In the laboratory, round goby from a relatively cleaner site showed greater activity and exploration than goby from two highly contaminated sites. Male fish were more active than females but the site effects were similar in both sexes. In contrast to laboratory findings, a field mark-recapture study of 881 round goby showed that fish from the cleaner site did not move greater distances or exhibit shorter residence times within the site than round goby from highly

contaminated sites. Our results indicate that while behavioral changes in the laboratory may be one of several useful diagnostics of toxicant exposure of wild-exposed animals, they do not necessarily translate readily into measurable differences in a natural context. Thus, the potential fitness consequences of toxicant exposure based on behavioral changes need to be assessed carefully.

5.2 INTRODUCTION

Locomotor movement is a fundamental component of many behaviors and the qualities of locomotion (frequency of movements, velocity, and diel or seasonal patterns of activity) can affect success in foraging, finding mates and avoiding predators (Dingle and Holyoak 2001). Higher levels of activity are also often correlated with increased exploration, aggression and boldness (Sih et al. 2004) and these behaviors are commonly used to predict larger-scale movement in the field (Fraser et al. 2001; Dingemanse et al. 2003; Kobler et al. 2009; Cote et al. 2010). Increased movement also increases encounter rates with, or visibility to predators (Werner and Anholt 1993; Martel and Dill 1995).

A very wide range of contaminants (including pesticides, heavy metals, hydrocarbon industrial byproducts, wastewater or industrial effluents) are known to affect locomotion in animals, especially in fishes, and as a consequence changes in activity or movement quality are some of the most widely measured behavioral biomarkers of contaminant exposure (Little and Finger 1990; Bayley 2002). Exposed individuals may increase activity (e..g. stickleback, *Gasterosteus aculeatus*,

exposed to the xenoestrogen EE_2; Bell 2004), or decrease (rainbow trout, *Oncorhynchus mykiss*, exposed to aluminum; Allin and Wilson 1999, rainbow trout exposed to copper; Campbell et al. 2002), a change in the timing of diel cycle activity (carp, *Cyprinus carpio*, exposed to PCBs and TBT; Schmidt et al. 2005), and in the location of activity (goldfish, *Carassius auratus*, exposed to the pesticide carbofuran; Bretaud et al. 2002). The reasons for these alterations vary. Toxicants to which animals are exposed may have neurotoxic effects (e.g., methylmercury or many pesticides), producing cognitive or motor deficits that can reduce or increase movement (Brewer et al. 2001; Bretaud et al. 2002). High doses of contaminants can also increase the metabolic burden of the body as the liver attempts to transform and excrete the foreign substance, or as the body attempts to repair toxicant damage, depleting energy reserves and reducing body condition (Campbell et al. 2002). Certain toxicants are known to reduce the oxygen-carrying capacity of the blood, or the oxygen intake of the gills through structural or physiological damage (Allin and Wilson 1999; Schmidt et al. 2005). This can affect the location in the water column where fish choose to be, as well as the amount of locomotion produced.

Despite the array of evidence that contaminants can affect behavior, the fitness effects of sublethal behavioral impacts remain unclear for wild populations (Peakall 1985; Heinz 1989; Peakall 1996; Peakall et al. 2002). In fact, studies of behavioral impacts on naturally-exposed individuals, or tests for consequences of exposures in more natural settings are rarely performed (Birtwell et al. 1999;

Scholz et al. 2000; Weis et al. 2001; Grue et al. 2002; Breckels and Neff 2010). Although some field exposures come in brief pulses, such as pesticides draining from agricultural regions after rainfall (Floyd et al. 2008), most are chronic or even life-long. The results of acute exposures in the laboratory do not always capture the consequences of the chronic exposures common in the wild. In some cases, laboratory exposed animals that show initial behavioral impacts may recover to baseline levels of activity over longer time periods (Schmidt et al. 2005). Laboratory studies also typically examine impacts of only one toxicant at a time; in nature, however, exposure to multiple toxicants simultaneously is the rule, not the exception.

Here we examine the impacts on movement by long-term exposure to pollutants in a population of round goby, *Neogobius melanostomus*, living in a highly contaminated Canadian harbour (Hamilton Harbour) in Lake Ontario, one of the Laurentian Great Lakes (**Figure 5.1**). The round goby is a small fish invasive in North America (Jude et al. 1995), with a benthivorous diet comprising largely invasive dreissenid mussels, prey known to accumulate persistent toxicants (Jude 1997). It has become an important prey species for many piscivores in higher trophic levels, such as double-crested cormorants (*Phalacrocorax auritus*; Somers et al. 2003) and is known as a pollution-tolerant species (Pinchuk et al. 2003). For these reasons, the round goby has been identified as an important vector for contaminant mobilization in Great Lakes foodwebs (Jude 1997; Morrison et al.

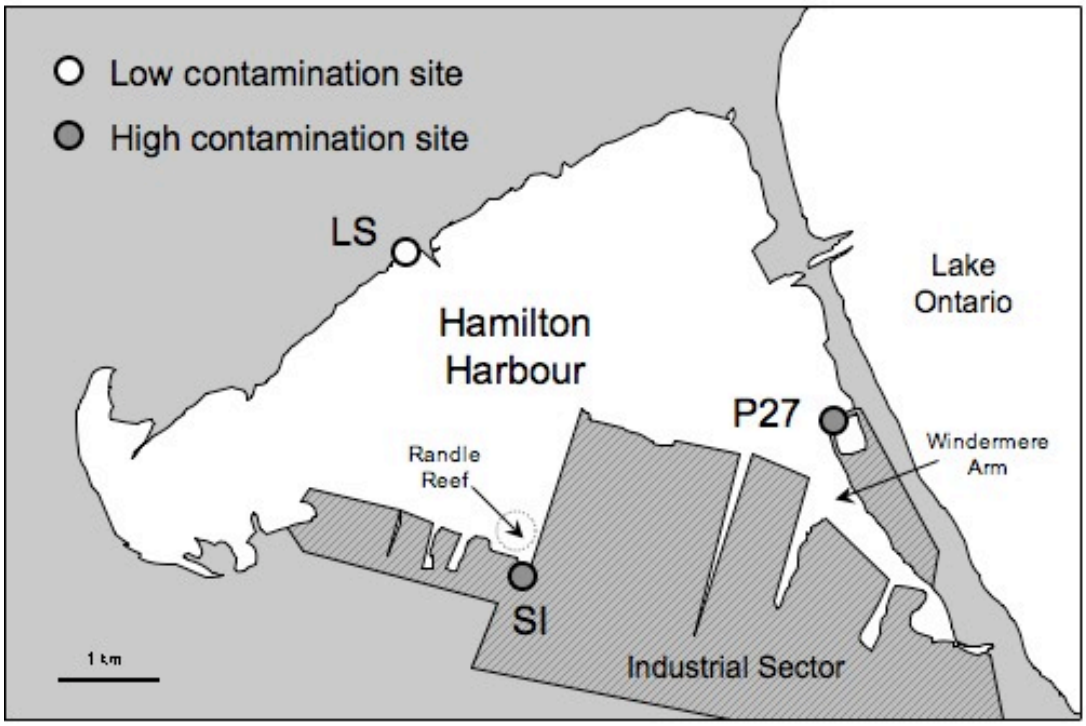

Figure 5.1. Map of Hamilton Harbour with the round goby collection sites indicated as a site of low contamination (LaSalle Park, or LS; white circle) or one of high contamination (P27 and SI; gray circle). The site known as Pier 27 (P27) is located near a channel called Windermere Arm, a region known to be contaminated with PCBs and many metals; the site known as Sherman Inlet (SI) is located at Pier 15 near a coal tar dump rich in PAHs and metals known as Randle Reef (Hamilton Harbour RAP 2003, Zeman 2009).

2000; Kwon et al. 2006; Hogan et al. 2007). Any behavioral changes, following contaminant exposure, that impair survival could affect the rate of this mobilization locally. Round goby are thought to have invaded our study site, Hamilton Harbour, a 2150 ha embayment on the western tip of Lake Ontario, over a decade ago (Young et al. 2010; Vélez-Espino et al. 2010). Hamilton Harbour is a Canadian Area of Concern designated by the International Joint Commission (IJC, 1999) due in large part to a long history of environmental contamination and degradation by urban and industrial sources, primarily historical byproducts of local steel mills (Hamilton Harbour RAP 1992; Murphy 2000). Contaminants known to be at problematic levels in the Harbour ("A list" contaminants) are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and metals including arsenic, cadmium, lead, iron, mercury and zinc, and the Harbour is also the recipient of discharges from four urban wastewater treatment plants (Hamilton Harbour RAP 2003). Given a presumed high site fidelity and small home range in this species (Ray and Corkum 2001; Cookingham and Ruetz 2008), round goby in Hamilton Harbour would be exposed over their lifetimes to high concentrations of multiple toxicants.

We examined differences in movement between round goby collected from areas of known contamination levels (highly contaminated versus relatively cleaner sites). Round goby collected from these areas have previously been shown to demonstrate corresponding differences in biomarker evidence of relative contaminant exposure (see Methods; Bowley et al. 2010; Sopinka et al. 2010,

Chapter 4). We investigated round goby activity level as they explored a novel environment in a laboratory behavioral assay. We also performed a mark-recapture study of round goby in the field, in areas of both high and low substrate contamination, to examine differences in residence times and distances moved between capture events. Based on the majority of studies of contaminant effects on locomotion (Little and Finger 1990, Bayley 2002), we predicted that round goby from highly contaminated areas would show reduced activity, and thus also have lowered exploration relative to fish from cleaner areas. We also predicted that activity level in the laboratory would correlate positively with home range size and dispersal capacity in the field (Cote et al. 2010). Specifically, we predicted that round goby in contaminated areas would move less (smaller distances travelled), and remain in the same area longer (increased residence times), than round goby in cleaner areas.

5.3 METHODS

5.3.1 Study areas and collection of fish

Fish used for both laboratory and field studies came from one area of low contamination (LaSalle Park, or LS) and two areas of very high contamination (Pier 27 and the mouth of Sherman Inlet in Pier 15, or P27 and SI), within Hamilton Harbour (**Figure 5.1**). Contamination of sediments and of water within the Harbour is not uniform, and regions of high and low contamination were based on

sediment distribution patterns of multiple contaminants (Hamilton Harbour RAP 1992; Hamilton Harbour RAP 2003; Zeman 2009). Compared to round goby from areas of low contamination, round goby collected from highly contaminated areas are smaller, have a higher frequency of intersex and vitellogenin production in males (indicating endocrine disruption), higher body burdens of copper and cadmium, greater hepatic EROD activity indicating exposure to AhR-binding contaminants such as PAHs (Bowley et al. 2010; **Chapter 4**). Sites were similar in water parameters such as turbidity, oxygen concentration and temperature (**Chapter 4**), and all three sites have substrates comprising a sand, cobble and boulder mix. Rugosity, or habitat complexity, was measured using a chain method (Saleh 1993); a 3 m (L1) chain was laid out over four different trap locations at each site at 1 m depth, and the resulting horizontal chain length measured (L2), to produce a dimensionless rugosity measure of L1/L2. One high contamination site, SI, had higher rugosity (mean 2.67) than either LS or P27 (means both 1.86; $F_{2,9}$ = 13.9, p = 0.002).

Fish were collected using baited minnow traps set for 24 h, up to 7 m from shore in < 1 m of water, and transported back to the laboratory within 4 hr of capture. Once in the laboratory, fish were housed in 60 L aquaria filled withdechlorinated tap water, equipped with an external box filter, two airstones, aquarium gravel to a depth of 2 cm, and 15 cm sections of PVC piping as shelter, and allowed to acclimate to these conditions for at least two days. Fish were housed separately by sex and site of origin. Round goby are externally sexed by an

examination of the urogenital papilla, which is pointed in males and blunt in females. Fish were fed once daily ad libitum with Nutrafin Staple fish flakes, except on the day of testing. All fish for behavioral studies were used within seven days of capture. The number of days spent in the laboratory did not influence our results (see Statistical Analyses section below).

5.3.2 Laboratory behavioural assay

Round goby (*N* = 198) were collected between 16 May and 25 July 2008 from three locations described above (LS, P27 and SI) and housed in sex- and site-specific 60 L holding tanks. Fish were held under a shifted 16L:8D light cycle, with the dark phase between 1200 and 2000 h, to facilitate behavioral observations performed in the nocturnal phase under red light (when round goby are most active; Dubs and Corkum 1996; Diana et al. 2006). Fish were divided into 66 groups of three fish, or 11 groups for each sex and site combination. We tested the fish in triads because a pilot study indicated that round goby were more active in triads than when tested alone (mean increase of 2.0 non-social movements/min, 95% CI of 0.8—3.1 movements/min, *N* = 81 fish), and this social effect was similar across sites and sexes.

Fish exploration and activity were measured in a large, segmented arena (2.5 m long x 0.75 m wide, divided lengthwise into 5 chambers 0.50 m long, 0.75 m wide, 0.15 m deep; **Figure 5.2a**). The chambers were separated by removable acrylic dividers, each with a doorway (25 cm long, 15 cm high) in the middle. All

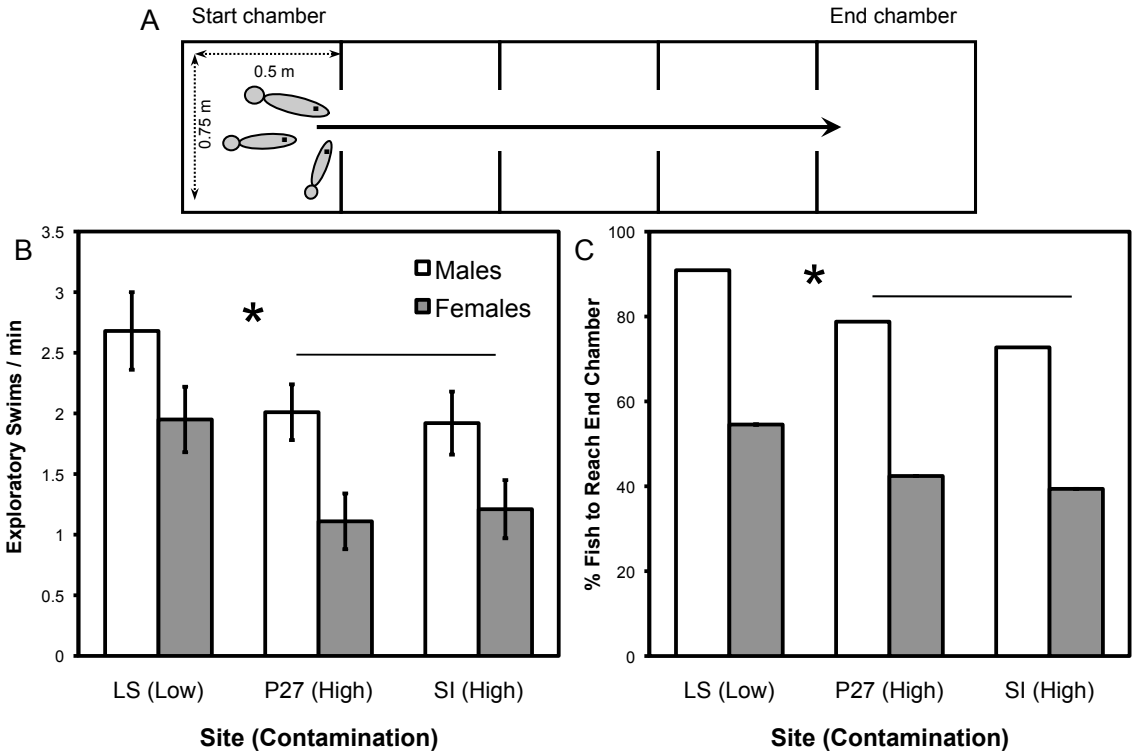

Figure 5.2. A) Top view of chambered arena used to test round goby for activity and exploration. Fish were introduced to either end of the arena, which became the Start chamber. **B)** The mean ± SE rate of exploratory swims/min exhibited by males (white bars) and females (gray bars) from areas of low and high contamination. **C)** More fish from low contamination sites reached the last chamber of the arena, versus fish from high contamination sites. More males (white bars) than females (gray bars) reached the last chamber as well. Linear contrasts revealed significant differences between fish from the low contamination site LS, and the two high contamination sites P27 and SI ($p < 0.05$), indicated with an asterisk (*). A line over the bars of P27 and SI indicates there were no significant differences between these sites ($p > 0.05$).

five chambers were equipped with three acrylic shelters, white sand 1 cm deep and one external box filter. Fish were placed in one end chamber of the arena in triads, in which fish were of the same sex and site, but not matched in size to facilitate fish identification by the observer during trials. Each group was given a unique ID. The observer was blind to the sex and site of the fish. Consecutively tested groups were started from alternating ends of the arena, and water was thoroughly mixed between trials to eliminate odor gradients or cues from previous groups. Water within the experimental apparatus was changed once daily.

Each group was allowed to acclimate for 30 min in the first chamber, with the entrance to the second chamber blocked by a removable divider. Observers were positioned behind a blind 1 m away from the testing arena. During the last 15 min of the acclimation period, the observer recorded all behaviors exhibited by each fish for 5 min for 60 of 66 groups, 10 of each sex and site combination. The order of fish observation (by size rank) was randomized for each group. Behaviors were counted and grouped by function: horizontal locomotion, exploration, and substrate-oriented behaviors and expressed as a rate per minute (**Table 5.1**). Social behaviors such as bites and chases were enumerated separately from these non-social movements. Following the acclimation period, the observer removed the divider blocking the entrance to the second chamber, and recorded all entries to chambers made by each fish during the 30 min test period. The time to begin exploration was defined as the time elapsed (in seconds) to first exit the starting chamber, and the time to furthest distance was defined as the time elapsed (in

Table 5.1. Categories of movement classification for round goby laboratory behavioral assays.

Category	Behavior	Description
Horizontal Locomotion	Hop	Fish movement of ≤ 1 body length
	Swim	Sustained horizontal movements in water column of > 1 body length
	Dart	Rapid swim of > 1 body length
Exploration	Swim	Sustained, repeated, frequently vertical movements in water column with mouth oriented at perimeter of aquarium
Social Interactions	Bite or Chase	Fish rapidly approaches another, with (Bite) or without (Chase) opening and closing its mouth on the body of the other
	Bitten, Chased or Displaced	Reciprocal of above. A fish rapidly departs from the approach of another. In the case of displacement, the approaching individual moves slowly and does not appear to initiate a Bite or a Chase

seconds) to enter the furthest chamber reached by the fish. Each fish was assigned a number of chamber switches, where fish that never left the starting chamber were assigned a 0. Fish were also classed as to whether they reached all five chambers during the test phase (yes or no). At the end of the test period, all tested fish were euthanized with an overdose of Benzocaine and their body size (total length, to 0.1 mm, and total mass, to 0.001 g) measured during dissection. Body condition was evaluated as Fulton's condition factor K (total mass:total length^3 x 100). The presence of eroded or damaged ventral fins, found most frequently in round goby from highly contaminated sites (see **Chapter 4**), was scored as present or absent.

5.3.3 Field mark-recapture study

Adult (N = 867) and juvenile (N = 14) round goby were tagged and released in five cohorts at the low contamination site LS and the high contamination sites P27 and SI between May 5 and August 21 2009. Six traps were set at each site along the shore at 6 m intervals. All fish collected in these traps between a) May 5–8 (N = 65 fish), b) June 2 and 5 (N = 148), c) June 23–25 (N = 208), d) July 22–24 (N = 142), and e) August 18– 21 (N = 319), were sexed, assigned a reproductive status and measured to obtain total length and total mass. Fish were then given four markings in any of 12 locations along the dorsolateral aspect of the body with a subcutaneous injection of VIE (Visible Implant Elastomer, Northwest Marine Technologies, Inc.) and allowed 5-15 min to recover before being released at the

location of capture. This method allowed for 495 unique codes to be used for each of two VIE colors (fluorescent orange and green).

From May 5 to November 6, 2009, weekly sampling was performed for recaptures. When VIE-tagged individuals were recaptured, they were identified, re-measured for length and mass, their reproductive status noted and the distance moved calculated and the number of days elapsed since the previous capture was assigned. Occasionally tagged fish were recovered in traps not part of the mark-recapture study. Distances moved by these fish were calculated by measuring the distance along the shoreline to the trap from which the fish was last seen. At the end of the study, each recaptured fish was assigned a maximum distance moved and a maximum known residence time (the days elapsed between first and last capture). From these, fish were assigned a travelling rate in m/week, which was calculated as the maximum distance moved by the fish divided by the number of weeks, with weekly units calculated as residence time in days/7.

5.3.4 Statistical analyses

Data were normalized by log or arcsine square root transformations when possible; otherwise, rank-based statistical tests were used. Spearman's rho non-parametric correlations were used to determine relationships between continuous non-normally-distributed variables. Binary data (yes/no classifications) were examined with nominal logistic regression followed by main effect likelihood ratio tests to establish differences among sites or sexes. Where individual fish were

tested as part of a triad, linear mixed models (using the residual maximum likelihood method) were used to examine behavioral data using Sex and Site as main factors and Group ID specified as a random effect, nested within Site and Sex. Sex-site interaction terms were not significant ($P > 0.1$) and were removed from models. Covariates (total body length, and number of days elapsed in the laboratory since capture) were incorporated into the models but removed when they did not prove significant. The number of days spent in the laboratory was never a significant covariate in ANCOVA models. Orthogonal linear contrasts were used to calculate post-hoc significant differences among sites: low contamination (LS) versus high contamination (P27 and SI), and also to detect any differences between the two highly contaminated sites, P27 and SI. Otherwise, Tukey HSD tests were used to identify post-hoc differences among groups. All data analysis was performed using the program JMP 5.0.1a for MacIntosh (SAS Institute, Inc., 2002).

5.4 RESULTS

5.4.1 Laboratory behavioural assay

5.4.1.1 Acclimation phase

Fish from the low contamination site (LS) showed more horizontal locomotion ($F_{60,119} = 1.8$, $P = 0.003$; site $F_{2,56} = 3.7$, $P = 0.03$; linear contrast $F_{1,56} = 7.3$, $P = 0.009$) and more exploratory swimming ($F_{59,120} = 2.7$, $P < 0.0001$; site $F_{2,56} =$

3.2, P = 0.049; linear contrast $F_{1,56}$ = 6.3, P = 0.01; **Figure 5.2b**) than fish from the two high contamination sites (P27 and SI). Irrespective of site, males showed greater exploration than females (sex $F_{2,56}$ = 6.7, P = 0.01; **Figure 5.2b**), but sexes showed similar rates of horizontal locomotion (sex $F_{1,56}$ = 1.3, P = 0.27).

5.4.1.2 Test phase

Fish from the low contamination site LS began to explore the novel arena sooner than fish the high contamination sites P27 and SI ($F_{61,84}$ = 1.6, P = 0.02; effect of site $F_{2,57}$ = 3.1, P = 0.05; linear contrast $F_{1,57}$ = 5.6, P = 0.02). Irrespective of site, males begin exploring earlier than females (sex $F_{1,57}$ = 8.0, P = 0.007). More fish from the low contamination site than the highly contaminated sites reached the fifth and last chamber (fish from P27 and SI pooled; χ^2_2 = 32, p < 0.0001; site χ^2_1 = 4.6, P = 0.03; **Figure 5.2c**). More males than females reached the fifth chamber as well (sex χ^2_1 = 28, P < 0.0001; **Figure 5.2c**). Over the entire test period, fish from the low contamination site LS also made a greater number of chamber switches than fish from either contaminated site ($F_{66,131}$ = 3.3, P < 0.0001; site $F_{2,62}$ = 4.3, P = 0.018; linear contrast $F_{1,62}$ = 8.5, P = 0.005), and males made more switches than females ($F_{1,62}$ = 16.3 = 7.1, P = 0.0001). Across all fish, a higher rate of exploration in the acclimation phase correlated with a quicker start to begin exploration (r_s = -0.22, P = 0.01, N = 44), a greater number of chamber switches (r_s = 0.45, P < 0.0001, N = 60) and farther chambers reached (i.e. distances travelled; r_s = 0.51, P < 0.0001, N = 60) during the test phase.

5.4.1.3 Physical differences among sites

Fish from the low contamination site LS were larger (mean ± SE total body length 87 ± 2 mm) than fish from the two highly contaminated sites, P27 (79 ± 2 mm) and SI (77 ± 2 mm). Male fish (96 ± 2 mm) were larger than females (75 ± 1 mm; $F_{3,194}$ = 11.3, P < 0.0001; site $F_{2,194}$ = 7.9, P = 0.0005, sex $F_{1,194}$ = 18.1, P < 0.0001). These site-related size differences are similar to findings from the same sites in previous years (**Chapter 4**). Fish total length was a significant covariate in models of horizontal locomotion, but not exploratory swimming. Fish total length also did not significantly correlate with time to start exploration (r_s = 0.13, P = 0.12 N = 44), number of chamber switches (r_s = 0.04, P = 0.54, N = 60), or the furthest chamber distance reached (r_s = 0.10, P = 0.16, N = 60).

Male and female fish from the three sites were similar in body condition as measured by Fulton's K ($F_{5,192}$ = 1.1, P = 0.35). Eroded ventral fins were only found in fish from highly contaminated sites (observed in N = 3, or 5% of fish from P27; N = 25, or 38% of fish from SI; (χ^2_2 = 49.4, P < 0.0001). We explored whether eroded fins affected locomotion in fish from SI, the site with the higher prevalence of fin loss. Fish with eroded fins showed significantly less horizontal movement than fish with normal fins ($F_{1,58}$ = 4.5, P = 0.037), but similar levels of exploratory swimming ($F_{1,58}$ = 0.4, P = 0.85) in the acclimation phase. In the test phase, however, fish with eroded fins were just as likely to leave the starting chamber (χ^2_1 = 0.45, P = 0.50), started exploring at similar times ($F_{1,46}$ = 0.01, P = 0.94), and in fact tended to make

more chamber switches than fish with normal fins in the test phase ($F_{1,58}$ = 3.7, P = 0.06).

5.4.2 Field mark-recapture study

Of the 881 round goby tagged in 2009, 167 or 19% were ultimately recaptured the same year. This represents 21.1% of fish at the low contamination site LS (N = 66 of 311), 14.5% of fish at P27 (N = 41 of 283) and 20.9% of fish at SI (N = 60 of 227), both highly contaminated sites. This recapture percentage did not differ across sites (χ^2_2 = 4.6, P = 0.10). Most recaptured fish were only recaptured once (113 of 167; 67.7%) although some fish were recaptured up to six times (N = 3) over the course of the study. There were no differences across sites in the proportions of males and females tagged (χ^2_2 = 0.9, P = 0.65) and males and females had equivalent recapture rates (χ^2_1 = 0.1, P = 0.80). The location of the traps along the shore of a given site (inner, intermediate or outer traps) did not affect the number of subsequent recaptures (χ^2_2 = 1.7, P = 0.42). Across all sites, the earlier fish were tagged in the study, the longer their known residence times were (r_s = -0.29, P = 0.0001) and the further they ended up (r_s = – 0.29, P = 0.0002); travelling rates in m/week, however, were not related to date of tagging (r_s – 0.11, P = 0.16).

Recaptured fish were last seen anywhere from 1 to 168 days after their initial capture, with a median known residence time of 29 days. Known residence times did not vary among sites (H_2 = 3.74, P = 0.15) or between sexes (Z = 0.72, P =

0.50). Most recaptured fish (N = 112, or 67.1%) were only caught at the trap where they were originally captured, and were therefore assigned a movement distance of 0 m. The proportion of fish that apparently never moved was similar across sites of both low and high contamination (χ^2_2 = 1.8, P = 0.40), and between the sexes (χ^2_1 = 0.001, P = 0.98). Fish that did move to a different trap between capture events (N = 55, or 32.9% of all recaptured fish) travelled distances ranging from 3 to 18 m along the shoreline. This translated to a median of 0.67 m/week (range 0.15 to 12 m/week) in travelling rates. Fish from sites of low and high contamination that moved between traps had similar travelling rates (H_2 = 0.18, P = 0.91; **Figure 5.3**), and so did males and females (Z = 1.24, P = 0.22; **Figure 5.3**).

A few tagged round goby (N = 13; N = 9 for LS, N = 2 for SI, N = 2 for P27) were recaptured in 2010, the second year of the study. There were no site differences in distances travelled (in m/week, fish from P27 and SI pooled; Z = 0.1, P = 0.94). Males (N = 9), however, moved more than females (N = 4; Z = 2.3, P = 0.02). After restricting the data only to males, there were no differences in distances travelled between fish from high (N = 3) and low contamination sites (N = 6; Z = 0.4, P = 0.70).

5.4.2.1 Physical differences among sites

As found for fish used in the laboratory experiment described above, and field data from other years (**Chapter 4**), round goby from the low contamination

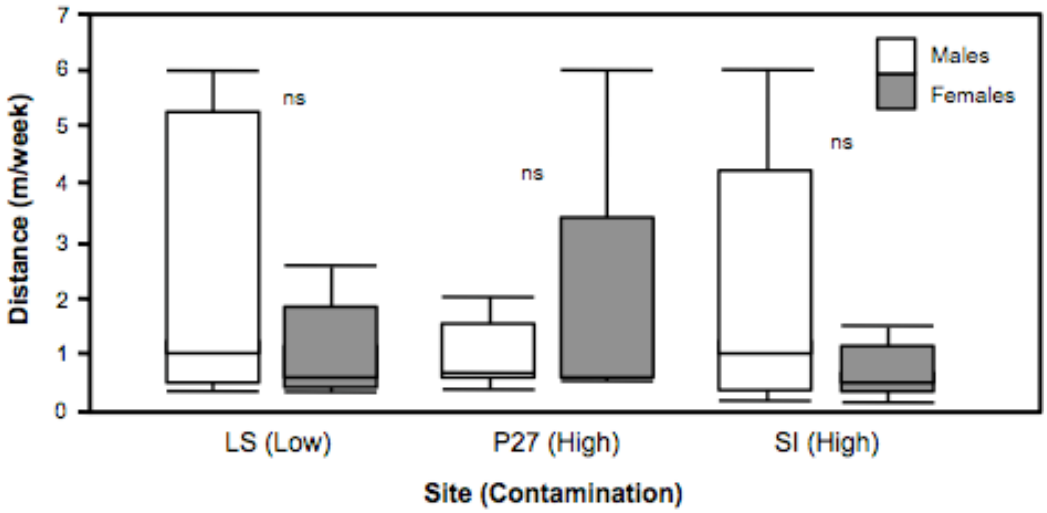

Figure 5.3. Boxplot distributions of travelling rates for male and female round goby in the field at each of three sites (LS, n = 9 males, 9 females; P27, n = 9 males, 5 females; SI, n = 17 males, 6 females). Boxplots represent medians, quartiles and 95th percentiles. Data represent *only* fish that moved along the nearshore monitored over the course of six months; fish that did not move are excluded. There were no differences in travelling among sites or between sexes.

site (LS) were larger than those from the high contamination sites (P27 or SI), and males were larger than females (juveniles excluded; $F_{3,863} = 40.8$, $P < 0.0001$; site $F_{2,863} = 46.0$, $P < 0.0001$, sex $F_{1,\ 863} = 32.4$, $P < 0.0001$). The total length of the fish when first caught and tagged did not correlate with the absolute distance moved, in m ($r_s = 0.06$, $P = 0.40$) or with the travelling rate, m/week ($r_s = 0.05$, $P = 0.50$).

5.5 DISCUSSION

Round goby from two highly contaminated sites (P27 and SI) show lower levels of activity and exploration in the laboratory compared to fish from a comparison site of relatively lower contamination (LS). This was true of both horizontal locomotion and exploratory swims. More active and exploratory fish moved further and faster in the laboratory environment. Males were more explorative than females, and dispersed further in a novel environment, but site effects were consistent across the sexes. There were no differences, however, in movement rates of fish from different sites measured in the field. There were also no differences in known residence times of fish from low and high contamination sites.

As discussed above, a change in activity has been frequently observed in animals exposed to a wide variety of contaminants. Which pollutant or pollutants in Hamilton Harbour might be responsible for an alteration in round goby behavior? Attributing the differences in laboratory-based activity observed in this study to any one contaminant would not be feasible. These small-bodied fish have

been exposed to a complex cocktail of interacting contaminants in their habitats, including PAHs, PCBs, urban wastewater treatment plant effluents, lead, cadmium arsenic, iron, mercury, zinc, copper and many other pollutants at varying degrees (Zeman 2009). Fish from these sites have shown signs of toxicant exposure including fin erosion and endocrine disruption (Bowley et al. 2010; **Chapter 4**). Previous work has demonstrated that PAHs (gilthead seabream, *Sparus aurata*, Goncalves et al. 2008; seabass, *Dicentrarchus labrax*; Gravato and Guilhermino 2009), PCBs (medaka, *Oryzias latipes*, Nakayama et al. 2005; carp, *Cyprinus carpio*, Schmidt et al. 2005), mercury (mummichog, *Fundulus heteroclitus*; Zhou and Weis, 1998), cadmium (matrinxã, *Brycon amazonicus*, Honda et al. 2008), copper (rainbow trout, Campbell et al. 2002), as well as complex combinations of contaminants (brown trout, *Salmo trutta*, Triebskorn et al. 1997; brown bullhead, *Ameiurus nebulosus*, Breckels and Neff 2010) can all affect locomotion in a range of taxa.

Why might fish show lower levels of activity in the laboratory after chronic exposure to contamination in the field? Round goby collected from contaminated areas often have demonstrated ventral (pelvic, anal and caudal) fin damage or erosion (**Chapter 4**; this study), but this type of fin damage in other species is not always known to impair movement (Hopkins et al. 2003). The ventral fins are not used as much as the pectoral and caudal fins in round goby locomotion (personal observation). Fish with eroded fins did not explore or disperse differently in a novel environment than fish with normal, intact fins, and so we do not think this is

likely to be a major driver of the lower activity in contaminated-site fish. Round goby from highly contaminated areas were not in worse body condition than those from our low contamination collection site (**Chapter 4**, this study); individual condition reflects body composition and energetic levels (Kaufman et al. 2007) and can be used as a biomarker in fish exposed to contaminants (Schlenk et al. 2008). Other physiological mechanisms, such as neurotoxic effects, chronic stress, or endocrine disruption may have been the proximate cause of the reduced activity here observed here, but these factors have yet to be explored in detail in this system.

Round goby from highly contaminated areas moved less than fish from a less contaminated area in the laboratory, but not in the field. Laboratory behaviors such as exploration of a novel environment and boldness have been used successfully to predict movement and dispersal patterns in the field (reviewed by Cote et al. 2010). Why did we not observe the reduction in travelling rate or increases in residence time in the field that would correspond with the results of our laboratory assay of movement? Laboratory exploration and activity patterns simply may not relate to movement, home range size and dispersal in and out of a site in the round goby. It may also be possible that behavioral differences in fish from clean and contaminated habitats only manifest in novel, stressful or changing environments (e.g., Breckels and Neff 2010). In a familiar environment with well-established home ranges, movement differences may not be apparent. Alternatively, our field study may not have had sufficient recapture events (sample

size) to detect real differences in movement, or we may have needed finer-scale spatial resolutions in recaptures (i.e., less than 6 m) to reveal differences in movement. The 6 m distance between traps was selected to represent an intermediate value for observed round goby movement distances over one hour (mean 5 m^2 area; Ray and Corkum 2001), and 48 hours (mean 7.3 m linear distances; Cookingham and Ruetz 2008) in the field. Based on these two previous reports, 6 m was not an unrealistic distance for these fish to move.

A major benefit of working with field-exposed individuals is that the animals have been exposed at realistic levels, routes of entry and timescales (a year or more) not easily replicated in the laboratory. Causal links between contaminant exposure and the differences in laboratory activity and exploration observed in our work are suggested but not addressed by the data. Other factors specific to the habitat of origin of these fish might contribute to the observed differences in activity. Although all three sites were similar in water parameters such as pH, turbidity, oxygen and temperature (**Chapter 4**), one site (the highly contaminated site SI) was more rugose, or rocky, than the others. Attributing behavioral differences to habitat complexity would not, however, explain why fish from SI, one contaminated site, had similar levels of activity to fish from P27, the second contaminated site, and both had lower levels of activity to the low contamination site LS. Round goby used in these studies were smaller, and thus possibly younger, at sites of high contamination than low contamination; however body size was rarely correlated to movement measured in the laboratory and did not affect

movement in the field. The three sites may be inhabited or visited at different rates by round goby predators, both piscine and avian. Higher levels of predation risk may favour shy, less active individuals over bolder ones (Huntingford 1982; but see Brown et al. 2005). If more predators are foraging at highly contaminated sites, this may contribute to the behavioral patterns observed, but differences in predation rates among sites are not clear. The high contamination site P27 is located near a colony of double-crested cormorants (*Phalacrocorax auritus*) and other waterbirds; however, these piscivores often forage many km from their nests, potentially affecting sites throughout the harbour (Stapanian et al. 2002). Piscivorous fish are in fact more prevalent within one km of the low contamination site LS than near the high contamination site P27; data on predator fish distributions for the high contamination site SI are not available (Brousseau and Randall 2008).

Much is known about the impacts of various classes of contaminants on animal behavior in the laboratory (Dell'omo 2002), and behavior is frequently evaluated as one of a suite of biomarkers of exposure in controlled experiments. In these contexts, behavior is often posited as a potentially useful diagnostic tool for predicting impacts of contaminant exposures on populations, because behavior conveniently integrates the whole-animal effects of multiple physiological processes, in ways that directly affect animal survival and reproduction. The challenge to this proposition is that considerably less is known about the ecological impact that contaminant-mediated behavioral changes have in natural settings (Bayley 2002). Similarly, behavior is a rarely-explored biomarker of field

exposures, where verification of biomarker predictions generated from laboratory studies must be made (Peakall 1996). Both of these areas are necessary research routes to pursue in the development of behavior as an ecotoxicological tool. In this paper, we were able to show that round goby collected from populations known to exhibit signs of contaminant exposure, also show differences in behavior that are not easily related to any other site-specific cause like habitat complexity and foraging or predator experiences. Thus, we propose that activity level in the round goby, and other species, may be a useful biomarker of exposure to complex contaminant mixtures in the field in conjunction with other physiological biomarkers. We were not able to show, however, that differences in laboratory activity predict measureable animal movement differences in the field. The absence of differences in field movement should not be interpreted to mean that there are no ecologically significant consequences for these fish in their natural habitat. It must be recognized that the pious hopes (Grue et al. 2002), or claims that all toxicant-induced behavioral changes are ecologically meaningful, found so frequently in the toxicology literature may not always be supported. The utility of behavioral metrics chosen in both laboratory and field, such as activity level, must be carefully evaluated. A more cautious inference is that establishing connections between contaminant exposure, laboratory observations of behavioral differences, and population-level consequences is not a simple matter. These connections need to be pursued more vigorously in future endeavors if more accurate predictions of population-level consequences are to be generated.

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Chapter 6

GENERAL DISCUSSION

6.1 THESIS SUMMARY

In the previous chapters, I provide evidence of alternative reproductive tactics in round goby and explore how sex and contaminant exposure influence movement patterns in this species. While a polygamous mating system with male-only parental care was well known to occur in the round goby, in **Chapter 2** I provide the first strong support (based on convergent lines of evidence) for two alternative male reproductive tactics in this species. I then examined round goby movement in both the laboratory and the field in light of predicted differences based on sex and male tactic. In **Chapter 3**, I show that males move more than females in both the lab and the field—and that males adopting the different reproductive tactics move to a similar extent. In **Chapter 4**, I switch topics entirely to look at anthropogenic influences on round goby. In each physical and physiological biomarker of contaminant exposure measured, differences due to sex and male tactic were taken into account where possible. Although biomarkers of contaminant exposure were present in males and females and both tactics, certain biomarkers (e.g. urogenital shape) was more useful in one sex, or one tactic, but not the other, demonstrating the importance of accounting for both sex and reproductive status in field studies. Finally, in **Chapter 5,** I returned to examine round goby movement in relation to hypothesized contaminant exposure – the

complementary study of **Chapter 3**. I showed that in the laboratory round goby from contaminated sites were less active than fish from a cleaner site, but this was ultimately not paralleled by differences in field movement.

6.2 FIRST AIM: ROUND GOBY REPRODUCTIVE BIOLOGY

In **Section 1.6,** I laid out three aims for my thesis. The first aim was to increase the understanding of the reproductive biology of an invasive fish, which I address in the first half of my thesis, **Chapters 2** and **3**. Using the species' mating system as a starting point, I expanded knowledge of round goby reproductive tactics and movement patterns. Round goby exhibit a polygamous mating system (where both males and females mate with multiple individuals each spawning season), and some reproducing males provide parental care. Paternal care is a costly resource that is exploited by sneaking males, and I was able to show that such sneaker males do exist in round goby (**Chapter 2**). In this sense, round goby males adhere to reproductive strategies similar to those found in some other gobiid species, other fishes and other non-fish taxa (Magnhagen 1992, Sinervo and Lively 1996, Emlen 1997, Mazzoldi and Rasotto 2002, Oliveira et al. 2008, Svensson 2004, Taborsky 1998).

The round goby's polygamous mating system also predicts a sex difference in both dispersal and home range size between the sexes, and between males of different reproductive tactics (**Sections 1.1 and 3.2**). Like other polygamous animals where males are larger, compete for access to mates, and female

reproductive rate is limited by resources, males were expected to disperse and range more than females (Pusey 1987, Perrin and Goudet 2001). However, during the breeding season, nest-guarding males were expected to be limited to small territories and move very little around their nests. I tackled these predictions with a combination of laboratory behavioural assays and field measurements (**Chapter 3**). My laboratory assay of movement was specifically designed to target behaviours known from other species to predict greater home range size and dispersal in the field (Fraser et al. 2001; Dingemanse et al. 2003; Rehage and Sih 2004; Kobler et al. 2009; Cote et al. 2010a and 2010b). I found that males, regardless of current reproductive tactic (guarding, sneaker or non-reproductive), moved more than did females while exploring a novel environment. Over a two-year period, males moved more than females in the field as well. I did not find a predicted reduction in movement by guarding males or the expected greater movement by sneaker males during the breeding season.

In **Appendix A**, I provide a summary of 31 papers based on 25 fish species that examine sex differences in movement (**Section A.1**), and an additional 7 papers that looked at male tactic-related differences in movement across fishes (**Section A.2**). I grouped the data from **Section A.1** by the temporal scale across which data were collected into: 1) studies that measured dispersal or home range size over the long term or outside of a breeding context, or 2) studies that examined home range size or locomotion within the breeding season. I also divided the species used in studies of sex differences according to whether they were

polygamous, strictly monogamous or sex-role reversed, and also by parental care (either no care, or with predominantly male care; **Figure 6.1**).

My results in **Chapter 3** are comparable to other studies (**Section A.1)**, where in five of six comparable species (i.e., polygamous, with generally male parental care, and typical sex roles where male potential reproductive rate exceeds that of females), greater movement by males has been reported (Osugi et al. 1999, Sunobe and Nakazono 1999, Natsumeda 2001, Stiver et al. 2007, Cano et al. 2008; but see Taru and Sunobe 2002; **Figure 6.1**). I did not find evidence that reproducing males during the breeding season moved less than females either in the laboratory (where they were actually observed to move more), or in the field. Five of seven papers on polygamous, generally male-caring taxa report greater movement by females during the breeding season (Natsumeda 2001, Santos and Almada 1988, Savitz et al. 1993), or at minimum on days when spawning was occurring (Sunobe and Nakazono 1999, Taru and Sunobe 2002), and two did not find sex differences (Natsumeda 2007, Takahashi 2000). As gobiid fishes or the ecologically similar cottid fishes were the focus of the studies that found females moved more only when spawning was actively occurring (Sunobe and Nakazono 1999, Taru and Sunobe 2002) and the studies reporting no sex differences (Natsumeda 2007, Takahashi 2000), it is perhaps not surprising that round goby, a gobiid, also lack sex differences in breeding season movements. When not specifically guarding eggs in a nesting territory, it is possible that guarding males

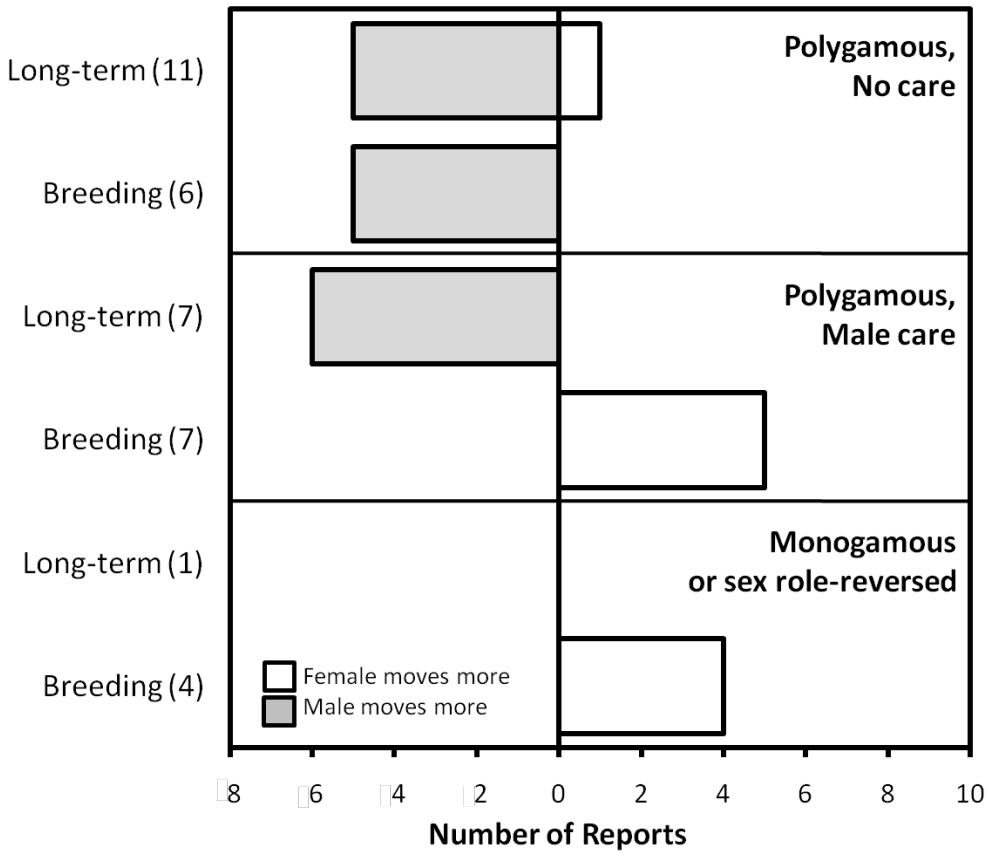

Figure 6.1. Summary of reports (n in parentheses) concerning sex differences in movement across fishes, compiled from of 31 papers listed in **Appendix A**. Each paper could contribute more than one report. Reports of no sex differences are included in the total number of reports, but are not graphed. The reports are grouped by the mating and parental care systems of the taxa involved, and are further subdivided by whether the study took place over the long term (one or more years, or outside of the breeding context; Long-term), or specifically while breeding was occurring (Breeding). Movement encompasses activity level, home range size, and dispersal.

may roam widely in their home range (Sunobe and Nakazono 1999, Taru and Sunobe 2002).

In **Chapter 3**, I showed that guarding and sneaker male round goby moved to a similar extent in the laboratory, and in the field (during the breeding season only). Guarding males moved more than sneakers over the longer term. Given that guarding males are larger, I had predicted these males would move more than sneakers over the long term due to greater energetic needs and lowered predation risk associated with moving (**Section 3.2**). Three papers in **Section A.2** explored movement in species with tactics that approximate guarding and sneaker male roles, like those observed in the round goby. These fish were: another goby, *Asterropteryx semipunctata* (Manabe et al. 2009), a cichlid, *Telmatochromis vittatus* (Ota and Kohda 2006) and a blenny, *Blennius sanguinolentus* (Santos and Almada 1988). In each of these systems, at least one tactic defends a nesting territory, courts females, and performs parental care, while at least one other sneaks fertilizations. All of these studies took place only during one breeding season. Movement patterns (home range size) differed with male tactic only in the cichlid (Ota and Kohda 2006), a fish with four male tactics (guarders and sneakers; two other tactics, pirates and satellites, were not considered here). However, in that study, sneakers moved less than guarders. Thus, it appears that round goby male reproductive tactics display a lack of difference in field movement patterns that is analogous to other, similar species. **Chapter 3** was the first study to look at male tactic differences in movements over longer timescales. Currently there is no

comparative evidence from other species, so it is difficult to say whether the pattern of guarding males moving more than sneaker males over the long term (which is an expectation from ecological and evolutionary theory) is in fact representative of a general pattern in fishes of similar mating systems. This is an obvious avenue for future research.

6.3 SECOND AIM: ROUND GOBY AS CONTAMINANT SENTINEL

6.3.1 Is the round goby a useful sentinel species?

The second major aim of my thesis was to investigate the practicality of using round goby as a sentinel of habitat contamination, while working exclusively within a single Area of Concern, Hamilton Harbour. In the second half of my thesis, **Chapters 4** and **5**, I examined both physical and behavioural biomarkers of contaminant exposure in naturally-exposed round goby populations.

In **Section 1.4**, I identified many *a priori* reasons why round goby might be an appropriate sentinel species. Prior to my thesis research, it was already understood that round goby are a benthic fish and thus subjected to two major routes of contaminant exposure (consuming contaminated benthic organisms and undergoing essentially constant exposure to sediments). Ecologically, they play important roles as predators and prey within the trophic web, are not generally subject to targeted human exploitation, mature and grow quickly and have high fecundity (MacInnis and Corkum 2000a and 2000b, Ray and Corkum 2001, Pinchuk

et al. 2003, Young et al. 2010). They are also an abundant fish throughout the Great Lakes and associated watersheds, are easy to capture, and have restricted mobility. These characteristics set up round goby as a good potential sentinel species. In **Chapter 4**, I was able to verify that round goby were abundant and easy to collect in Hamilton Harbour, in regions of both high and low contamination. In **Chapters 3** and **5**, I confirmed that round goby have very low mobility, moving a median of 0 m and a maximum of 18 m along the shoreline over time periods spanning two years (although long-distance travelers have been reported in this fish, these are rare events; **Chapter 3, Chapter 5**, Wolfe and Marsden 1998). Limited mobility ensures that fish collected in a given site accurately reflect the conditions of that site. Further supporting the utility of round goby as a sentinel was the demonstration that round goby from different sites were physically distinguishable. A variety of physical biomarkers examined in **Chapter 4** reliably differentiated round goby collected from regions of low and high contamination. These included both simple measures of morphological change (ventral fin erosion, urogenital papilla shape), body size, sex ratio, indicators of endocrine disruption (urogenital papilla shape and intersex gonads) as well as more direct measures of contaminant concentrations (hepatic burdens of copper and cadmium) or physiological alterations (EROD activity).

There are also a few reasons why round goby are not an ideal sentinel species for habitat contamination. While small-bodied fish are generally considered good sentinels (Gibbons 1997), a small body size precludes the collection and

analysis of large tissue samples per individual. This may necessitate pooling of samples from multiple individuals in order to perform certain assays. A second problem may be that round goby are, ironically, perhaps too pollution-tolerant (Pinchuk et al. 2003) for some common biomarkers to be useful. Round goby may have arrived from polluted Eurasian waters (especially the Dneiper River; Sansone et al. 1996, Maldonado et al. 1999, Brown and Stepien 2009) pre-adapted to tolerate high levels of contamination (**Chapter 4**). Round goby in areas of high contamination were not in worse body condition, nor did they demonstrate certain other predicted patterns of contaminant exposure (e.g., reduced investment in reproductive organs) typical of populations exposed to toxicant stressors (Gibbons and Munkittrick 1994). While intersex males were present, the frequency of intersex was lower for round goby (13%) in comparison to other species from similar areas (e.g., 83% of white perch, *Morone Americana*) but higher than that of common carp (0%, *Cyprinus carpio*) or brown bullhead (0%, *Ameiurus nebulosus*; Kavanagh et al. 2004, Bowley et al. 2010). Furthermore, round goby were highly and nearly equally abundant in most sites, perhaps indicating that mortality rates due to contaminant exposure in even some of the most toxic locations in Canada are unexpectedly low. A sentinel species whose survival and population demographics do not respond sensitively enough to contaminant gradients may not always be the most appropriate species to use, depending on the specific contaminants or biomarkers of interest. Regardless, the results of **Chapter 4** do

indicate that the round goby functions well as a sentinel species within contaminated harbours.

6.3.2 Sex, reproductive status, and physical biomarkers

Throughout this thesis, I accounted for variation in contaminant biomarker patterns while considering fish sex, and especially male tactic and reproductive status, something few studies take into account. Round goby of both sexes, particularly in the breeding season, are known to vary in their reproductive readiness. Interestingly, the vast majority of adult males collected in any given month during the breeding season do not have developed testes or accessory glands, and these fish are termed non-reproductive males; females cycle in and out of reproductive readiness with each sequential spawning event (Young et al. 2010). Males ready to spawn can be divided into guarding and sneaker males (**Chapter 2**). Sex ratios and the proportions of individuals in spawning condition also varied as a function of habitat contamination (**Chapter 4**).

While many field-based studies of contaminant exposure do account for sex differences in biomarkers, plan the timing of studies to minimize variance in reproductive readiness, or limit study of some biomarkers to only one sex (e.g., vitellogenin expression or intersex gonads in males), very few studies take into account male reproductive tactic even though species frequently used in toxicology, such as salmonids and centrarchids, do have alternative reproductive tactics (Taborsky 1998). As male tactics can differ substantially in their morphology and

physiology, accounting for these differences may be critical in correctly interpreting contaminant impacts on wild populations. In **Chapter 4**, I demonstrate the relatively novel finding that the ratio of male tactics to each other may vary with habitat contamination. Additional research in which I have been involved has shown that certain biomarkers, such as vitellogenin expression as a measure of endocrine disruption, may manifest in only one tactic (NRMs; Bowley et al. 2010).

6.4 THIRD AIM: MOVEMENT MODULATED BY SEX AND CONTAMINATION

In a laboratory assay designed to elicit exploratory behaviour in a novel environment, male round goby moved more than females (**Chapter 3**), and round goby from highly contaminated habitats moved less than round goby from a less contaminated site (**Chapter 5**). The effects of sex and contamination (low versus high) were additive, similar in magnitude (effect sizes measured as Cohen's f-hat of 0.180 and 0.173, respectively), and as main factors in statistical models, did not interact, allowing their separate consideration (**Figure 6.2**). The laboratory assay did not reveal differences in exploration tendencies between males of alternative reproductive tactics.

In **Appendix B**, I summarize 66 papers spanning four decades of research (1973-2011) that demonstrated toxicant-mediated changes in simple, laboratory-measured spontaneous locomotion across fishes. These papers represent 97 separate experiments on 34 species, primarily salmonids (25 studies), cyprinids (18 studies) and Japanese medaka (14 studies), using 45 different contaminants or

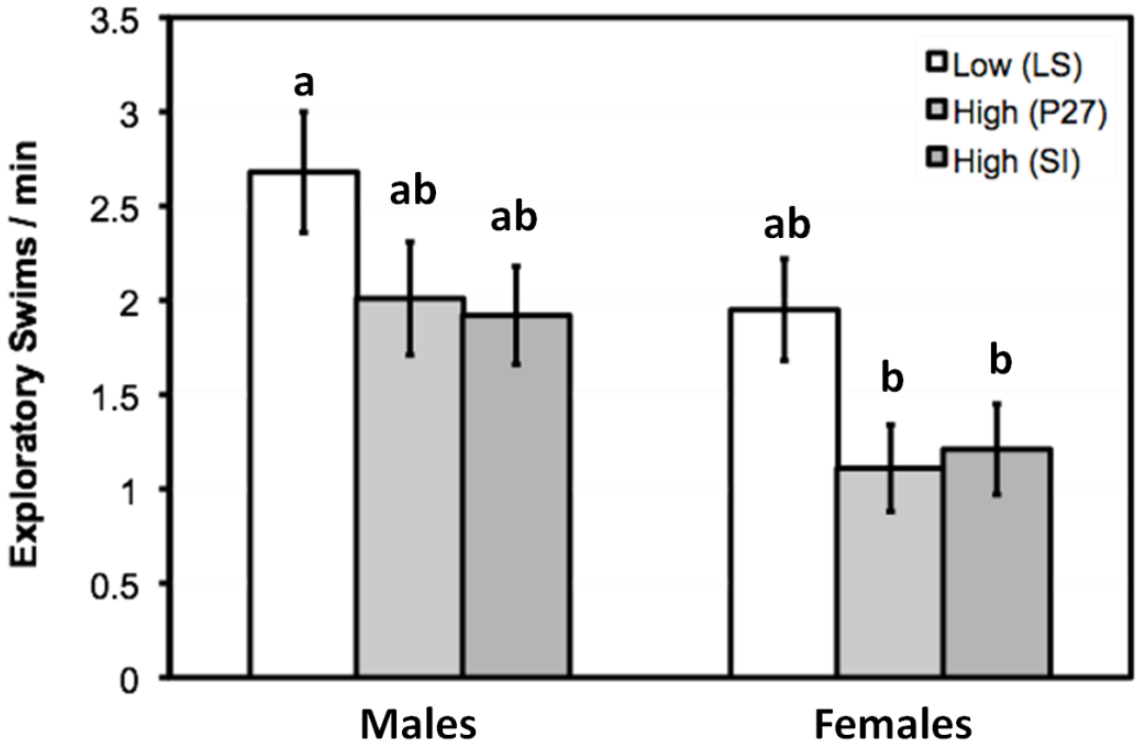

Figure 6.2. Mean (standard error) rates of exploratory swimming for male and female round goby from sites of low (LS) and high (P27, SI) contamination during a behavioural assay of exploration in a novel environment. LS = LaSalle Park. P27 = Pier 27. SI = Sherman Inlet. Data are combined from **Chapters 3** and **5**, and demonstrate that the same sex differences are apparent within each site, and the same site differences are apparent within both sexes. Letters indicate significant differences among groups (Tukey HSD, $p < 0.05$).

complex mixtures. The majority of studies were acute in duration, lasting at most 96 hours (62 studies). Only 11 studies looked at the effect of long-term exposure (greater than one month, up to lifetime; comparable to **Chapter 5**), or the consequences of parental or developmental exposures. Nearly one half (45 studies) reported a decrease in spontaneous locomotion compared to control animals. Approximately one quarter (25) reported an increase, while 15 reported no effect on activity compared to controls and 12 revealed that a given contaminant could produce both increases and decreases in activity that varied with exposure level, duration and the time at which measurements were taken. A comparison of changes in locomotion by contaminant class is given in **Figure 6.3**, showing the extent of variation in behavioural patterns even within studies using the same contaminant.

Round goby from contaminated habitats show lower levels of exploration activity in the laboratory, compared to fish from a reference site (**Chapter 5**). Four comparable studies from **Appendix B** examined the consequences of long-term contaminant exposure to complex mixtures of contaminants either directly or indirectly through parental (i.e., transgenerational) exposure. Three of these also reported decreases in activity (Triebskorn et al. 1997, Zhou and Weis 1999, Candelmo et al. 2010; but see Breckels and Neff 2010 where an increase was reported).

What contaminants might be causing the reduction in round goby activity? Untangling the confounding impacts of multiple long-term contaminant exposure is

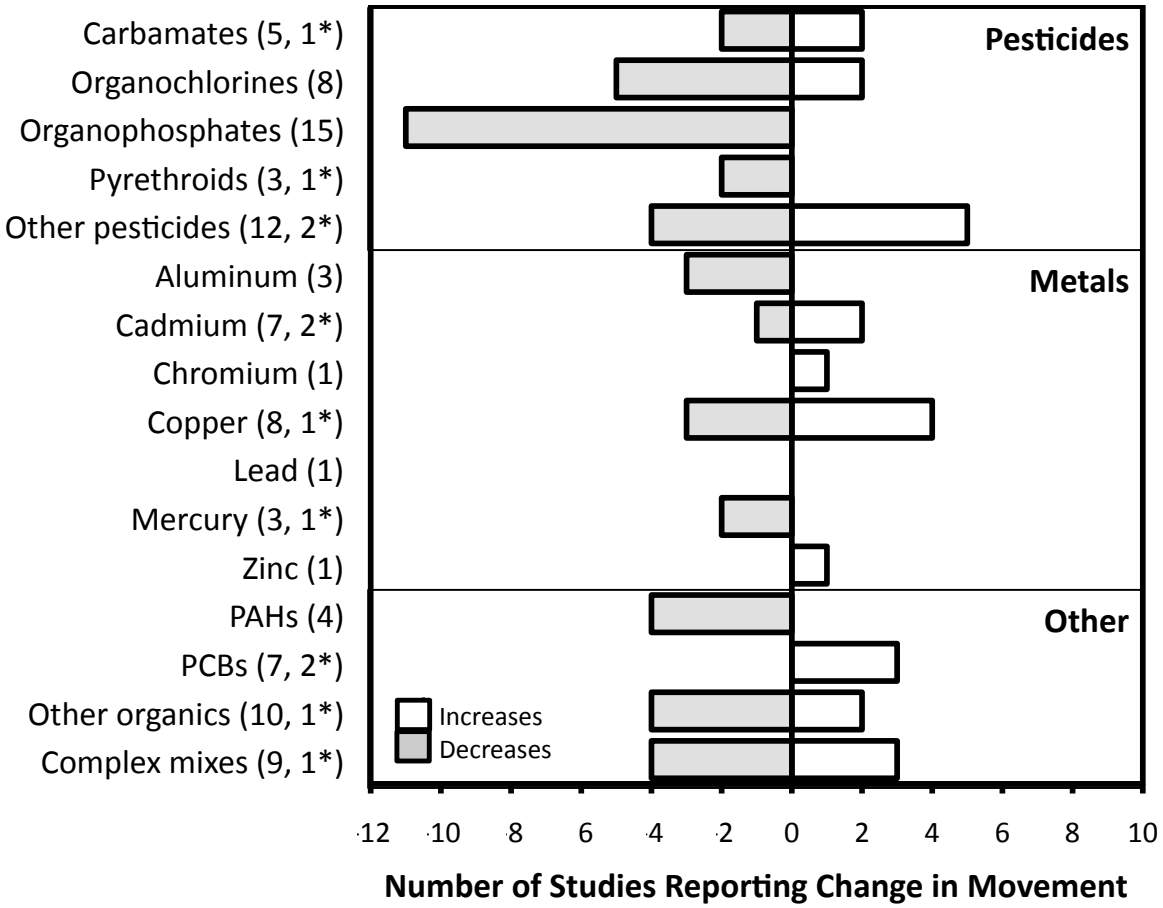

Figure 6.3. Summary of results on movement changes in fishes following contaminant exposure, compiled from of 97 experiments detailed in 66 papers listed in **Appendix B**. Contaminants are grouped broadly by class (pesticides, metals and all other contaminants), and by subclass where appropriate (e.g., carbamate pesticides). Numbers in parentheses indicate the total number of experiments represented in that chemical class, and the number of experiments that indicated context-dependent results (i.e., both increases and decreases in activity level, depending on concentration or duration) are marked with an asterisk (*). Reports of no effect are included in the total number of reports, but are not graphed. Complex mixes: contaminants derived from polluted environments (e.g., sediments) or laboratory-derived combinations of multiple chemical classes.

likely to be a challenging task. However, it is worth noting that of the contaminants known to be at problematic levels in Hamilton Harbour ("A" list contaminants, Hamilton Harbour RAP 2003), PAHs and mercury have been shown to generally reduce activity (Ostrander et al. 1988; Zhou and Weis 1998; Alvarez et al. 2006; Jakka et al. 2007; Gonçalves et al. 2008), while PCBs, cadmium, lead and zinc generally increase or do not affect activity (Ellgaard et al. 1978; Fingerman and Russell 1980; Ellgaard and Rudner 1982; Grillitsch et al. 1999; McCarthy et al. 2003; Scott et al. 2003; Nakayama et al. 2004, 2005; Schmidt et al. 2004, 2005; Honda et al. 2008; Eissa et al. 2010; Couillard et al. 2011).

Simple assays of locomotion, boldness and exploration, such as the one I used in **Chapters 3** and **5** have been used to predict movement patterns (in both home range size and dispersal) over much larger spatiotemporal scales in nature (as discussed in **Section 6.2**). Thus, I had predicted that similar patterns would manifest in the field, in a mark-recapture study, for both sex and contamination. To my knowledge, this represents the first time that contaminant-related changes in locomotion in the laboratory have been used to test predictions about changes to the distance of animal movement in the field. I expected reduced field movements within the nearshore habitats for females compared to males, and for fish in high contamination compared to low contamination areas. While my predictions were met for sex differences across years, they were not for contaminant-related differences.

Why might this have happened? One possibility may lie with the source information on which predictions were originally generated. The prediction for sex differences, that males should move more females, was rooted in evolutionary theory and in comparative studies—that there are certain costs and benefits of movement for sexes across species, and thus selective forces have shaped male and female behaviour differently. The prediction for site contamination differences, that animals from highly contaminated habitats should move less than those from cleaner areas, assumes that the strength of contaminant impairment of behaviour is analogous, at minimum, to the strength of natural selection on behaviour. It ignores the fact that animals may physiologically acclimate to chronic (long-term) toxicant exposure, allowing them to maintain similar scales of behaviour as that for which they have evolved, possibly after an initial period of adaptation. If populations are maintained in a contaminated region for multiple generations, they may also demonstrate an evolved capacity to tolerate levels of contaminants that would impair or kill conspecifics from other regions (e.g., Nacci et al. 1999). A second possibility is that behavioural impairments due to toxicant exposure may only manifest during unusual circumstances, such as a stress challenge or a laboratory assay under highly artificial conditions (see Breckels and Neff 2010). A third possibility is that the scale of field measurements may have been appropriate to distinguish among individuals for one movement context (i.e., sex differences), but not the other (site differences in contaminant exposure). With a larger sample

size, or data collected on either finer or coarser spatial or temporal scales, a different pattern may have been uncovered.

6.5 FUTURE DIRECTIONS

6.5.1 Role of male reproductive tactic in contaminant biomarker expression

One interesting finding in **Chapter 4** was that the ratio of alternative male reproductive tactics in round goby, first described in **Chapter 2**, varied among sites, with the proportion of sneakers increasing with habitat contamination. In addition, expression of the biomarker of endocrine disruption, vitellogenin, was markedly different in guarding versus sneaker males (Bowley et al. 2010), possibly due to their different endocrinological profiles (Bowley et al. 2010, **Chapter 2**). Many fishes used in contaminant monitoring are known to possess alternative male reproductive tactics (e.g., salmonids and centrarchids; Taborsky 1998, Oliviera et al. 2008). Taking the reproductive tactic of males into account in both field and laboratory studies for many species may yield new results and shed light on additional ways that contaminant exposure may impact population dynamics (Young 2009).

6.5.2 Implications of movement differences

Using the conceptual framework for movement ecologists suggested by Nathan and others in 2008, several areas for future research can be identified (**Figure 6.4**). In this dissertation, I looked at the movement of round goby as it related to both external factors (habitat contamination), and internal factors (sex differences; **Figure 6.4a**). However, movement in turn can feed back to affect both internal and external factors (Nathan et al. 2008; **Figure 6.4b**). For example, animals that move out of a contaminated habitat would no longer be continually exposed to contaminants, and individuals that stay within that habitat would experience prolonged exposure. Dispersal could also influence the number of individuals of the same or opposite sex a focal animal experiences. The first half of my thesis, **Chapter 3**, examines one possible driver of the composition of round goby invasion fronts—behavioural differences between sexes. Behavioural correlates predicting dispersal patterns, and hence, invasion fronts, are a newly developing area of interest (Cote et al. 2010b). For the round goby in particular, what proportion of the genetic exchange among populations, and thus also the individuals advancing the round goby invasive range, is affected by sex differences in movement? What scale of movement truly represents a round goby dispersal event? Do other factors, such as personality (i.e., coping style or temperament), adult versus juvenile dispersal, or natural versus human-mediated "dispersal," play more important roles? Do male alternative reproductive tactics (**Chapter 2**) vary in frequency in newly colonized areas compared to well-established populations?

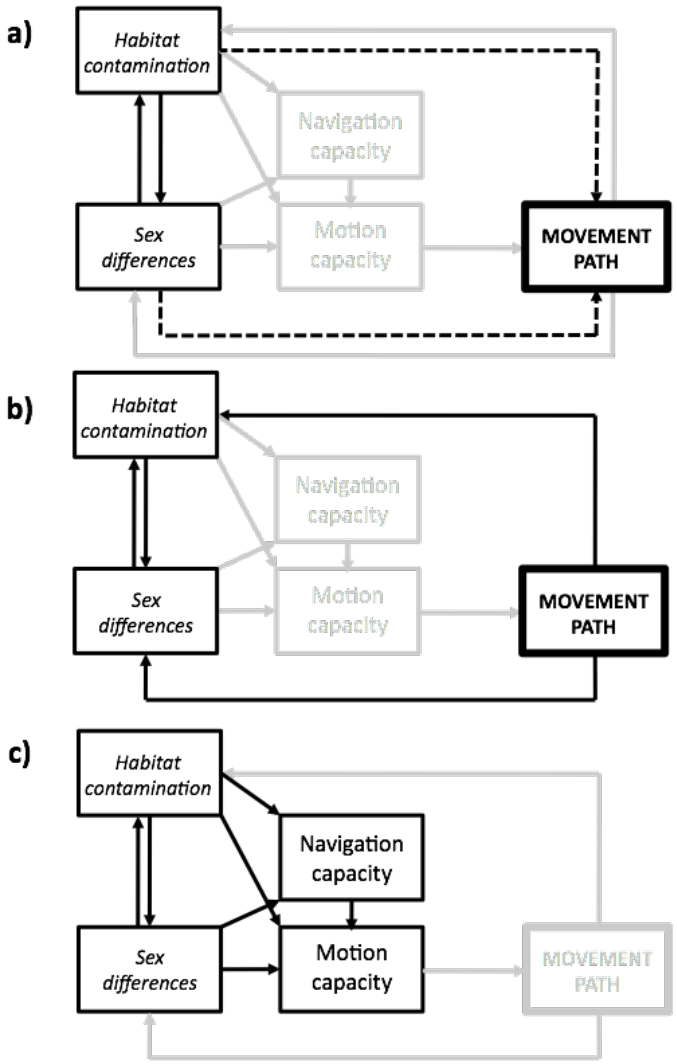

Figure 6.4. **a)** The third aim of this thesis, understanding the dual effects of sex and contamination on movement, placed in the context of the conceptual framework for movement ecology proposed by Nathan et al. 2008. This is identical to **Figure 1.1b**. Arrows indicate the direction of effects; dashed arrows indicate indirect effects. Gray areas indicate sections of the framework not examined in this dissertation. **b)** and **c)** Questions for future research, suggested by this dissertation. **b)** What are the implications of movement differences that feed back to affect internal factors (such as sex) or external factors (such as exposure to contamination)? **c)** Through what aspects of sensory systems, neurology, physiology, morphology and development (together, the navigation and motion capacity) do sex- and contaminant-related variation in round goby movement occur? See text for details.

What effect might this have on population dynamics and sexual selection? My thesis has laid the groundwork to address these questions with round goby.

6.5.3 General mechanisms of contaminant influence on movement

Why do round goby, and other fishes, reduce laboratory activity after long-term exposure to a complex mixture of contaminants (**Figure 6.4c**)? The mechanism(s) underlying the reduced activity and exploration levels seen in **Chapter 5** are still to be elucidated. Untangling the separate effects of individual contaminants may not be a feasible approach, as each location in the field will possess its own unique cocktail with a composition that may vary annually, seasonally or even daily among different sampling events. It may be more appropriate to search for common ground, a "movement impairment syndrome" among different field conditions and different taxa. Is a reduction in movement associated most frequently with structural damage to fins or gills? Is it associated with a change in metabolic rate or increased cortisol levels? What role might sensory impairment play in altering locomotor patterns? These are all questions worthy of future inquiry.

6.5.4 Movement: biomarker, pious hope?

While changes in activity level are one of the most frequently reported behavioural impacts in toxicology, and appear to be an easily-measured, reliable biomarker of contaminant exposure in many studies (Little and Finger 1990,

Bayley 2002; **Appendix B**), the utility of this measure in understanding impacts on wild animals, and therefore on populations, has yet to be determined. The inference that all changes to behaviour are detrimental to survival, because they are by definition deviations from an adaptive optimum (Peakall 1996), is not likely to be valid for all behaviour and perhaps not for movement behaviour specifically. In **Chapter 5**, I examined one way that spontaneous locomotion should affect movement of individuals in the field, using theory and methodology derived from traditional behavioural ecology. My approach in this thesis is not the only way forward, however. Spontaneous locomotion should also affect foraging success, ability to avoid or escape predators, or move in spatial scales both smaller and larger than I was able to address in **Chapter 5** with a mark-recapture study. Alternative methodologies such as PIT tags or acoustic tags may yield contaminant-related differences in long-distance dispersal or more finely-measured home ranges, not just in round goby but a range of sentinel taxa.

6.6 GENERAL CONCLUSIONS

Round goby possess a polygamous mating system characterized by male parental care, at least two alternative male reproductive tactics, and greater long-term movement in males than females. Although natural adult dispersal may play a limited role in round goby population dynamics compared with human-mediated or passive larval dispersal, it does imply that invasion fronts moving upriver would be biased toward adult males. The same laboratory behavioural assays that work

well in understanding sex differences in movement may or may not allow us to probe how contaminant-induced impairments in simple locomotion act to impair movement in the field, and thus affect populations. Round goby are a hardy, pollution-tolerant fish that could serve as a useful sentinel species of habitat contamination in many areas around the Great Lakes.

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Appendix A

A.1 SEX DIFFERENCES IN FISH MOVEMENT

Sex differences in movement and space use across fishes, with respect to whether the study occurred during or outside the breeding season (BRS), or whether it occurred over multiple seasons or years (long-term). LB = livebearing; SH = simultaneous hermaphrodite; SRR = sex-role reversed species. *depends on male reproductive tactic.

Duration of Study	Measured	Mating System (Parental Care)	Who Moves More?	Species	Citation
long-term	movement during colonization	polygamous (none)	males	Coho salmon (*Oncorhyncus kisutch*)	Anderson and Quinn 2007
	relatedness (proxy for dispersal)	polygamous (males)	males	Threespine stickleback (*Gasterosteus aculeatus*)	Cano et al. 2008
	relatedness (proxy for dispersal), dispersal	polygamous (none)	neither	Atlantic salmon (*Salmo salar*)	Consuegra and García de Leániz 2007
	relatedness (proxy for dispersal)	polygamous (none)	neither or trend to males	Walleye (*Sander vitreus*)	Dupont et al. 2007
	dispersal	polygamous (none)	neither	Trinidad killifish (*Rivulus hartii*)	Fraser et al. 2001, Gilliam and Fraser 2001
	relatedness (proxy for dispersal)	polygamous (none)	males or females; scale-dependent	Brook trout (*Salvelinus fontinalis*)	Fraser et al. 2004
	dispersal	polygamous (none)	males	Brook trout (*Salvelinus fontinalis*)	Hutchings and Gerber 2002

Duration of Study	Measured	Mating System (Parental Care)	Who Moves More?	Species	Citation
	home range	monogamous (male)	females (during BRS); uncertain (outside BRS)	Gobiid *Paragobidon echino-cephalus*	Kuwamura et al. 1993
	dispersal	polygamous (none)	females	Sablefish (*Anoplopoma fimbria*)	Morita et al. 2010
	movements between shelters	monogamous (SRR)	females (during BRS); neither (outside BRS)	Black cardinalfish (*Apogon niger*)	Okuda 1999
	relatedness (proxy for dispersal)	cooperative breeding; monogamous and polygamous (biparental)	large males	Cichlid (*Neolamprologus pulcher*)	Stiver et al. 2004; Stiver et al. 2007
	home range	polygamous (none)	neither or trend to males	Coral trout (*Plectropomus leopardus*)	Zeller 1997
during BRS	dispersal (in lab)	polygamous (none; LB)	males	Mosquitofish (*Gambusia affinis*)	Cote et al. 2010
	emigration among pools	polygamous (none; LB)	males	Guppy (*Poecilia reticulata*)	Croft et al. 2003
	home range	polygamous (none)	territorial males	Sharpnose puffer (*Canthigaster valentini*)	Gladstone 1987
	home range	polygamous (male)	females (once males occupy nests)	Fluvial sculpin (*Cottus pollux*)	Natsumeda 2001
	home range	polygamous (male)	neither	Fluvial sculpin (*Cottus pollux*)	Natsumeda 2007

Duration of Study	Measured	Mating System (Parental Care)	Who Moves More?	Species	Citation
	home range	polygamous (none; SH)	fish acting exclusively male	Barred serrano (*Serranus fasciatus*)	Petersen 1987
	home range	polygamous (none; SH)	fish acting predominantly male	Tobaccofish (*Serranus tabacarius*)	Petersen 1995
	home range	polygamous (none; SH)	fish acting exclusively male	Lantern bass (*Serranus baldwini*)	Petersen and Fischer 1986
	activity	monogamous (male; SRR)	females	Northern pipefish (*Syngnathus fuscus*)	Roelke and Sogard 1993
	pool constancy (proxy for home range)	polygamous (male)	females	Blenniid (*Blennius sanguine-lentus*)	Santos and Almada 1988
	home range stability	polygamous (male)	females	Smallmouth bass (*Micropterus dolomieu*)	Savitz et al. 1993
	home range	polygamous (male)	males* (non-spawning days), females* (spawning days)	Gobiid (*Eviota prasina*)	Sunobe and Nakazono 1999
	home range	polygamous (male)	neither	Gobiid *Rhinogobius* sp. DA	Takahashi 2000
	home range	polygamous (male)	neither; females (once males occupy nests)	Gobiid (*Eviota abax)*	Taru and Sunobe 2002
	home range	monogamous (male; SRR)	females	Australian seahorse (*Hippocampus whitei*)	Vincent et al. 2005

Duration of Study	Measured	Mating System (Parental Care)	Who Moves More?	Species	Citation
outside BRS	activity	polygamous (none)	neither	Atlantic salmon juveniles (*Salmo salar*)	Martin-Smith and Armstrong 2002
	home range	polygamous (male)	males	Fluvial sculpin (*Cottus pollux*)	Natsumeda 2001
	home range	polygamous (male)	males	Gobiid (*Rhinogobius* spp.)	Osugi et al. 1998

A.2 MALE TACTIC DIFFERENCES IN MOVEMENT IN FISHES

Differences in movement and space use by male reproductive tactic across fishes, with respect to whether the study occurred during or outside the breeding season (BRS), or whether it straddled both the breeding and non-breeding seasons, or multiple years (long-term). LB = livebearing; SRR = sex-role reversed species. Species-specific terms for male tactics are used in each study. *indicates a tactic that exploits the parental care or courtship efforts of other males. ζ difference inferred but statistical results not given.

Duration of Study	Measured	Mating System (Parental Care)	Who Moves More?	Species	Citation
during BRS	home range	polygamous (none)	territorial males > bachelor males*	Sharpnose puffer (*Canthigaster valentini*)	Gladstone 1987
	home range	Polygamous (male)	nesting = sneakerζ	Gobiid (*Asterro-pteryx semi-punctata*)	Manabe et al. 2009
	home range	monogamous (biparental)	pirate males* > pairing males	Cichlid (*Telmato-chromis temporalis)*	Mboko and Kohda 1999
	home range	polygamous (female)	Pirates* > territorial & satellite > sneaker*ζ	Cichlid (*Telmato-chromis vitattus)*	Ota and Kohda 2006

Duration of Study	Measured	Mating System (Parental Care)	Who Moves More?	Species	Citation
	home range	polygamous (none; SH)	males > hermaphrod -ites*	Barred serrano (*Serranus fasciatus*)	Petersen 1987
	pool constancy (proxy for home range)	polygamous (male)	parental males = small mature males*	Blenniid (*Blennius sanguino-lentus*)	Santos and Almada 1988
	home range	polygamous (male)	nest-holders > trappers (non-spawning days), trappers > nest-holders (spawning days)	Gobiid (*Eviota prasina*)	Sunobe and Nakazono 1999

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Appendix B

B.1 CONTAMINANT EFFECTS ON FISH MOVEMENT

Contaminant effects on the quantity of fish spontaneous locomotion. A = adult, J = juvenile, L = larvae and SA = subadult. * denotes exposures that are representative of environmentally relevant levels. Exposure durations range from min (minutes), h (hours), d (days), w (weeks) and mo (months).

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
decrease	% time still, burst, slow swimming	Rainbow trout (*Oncorhynchus mykiss)*	J	Al (30 ug/L*)	32 d	Allin and Wilson 1999
decrease	% time still, burst, slow swimming	Rainbow trout *(O. mykiss)*	J	Al (36 ug/L*)	4 d + pre-acclim.	Allin and Wilson 2000
decrease	velocity	European seabass (*Dicentrarchus labrax*)	J	Fenitrothion (2 mg/L)	96 h	Almeida et al. 2010
increase	velocity (overall and active), % time active, path complexity	Red drum (*Sciaenops ocellatus)*	L	Atrazine (40 ug/L*)	96 h	Alvarez and Fuiman 2005
none	velocity (overall and active), % time active, path complexity	Red drum *(S. ocellatus)*	L	Malathion (n/a*)	96 h	Alvarez and Fuiman 2006
decrease	velocity (overall and active), % time active, path complexity	Atlantic croaker (*Micropogonias undulatus)*	L	MeHg (0.05 mg/kg* (maternal))	Parental (1 mo)	Alvarez et al. 2006

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
decrease	velocity	Zebrafish (*Danio rerio)*	A	Microcystin (15 ug/L)	17 d	Baganz et al. 2005
increase	velocity at night only	Sunbleak (*Leucaspius delineatus)*	A	Microcystin (0.5 ug/L)	17 d	Baganz et al. 2005
decrease	velocity, % time moving (in day only)	Rio de la Plata onesided livebearer (*Jenynsia multidentata)*	A	Endosulfan (0.072 ug/L*)	48 h	Ballesteros et al. 2009
decrease	velocity, distance, no. of turns	Rainbow trout *(O. mykiss)*	L	Diazinon (250 ug/L), malathion (20 ug/L)	24-96 h + recovery	Beauvais et al. 2000
increase	movement/ min	Threespine stickleback (*Gasterosteus aculeatus)*	SA	EE_2 (100 ng/L*)	lifetime (175-245 d)	Bell 2004
increase	Volitional distance	Brown bullhead (*Ameiurus nebulosus*)	A and J	River sediment (n/a*)	Lifetime and/or 24 h	Breckels and Neff 2010
decrease	no. of fish moves between zones	Goldfish *(Carassius auratus)*	J	Carbofuran (500 ug/L)	24-48 h	Bretaud et al. 2002
decrease	velocity, turns and distance	Rainbow trout *(O. mykiss)*	J	Malathion (20 ug/L) diazinon (250 ug/L)	24-96 h	Brewer et al. 2001
none	% fish swimming	Guppy (*Poecilia reticulata)*	A	PCP (n/a*)	30 d	Brown et al. 1985
decrease	feeding, social and comfort movements	Coho salmon (*Oncorhynchus kisutch)*	J	fenitrothion (Sumithion) (0.1 ppm)	96 h	Bull and McInerney 1974

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
decrease	velocity (at night)	Rainbow trout *(O. mykiss)*	J	Cu (726 mg/kg*)	12 w	Campbell et al. 2002
decrease	velocity	Bluefish (*Pomatomus saltatrix*)	J	Prey from polluted river	4 mo	Candelmo et al. 2010
increase (night and day) or decrease (day)	velocity, % time moving	Rio de la Plata onesided livebearer (*J. multidentata)*	A	Microcystin (0.01 ug/g)	24 h	Cazenave et al. 2008
decrease	observer described	Rainbow trout *(O. mykiss)*	J	PCAs (12 ug/g)	21 d	Cooley et al. 2001
increase	velocity	Mummichog (*Fundulus heteroclitus*)	L	PCB126 (50 pg/egg*)	egg stage (12 d)	Couillard et al. 2011
increase and none (*A. fasciatus*)	velocity and no. of moves	Common carp (*Cyprinus carpio*), *Australoheros facetum*, *Astyanax fasciatus*	J	Cd (0.6 mg/L)	96 h	Eissa et al. 2010
increase, no recovery	rate constant calculated from dispersal between chambers A and B	Bluegill (*Lepomis macrochirus)*	J	DDT (0.008 ppb)	16 d + 2 w recovery	Ellgaard et al. 1977
increase (all), and decrease (Cd)	rate constant calculated from dispersal between chambers A and B	Bluegill *(L. macrochirus)*	J	Cd (0.1 mg/L) Cr (0.05 mg/L) Zn (0.1 mg/L)	2 w	Ellgaard et al. 1978

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
none	rate constant calculated from dispersal between chambers A and B	Bluegill *(L. macrochirus)*	J	Pb (n/a)	96 h	Ellgaard and Rudner 1982
decrease	velocity, amt. time active	Atlantic croaker *(M. undulatus)*	L	DDT (20 ug/kg*)	Parental (1 mo)	Faulk et al. 1999
decrease	movements	Striped bass (*Morone saxatilis)*	L	river water (n/a*)	96 h	Finger and Bulak 1988
increase	lines crossed	Gulf killifish (*Fundulus grandis)*	A	PCBs (4 ug/mL)	24 h	Fingerman and Russell 1980
decrease	% abnormal	Fathead minnow (*Pimephales promelas)*	L	Esfenvalerate (0.455 ug/L*)	4 h	Floyd et al. 2008
decrease	% time active	Gilthead seabream (*Sparus aurata*)	J	PAHs fluorene, phen-anthrene, pyrene (0.04 to 0.26 uM)	96 h	Gonçalves et al. 2008
decrease	distance travelled	Japanese medaka (*Oryzias latipes)*	L	Endosulfan (0.1 ug/L*)	24 h	Gormley and Teather 2003
increase and decease	velocity	Zebrafish *(D. rerio)*	SA	Cd (0.01 mg/L)	96 h	Grillitsch et al. 1999
none or increase	lines crossed	Japanese medaka (*O. latipes)*	L	methyl parathion, molinate, carbofuran (4050 ug/L (molinate & mix)*)	96 h	Heath et al. 1993

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
decrease	% time active	Matrinxã (*Brycon amazonicus*)	J	Cd (9 ug/L)	96 h	Honda et al. 2008
decrease	observer classified	Florida pompano (*Trachinotus carolinus)*	J	ethylene glycol (2.1%*)	24 h + 15 h recovery	Hymel et al. 2002
decrease	velocity, distance	Mosquitofish (*Gambusia affinis)*	A	$HgCl_2$ (20 ug/L)	4 w	Jakka et al. 2007
decrease	distance	Zebrafish (*D. rerio*)	L	Bifenthrin (10 ug/L)	30 min	Jin et al. 2010
increase	velocity	Atlantic silverside (*Menidia menidia)*	A and SA	Cu (100 ug/L)	1.5 h	Koltes 1985
decrease	velocity, movement between fresh, saltwater	Pacific salmon (*Oncofrhynchus* spp.)	J	TCMTB (10 ug/L*)	36-96 h	Kruzynski et al. 1994
decrease	amt. time active	Rainbow trout *(O. mykiss)*	J	Carbaryl (1 mg/L) chlordane (0.0002 mg/L) 2,4-DMA (5 mg/L) DEF (0.005 mg/L) methyl parathion (0.01mg/L) PCP (0.002 mg/L)	96 h	Little et al. 1990
none	velocity (overall and active), % time active	Atlantic croaker *(M. undulatus)*	L	PCBs (n/a*)	Parental (2 w)	McCarthy et al. 2003

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
none	velocity, frequency of swimming, entropy	Japanese medaka *(O. latipes)*	A	TBTO, PCBs (1 ug/g each)	3 w	Nakayama et al. 2004
increase	velocity, frequency of swimming, entropy	Japanese medaka *(O. latipes)*	J	PCBs (25 ug/g)	21 d	Nakayama et al. 2005
decrease	% fish inactive	Rainbow trout (*O. mykiss*)	J	Al (500 ug/L)	26 h	Ogilvie and Stechey 1983
increase	displays and forages	Coho salmon *(O. kisutch)*	L	PAH (B[*a*]P) (25 ug/mL*)	24 h	Ostrander et al. 1988
decrease	compartment entries, orientation angles	Goldfish *(C. auratus)*	A	Parathion (0.33 ppm)	24 h	Rand 1977
decrease	distance and velocity	Mosquitofish *(G. affinis)*	A	Chlorpyrifos (60 ug/L)	20 d	Rao et al. 2005
decrease	Grid lines, time cross line	Zebrafish (*D. rerio*)	A	EE2 (25 ng/L)	2 w	Reyhanian et al. 2011
increase (per, phen), decrease (chl, str, per, phen) and none (DNP)	observer classified	Japanese medaka *(O. latipes)*	J	Chlorpyrifos (0.2 mg/L) Permethrin (0.01 mg/L) Phenol (15 mg/L) Strychnine (1 mg/L) 2,4-DNP	48 h	Rice et al. 1997
increase	burst swim frequency	Goldfish *(C. auratus)*	J	atrazine, diuron (0.5 ug/L each)	24 h	Saglio and Trijasse 1998
increase	burst swim frequency	Goldfish *(C. auratus)*	J	Carbofuran (1 ug/L*)	4-12 h	Saglio et al. 1996
decrease	velocity	Coho salmon *(O. kisutch)*	J	Chlorpyrifos (0.05 ug/L*)	96 h	Sandahl et al. 2005

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
decrease	chamber entries, time in compartments, velocity	Pinfish (*Lagodon rhomboids)*	A	Cu (0.1 mg/mL)	72 h	Scarfe et al. 1982
decrease	chamber entries, time in compartments, velocity	Atlantic croaker (*M. undulatus)*	A	Cu (0.1 mg/mL)	72 h	Scarfe et al. 1982
increase	chamber entries, time in compartments, velocity	Sheepshead (*Archosargus probatocephalus)*	A	Cu (0.1 mg/mL)	72 h	Scarfe et al. 1982
increase	chamber entries, time in compartments, velocity	Hardhead sea catfish (*Ariopsis felis)*	A	Cu (0.1 mg/mL)	72 h	Scarfe et al. 1982
Decrease (day) and increase (night)	velocity	Common carp (*Cyprinus carpio)*	J	PCBs (14 ug/L*) TBT (0.3 ug/L*)	21 d	Schmidt et al. 2004
decrease (day) and increase (night)	velocity	Common carp *(C. carpio)*	J	PCBs, TBT (mix of 10 and 4 ug/L respectively)	1-2 d	Schmidt et al. 2005
none	% time active	Chinook salmon *(Oncorhynchus tshawytscha)*	J-A	Diazinon (n/a*)	2 h	Scholz et al. 2000
none	no. line crosses, time in shelter, latency to feed, feeds	Rainbow trout *(O. mykiss)*	J	Cd (n/a*)	1-7 d	Scott et al. 2003

Change in Activity	Measured	Species	Age	Toxicant (Minimum effective exposure)	Exposure Duration	Citation
increase and decrease	chamber entries, time in compartments, velocity	Hardhead sea catfish (*A. felis)*	A	Cu (0.01 mg/L*)	72 h	Steele 1983
increase	chamber entries (day)	Hardhead sea catfish (*A. felis)*	A	Cu (0.1 mg/L*)	72 h	Steele 1989
none	distance moved	Atlantic salmon (*Salmo salar)*	J	fenitrothion (Sumithion) (0.1 ppm)	15-16 h	Symons 1973
increase	time and distance at each of three speeds	Rainbow trout *(O. mykiss)*	J	TBTO (0.5 ug/L)	7-21 d	Triebskorn et al. 1994
decrease	time and distance at each of three speeds	Brown trout (*Salmo trutta)*	J	stream water (n/a*)	8-21 w	Triebskorn et al. 1997
none	velocity	Rainbow trout *(O. mykiss)*	J	4-nonylphenol (n/a*)	5 d	Ward et al. 2006
increase	distance, no. of turns	Goldfish *(C. auratus)*	J	DDT (1 ppb*)	1 - 7 d	Weis and Weis 1974a
increase	distance travelled	Atlantic silverside (*M. menidia)*	J	Sevin (carbaryl) (100 ppb)	24-72 h	Weis and Weis 1974b
increase and decrease (depending on site)	lines crossed, time in motion	Mummichog (*F. heteroclitus)*	L	MeHg (6 ug/L*)	embryonic and/or larval	Zhou and Weis 1998
decrease	grid lines crossed, time moving	Mummichog *(F. heteroclitus)*	L	polluted parental habitat (n/a*)	parental	Zhou and Weis 1999

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