IMPACT OF ENVIRONMENTAL CONTAMINANTS ON SPERM

IMPACT OF ENVIRONMENTAL CONTAMINANTS ON SPERM

By

NATALIE M. SOPINKA, BSc

A Thesis

Submitted to the School of Graduate Studies

In Partial Fulfilment of the Requirements

For the Degree

Master of Science

McMaster University

MASTER OF SCIENCE (2010) McMaster University

(Psychology) Hamilton, Ontario

TITLE: Impact of Environmental Contaminants on Sperm

AUTHOR: Natalie M. Sopinka, B.Sc. (McMaster University)

SUPERVISOR: Professor S. Balshine

NUMBER OF PAGES: i-xi, 79

ABSTRACT

Aquatic contaminants are known to negatively impact reproductive behaviour and physiology. However, the influence of chronic exposure to aquatic contaminants on gamete traits has largely been explored using species with simple mating systems. In this thesis I explored how sperm is impacted using wild caught round gobies (*Neogobius melanostomus*) and plainfin midshipman (*Porichthys notatus*), two species that inhabit bodies of water polluted with industrial and urban contaminants, and also exhibit complex mating systems with male alternative reproductive tactics (MARTs). Species with MARTs generally have two male morphs: a guarding male morph that courts females and aggressively defends a territory, females and/or young, and a sneaking male morph that does not court females or defend a territory but instead parasitizes territories held by guarding males and simply sheds sperm within the nests. Guarding males are larger, older and remain fairly stationary in/near their nests for long periods of time are likely to have had greater exposure to contaminants compared with non-sedentary younger, smaller sneaker males that roam from nest to nest. These behavioural and physiological differences may result in differential impacts or exposure to contaminants between male mating tactics. Thus, species with MARTs living in polluted environments make excellent models to investigate interactive effects of contaminant exposure and mating strategy on gametes. Using round gobies from clean and contaminated areas of Hamilton Harbour, Lake Ontario I show that interactions between sites (clean versus contaminated) and male morphs (guarding versus sneaker) on testicular investment, relative sperm tail length and sperm velocity are limited. Few interactions between site and tactic suggest that guarding and sneaker male round goby sperm are equally affected by

environmental factors or that both tactics are not influenced by contaminants. Both guarding and sneaker round gobies appear resilient to complex combinations of anthropogenic based pollutants. Using plainfin midshipman populations that inhabit areas close versus distant to sources of industrial and urban effluents, I show that living in contaminated areas influences gametic quality of guarding males. Guarding males from contaminated areas occupied nests with greater proportions of dead eggs, had greater testicular asymmetry and shorter sperm heads. Exploration of how these findings correlate with sperm velocity is forthcoming. Collectively my results show that extended exposure to aquatic contaminants can alter gametic characteristics but that these alterations appear to have similar effects across male mating tactics.

ACKNOWLEDGEMENTS

I first thank my supervisor Dr. Sigal Balshine whose passion for research will forever inspire me; thank you for your encouragement and support. Many thanks to Dr. John Fitzpatrick who (despite being 25 000 km away) taught me everything I know about imaging sperm and was always willing to share invaluable advice on all things sperm related. Thanks to Mathew Voisin for graciously measuring sperm and Stephanie Tong and Grace Wang for collecting round gobies. Thank you Julie Marentette and Vicky Mileva for listening to my banter. To Susan Marsh-Rollo whose immeasurable knowledge of the lab and life made each day of this degree possible. To Dr. Joanna Wilson and Dr. Dave Feinberg for serving on my committee. Thank you Jonathan Taves for making sure I returned home from Vancouver Island in one piece. Thank you Matt Taves for making sure I stayed in one piece upon arriving home, undoubtedly a more challenging feat than expected. I am indebted to my mother, Alicia Sopinka, who bravely coped with the moodiest grad student to ever exist. And many thanks to Michael Sopinka, Halina Szczawinski, Dave Rollo, Mary Telford, Kay Palmer, Sarah Bazinet, Nicole Staresina, Lindsay Dores, Bill Morrison, Elenor and Don Taves, Diana Carbone, Christina Gould and Jan Lewandowski for always giving me reasons to smile.

THESIS ORGANIZATION AND FORMAT

The organization of this thesis consists of two chapters. **Chapter 1** introduces the background theory and experimental evidence motivating this study, the study species, research aims, results and discussion. **Chapter 2** includes a theory focussed introduction, a description of my second study species, methods and a preliminary results section. The thesis concludes with a brief overview of how my results relate to other studies within the field of behavioural environmental toxicology and with suggestions for promising avenues of future research that would provide further support for my predictions.

CHAPTER 1: **Male alternative reproductive tactics and contaminants: the impacts of environmental toxicants on round goby sperm**

Authors: Natalie M. Sopinka, Mathew R. Voisin, Susan E. Marsh-Rollo, J.R. Marentette and Sigal Balshine

Publication: Manuscript will be submitted to Canadian Journal of Zoology

Comments: This manuscript is based on work by NMS and MRV. NMS collected sperm samples, analyzed sperm velocity and was responsible for writing the manuscript under the supervision of SB. MRV measured sperm morphology under the supervision of NMS. SMR and JRM provided assistance with data collection during all field seasons (2006-2009).

CHAPTER 2: **Living in contaminated areas: gametic consequences in the plainfin midshipman**

Authors: Natalie M. Sopinka, John L. Fitzpatrick, Jonathan E. Taves, Aaron Chang, Susan E. Marsh-Rollo and Sigal Balshine

Publication: Manuscript will be submitted to Environmental Biology of Fishes

Comments: This manuscript is based on work by NMS, JET and AC. NMS and JET collected sperm and egg samples. JLF provided guidance on data collection and analyses. AC analyzed egg number and quality under the supervision of NMS and SB. NMS measured and analyzed sperm morphology and was responsible for writing the manuscript under the supervision of SB. SMR provided remote field coordination and assistance with field season logistics.

TABLE OF CONTENTS **Page**

LIST OF FIGURES **Page**

LIST OF TABLES

CHAPTER 1

MALE ALTERNATIVE REPRODUCTIVE TACTICS AND CONTAMINANTS: THE IMPACTS OF ENVIRONMENTAL TOXICANTS ON ROUND GOBY SPERM

1.1 SPERM COMPETITION THEORY

A male's reproductive success is dependent on his sperm fertilizing a female's egg(s). Therefore males are favoured by natural selection if they produce a sufficient number of motile sperm capable of successfully reaching and fertilizing eggs. However, in many species male-male competition is prevalent, and sexual selection is further exaggerated as a result of sperm competition (when sperm from different males compete for access to a female's eggs; Parker 1970). As a result, in species were the risk of sperm competition is great, the evolution of sperm traits are influenced by both natural and sexual selection. For example, males experiencing sperm competition are expected to produce more competitive ejaculates that are made up of greater numbers of larger, faster swimming sperm (Parker 1990a, b; Ball & Parker 1996; Parker & Ball 2005) than males that do not experience sperm competition. This is because sperm number, size and swimming speed influence the probability of out-competing sperm from rival males (Lahnsteiner et al. 1998; Levitan 2000; Gage & Morrow 2003; Gage et al. 2004; Snook 2005; Casselman et al. 2006). Larger sperm, with longer flagella (tails, indicating more propulsive force) and midpieces (indicating more mitochondria and energy production, Cardullo & Baltz 1991; Cummins 1999) are expected to swim faster than are smaller sperm (Gomendio & Roldan 1991). These theoretical predictions have prompted

numerous empirical studies across taxa yielding important insights on the interconnectedness between sperm competition and the functional significance of sperm traits.

Evidence for the aforementioned theoretical predictions that link sperm competition, morphology and motility is not without criticism. Empirical studies demonstrate that high sperm competition selects for more, longer, faster moving sperm (reviewed in Stockley et al. 1997; Balshine et al. 2001; Burness et al. 2004; Fitzpatrick et al. 2007; 2009; 2010; Gomendio & Roldan 2008). However, Humphries et al. (2008) argue that the link between sperm morphology and speed is not as straightforward as Gomendio & Roldan (1991) suggest in their study (see above) that did not control for phylogeny and was limited to only a few species. Using biophysical models Humphries et al. (2008) argue that absolute lengths of sperm components may not predict sperm swimming speed as accurately as do relative lengths, which are more likely to be targeted by selection. Specifically, the ratio between sperm tail length and sperm head length would be the most appropriate metric to use when studying the correlation between sperm morphology and motility in external fertilizers (Humphries et al. 2008). Despite Humphries et al. (2008)'s criticisms, comparative and intra-specific studies across a wide range of species have managed to show links between sperm morphology and motility. Sperm with longer heads (Malo et al. 2006), longer midpieces (Lüpold et al. 2009; Firman & Simmons 2010) and longer sperm tails (Fitzpatrick et al. 2009; 2010; Lüpold et al. 2009; Mossman et al. 2009) swim faster. Therefore, recent papers provide strong support for the theory that predicts sperm morphology will relate to sperm velocity and hence, predictions on what factors might influence these relationships can be logically

made.

1.1.2 EXPERIMENTAL EVIDENCE OF CONTAMINANT MEDIATED EFFECTS ON MALE GONADS AND SPERM

Aquatic contaminants can have reverberating effects on the development of male testes and gametes (Kime 1995; Kime 1999; Vos et al. 2000; Jobling & Tyler 2003). Evidence for intersex (presence of eggs in testicular tissue) is found in Mozambique tilapia (*Oreochromis mossambicus*) collected from areas sprayed with the pesticide DDT (di-chloro-diphenyl-trichloroethane, Marchand et al. 2008) and in white sucker (*Catostomus commersonii*) collected downstream from a wastewater treatment plant (Vajda et al. 2008). White sucker collected from these same waters (Vajda et al. 2008), and areas contaminated with bleached kraft mill effluent (BKME, McMaster et al. 1992) also had reduced gonad size. Yellow perch (*Perca flavescens*) collected from metal contaminated lakes exhibited reductions in relative gonad size (Levesque et al. 2002). Additionally, contaminant-mediated changes in gonad development, the site of sperm production, are also associated with altered sperm characters (Gill et al. 2002). Highly motile, normally shaped sperm have much better chances of fertilizing eggs, making sperm morphology and motility excellent tools for measuring toxicant mediated changes in male reproductive success (Kime 1996; Kime & Nash 1999). Exposures of contaminants reduce sperm counts (Haubruge et al. 2000; Bayley et al. 2002) and impair sperm morphology and sperm motility (Table 1). Males from a wide variety of aquatic species exposed to contaminated water produce sperm with abnormally shaped heads, structurally damaged mitochondria located in the midpiece, shorter flagellum and reduced velocity (Table 1). Such dramatic changes in sperm morphology and

performance are evident after relatively long or even short exposure to contaminants. For example, in the sea urchin *Anthocidaris crassispina*, males chronically exposed for 4 weeks to 0.1 mg/L of cadmium exhibited reduced sperm motility (Au et al. 2001b) and short or broken sperm tails (Au et al. 2001a). A brief 60-minute exposure to cadmium (5-10 mg/L) or phenol (500 mg/L) resulted in irregular sperm midpiece shape and disorganization of mitochondrial cristae within the midpiece (Au et al. 2000). For males experiencing sperm competition such alterations in sperm morphology and motility can compromise the ability of sperm to out compete the sperm from rival males.

1.1.3 MOTIVATION FOR THE INTERACTIVE EFFECTS OF POLLUTION AND MARTs ON SPERM

Investigations on how sperm traits are affected by "real world exposures" or polluted environments, where animals may experience chronic exposures to cocktails of contaminants, are limited (but see McMaster et al. 1992; Gill et al. 2002; Kleinkauf et al. 2004; Abdelmeguid et al. 2007 for notable exceptions). Furthermore, the extent to which contaminants influence sperm in the context of sperm competition has yet to be explored. Among fish, species with male alternative reproductive tactics (MARTs) are a convenient model for studying the interactive effects of sperm competition and differential exposure to contaminants. In species with MARTs, males adopt different reproductive tactics when mating: large conventional males court and guard females and repel competitors, while smaller sneakers males do not court females but instead simply release sperm into the nests of conventional males. These differences in male mating behaviours create an asymmetry in the risk of sperm competition. Conventional guarding males, who mate in

1.6 TABLE 1

Effects on sperm morphology and motility following field and laboratory exposures to various toxicants in 17 teleost species, an urchin, oyster and mussel. For comparison, sediment concentrations of toxicants found in contaminated areas of Hamilton Harbour are 1.71-6.17 μg/g (PCBs), 157-499 μg/g (PAHs), and greater than 197 μg/g (copper), 820 μg/g (zinc), 250 μg/g (lead), and 3.5 μg/g (cadmium) and 2 μg/g (mercury; Milani & Grapentine 2006). Substances listed under Toxicants refer to the contaminants used during laboratory exposures or the contaminants wild populations were exposed to in their environments. When more than one toxicant is listed this indicates that separate exposures were conducted for each toxicant.

Toxicants	Exposure Concentration/Period	Species	Sperm Morphology	Sperm Velocity	Reference
Metal					
methyl-mercury	0.01-0.05 mg/L 2 or 5 minutes	Killifish (*Fundulus heteroclitus*)	•no differences	decreased but initial speed faster in contaminated fish	Khan & Weis 1987
cadmium, zinc	0-2000 mg/L instant, 24 h	African catfish (*Clarias gariepinus*)	•not reported	decreased	Kime et al. 1996
cadmium	0.1-0.01 mg/L 4 weeks	Sea urchin (*Anthocidaris crassispina*)	•short, broken tails •mitochondria cristae malformed •plasma membrane convoluted	decreased	Au et al. 2001a; Au et al. 2001b
mercury	0.001-0.1 mg/L instant 24 h	Goldfish (*Carassius auratus*)	•increased head length, width and area	decreased	VanLook & Kime 2003
mercury, copper, lead	0.01-100 mg/L Instant	Sea bass (*Dicentrarchus labrax*)	•short, broken tails at 0.4 ppm	no difference but complete arrest at 100 ppm	Abascal et al. 2007

Toxicants	Exposure Concentration/Period	Species	Sperm Morphology	Sperm Velocity	Reference
copper	100 ug/L 3 minutes	Blue mussel (*Mytilus trossulus*)	•no differences	decreased	Fitzpatrick et al. 2008
Organic					
bleached kraft mill effluent (BKME)	wild populations	White Sucker (*Catostomus commersoni*)	•not reported	decreased	McMaster et al. 1992
tributylin (TBT) bisphnol A (BPA)	11.2-22.3 ng/L TBT 274-549 ug/L BPA 21 days	Guppies (*Poecilia reticulata*)	•no differences	not reported	Haubruge et al. 2000
genistein	0-1000 μg/g in diet 365 days	Rainbow trout (*Oncorhynchus mykiss*)	•not reported	decreased	Bennetau-Pelissero et al 2001
tributylin (TBT)	27 ug/L 24 h	African catfish (*Clarias gariepinus*)	•ATP content decreased	decreased	Rurangwa et al. 2002
		Common carp (*Cyprinus carpio*)			

Toxicants	Exposure Concentration/Period	Species	Sperm Morphology	Sperm Velocity	Reference
estradiol (E2)	50 ng/L 10 weeks	Goldfish (*Carassius auratus*)	•not reported	decreased	Schoenfuss et al. 2002
TBT	0.1, 1 ng/L 70 days	Zebrafish (*Danio rerio*)	•missing tails	decreased	McAllister & Kime 200
PAHs	0, 50 100, 200 μg/L 30 days	Pacific oyster (*Crassostrea gigas*)	•not reported	decreased	Jeong & Cho 2005
DDT	wild populations	*Clarias gariepinus* *Oreochromis mossambicus*	•not reported	decreased	Marchand et al. 2008
17α-ethinylestradiol (EE2)	60–480 ng/L 21 days	Japanese medaka (*Oryzias latipes*)	•not reported	increased	Hashimoto et al. 2009
genistein, β-sitosterol	1 μg/L 21 days	Siamese fighting fish (*Betta splendens*)	•not reported	no differences	Stevenson et al. 2010

Toxicants	Exposure Concentration/Period	Species	Sperm Morphology	Sperm Velocity	Reference
Metal and/or Organic					
cadmium, phenol	5-10 mg/L Cd 500 mg/L phenol 60 minutes	Sea urchin (*Anthocidaris crassispina*)	•disorganized mitochondria with fewer cristae •irregular, rigid and less electron dense midpieces	decreased	Au et al. 2000
raw sewage, industrial effluent, metals, PAHs, PCBs	wild populations	Flounder (*Platichthys flesus*)	•big heads and fuzzy tails	not reported	Gill et al. 2002
sewage effluent	flow through 10 weeks	Goldfish (*Carassius auratus*)	•not reported	no differences	Schoenfuss et al. 2002
raw sewage, industrial effluent, metals, PAHs, PCBs	wild populations	Flounder (*Platichthys flesus*)	•not reported	increased	Kleinkauf et al. 2004
copper, cadmium, zinc, cyclohexane	5,25,75,100 mg/L respectively instant	Brown trout (*Salmo trutta fario*)	•not reported	increased	Lahnsteiner et al. 2004

Toxicants	Exposure Concentration/Period	Species	Sperm Morphology	Sperm Velocity	Reference
mercury, cyclohexane, 2,4-dichlorophenol	50,100,100 mg/L respectively instant	Chub (*Leuciscus cephalus*)	•not reported	increased mercury decreased	Lahnsteiner et al. 2004
mercury, cyclohexane, lead	25,100,300 mg/L respectively instant	African catfish (*Clarias gariepinus*)	•not reported	decreased cyclohexane increased	Lahnsteiner et al. 2004
raw sewage, industrial effluent, metals, PAHs, PCBs	wild populations	Tilapia (*Oreochromis niloticus*)	•malformed heads •mitochondrial degradation •degeneration of tails	not reported	Abdelmeguid et al. 200
wastewater effluent	sperm activated with 10, 50,100% effluent	Goldfish (*Carassius auratus*)	•not reported	decreased	Schoenfuss et al. 2008

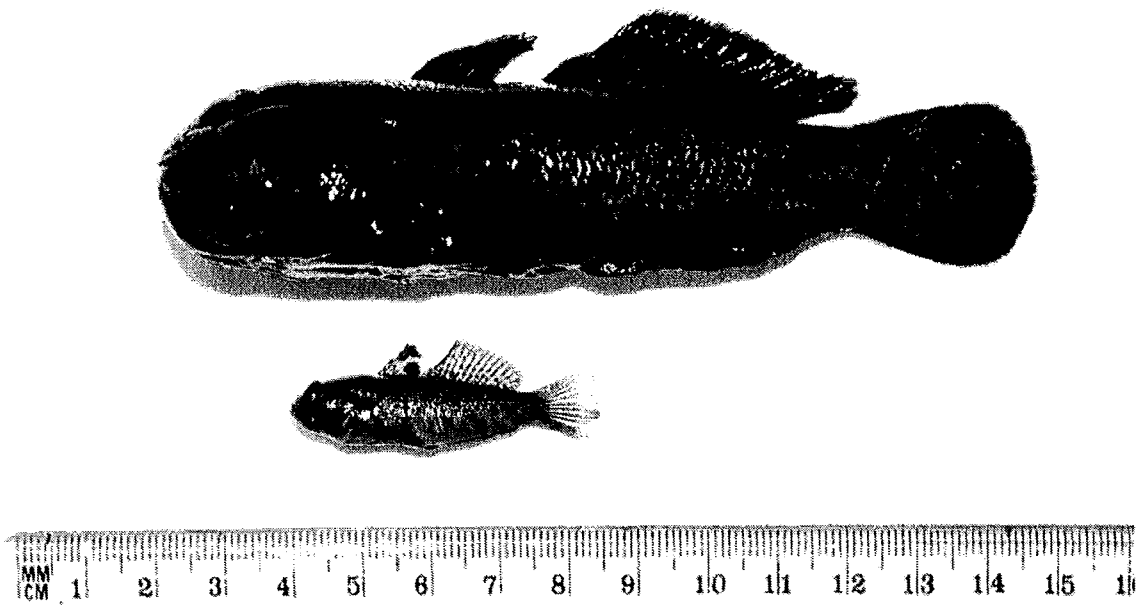
both the presence and absence of sneakers, experience a relatively lower risk of sperm competition compared to sneaker males who always reproduce in the presence of competitors and therefore experience a constant and thus greater risk of sperm competition (Snook 2005). Moreover, the behavioural differences between male morphs are also likely to influence the degree of contaminant exposure and ultimately degree of reproductive abnormality observed in their sperm. This is because guarding male morphs are larger, older, and relatively sedentary while they protect their territory and provide parental care to young throughout the breeding season (Taborsky 2008). In contrast, sneaker males are smaller and often younger, they also move frequently among nests occupied by a guarding male (Waltz 1982). Important physiological differences are also evident between male tactics. Guarding males have larger relative liver investment at the beginning of the breeding season (Malavasi et al. 2004; this study) and toxicants (PCBs, trace metals) readily accumulate in fatty tissues of this organ (Newman & Clements 2008). Guarding males are thought to cease feeding during territory guarding (Miller 1984), and such starvation can initiate the mobilization of energy stores from the liver, potentially concentrating tissue burden further (Jørgensen et al. 1999). All of these factors would suggest that guarding males would have higher contaminant burdens than sneakers. Thus, both the risk of sperm competition and contaminant-mediated changes to sperm morphology and motility are likely to be different in males adopting the different mating tactics.

1.1.4 NATURAL HISTORY OF THE ROUND GOBY, *NEOGOBIUS MELANOSTOMUS*

The model species used in this study, the round goby, is an invasive benthic fish

FIGURE 1

Large, guarding male (top fish) and small sneaker male (bottom fish). Photograph by J.R. Marentette.



native to the Ponto-Caspian region of Europe (Jude et al. 1992) that exhibits male alternative reproductive tactics (Marentette et al. 2009; Figure1). Two types of male morphs have been identified in populations invading the Laurentian Great Lakes; 1) a large, darker morph, that has secondary sexual characteristics such as a swollen head, and guards nests and young (Miller 1984; Marentette & Corkum 2008; Marentette et al. 2009), and 2) a smaller, lighter morph called a sneaker because it does not exhibit secondary sexual characteristics (Marentette et al. 2009; Figure 1), and sneaks fertilizations instead of guarding females/nests (C. Murphy, University of Alberta, personal communication). Sneaker males have relatively larger investment in testicular tissue (Marentette et al. 2009). In the laboratory the dark guarding morph readily occupies and controls a shelter and protects young (Meunier et al. 2009, Marentette personal communication). Note, that while there is ample morphological and endocrine support for the existence of these two morphs, the behaviour of guarding and sneaker males in the wild has yet to be fully investigated.

Population modelling indicates that round gobies have inhabited Hamilton Harbour, an International Joint Commission (IJC) Area of Concern, since 1994 (Vélez-Espino et al. 2010). As a result of their limited mobility and small home range (5 m^2 Wolfe & Marsden 1998; Ray & Corkum 2001), round gobies living in areas of the Harbour near the steel industry and wastewater treatment facilities are exposed to a combination of PCBs (poly-chlorinated biphenyls), PAHs (polynuclear aromatic hydrocarbons), other polychlorinated compounds, as well as numerous metals (arsenic, copper, iron, lead, zinc, cadmium) found in sediments, water and their diet (Hamilton RAP 1992; IJC 1999; Hamilton RAP 2003; Zeman & Patterson 2003; Hamilton RAP

2008; Hamilton RAP 2009; Zeman 2009). Chronic exposure to these toxicants has resulted in populations of smaller fish with higher metal contaminant body burdens, and a higher incidence of fin erosion (Marentette et al. in press). Fish from these highly contaminated areas also demonstrate disrupted locomotion, altered predator avoidance and lower rates of active aggression (Marentette unpublished data, Sopinka et al. in press). Compared to sneakers, older, larger guarding round goby males may move around less (although see Discussion) and are likely to have been in contact with contaminated sediment for longer periods of time while defending their nests. Round gobies inhabiting Hamilton Harbour thus make excellent models to study how exposure to metal and organic contaminants influence sperm dynamics in a species with MARTs.

1.1.4 AIM AND PREDICTIONS OF THESIS

To date, no studies have investigated whether environmental toxicants modify the quality of sperm in species with male alternative reproductive tactics (MARTs). Establishing whether male morphs vary in relation to contaminants could provide critical insights on how environmental conditions can impact population dynamics for round gobies as well as for other species with MARTs. Hence, the aim of this thesis was to examine the impact of contaminant exposure on sperm morphology and motility of large guarding and small sneaker round gobies collected from clean and contaminated study sites. Sperm motility was assessed using computer-assisted sperm tracking analysis (CASA) and morphological measures quantified from digital images. We predicted that 1) contaminated males would have reduced reproductive investment compared to clean males, 2) males from contaminated areas would have shorter sperm tails (relative to sperm

head length) and shorter sperm midpieces resulting in slower swimming sperm and 3) that sedentary, larger guarding morphs from contaminated areas would show greater sperm impairment and a greater reduction in testicular investment patterns compared with the roving sneaker males.

1.2 METHODS

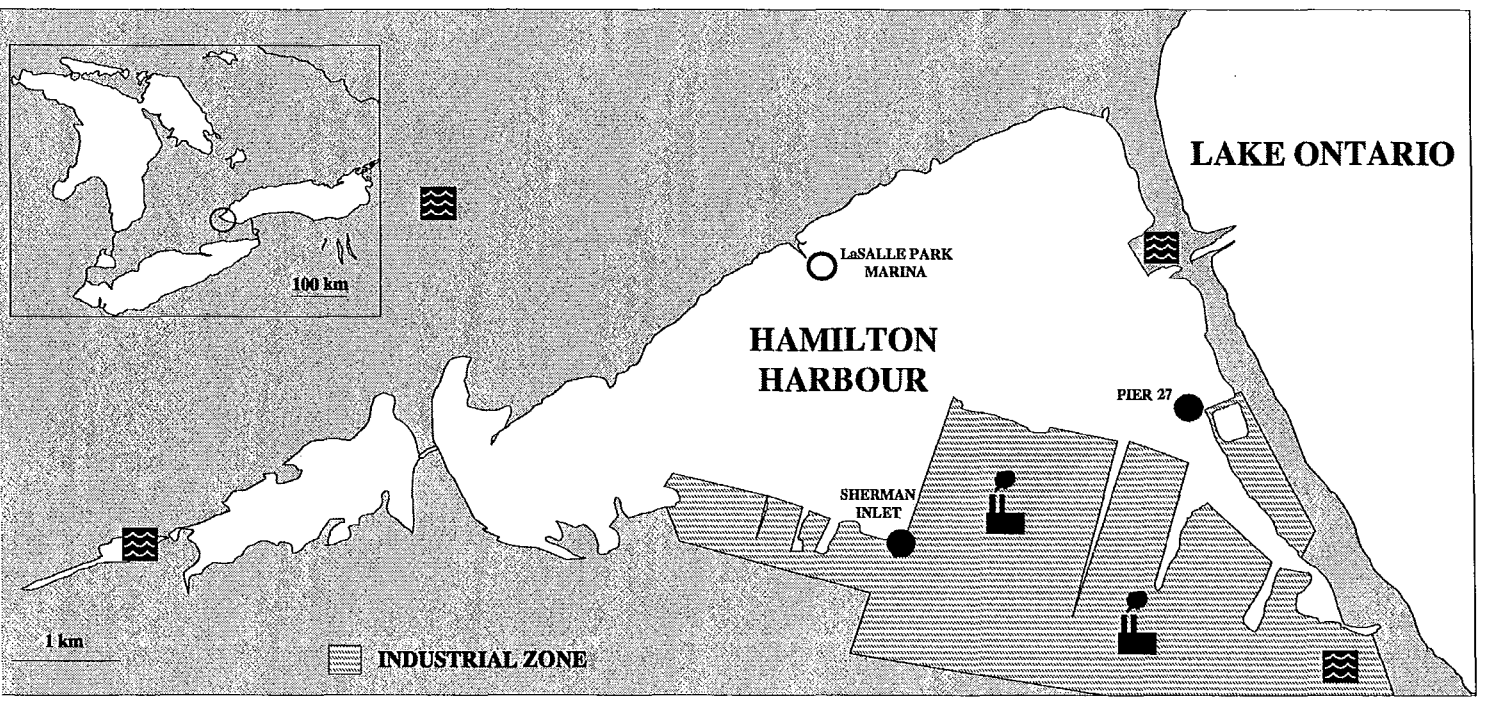
1.2.1 ANIMAL COLLECTION

Between 3 May and 21 August 2009, 84 reproductive male round gobies were caught with baited minnow traps from three sites in Hamilton Harbour, Lake Ontario: LaSalle Park Marina (43°18'03N, 079°50'45W), Pier 27 (43°16'53N, 079°47'32W) and Sherman Inlet (43°16'11N, 079°50'02W). LaSalle Park Marina is located on the northern shore of the Harbour across the bay (~ 3 km) from the industrial sector and was designated as the cleaner site (see Figure 2). Pier 27 along the eastern shore of the Harbour, is an area exposed to waste water treatment plant effluents and high levels of PCBs in water and sediment. Contamination in this area is also significant as a result of ship traffic dredging up historical contaminants from the nearby Harbour's steel and iron industries (Zeman & Patterson 2003; Hamilton RAP 2008; Zeman 2009). Sherman Inlet is located on the southern industrial shoreline (Figure 2), in close proximity to Randle Reef (~ 200 m), an area characterized by a PAH laden coal tar discharge and identified as Canada's second most PAH-contaminated sediment site (Hamilton RAP 1992). For further details about the sites and trapping procedures see Young et al. (2010) and Marentette et al. (in press).

Fish collected were sexed externally based on examination of the urogenital papillae (Miller 1984). All males were brought back to McMaster University and held in

FIGURE 2

Contaminated (filled circles) and cleaner (open circle) sites in Hamilton Harbour, Lake Ontario. Contaminated sites are located within the industrial areas of the Harbour (hatched shading). Steel and iron mill industry ( ), waste-water treatment plants ( ) and 23 combined sewer overflow facilities characterize this area.



site-matched male-only groups in tanks (61.0 cm long x 30.5 cm wide x 35.6 cm high) for an average of 2 days (range 2-7 days). Compared to slowly metabolized PCBs (whole body half-life = 55-145 days, Buckman et al. 2006) and metals (liver half-life of zinc > 75 days, cadmium = 75 days, Kraemer et al. 2005), PAHs are metabolised and eliminated quickly (whole body half life of PAHs <1 day, Djomo et al. 1996). As the fish were typically held for two days prior to processing concentrations of metals or PCBs were not likely to significantly decrease throughout the holding duration. Any negative impacts on sperm uncovered would have been attributed to differential burdens of metals and PCBs but not PAHs, which are rapidly eliminated. Note, round gobies spawn multiple times within a breeding season (April to September, Jude et al. 1992) suggesting that spermatogenesis was occurring throughout the collection period. Spermatogenesis takes approximately 30 days in some teleost fish (Billard 1986), therefore the sperm sampled in this study were likely produced while fish were still in their exposed habitats. There was a maximum of six fish per holding tank and the fish experienced a 15:8 hour light:dark cycle and water temperatures between 22 -24°C. Fish were fed commercial fish flakes (Nutrafin Basix Staple Food) daily up until the day of processing.

The male reproductive morphs were distinguished using a combination of body colouration (black versus mottled), head width (wide versus narrow), relative length of the genital papilla (short versus long) and relative testicular mass (small versus large) and accessory gland (large versus small) somatic index. Compared to dark guarding males, sneaker males are mottled, smaller (total body length), with narrower head widths and relatively longer papilla (Marentette et al. 2009). The testicular (TSI) and accessory gland somatic index (AGSI) were calculated upon dissection for each fish (see below), where

TSI=total testes mass (g) /total body mass (g)-total testes mass (g) x 100% and AGSI = total accessory gland mass (g)/ body mass (g) – total testes mass (g) x 100%. We assigned guarding male status to any male in this study with a TSI of approximately 1.25 % and AGSI of 0.78% or greater, following Marentette et al. (2009). Sneaker male status was assigned to males with a TSI of 4.31% or greater and AGSI of approximately 0.34%. Guarding morphs were on average larger (mean standard length of fish = 8.2±0.2 cm, range 5.6-11.7 cm; mean body mass = 13.37±0.97 g, range 4.66-33.99 g) than sneaker males (5.3±0.1 cm, range 4.1-7.3 cm; 3.72±0.36 g, range 1.85-9.55 g, t-test: length, $t=131.24$, $N=84$, $P<0.0001$; mass, $t=131.20$, $N=84$, $P<0.0001$).

1.2.2 SPERM COLLECTION

All reproductively active male round gobies (57 guarding morphs and 27 sneaker morphs) were weighed to the nearest 0.001 g and measured for total length, standard length, head width and papilla length to the nearest 0.01 cm. Following submersion in ice water, fish were rapidly decapitated. The liver, testes and accessory glands were removed and weighed to the nearest 0.001 g. A testicular somatic index (TSI) and accessory gland somatic index (AGSI) were calculated for each fish (see above). To obtain sperm an incision was made in the anterior portion of the testis and dabbed with a 10μl microcapillary tube.

1.2.3 SPERM MORPHOLOGY

To analyze sperm morphology, a drop of sperm from 29 guarding morphs and 20 sneaker morphs was placed in 1.5 ml microcentrifuge tubes, diluted with 1 ml of filtered water and fixed with 20 μl of 10% formalin buffer. Using a Prosilica EC-650 digital camera mounted on a light microscope (400x magnification), an image was taken of

visible spermatozoa (20 sperm images per male). Sperm head, midpiece and tail/flagellum length (end of midpiece to end of tail) were measured to the nearest 0.1 μm using ImageJ (v. 1.42q, available at http://rsb.info.nih.gov/ij/). Measurements were calculated by drawing a freehand line over each sperm section using an Intuos graphic table (Wacom Co. Ltd., Japan). For each male, an average length of each sperm piece was calculated from the 20 images.

1.2.4 VIDEO RECORDING AND ANALYSIS

To analyze sperm velocity, a drop of sperm was immediately activated with 100μl of filtered water in 1.5 ml microcentrifuge tube. Water used for all analyses was obtained from LaSalle Park Marina (the cleaner site) on 21 July 2009 and filtered with a 0.22μm filter. Clean water was used in order to examine effects of long-term exposure on sperm rather than immediate effects of polluted water on sperm. A 60μl sub-sample of the sperm and water mixture was extracted and immediately dispensed on a welled slide (1-mm deep) covered by half a cover slip. The welled slide was then placed under the microscope.

Video capture of sperm under pre-focused 200X magnification began at the instance of sperm activation. Videos were recorded under phase contrast with a Prosilica EC-650 digital camera (Prosilica, Burnaby, British Columbia, Canada) mounted on an Olympus CX41 microscope (Olympus America Inc.). Videos were recorded using Astro IIDC (v. 4.04.00) software at 60 frames/second. Sperm velocity was measured using the CEROS (v.12) video analysis program (Hamilton-Thorne Research, Beverly, Maine, USA). Smooth path velocity (VAP, velocity of the smoothed path of sperm cell) and curvilinear velocity (VCL, point by point track followed by sperm cell) for each male

round goby was calculated (μm/s) at 10 one-second intervals; 20, 30, 45, 60, 90, 120, 180, 240, 360 and 480 seconds after sperm began swimming.

1.2.5 STATISTICAL ANALYSES

Statistical analyses were performed using the statistical package JMP (version 5.0.1, 2002; SAS Institute Inc., Cary, NC). All data were tested for normality and body, soma, testicular, accessory gland and liver mass were log transformed to achieve normality. Parametric statistics were used throughout. Preliminary analyses revealed that relative testicular investment (ANCOVA: : $F_{1,515}$=0.52, P=0.47), sperm size (t-test: t=2.50, N=29, P=0.13) and velocity (Repeated Measures ANOVA: $F=_{1,349}$=0.01, P=0.90) did not vary between the two contaminated sites, Sherman Inlet and Pier 27, and thus the two sites were combined for all further analyses. Relative testicular, accessory gland and liver investment was calculated between guarding and sneaker males from clean and contaminated sites for each male tactic using an ANCOVA with Site (clean versus contaminated) and Tactic (guarding versus sneaker) as fixed effects and soma mass as a covariate. Soma mass was calculated as total body mass (g) – tissue (testicular, accessory gland or liver) mass (g) x 100%. Long-term relative testicular investment was also investigated by using a dataset for comparison of guarding and sneaker males collected from clean and contaminated areas during July and August in 2006, 2007 and 2008. A two-way ANOVA was used to calculate body condition across Site and Tactic. Body condition was calculated using the formula, Fulton's body condition index = body mass/total body length^3. Following Humphries et al. (2008), a ratio of sperm tail length to sperm head length was calculated. A two-way ANOVA was then used to evaluate Site (clean versus contaminated) and Tactic (guarding versus sneaker) differences in the ratio

of tail:head and absolute sperm length measures (head, midpiece and flagellum). In other external fertilizers, sperm VAP and VCL are highly correlated and both positively relate to fertilization success (Au et al. 2002). A principal components analysis (PCA) was used to obtain scores based on these two measures. The PCA returned two principal Components; PC1 explained 99% of the variance and PC2 explained 1% of the variance, thus PC1 was used as the independent variable in subsequent analyses. A repeated measures mixed model with Site, Tactic and Time as fixed effects and Fish ID as a random effect was used to assess site and tactic differences in sperm velocity. The number of days a fish spent in the lab did not affect sperm velocity (ANOVA: $P>0.30$). Non-significant interactions ($P>0.05$) were removed from all models. Correlations between sperm velocity and sperm morphology were investigated using Pearson correlations. Tukey's HSD tests were used to determine post-hoc differences between sites. Different letters on graphs are based on theses analyses and denote statistical differences ($P<0.05$).

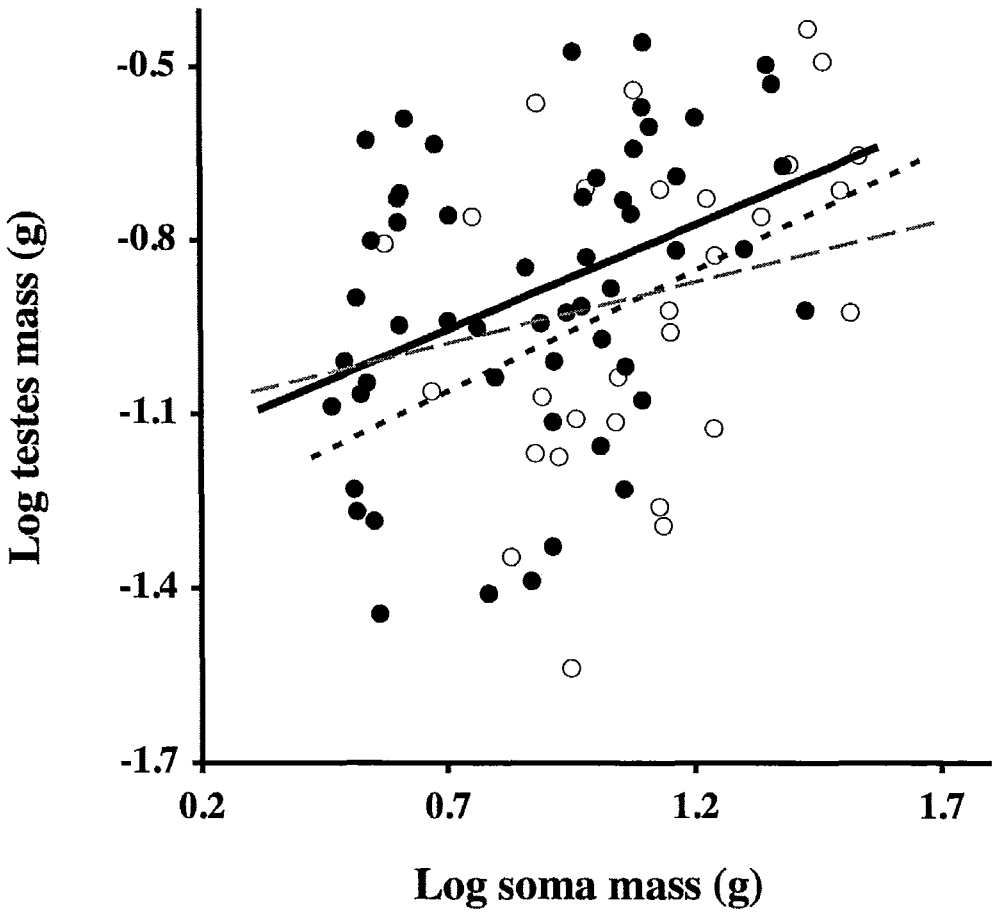
1.3 RESULTS

1.3.1 REPRODUCTIVE INVESTMENT

Guarding males were heavier (Two-way ANOVA: Tactic, $F_{1,81}=117.96$, $P<0.0001$) and longer (Tactic, $F_{1,81}=119.43$, $P<0.0001$) than sneaker males. Males from cleaner sites were heavier (Site, $F_{1,81}=7.81$, $P=0.01$) and longer (Site, $F_{1,81}=10.60$, $P=0.002$) than males from contaminated sites. Controlling for body size, males collected from contaminated areas had greater testicular investment compared to males from clean areas (ANCOVA: Site, $F_{1,80}=4.93$, $P=0.03$; Figure 3). As expected, sneaker males had greater testicular investment than guarding males (Tactic, $F_{1,80}=26.65$, $P<0.0001$; Soma Mass, $F_{1,80}=47.46$, $P<0.0001$). Guarding males had greater accessory gland investment (Tactic, $F_{1,74}=5.26$,

FIGURE 3

Regression of log transformed testicular investment in fish (controlling for male tactic) collected from clean (open circles, dotted line) and contaminated (filled circles, solid line) sites. Three year average investment for guarding and sneaker male fish from clean and contaminated areas in Hamilton Harbour is denoted by a grey dashed line and was calculated from a multi-year population monitoring dataset (Marentette et al. unpublished data). These testicular mass data were collected during the months of July and August from 2006 to 2008.



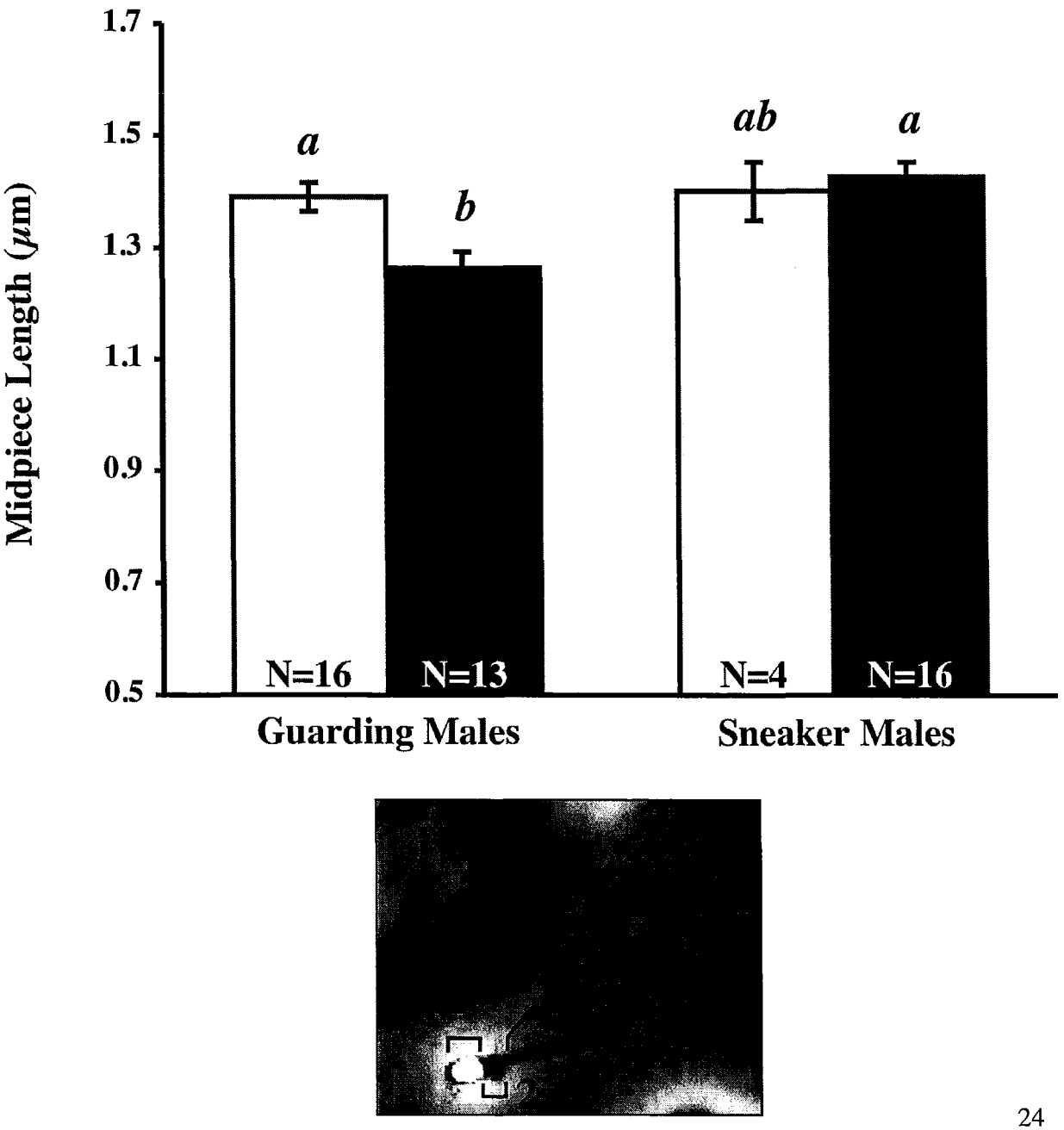
P=0.02; Soma Mass, $F_{1,74}$=53.93, P<0.0001) but this investment did not differ across sites (Site, $F_{1,74}$=0.02, P=0.90). Liver investment (controlling for body size) did not vary between males from clean and contaminated sites (Site, $F_{1,76}$=1.44, P=0.23). Compared to sneaker males, guarding males had relatively larger livers (Tactic, $F_{1,76}$=6.02, P=0.02; Soma Mass, $F_{1,76}$=64.65, P<0.0001). Sneakers (Two-way ANOVA: $F_{1,81}$=7.28, P=0.09) and in general contaminated fish ($F_{1,81}$=5.58, P=0.02) were in better body condition compared to guarding males and cleaner fish, respectively. For each of these models the interaction between site and tactic was not significant (P>0.05) indicating that males adopting one or the other reproductive tactic responded in a similar manner.

1.3.2 SPERM MORPHOLOGY

Interestingly, guarding males collected from contaminated areas had smaller midpieces than guarding males from clean sites (ANOVA: Site, $F_{1,46}$=2.04, P=0.16; Tactic, $F_{1,46}$=6.11, P=0.02; Site x Tactic, $F_{1,45}$=4.68, P=0.04; Figure 4). Sperm midpiece size did not correlate with absolute or relative (sperm tail length: head length) sperm tail length (all Ps>0.05). Sneaker males had longer sperm heads than guarding males (Tactic, $F_{1,46}$=3.96, P=0.05) but head length did not differ across sites (Site, $F_{1,46}$=0.01, P=0.94). Absolute tail length did not vary between tactics or site (Tactic, $F_{1,46}$=0.16, P=0.69; Site, $F_{1,46}$=0.29, P=0.59). Perhaps most importantly, males (guarding or sneaker) collected in clean versus contaminated areas also did not differ in their ratio of sperm head to tail length (ANOVA: Site, $F_{1,45}$=0.18, P=0.67; Tactic, $F_{1,45}$=1.22, P=0.27). Again, no significant interaction was observed between site and tactic again suggesting that guarding and sneaker males are either not impacted by the contaminants found at these sites, or respond in a similar way to the environmental differences between collection sites.

FIGURE 4

Comparison of mean sperm midpiece length (μm, ±SE) of guarding and sneaker male fish collected from clean (open bars) and contaminated (filled bars) areas of Hamilton Harbour, Lake Ontario. Bottom image depicts round goby sperm 1) head, 2) midpiece and 3) flagellum.



1.3.3 SPERM VELOCITY

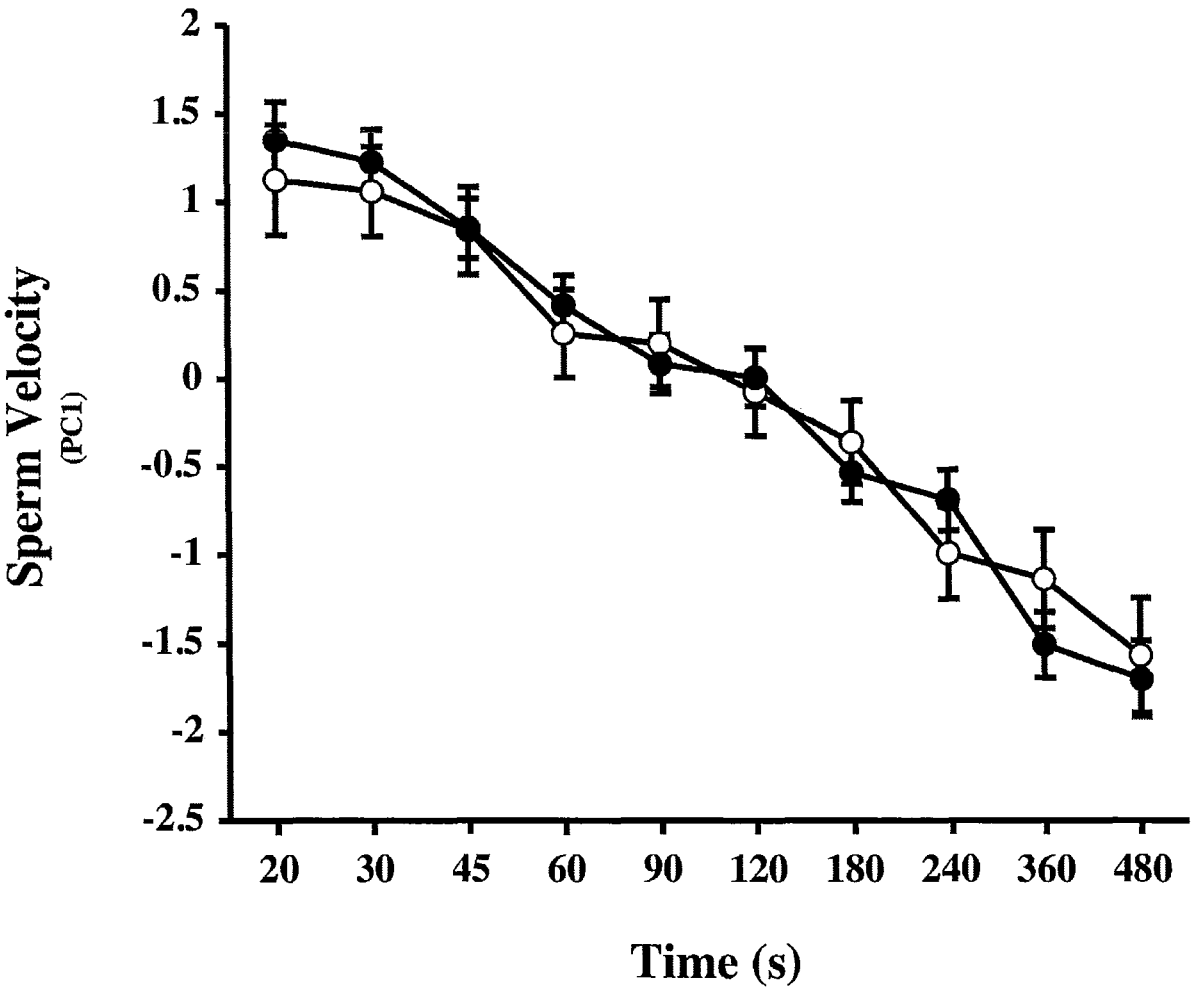
Sperm velocity decreased with time (Repeated Measures ANOVA: Time, $F_{1,515}$=70.38, P<0.0001). Sperm velocity showed a decrease toward the end of the breeding season (Julian Date $F_{1,515}$=6.35, P=0.01) but sperm velocity did not differ between clean and contaminated guarding or sneaker males (Site, $F_{1,515}$=0.01, P=0.93; Figure 5). Guarding male sperm velocity also did not differ from sneaker male sperm velocity (Tactic, $F_{1,515}$=0.30, P=0.59). Sperm head, midpiece and tail length did not correlate with sperm velocity (all Ps>0.05).

1.4 DISCUSSION

Guarding males from contaminated areas had smaller sperm midpieces relative to guarding males from cleaner areas. These differences in midpiece size could have been important because this is the part of sperm that supplies energy to the cell in the form of ATP (adenosine triphosphate), however no site differences in overall sperm velocity were observed. Theoretical models predict that mitochondria volume in mammals is positively correlated with sperm tail length (Cardullo & Baltz 1991) and midpiece size predicts sperm swimming speed in house mice (*Mus domesticus*, Firman & Simmons 2010). In our study, sperm midpiece size did not correlate with sperm velocity or sperm tail length (absolute and relative). Assessing sperm ATP content, rather than relying on midpiece size as a proxy for ATP, is likely to provide a better quantification of the cell's energy supply (Perchec et al. 1995). In Atlantic salmon (*Salmo salar*), midpiece ATP content positively correlated with sperm tail length (Vladić et al. 2002). Also, Burness et al. (2004) found that ATP content of sperm correlated with initial sperm swimming speed in bluegill

FIGURE 5

Controlling for male tactic, mean sperm velocity (±SE) for fish collected from cleaner (open circles) and contaminated (filled circles) areas of Hamilton Harbour, Lake Ontario. Sperm velocity values were obtained using principal components analysis.



sunfish (*Lepomis macrochirus*). Future studies should also quantify sperm density and longevity in clean versus contaminated round gobies, as these valuable parameters would provide a better understanding of the trade-offs between sperm morphology and velocity (Petersen & Warner 1998).

Other than midpiece length, statistical interactions between Site and Tactic were seldom observed suggesting that, in contrast to our predictions, guarding and sneaker male sperm appear equally altered or similarly unchanged in relation to exposure to the different environmental regimes. It is possible that the contaminated sites did not contain a threshold level of toxicants that would influence sperm. We had originally predicted that tactic differences in behaviours would generate different exposure regimes, however, it is likely that parental and sneaker males do not differ in movement/activity to a degree that would elicit differences in contaminant burden. Studies stemming from our laboratory have not revealed the expected tactic related differences in locomotion (Marentette et al. unpublished data). However, guarding males are larger (Marentette et al. 2009; this study) and older (Marentette et al. unpublished data) and thus would have had longer periods of contaminant exposure and accumulation compared to smaller and younger sneaker males. Although the measures that we recorded did not differ between male tactics, there could be tactic differences in ejaculate investment patterns (e.g. sperm trails). Most male Gobiid fish typically release sperm in viscous, sticky trails on the nest surface, prior to egg release (Marconato et al. 1996). Over periods of time mucins (produced in the accessory glands) in the trail break down and sperm are released into the water and fertilize eggs. Guarding male grass gobies (*Zosterisessor ophiocephalus,* Mazzoldi et al. 2000) and black gobies (*Gobius niger*, Rasotto et al. 2002) produce trails that are more mucin rich, last longer and

release sperm more slowly compared to sneaker male trails that dissolve and release more sperm quickly. Given that mucin producing accessory glands are relatively larger in guarding round gobies (Marentette et al. 2009; this study) and other Gobiid species (Mazzoldi et al. 2000; Rasotto et al. 2002) and their trails are mucin-rich, it is possible that the production and composition of mucins in the accessory glands of these males and the longevity of their sperm trails would have been influenced by contaminant exposure had they been allowed to ejaculate freely. An interaction between collection site and male tactic may have been masked in this study by the use of sperm dissected from the testes and thus, not enriched by mucins. Investigating the velocity of sperm extracted from such sperm trails would further our understanding of the intricate dynamics of sperm velocity in relation to the fertilization success of round gobies mating tactics.

To date, studies of laboratory exposures to contaminants have mainly found reductions or inhibitory effects on sperm velocity (see Tables 1 and 4). Our results were unexpected and are in contrast to most laboratory studies (Table 1). Interestingly, other field studies using wild caught fish have also found contrasting results. First, stripped sperm of wild caught flounder (*Platichthys flesus*) from the contaminated Mersey estuary unexpectedly swam faster relative to sperm from uncontaminated males (Kleinkauf et al. 2004). Like Hamilton Harbour, the Mersey estuary has a history of input from sewage and industrial effluent. Levels of metal contaminants (Collings et al. 1996) and PCB congeners (Leah et al. 1997) detected in fish collected from the Mersey estuary exceeded standard food limits. However, the muscle PCB burden in flounder from the Mersey estuary were considerably lower (0.03 μg/g) than levels found in round gobies from Hamilton Harbour (0.36 μg/g, Hynes/Slater unpublished data). Second, Khan & Weis (1987) report that

baseline sperm velocity collected from killifish (*Fundulus heteroclitus*) inhabiting areas with high levels of methylmercury (meHg, 0.05 mg/L) was higher than the sperm from killifish collected from clean areas. The inconsistencies between field and laboratory results (Table 4) may reflect the complexity and possibly interactive effects of chronic exposure to cocktails of metal and organic contaminants.

There are many interacting factors that could influence the way males invest in testes and production of sperm. First, an animal's social environment could alter reproductive investment. Up-regulation of the reproductive axis could be driven by increases in sperm competition, masking any initial contaminant mediated reductions in testicular and sperm investment. Given that the ratio of sneaker males to guarding males is greater at contaminated sites (SM:GM, clean sites: 1:1.7; contaminated sites; 1:1, Marentette et al. 2009), contaminated fish likely experience greater sperm competition and would have been expected to increase investment in testes, sperm number and sperm velocity (Parker 1990a, b; Ball & Parker 1996; Stockley et al. 1997). Alternatively, up-regulation of the reproductive axis could be directly influenced by contaminant exposure. Miller et al. (1999) found that the gonads of Antarctic fish (*Trematomus bernacchii*) that live in Winter Quarters Bay, a body of water with cargo ship traffic and sediments highly contaminated with PAHs and PCBs, were enlarged relative to the gonads of fish from more remote areas. Similarly, mosquitofish (*Gambusia holbrooki*) collected downstream from a pulp and paper mill exhibited enlarged gonads (Toft et al. 2004), while in mammals, exposure to PCBs (0.4–3.2 mg/day from birth to Day 25) also resulted in enlarged testes (Cooke et al. 1996).

Social environment and contaminant exposure could also simultaneously shape gonadal and sperm traits. Testicular investment was greater in contaminated round gobies suggesting that the negative impacts of contaminants on testis investment were not as pronounced as the potential impacts of reproductive competition. Sperm velocity however did not differ between clean and contaminated round gobies. Contaminant induced reductions in sperm velocity could be counterbalanced via a physiological mechanism induced by increased sperm competition. Miura et al. (1992) show that up-regulation of the reproductive axis in masu salmon (*Oncorhynchus masou*) involves release of gonadotropin which stimulates production of 17α,20β-DP (7α,20β-dihydroxy-4-pregnen-3-one). Increases of 17α,20β-DP increase sperm duct pH which then increases the cAMP (cyclic adenosine monophosphate, derived from the energy source adenosine triphosphate/ATP) content of sperm, thus increasing sperm velocity (Miura et al. 1992). It is possible that this hormonal pathway is up-regulated in contaminated sites due to a higher risk of sperm competition. To further elucidate the impact of reproductive competition on sperm traits, future studies will explore how reproductive social environment changes sperm investment (number, density, morphology, velocity).

A similar sperm velocity in contaminated fish, and cleaner fish, suggests that round gobies are particularly resilient to environmental degradation, perhaps contributing to the success of this invasive species. It remains unclear however if the activation environment influenced sperm velocity. As Weis (2002) notes, the environmental milieu in which a sperm is active in is extremely important to consider. In our study, sperm from round gobies from contaminated and clean were always tested in filtered water collected from the clean site (see Methods). It is possible that sperm from contaminated round

gobies swam faster because they were activated in cleaner water. Compared to lipophilic PAHs and PCBs, highly aqueous metal toxicants (e.g. zinc, cadmium, Birmili et al. 2006) found in high concentrations in water from contaminated sites may interfere with sperm movement. When activated in controlled clean seawater, sperm from contaminated killifish actually had greater overall fertilization success and were capable of fertilizing eggs after longer periods of time than were sperm from clean killifish in the same activation medium (Khan & Weis 1987). Ideally we would have activated sperm from the two sites with water from each site. There are likely differences in the water quality in which the fish normally spawn, and ionic/osmotic factors (Alavi & Cosson 2006) and pH/temperature (Alavi & Cosson 2005) need to be further considered and investigated to determine the impact of activation fluid on round goby sperm motility.

We have demonstrated that variation in sperm morphology and velocity is limited in a fish with alternative reproductive tactics that chronically resides in a polluted ecosystem. Though sperm velocity did not vary between clean and contaminated fish, it is unclear whether contaminated sperm would be more or less viable overall. Further work assessing sperm viability via assays (SYBR-14, Evans 2009), additional sperm morphology measures (such as head and piece volume), and quantification of sperm swimming speed in *in vitro* fertilizations should reveal if sperm from contaminated and clean males indeed have equal reproductive success. In general, the results of this study suggest that the interplay between contaminant exposure and reproductive strategies does not appear to severely impact fundamental physiological processes of reproduction in round gobies.

1.4 ACKNOWLEDGEMENTS

We thank J. Fitzpatrick for providing guidance on data collection and statistical analyses and helpful manuscript comments. F. Chain for providing assistance with data organization, S. Tong and G. Wang for help collecting fish in 2009 and C. Gross, A. Schermel, D. Re, C. Schiller and M. Taves for help in field collections from 2006 to 2008. All research conformed to the protocols approved by the Animal Research Ethics Board of McMaster University (AUP # 06-10-61) and met the Canadian Council for Animal Care guidelines. This research was conducted with the permission and cooperation of the Hamilton Port Authority and the Royal Botanical Gardens. SB is funded by a New Investigator and a New Opportunities award from the Canada Foundation for Innovation (CFI), Ontario Innovation Trust (OIT), Ontario Ministry of Research and Innovation and by a Discovery and Research Tools Grant from the National Science and Engineering Council of Canada (NSERC). NMS and JRM are funded by NSERC CGS awards.

1.5 REFERENCES

Abascal FJ, Cosson J, Fauvel C. Characterization of sperm motility in sea bass: the effect of heavy metals and physicochemical variables on sperm motility. J Fish Biol 2007; 70: 509-522.

Abdelmeguid NE, Kheirallah AM, Matta CA, Abdel-Moneim, AM. Environmental contaminant-induced spermatozoa anomalies in fish inhabiting Lake Mariut, Alexandria Egypt. Int J App Environ Sci 2007; 2:1-18.

Alavi SMH, Cosson J. Sperm motility in fishes. I. Effects of temperature and pH: a review. Cell Biol Int 2005; 29: 101-110

Alavi SMH, Cosson J. Sperm motility in fishes. (II) Effects of ions and osmolality: A review. Cell Biol Int 2006; 30: 1-14.

Au DWT, Chiang MWL, Wu RSS. Effects of cadmium and phenol on motility and ultrastructure of sea urchin and mussel spermatozoa. Arch Environ Contam Toxicol 2000; 38: 455-463.

Au DWT, Lee CY, Chan KL, Wu RSS Reproductive impairment of sea urchins upon chronic exposure to cadmium. Part I: Effects on gamete quality. Environ Pollut 2001a; 111: 1-9.

Au DWT, Reunov AA, Wu RSS. Reproductive impairment of sea urchin upon chronic exposure to cadmium. Part II: Effects on sperm development. Environ Pollut 2001b; 111: 11-20.

Ball MA, Parker GA. Sperm competition games: external fertilization and "adaptive" infertility. J Theor Biol 1996; 180: 141-150.

Balshine S, Leach B, Neat F, Werner NY Montgomerie R. Sperm size of African cichlids in relation to sperm competition. Behav Ecol 2001; 12: 726-731.

Bayley M, Junge M, Baatrip E. Exposure of juvenile guppies to three antiandrogens causes demasculinzation and a reduced sperm count in adult males. Aquat Toxicol 2002; 56: 227-239.

Bennetau-Pelissero C, Breton BB, Bennetau B, Corraze G, Le Menn F, Davail-Cuisset B, Helou C, Kaushik SJ. Effect of genistein-enriched diets on the endocrine process of gametogensis and on reproduction efficiency of the rainbow trout (*Oncorhynchus mykiss*). Gen Comp Endocr 2001; 121: 173-187.

Billard Reprod Nutr Develop 1986; 26: 877-920.

Birmili W, Allen AG, Bary F, Harrison RM. Trace metal concentrations and water solubility in size-fractionated atmospheric particles and influence of road traffic. Environ Sci Technol 2006; 40: 1144-1153.

Buckman AH, Wong CS, Chow EA, Brown SB, Solomon KR, Fisk AT. Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBS in fish. Aquat Toxicol 2006; 78: 176-185.

Burness G, Casselman SJ, Schulte-Hostedde AI, Moyes CD, Montgomerie R. Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). Behav Ecol Sociobiol 2004; 56: 65-70.

Cardullo RA, Baltz JM.Metabolic regulation in mammalian sperm: Mitochondrial volume determines sperm length and flagellar beat frequency. Cell Motil Cytoskel 1991; 19: 180-188.

Casselman SJ, Schulte-Hostedde AI, Montgomerie R. Sperm quality influences male fertilization success in walleye (*Sander vitreus*). Can J Fish Aquat Sci 2006; 63: 2119-2125.

Collings SE, Johnson MS, Leah RT. Metal contamination of angler-caught fish from the Mersey estuary. Mar Environ Res 1996; 41: 281-297.

Cooke PS, Zhao Y, Hansen LG. Neonatal polychlorinated biphenyl treatment increases adult testis size and sperm production in the rat. Toxicol Appl Pharm 1996; 136: 112-117.

Cummins J. 2009. Sperm motility and energetics In: Sperm Biology: An Evolutionary Perspective (eds. Birkhead TR, Hosken DJ, Pitnick S) Elsevier: London, pp 185-206.

Djomo JE, Garrigues P, Narbonne JF. Uptake and depuration of polycyclic aromatic hydrocarbons from sediment by the zebrafish (*Brachydanio rerio*). Environ Toxicol Chem 1996; 15: 1177-1181.

Evans JP. No evidence for sperm priming responses under varying sperm competition risk or intensity in guppies Naturwissenschaften 2009; 96: 771–779.

Firman RC, Simmons LW. Sperm midpiece length predicts sperm swimming velocity in house mice. Biol Let 2010; 6: 513-516.

Fitzpatrick JL, Desjardins JK, Milligan N, Montgomerie R, Balshine S. Reproductive-tactic-specific variation in sperm swimming speeds in a shell-brooding cichlid. Biol Reprod 2007; 77: 280-284.

Fitzpatrick JL, Nadella S, Bucking C, Balshine S, Wood CM. The relative sensitivity of sperm, eggs and embryos to copper in the blue mussel (*Mytilus trossulus*). Comp Biochem Phys C 2008; 147: 441-449.

Fitzpatrick JL, Montgomerie R, Desjardins JK, Stiver KA, Kolm N, Balshine, S. Female promiscuity promotes the evolution of faster sperm in cichlid fishes. P Natl Acad Sci USA 2009; 106: 1128-1132.

Fitzpatrick JL, Garcia-Gonzalez F, Evans JP. Linking sperm length and velocity: the importance of intramale variation. Biol Let 2010; DOI:10.1098/rsbl.2010.0231

Gage MJG, Morrow EH. Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. Curr Biol 2003; 13: 754-757.

Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB, Parker GA. Spermatozoal traits and sperm competition in atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. Curr Biol 2004; 14: 44-47.

Gill ME, Spiropoulos J, Moss C. Testicular structure and sperm production in flounders from a polluted estuary: a preliminary study. J Exp Mar Biol Ecol 2002; 281: 41-51.

Gomendio M, Roldan ERS. Sperm competition influences sperm size in mammals. Proc R Soc Lond 1991; 243: 181-185.

Gomendio M, Roldan ERS. Implications of diversity in sperm size and function for sperm competition and fertility. Int J Dev Biol 2008; 52: 439-447.

Hamilton Harbour Remedial Action Plan (RAP). 1992. Hamilton Harbour Stage 1 Report: Environmental conditions and problem definition. Burlington, Ontario, Canada.

Hamilton Harbour Remedial Action Plan (RAP). 2003. The Remedial Action Plan for Hamilton Harbour. Goals, options and recommendations. Stage 2 Update 2002. Burlington, Ontario, Canada.

Hamilton Harbour Remedial Action Plan (RAP). 2008. An assessment of polychlorinated biphenyls (PCBs) in the Hamilton Harbour Area of Concern (AOC) in support of the beneficial use impairment. (BUI): Restrictions of fish and wildlife consumption. Burlington, Ontario, Canada.

Hamilton Harbour Remedial Action Plan (RAP). 2009. 2007 Field Season in the Hamilton Harbour Area of Concern: PCB and PAH water monitoring undertaken by the Ontario Ministry of the Environment to support mass balance work by theHamilton Harbour Remedial Action Plan (RAP) on PAH contamination at Randle Reef and PCB contamination in Windermere Arm. Burlington, Ontario, Canada.

Hashimoto S, Watanabe E, Ikeda M, Terao Y, Strüssmann, Inoue M, Hara A. Effects of ethinylestradiol on medaka (*Oryzias latipes*) as measured by sperm motility and fertilization success. Arch Environ Contam Toxicol 2009; 56: 253-259.

Haubruge E, Petit F, Gage MJ. Reduced sperm counts in guppies (*Poecilia reticulata*) following exposure to low levels of tributyltin and bisphenol A. Proc Biol Sci 2000; 267: 2333-2337.

Humphries S, Evans JP, Simons LW. Sperm competition: linking form to function. BMC Evol Biol 2008; 8: 319-329.

International Joint Commission (IJC). 1999. Hamilton Harbour Area of Concern Status Assessment. Windsor, Ontario, Canada.

Jeong W, Cho S. The effects of polycyclic aromatic hydrocarbon exposure on the fertilization and larval development of the pacific oyster, *Crassostrea gigas*. J Shellfish Res 2005; 24:209-213.

Jobling S, Tyler CR. Endocrine disruption, parasites and pollutants in wild freshwater fish. Parasitology 2003; 126:S103-S107.

Jørgensen EH, Bye BE, Jobling M. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (*Salvelinus alpinus*). Aquat Toxicol 1999; 44: 233-244.

Jude DJ, Reider RH, Smith GR. Establishment of Gobiidae in the Great Lakes basin. Can J Fish Aquat Sci 1992; 49: 416–421.

Khan AT, Weis JS. Effects of methylmercury on sperm and egg viability of two populations of killifish *(Fundulus heteroclitus*). Arch Environ Contam Toxicol 1987; 16: 499-505.

Kime DE. The effects of pollution on reproduction in fish. Rev Fish Biol Fisher 1995; 5: 52-96.

Kime DE, Ebrahimi M, Nysten K, Roelants I, Rurangwa E, Moore HDM, Ollevier F. Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to the effects of heavy metals Aquat Toxicol 1996; 36: 223-237.

Kime DE. A strategy for assessing the effects of xenobiotics on fish reproduction. Sci Total Environ 1999; 225: 3-11.

Kime DE, Nash JP. Gamete viability as an indicator of reproductive endocrine disruption in fish. Sci Total Environ 1999; 223: 123-129.

Kleinkauf A, Macfarlane C, Yeates S, Simpson MG, Leah RT. A biomarker approach to endocrine disruption in founder – estrogen receptors, hepatocyte proliferation, and sperm motility. Ecotox Environ Saf 2004; 58: 324-334.

Kraemer LD, Campbell PGC, Hare L. A field study examining the metal elimination kinetics in juvenile yellow perch (*Perca flavescens*). Aquat Toxicol 2005; 75: 108-126.

Lahnsteiner F, Berger B, Weismann T, Patzner RA. Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. Aquaculture 1998; 163: 163-181.

Lahnsteiner F, Mansour N, Berger B. The effect of inorganic and organic pollutants on sperm motility of some freshwater teleosts. J Fish Biol 2004; 65: 1283-1297

Leah RT, Johnson MS, Connor L, Levene C. Polychlorinated biphenyls in fish and shellfish from the Mersey Estuary and Liverpool Bay. Mar Environ Res 1997; 43: 345-358.

Levesque HM, Moon TW, Campbell PGC, Hontela A. Season variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. Aquat Toxicol 2002; 60: 257-267.

Levitan DR. Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegates*. Proc R Soc Lond B 2000; 267: 531-534.

Lüpold S, Calhim S, Immler S, Birkhead TR. Sperm morphology and sperm velocity in passerine birds. Proc Biol Sci 2009; 276: 1175–1181.

Malavasi S, Fiorin R, Franco A, Torricelli P. Somatic energy storage and reproductive investment in the grass goby *Zosterisessor ophiocephalus*. J Mar Biol Ass UK 2004; 84: 455-459.

Malo AF, Gomendio M, Garde J, Lang-Lenton B, Soler A, Roldan ERS. Sperm design and sperm function. Biol Lett 2006; 2: 246-249.

Marchand MJ, Pieterse GM, Barnhoorn IEJ. Preliminary results on sperm motility and testicular histology of two feral fish species, *Oreochromis mossambicus* and *Clarias gariepinus*, from a currently DDT-strapyed area, South Africa. J Appl Ichthyol 2008; 24:

423-429.

Marconato A, Rasotto MB, Mazzoldi C. On the mechanism of sperm release in three gobiid fishes (Teleostei: Gobiidae). Environ Biol Fishes 1996; 46: 321-327.

Marentette JR, Corkum LD. Does the reproductive status of male round gobies (*Neogobius melanostomus*) influence their response to conspecific odours? Environ Biol Fish 2008; 81: 447-455.

Marentette JR, Fitzpatrick JL, Berger RG, Balshine S. Multiple male reproductive morphs in the invasive round goby (*Apollonia melanostoma*). J Great Lakes Res 2009; 35: 302-308.

Marentette JR, Gooderham KL, McMaster ME, Ng T, Parrott JL, Wilson JY, Wood CM, and Balshine, S. Signatures of contamination in invasive round gobies (*Neogobius melanostomus*): A double strike for ecosystem health? Ecotox Environ Safe 2010; DOI:10.1016/j.ecoenv.2010.06.007.

Mazzoldi C, Scaggiante M, Ambrosin E, Rasotto MB. Mating systems and alternative male mating tactics in the grass goby *Zosterisessor ophiocephalus* (Teleostei: Gobiidae). Mar Biol 2000; 137: 1041-1048.

McAllister BG, Kime DE. Early life exposure to environmental levels of the aromatase inhibitor tributyltin causes masculinisation and irreversible sperm damage in zebrafish (*Danio rerio*). Aquat Toxicol 2003; 65: 309-316.

McMaster ME, Portt CB, Munkittrick KR, Dixon DG. Milt characteristics, reproductive performance, and larval survival and development of white sucker exposed to bleached kraft mill effluent. Toxicol Environ Safety 1992; 23: 103-117.

Meunier B, Yavno, S, Ahmed S, Corkum LD. First documentation of spawning and nest guarding in the laboratory by the invasive fish, the round goby (*Neogobius melanostmus*). J Great Lakes Res 2009; 35: 608-612.

Miller HC, Mills GN, Bembo DG, Macdonald JA, Evans CW. Induction of cytochrome P4501A (CYP1A) in *Trematomus bernacchii* as an indicator of environmental pollution in Antarctica: assessment by quantitative RT-PCR. Aquat Toxicol 1999; 183-193.

Miller PJ. 1984. The tokology of gobioid fishes. In: Fish reproduction strategies and tactics (eds. Potts GW, Wooton RJ) Academic Press: London, pp 199-153.

Miura T, Yamauchi K, Takahashi H, Nagahama Y. The role of hormones in the acquistion of sperm motility in salmonid fish. J Exp Zool 1992; 261: 359-363.

Mossman J, Slate J, Humphries S, Birkhead T. Sperm morphology and velocity are genetically codetermined in the zebra finch. Evolution 2009; 63: 2730-2737.

Newman MC, Clements WH. 2008. Ecotoxicology: A Comprehensive Treatment. CRC Press: New York.

Oliveira RF. Ros AFH, Gonçalves DM. Intra-sexual variation in male reproduction in teleost fish: a comparative approach. Horm Behav 2005; 48: 430-439.

Parker GA. Sperm compctition and its evolutionary consequences in the insects. Biol Rev Camb Philos Soc 1970; 45: 525–567.

Parker GA. Sperm competition games: raffles and roles. Proc R Soc Lond B, 1990a; 242: 120-126.

Parker GA. Sperm competition games: sneaks and extra-pair copulations. Proc R Soc Lond B, 1990b; 242: 127-133.

Parker GA, Ball MA. Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. Biol Lett 2005; 1: 235-238.

Perchec G, Jeulin C, Cosson J, André F, Billard R. Relationship between sperm ATP content and motility of carp spermatozoa. J Cell Sci 1995; 108: 747-753.

Petersen CW, Warner RR. 1998. Sperm Competition in Fishes In: Sperm Competition and Sexual Selection (eds. Birkhead TR, Møller AP) Academic Press: San Diego, pp 435-464.

Rasotto MB, Mazzoldi C. Male traits associated with alternative reproductive tactics in *Gobius niger*. 2002; J Fish Biol 61: 173-184.

Ray WJ, Corkum LD. Habitat and site affinity of the round goby. J Great Lakes Res 2001; 27: 329-334.

Rurangwa E, Biegniewska A, Slominska E, Skorkowski EF, Ollevier F. Effect of tributyltin on adenylate content and enzyme activities of teleost sperm: a biochemical approach to study the mechanisms of toxicant reduced spermatozoa motility. Comp Biochem Phys C 2002; 131: 335-344.

Schoenfuss HL, Levitt JT, Van Der Kraak G, Sorensen PW. Ten-week exposure to treated sewage discharge has relatively minor, variable effects on reproductive behavior and sperm production in goldfish. Environ Toxicol Chem 2002; 21: 2185-2190.

Schoenfuss HL, Levitt JT, Rai R. Treated wastewater effluent reduces sperm motility along an osmolality gradient. Arch Environ Contam Toxicol 2008; 56: 397–407.

Snook RR. Sperm in competition: not playing by the numbers. Trends Ecol Evol 2005; 20: 46-53.

Sopinka NM, Marentette JR, Balshine S. Impact of contaminant exposure on resource contests in an invasive fish. Behav Ecol Sociobiol 2010; DOI:10.1007/s00265-010-1005-1.

Stevenson LM, Brown AC, Montgomery TM, Clotfelter ED. Reproductive consequences of exposure to waterborne phytoestrogens in male fighting fish *Betta splendens*. Arch Environ Contam Toxicol 2010; DOI: 10.1007/s00244-010-9561-y.

Stockley P, Gage MJG, Parker GA, Møller AP. The evolution of testis size and ejaculate characteristics. Am Nat 1997; 149: 933-954.

Taborsky M. 2008. Alternative reproductive tactics in fish In: Alternative Reproductive Tactics: An Integrative Approach (eds. Oliveira RF, Taborsky M, Brockmann HJ) Cambridge University Press: New York, pp 251-299.

Toft G, Baatrup E, Guillette LJ. Altered social behavior and sexual characteristics in mosquitofish (*Gambusia holbrooki*) living downstream of a paper mill. Aquat Toxicol 2004; 70:213-222.

Vajda AM, Barber LB, Gray JL, Lopez EM, Woodling JD, Norris DO. Reproduction disruption in fish downstream from an estrogenic wastewater effluent. Environ Sci Technol 2008; 42: 3407-3414.

VanLook KJW, Kime DE. Automated sperm morphology analysis in fishes: the effect of mercury on goldfish sperm. J Fish Biol 2003; 63: 1020-1033.

Vélez-Espino LA, Koops MA, Balshine S. Invasion dynamics of round goby (*Neogobius melanostomus*) in Hamilton Harbour, Lake Ontario. Biol Invas 2010; DOI: 10.1007/s10530-010-9777-9.

Vos JG, Dybing E, Greim HA, Ladefoged O, Lambre C, Tarazona JV, Brandt I, Vethaak AD. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit Rev Toxicol 2000; 30: 71-133.

Vladić TV, Afzelius BA, Bronnikov GE. Sperm quality reflected through morphology in salmon alternative life histories. Biol Reprod 2002; 66; 98-105.

Waltz EC. Alternative mating tactics and the law of Diminish Returns: The Satellite Threshold Model. Behav Ecol Sociobiol 1982; 10:75-83.

Weis J. Tolerance to environmental contaminants in the mummichog, *Fundulus heteroclitus*. Hum Ecol Risk Assess 2002; 8: 933-953.

Wolfe RK, Marsden JE. Tagging methods for the round goby (*Neogobius melanostomus*). J Great Lakes Res 1998; 24: 731-735.

Young JAM, Marentette JR, Gross C, McDonald JI, Verma V, Marsh-Rollo SE, Macdonald PDM, Earn DJD, Balshine S. Demography and substrate affinity of the round goby (*Neogobius melanostomus*) in Hamilton Harbour. J Great Lakes Res 2010; 36: 115-122.

Zeman AJ, Patterson TS. Characterization and mapping of contaminated sediments, Windermere Arm, Hamilton Harbour, Ontario, Canada. Soil Sed Contam 2003; 12: 619-629

Zeman AJ. 2009. Contaminated sediments in Hamilton Harbour: compilation and evaluation of sediment databases, publications and reports, 1975-2008. Environment

Canada Water Science and Technology Directorate WSTD Contribution No. 09-263

CHAPTER 2

LIVING IN CONTAMINATED AREAS: GAMETIC CONSEQUENCES IN THE PLAINFIN MIDSHIPMAN

2.1.1 SPERM COMPETITION: THEORY AND EVIDENCE

The fusion of a sperm and egg cell is the most fundamental aspect of sexual reproduction. However, this fusion often involves competition between rival sperm for fertilization of the egg, known as sperm competition (Parker 1970). As a result, males must not only be capable of mating with a female but must also produce enough sperm of sufficiently high quality to ensure fertilizations in the face of sperm competition (Birkhead & Møller 1998). Theoretical models predict that sperm competition will drive the production of larger gonads (Stockley et al. 1997; Parker & Ball 2005), more sperm (Parker 1990; Stockley et al. 1997) and faster swimming sperm (Gomendio & Roldan 1991; Ball & Parker 1996) to counter the presence of competing sperm. Indeed, males experiencing high sperm competition have larger gonads, produce denser ejaculates and faster swimming sperm (Stockley et al. 1997; Vladić & Järvi 2001; Neff et al. 2003; Burness et al. 2004; Fitzpatrick et al. 2007; 2009; 2010; Gomendio & Roldan 2008; Marentette et al. 2009). These increases in sperm number and quality are adaptive as greater numbers of sperm that swim faster are more likely to succeed in fertilizing an egg (Lahnsteiner et al. 1998; Levitan 2000; Gage & Morrow 2003; Gage et al. 2004; Casselman et al. 2006; Tuset et al. 2008). To facilitate faster swimming sperm, selection is expected to act on sperm size, particularly sperm midpiece and flagellum length,

because sperm with longer flagellum and midpieces are expected to swim faster relative to smaller sperm with shorter flagellum and midpieces (Cardullo & Baltz 1991; Gomendio and Roldan 1991; but see Humphries et al. 2008 and the Introduction and Discussion of Chapter 1 of this thesis). This link between sperm morphology and velocity is supported by many studies and across a diverse range of taxa (fish: Burness et al. 2004; Fitzpatrick et al. 2009; 2010; Pitcher et al. 2009; mammals: Gomendio & Roldan 1991; Firman & Simmons 2010; birds: Lüpold et al. 2009; Mossman et al. 2009). Hence, factors like aquatic pollution, that are known to cause reductions in sperm number and velocity, may be disadvantageous for males experiencing high sperm competition and compromise male reproductive success.

2.1.2 CONTAMINANT-MEDIATED CHANGES IN SPERM

Toxicological studies demonstrate multiple lines of evidence that exposure to aquatic contaminants impairs gametic quality. Exposure to metal and organic contaminants reduces sperm production (Haubruge et al. 2000; Schoenfuss et al. 2002), and alters sperm morphology (Au et al. 2000; Au et al. 2001b; Gill et al. 2002; VanLook & Kime 2003) and velocity (Khan & Weis 1987; Kime et al. 1996; Au et al. 2001a; VanLook & Kime 2003; Lahnsteiner et al. 2004; Abascal et al. 2007; Fitzpatrick et al. 2008). For example, goldfish (*Carassius auratus*) sperm exposed for 24 hours to 0.1 mg/L mercuric chloride had shorter sperm tails and sperm swimming speeds were reduced (VanLook & Kime 2003). Schoenfuss et al. (2002) found that milt (sperm and seminal fluid) was not produced at all in half of the goldfish exposed to 30 ng/L of estradiol (E2) for a 10-week period and the percentage of motile sperm was reduced in those males that did produce milt. Similarly, females exposed to aquatic contaminants

exhibit reduced fecundity (number of eggs spawned, Westernhagen 1988; Kime et al. 1995; Hewitt et al. 2008) and abnormal egg morphology (Westernhagen 1988; Khan & Weis 1993; Westernhagen & Dethlefsen 1997; Westernhagen et al. 2001; Klumpp et al. 2002). Together, the effects of contaminants on sperm and eggs can have a synergistic effect as disruption of egg production and development can exasperate the drawbacks of the production of damaged sperm causing detrimental consequences for the successful fertilization of eggs. Unfortunately, while laboratory evidence for the biological effects of aquatic toxicants on gametes is abundant, how these effects impact the gametes of wild caught fish experiencing sperm competition in naturally exposed areas needs further attention.

2.1.3 EXPOSURE TO ENVIRONMENTAL CONTAMINANTS IN SPECIES WITH ALTERNATIVE MATING TACTICS

Male alternative reproductive tactics (MARTs) are common among fish (e.g. plainfin midshipman, *Porichthys notatus*; black goby, *Gobius niger*; rainbow trout, *Oncorhynchus mykiss*; shell-brooding cichlid, *Lamprologus callipterus*, Sato et al. 2004). In species with MARTs, males adopting different tactics experience varying degrees of sperm competition. Large, dominant males defend territories, attract females and provide brood care but their reproductive success is jeopardized by small males that do not occupy territories but instead sneak fertilizations (Taborsky 1994; 1998; Oliveira et al. 2008). Thus, within a species, sneaking males always experience sperm competition while fertilizing in a nest guarded by dominant males (Snook 2005), and produce more, faster swimming sperm with longer flagella (Vladić & Järvi 2001; Neff et al. 2003; Burness et al. 2004; Fitzpatrick et al. 2007). Guarding males on the other hand experience

sperm competition less frequently than sneaker males (Snook 2005). How will contaminant-induced gametic impairment impact species with such complex mating systems? Guarding males, in particular, exhibit behavioural traits that could increase the impact of contaminant exposure. Guarding males are larger, older and sedentary (versus roving sneakers) while providing parental care to developing offspring. Such reduced mobility could increase exposure to contaminated sediment. Importantly, guarding males are also thought to fast throughout the breeding season (Arora 1948; Rohwer 1978; Marconato et al. 1993). Fasting can mobilize contaminants that are stored within fatty tissues (Jørgensen et al. 1999), and guarding males typically have greater fat reserves compared to sneaker males (e.g. body condition; Sato et al. 2004). Hence, pollutant induced reductions in sperm quality are predicted to be more severe in guarding males and faster swimming sperm of sneaker males could be at an advantage for obtaining greater paternity while under high sperm competition.

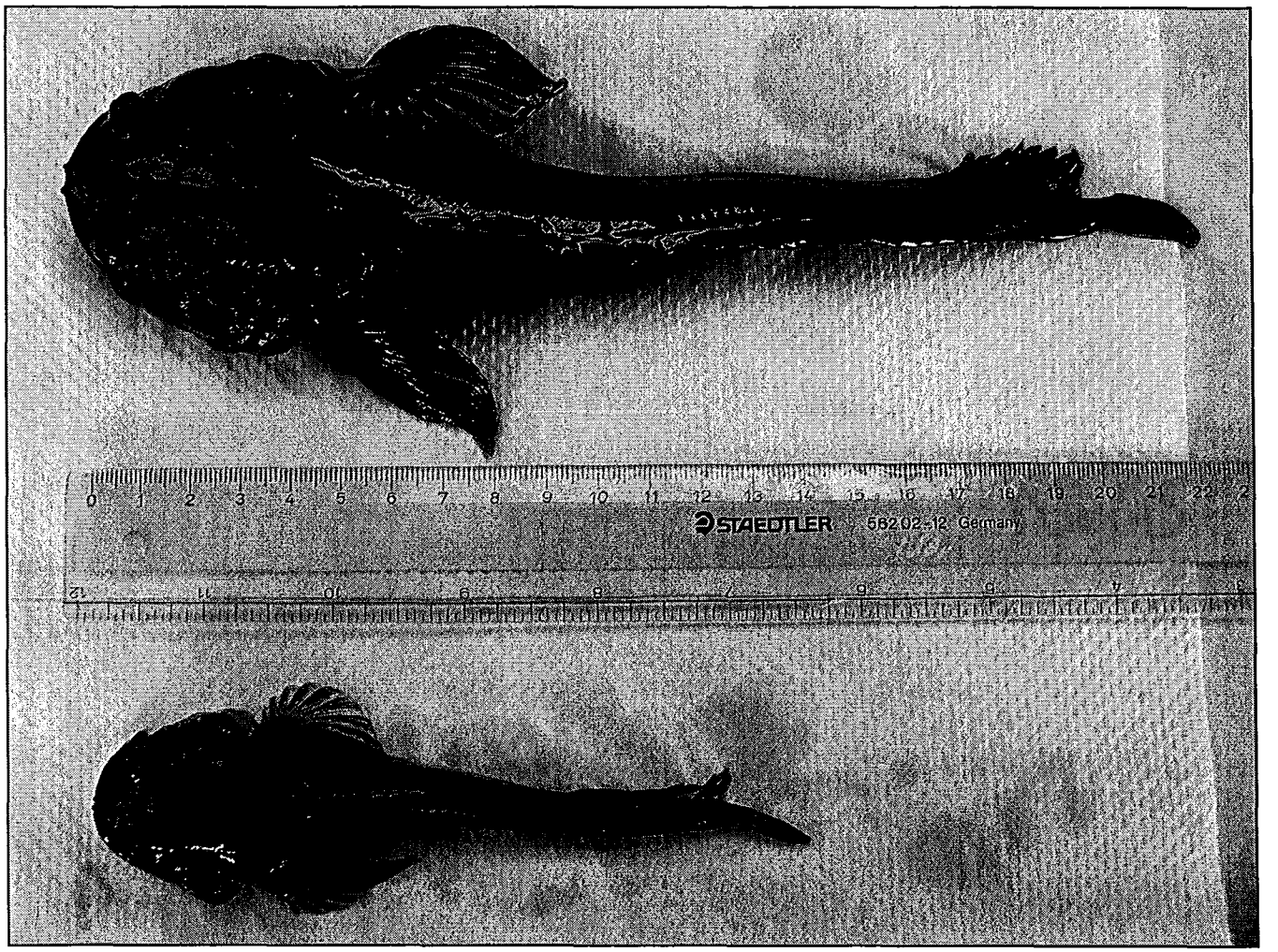
2.1.4 NATURAL HISTORY OF THE PLAINFIN MIDSHIPMAN

The plainfin midshipman, *P notatus*, is an excellent model for exploring novel interactive effects of contaminant exposure and MARTs on reproductive success. First, two male morphs occur in this species (Brantley & Bass 1994; Figure 6). A large territorial male (Type I) attracts females acoustically (McKibben & Bass 1998) and visually (Crane 1965), and then guards nests from intruding conspecifics and predators while providing parental care (egg fanning, Crane 1981). The small sneaking morph (Type II) does not hold territories or attract females (Brantley & Bass 1994) and instead invests in reproductive tissue. Type II males have relatively larger testes (GSI 900% larger than Type I males, Brantley & Bass 1994) but smaller accessory structures (Barni

FIGURE 6

Large, territorial Type I male (top fish) and small sneaking Type II male (bottom fish).

Photograph by J.L. Fitzpatrick.



et al. 2001). Type II males are often found in nests where a female is present and are aggressed on by the territorial Type I male (Brantley & Bass 1994). In agreement with sperm competition theory Type II males have faster swimming sperm compared to Type I males (Fitzpatrick 2008). Sperm tail length was not found to vary between morphs, but Type II males have larger sperm midpieces (Fitzpatrick, 2008), suggesting more mitochondria and larger energy reserves (Cummins 2009).

Plainfin midshipman inhabit intertidal areas along the Pacific coast (Aror 1948), some of these areas are characterized by current and historical pulp and paper/saw mill activity and the release of urban waste and agricultural effluents (Spies 1984; Bard 1998). Plainfin midshipman are classified as a species sensitive to pulp and paper mill effluent according to the literature as they are found only at distances greater than 10 km from mills (Bard 1998), but show neither avoid or are attracted to sewage outflow (Spies 1984). Aspects of the natural history of *P. notatus* make Type I males particularly susceptible to contaminant exposure. Type I males remain in their nests for at least 3 consecutive months (Crane 1981) and thus, it is possible that they are exposed to contaminated waters and sediments for extended periods of time. Egg cannibalism is also documented in the laboratory (Harper & Case 1999) and in field (eggs present in Type I male gut, personal observations). A dietary exposure to contaminants may be possible given that females shunt considerable contaminant burden to developing eggs (Johnston 2005). With many potential routes of contaminant exposure, contaminant mediated impairment to sperm and egg quality may influence the rate of successful fertilizations by Type I males.

2.1.5 AIMS AND PREDICTIONS

Here, we investigate how living in polluted water impacts the sperm and egg quality of wild caught fish with alternative mating strategies. Given the probable increased susceptibility of Type I males to aquatic contaminants, sperm of Type I plainfin midshipman were sampled from areas near and distant to sources of pollution in British Columbia, Canada. Indirect evidence for many important effects of contaminants (e.g. damaged sperm, impaired parental care, maternal shunting of contaminants to eggs) can be assessed by examination of egg quality and nest abandonment rates therefore we sampled the eggs from nests these Type I males were guarding. We predicted that during the breeding season guarding Type I males collected from contaminated sites would 1) have reduced gonadal investment, 2) have sperm with shorter heads, midpieces and tails, 3) have slower swimming sperm and 4) be guarding fewer eggs of lower quality.

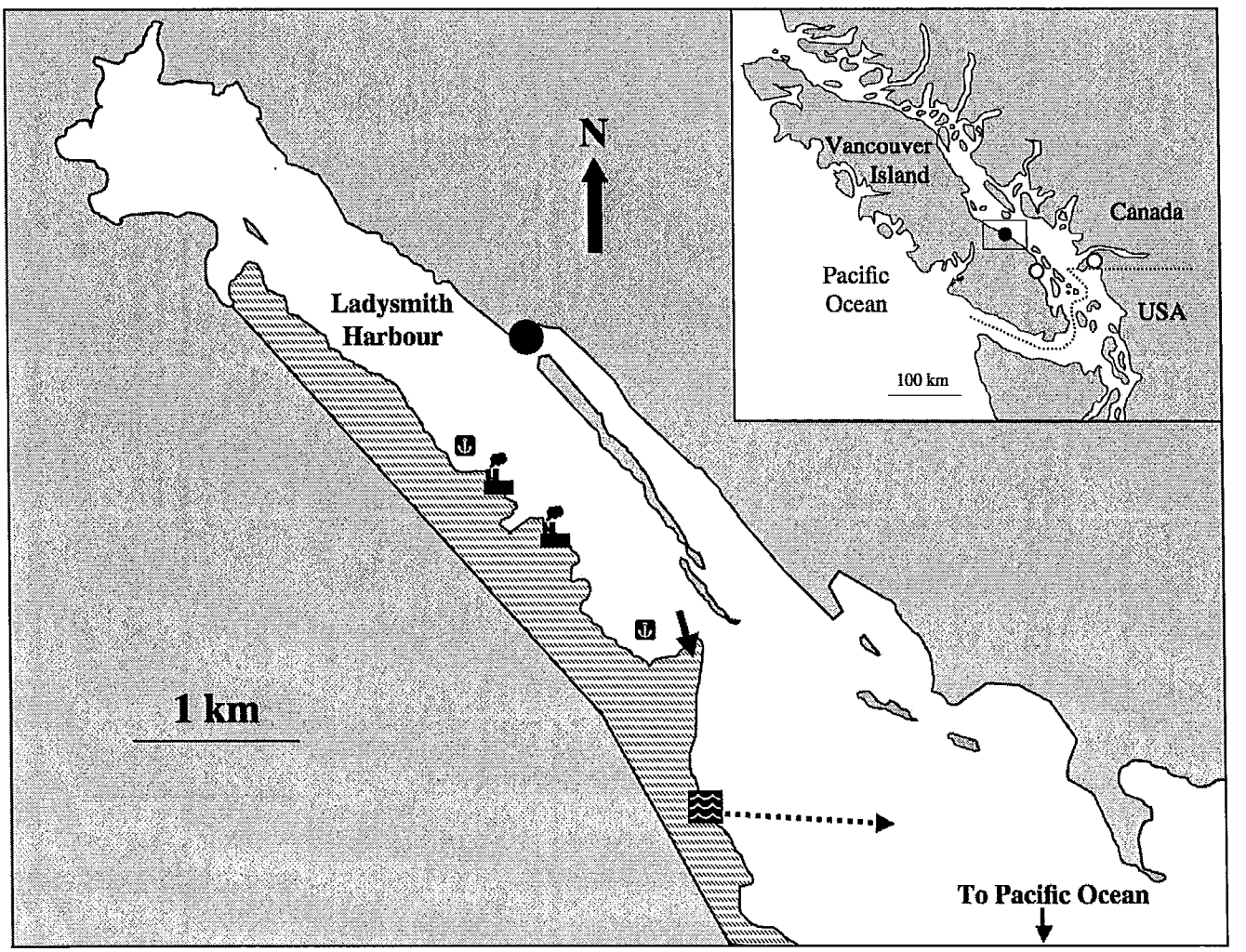
2.2 METHODS

2.2.1 SITE DESCRIPTION AND SURVEYING

During two low tide events from 17 May to 28 May and 18 June to 28 June 2009 plainfin midshipman nests were surveyed at two sites on the eastern coast of Vancouver Island (Mill Bay, MB, 48° 63′ N, 123° 53′ W and Ladysmith Harbour, LSH, 49° 01′ N, 123° 83′ W), and from one site on the mainland (Crescent Beach, CB, 49° 04′ N, 122° 88′ W), in British Columbia, Canada. Ladysmith Harbour (LSH) is characterized by historical and current pulp, paper and saw mill industry, recreational marinas, and an endpoint for agricultural and urban sewage effluent (Alava et al. 2009; Alava et al. 2010). At the mouth of the Harbour is Slag Point, a Brownfield area of concern identified by the government of British Columbia (Ministry of Agriculture and Lands 2005; Figure 7).

FIGURE 7

Inset: Clean (open circles) and contaminated (filled circle) sites on Vancouver Island and mainland British Columbia, Canada. **Full:** The contaminated site (filled circle) located in Ladysmith Harbour on the eastern coast of Vancouver Island. The hatched shading represents industrial activity (pulp and paper/saw mill;), recreational marinas (), and agriculture and urban sewage discharge locations. Within this hatched area is Slag Point (bold arrow), a coal fill with areas containing buried refuse, dredged wood waste, sand and gravel. Ladysmith Wastewater Treatment Plant (; sewage outfall indicated by dotted arrow) is located south of Slag Point.



Slag Point sediment is primarily composed of coal fill with other areas containing buried refuse, dredged wood waste, sand and gravel (Ministry of Agriculture and Lands 2005). Sediment levels of PAHs, petroleum hydrocarbons, metals (zinc, molybdenum, nickel and tin) and water levels of PAHs detected at Slag Point exceed residential, park and commercial land standards (B.C. Ministry of Agriculture and Lands 2005). Mill Bay (MB) and Crescent Beach (CB) were designated as unexposed open water sites, distant to sources of pollution (Figure 7). It should be noted that despite great distances between populations, there were no detectable site differences in 8 polymorphic microsatellite loci, indicating little genetic variance between collection sites on Vancouver Island (Suk et al. 2009). These results suggest little natal philopatry in midshipman and that any observed physiological differences between fish from clean and contaminated sites are more likely the result of contaminant exposure than underlying genetic differences among populations.

During these two low tide events, 50 clean and 53 contaminated nests were located beneath rocks in the intertidal zone and surveyed. All fish occupying the nests were sexed, weighed to the nearest 0.001 g and measured for standard and total length to the nearest 0.01 cm. Fish were sexed using a combination of ventral colouration (metallic golden colour in females) and shape of urogenital papilla (elongated in males, Brantley & Bass 1994). For each nest, the presence or absence of eggs was noted and proximity to neighbouring nests was measured (to the nearest 0.01 m) using a tape measure. A digital photograph was taken of the nests and eggs in it using a Canon Digital Rebel camera (EOS 300D). All males and rocks were carefully replaced upon completion of nest survey.

2.2.2 QUANTIFICATION OF EGGS

Egg counts were used to estimate each Type I guarding male's reproductive success. Eggs were counted for each nest using ImageJ. Five types of eggs were identified and counted separately: 1) live eggs were any golden-orange coloured, spherical eggs, 2) dead eggs were opaquely-white coloured, punctured and dull, 3) egg scars were defined by a leftover ring present on the rock or white egg fragments that had ripped or ruptured, 4) pink embryos still attached to an egg sac were classified as hatched juveniles and 5) fully formed silver juveniles were attached to the rock surface without any visible yolk sac present (Figure 8).

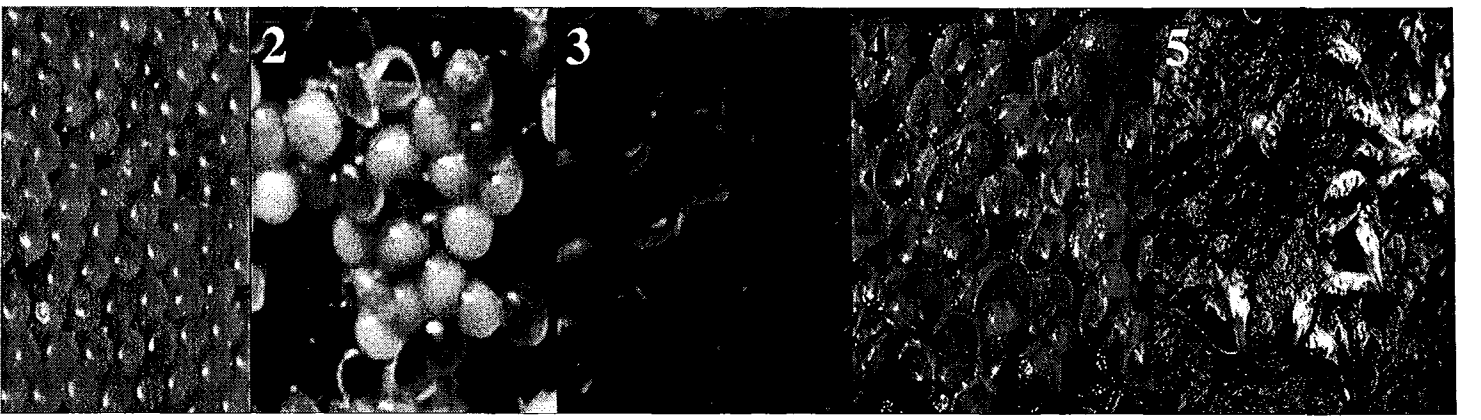
2.2.3 SPERM COLLECTION

Thirty-eight Type I guarding males (22 from clean nests, 16 from contaminated nests) were collected and brought to the laboratory in plastic containers of seawater within one hour of collection for sperm analyses. Fish were given a lethal overdose of benzocaine (Sigma Aldrich Canada Ltd., Oakville, Canada) prior to dissection. The left and right testes were dissected and weighed separately to the nearest 0.001 g. The liver, sonic muscle and bile were also weighed. A measure of testis asymmetry was calculated as the absolute mass of the left testis (g) – right testis (g).

To analyze sperm morphology, a drop of sperm from 38 males was placed in a 1.5 mL microcentrifuge tube, diluted with 1 mL of seawater and fixed with 20 μL of formalin buffer. Six males were excluded from the morphology dataset (N=32) because it was not possible to find any sperm to photograph. Using a Prosilica EC-650 digital camera mounted on a light microscope (400x magnification), an image was taken of visible spermatozoa (15 images per male). Sperm head, midpiece (when visible) and

FIGURE 8

Classification of plainfin midshipman eggs 1) live eggs, 2) dead eggs, 3) egg scars, 4) pink embryos and 5) silver juveniles.



length of both flagella (plainfin midhipman possess unusual biflagellate sperm, Stanley 1965) were measured to the nearest 0.1 μm using ImageJ. Measurements were calculated by drawing a freehand line over each sperm section using an Intuos graphic table (Wacom Co. Ltd., Japan). An average for each measured section was taken from the 15 images per male and used for statistical analyses. As discussed in Humphries et al. (2008), relative measures of sperm components are likely targeted by selection. Thus, in addition to absolute sperm head, midpiece and tail size, a ratio was calculated between sperm tail length and head length.

Videos of sperm were captured for a total of 38 Type I guarding males. Sufficient video capture (greater than 10 minutes) could not be obtained from 5 males and hence, 33 videos went into a velocity dataset. First, an incision was made in the anterior testis and sperm was collected using a 10μL microcapillary tube. The microcapillary tube was dabbed on the side of a 1.5mL microcentrifuge tube dispensing a drop of sperm. Sperm were immediately activated with 200μL seawater. Water was obtained the day of processing from the collection site of the fish (MB, LSH or CB). A 60μL sub-sample of the sperm and water mixture was placed on a welled slide (1-mm deep) covered by half a cover slip. Video capture of sperm under pre-focused 200X magnification began at the instance of sperm activation. Videos were recorded under phase contrast with a Prosilica EC-650 digital camera (Prosilica, Burnaby, British Columbia, Canada) mounted on an Olympus CX41 microscope (Olympus America Inc.). Videos were recorded using Astro IIDC (v. 4.04.00) software at 60 frames/second. The elongated head morphology of midshipman sperm prohibited the use of computer assisted sperm analysis software. Sperm velocity was measured using NIH ImageJ software (v. 1.42q, available at

http://rsb.info.nih.gov/ij/) and the CASA plugin (http://rsbweb.nih.gov/ij/plugins/casa.html). Smooth path velocity (VAP) was calculated for each male at 8 one-second intervals; 30, 45, 90, 180, 360, 600, 840 and 1080 seconds.

2.2.4 STATISTICAL ANALYSES

All statistical analyses were performed using the statistical package JMP (version 5.0.1, 2002; SAS Institute Inc., Cary, NC). All data were tested for normality. Soma, testicular, liver, sonic muscle and gall bladder mass were log-transformed and proportions of live eggs (versus dead eggs) were arcsine root transformed to achieve normality. When data were not normally distributed nor could they be transformed, non-parametric tests used. To explore differences in body mass and length between clean and contaminated fish Wilcoxon rank-sum tests was used. Two males were excluded from all statistical analyses related to testicular investment as the gonads of these fish were not measured. Using an ANCOVA, with soma mass (body mass (g) – tissue mass (g) x 100%) as a covariate, relative testicular, liver, sonic muscle (sound producing organ used to attract females, Ibara et al. 1983) and gall bladder (an organ that can accumulate toxicants and is enlarged when animals are starved, Newman & Clements 2008) investment was analyzed between clean and contaminated fish. Testicular asymmetry at clean and contaminated sites was analyzed using a t-test. An ANCOVA controlling for body mass (g) was used to calculate differences in the number of eggs (live and dead). A t-test was used to determine percentage of live eggs in nests at clean and contaminated sites. Percentage of live eggs was arcsine square root transformed prior to analysis. Spearman rank correlations were used to determine relationships between male body mass, number of eggs and male type (Type I versus Type II). T-tests were used to

evaluate sperm morphological differences between Type I males collected from clean versus contaminated sites. Non-significant interactions ($P>0.05$) were removed from all models. Tukey's HSD tests were used to determine post-hoc differences ($P<0.05$) between sites and these were denoted as different letters on graphs.

2.3 RESULTS

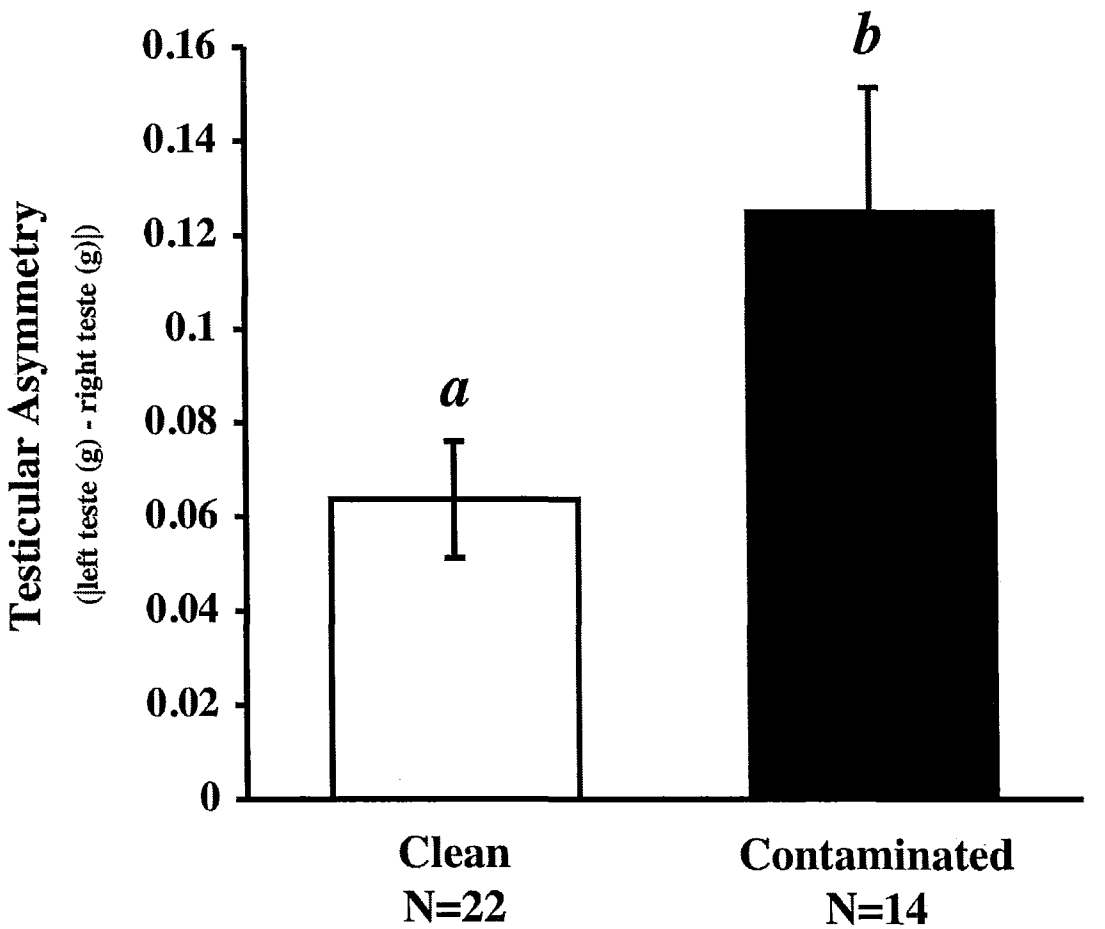
2.3.1 MALE TRAITS

Larger guarding males tended to occupy nests with bigger females (Spearman rank: $\rho=0.42$, $N=22$, $P=0.05$). Males and females collected from contaminated nests were heavier (Type I: Wilcoxon rank-sum: $Z=4.41$, $N=82$, $P<0.0001$; Female: $Z=2.54$, $N=22$, $P=0.01$) and longer (Type I: $Z=4.76$, $N=82$, $P<0.0001$; Female: $Z=2.74$, $N=22$, $P=0.01$) than fish collected from nests in cleaner areas. Sneaker males (Type II) were longer ($Z=2.06$, $N=21$, $P=0.04$) but not heavier in contaminated areas compared to cleaner areas ($Z=1.53$, $N=21$, $P=0.13$).

Across all fish sampled for sperm, larger, heavier males had greater testicular investment (Spearman rank: $\rho=0.8042$, $N=36$, $P<0.0001$). Relative testicular investment (sum of right testis and left testis) did not differ between male fish collected from clean and contaminated sites (ANCOVA: Site, $F_{1,33}=2.83$, $P=0.10$; Soma mass: $F_{1,33}=58.92$, $P<0.0001$). However, the degree of testis asymmetry ($|$right testis – left testis$|$) was greater in fish collected from contaminated sites than in fish collected from clean sites (t-test: $t=5.48$, $N=36$, $P=0.03$; Figure 9). Controlling for body size, investment in liver, sonic muscle and gall bladder did not vary between Type I males from clean and contaminated areas (ANCOVA: Sonic Muscle, $F_{1,33}=1.16$, $P=0.29$; Bile, $F_{1,32}=0.23$, $P=0.63$; Liver, $F_{1,33}=0.04$, $P=0.84$; Soma mass: all $Ps<0.05$). In absolute terms, sperm

FIGURE 9

Testicular asymmetry (|mass right testis (g) – mass left testis (g)|) in males from clean and contaminated sites.



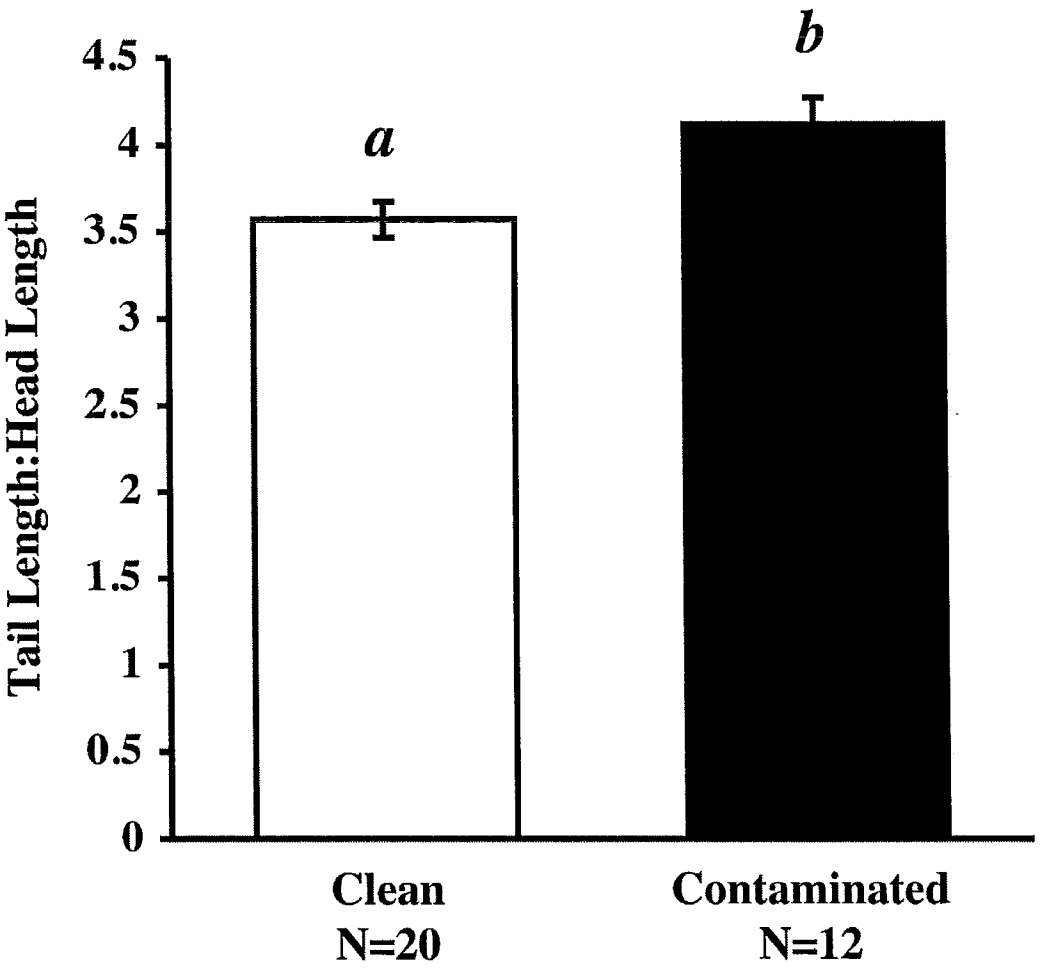
from contaminated males had shorter heads (T-test: t=7.76, N=32, P=0.01) and tended to have longer midpieces (t=3.04, N=32, P=0.09). Absolute sperm tail length did not vary between clean and contaminated males (t=0.14, N=32, P=0.71). The ratio between sperm tail length and head length was greater in males collected from contaminated areas (t=9.55, N=32, P=0.004; Figure 10).

2.3.2 NEST CHARACTERISTICS

Larger guarding Type I males with large sonic muscles had more eggs in their nests (Spearman rank: Mass: ρ=0.54, N=82, P<0.0001; Sonic Muscle: ρ=0.46, N=32, P=0.009), than smaller guarding males. Larger females were found in nests with more eggs (ρ=0.48, N=22, P=0.02). Controlling for male body mass, contaminated nests contained overall more eggs (sum of live and dead eggs, ANCOVA: Site: $F_{1,76}$=0.001, P=0.98; Body mass: $F_{1,76}$=15.27, P=0.0002; Site x Body mass: $F_{1,76}$=5.06, P=0.03; Figure 11 Inset) than unexposed nests in clean sites, but the nests in clean sites had a higher proportion of live eggs (T-test: t=14.06, N=102, P=0.0003; Figure 11).

FIGURE 10

Comparison of sperm tail length:sperm head length of Type I males collected from clean (open bar) and contaminated (filled bar) collection sites. Bottom image depicts midshipman sperm 1) head, 2) midpiece and 3) flagella.



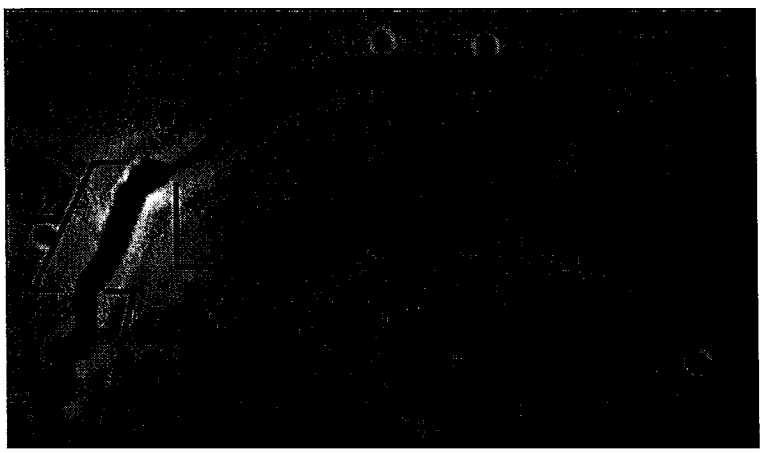
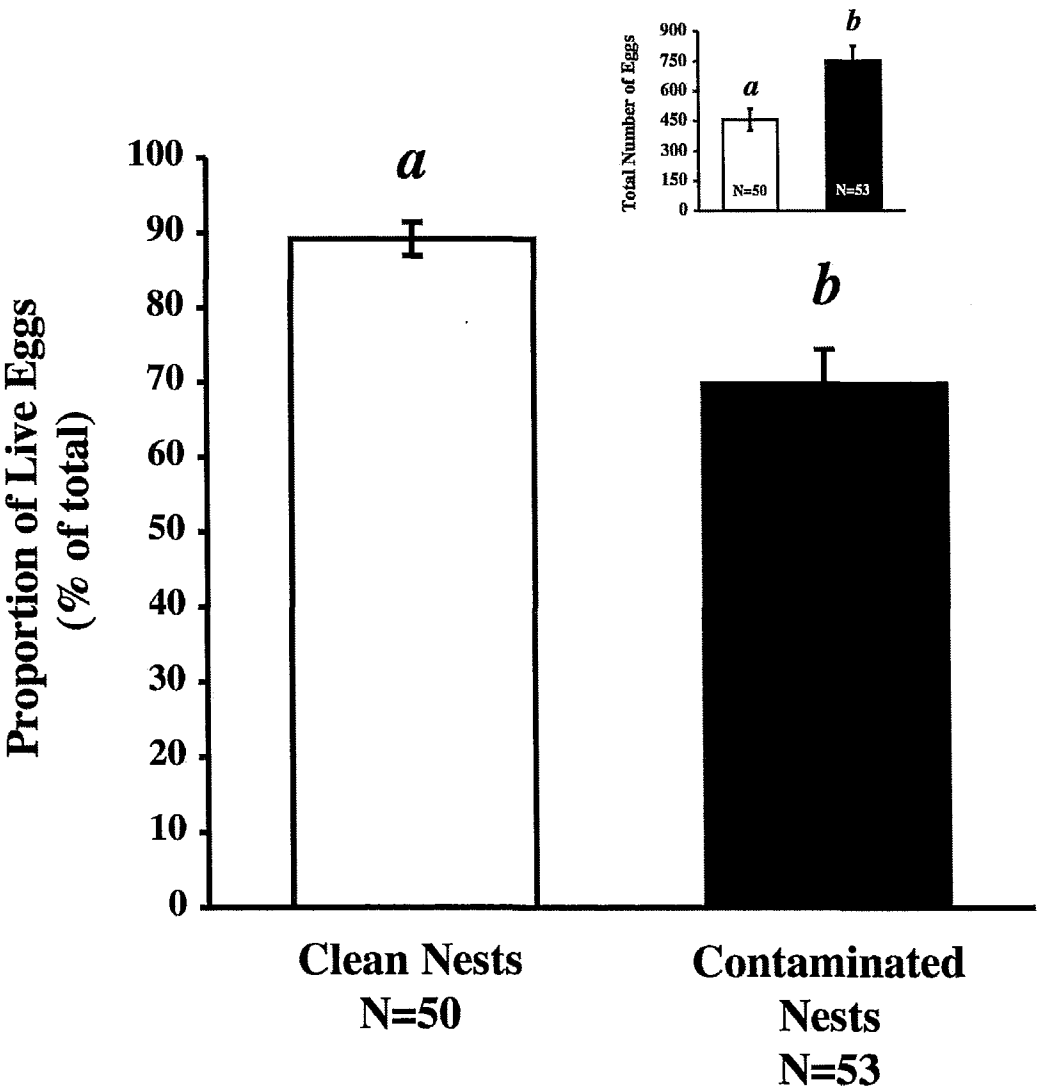


FIGURE 11

Proportion of live eggs (sum of intact eggs + hatched juveniles + juveniles) in surveyed nests at clean (open bar) and contaminated (filled bar) sites. **Inset**: Total number of eggs (mean ± SE) in clean (open bar) and contaminated (filled bar) nests.



2.4 DISCUSSION

The results of my study showed that plainfin midshipman from areas in close proximity to sources of pollution had smaller sperm heads, more dead eggs per nest and greater testicular asymmetry. The sperm head houses the cell's genetic material (Kunz 2004) and sperm cells are particularly susceptible to DNA damage (Lewis & Aitken 2005). Humans exposed to high levels of air pollution had more abnormally shaped sperm heads and these heads had an increased incidence of abnormal chromatin structure (Selevan et al. 2000). Furthermore, DNA damage in sperm was a major contributor to decreases in fertilization rate in rainbow trout *Oncorhynchus mykiss* (Labbe et al. 2001). Type I males from contaminated areas had smaller sperm heads, which could represent DNA damage and/or degradation and potentially account for the higher percentage of dead eggs in contaminated nests. Urbach et al. (2007) found that testicular asymmetry correlated with increased sperm velocity in alphine whitefish (*Coregonus fatioi*). Velocity data for this study is presently being analyzed. Based on Urbach et al. (2007)'s result and the tendency for sperm from clean males to have smaller midpieces and potentially fewer mitochondria (Cardullo & Baltz 1991), one might predict that sperm from clean males would swim slower compared to sperm from contaminated males. Type I males from contaminated areas had shorter sperm heads, which could represent DNA damage and/or degradation and potentially account for the higher percentage of dead eggs in contaminated nests. The patterns revealed upon analysis of sperm velocity and how sperm morphology relate to sperm swimming speed will provide either supporting or opposing empirical evidence for the theoretically predicted correlations between sperm form and function. Furthermore, this study will contribute to the presently limited

knowledge of the gametic consequences in species with MARTs exposed to aquatic contaminants.

Type I males in contaminated areas had more eggs in their nests suggesting that, in contrast to previous findings (Westernhagen 1988; Kime et al. 1995; Hewitt et al. 2008), females could be more fecund in contaminated areas or that there might be more females in these areas. A population census in these clean and contaminated areas has recently been completed and will be able to reveal whether more females are indeed captured at contaminated sites. However, a larger percentage of the eggs in contaminated nests were dead compared to the eggs counted in nests from clean areas. Future behavioural observations will shed light on whether poor egg quality is a result of direct exposure to contaminants, reduced parental care, increased predation, higher rates of egg cannibalism, maternal effects (e.g. shunting of contaminants, increased cortisol) or some combination of all these factors. A higher proportion of dead eggs could indicate that eggs failed to be fertilized as has been demonstrated in previous research. For example, eggs stripped from *Fundulus heteroclitus* and exposed to methylmercury for 20 minutes revealed that the micropyle (the opening on an egg's surface that a sperm enters) was blocked, preventing sperm from entering and fertilizing the egg (Khan & Weis 1993). Exposure to inorganic mercuric chloride caused a swelling of the outer lip of the micropyle, reducing the diameter of the sperm entryway (Khan & Weis 1993), which too could have negative consequences on the success of a sperm fertilizing an egg. Alternatively, a higher proportion of dead eggs in contaminated nests could be the result of reduced oxygen due to higher egg densities in these same nests. Indeed, higher densities of egg reduce available oxygen (Moran & Woods 2007) and exposure of

embryos to reduced oxygen increased mortality (Keckeis et al. 1996). Examination of the structure of contaminated eggs versus uncontaminated eggs and *in vitro* fertilizations, controlling for egg density, can reveal how impairment of sperm and/or the egg micropyle influences embryo development.

2.5 ACKNOWLEDGMENTS

All research was conducted within the protocols approved by the Animal Research Ethics Board of McMaster University (AUP #06-10-61) and the Canadian Council for Animal Care guidelines. This research was conducted with the permission of the Department of Fisheries and Oceans Canada and the Department of Natural Resources Chemainus First Nation. We thank W. and R. Cogswell and D. and S. Weatherell for providing access to field sites. This work was funded by the Canada Foundation for Innovation (CFI), Ontario Innovation Trust (OIT), National Science and Engineering Council of Canada (NSERC) in the form of a New Investigator award and a Discovery grant to SB, and an NSERC CGS scholarship to NMS.

2.6 REFERENCES

Abascal FJ, Cosson J, Fauvel C. Characterization of sperm motility in sea bass: the effect of heavy metals and physicochemical variables on sperm motility. J Fish Biol 2007; 70: 509-522.

Alava JJ, Lambourn D, Olesiuk P, Lance M, Jeffries S, Gobas F, Ross PS. 2009. Polychlorinated biphenyls (PCBs) and Polybrominated Diphenyl Ethers in Steller sea lions (*Eumatopias jubatus*) wintering off Vanocuver Islands, BC, Canada. In Abstracts, 18th Annual Meeting Pacific Northwest Chapter (PNW)-Society of Environmental Toxicology and Chemistry (SETAC), Port Townsend, WA, April16-18, 2009. p. 23.

Alava JJ, Lachmuth C, Ross PS, Gobas F. 2010. Modeling PCB bioaccumulation in Chinook salmon (*Oncorhynchus tshawytscha*) from British Columbia, Canada: Health risk implications for top predators. In Abstracts 2010 Ecological and Evolutionary Ethology of Fishes Biennial Conference, May 16 - 20, 2010. Simon Fraser University, Burnaby-Vancouver, BC Canada. p. 2.

Arora HL. Observations on the habits and early life history of the Batrachoid fish, *Porichthys notatus* Girard. Copeia 1948; 1948: 89-93.

Au DWT, Chiang MWL, Wu RSS. Effects of cadmium and phenol on motility and ultrastructure of sea urchin and mussel spermatozoa. Arch Environ Contam Toxicol 2000; 38: 455-463.

Au DWT, Lee CY, Chan KL, Wu RSS Reproductive impairment of sea urchins upon chronic exposure to cadmium. Part I: Effects on gamete quality. Environ Pollut 2001a; 111: 1-9.

Au DWT, Reunov AA, Wu RSS. Reproductive impairment of sea urchin upon chronic exposure to cadmium. Part II: Effects on sperm development. Environ Pollut 2001b; 111: 11-20.

Ball MA, Parker GA. Sperm Competition Games: External Fertilization and "Adapative" Infertility. J Theor Biol 1996; 180: 141-150.

Bard SM. A Biological index to predict pulp mill pollution levels. Water Environ Res 1998; 70: 108-122.

Barni A, Mazzoldi C, Rasotto MB. Reproductive apparatus and male accessory structures in two batrachoid species (Teleostei, Batrachoididae). J Fish Biol 2001; 58: 1557-1569.

Birkhead TR, Møller AP. 1998. Sperm Competition and Sexual Selection. (eds. Birkhead TR, Møller AP) Academic Press, London.

Brantley RK, Bass AH. Alternative male spawning tactics and acoustic signals in the plainfin midshipman fish *Porichthys notatus* Girard (Teleostei, Batrachoididae). Ethology 1994; 96: 213-232.

British Columbia Ministry of Agriculture and Lands (Crown of Contaminated Sites Branch). 2005. Supplemental Stage 1 Preliminary Site Investigation and Detailed Site Investigation Lot 16G Ladysmith Harbour Ladysmith, BC. Report Number: 05-1412-033. Burnaby, British Columbia, Canada.

Burness G, Casselman SJ, Schulte-Hostedde AI, Moyes CD, Montgomerie R. Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). Behav Ecol Sociobiol 2004; 56: 65-70.

Cardullo RA, Baltz JM. Metabolic regulation in mammalian sperm: Mitochondrial volume determines sperm length and flagellar beat frequency. Cell Motil Cytoskel 1991; 19: 180-188.

Casselman SJ, Schulte-Hostedde AI, Montgomerie R. Sperm quality influences male fertilization success in walleye (*Sander vitreus*). Can J Fish Aquat Sci 2006; 63: 2119-2125.

Crane JM. Bioluminscent courtship display in the teleost *Porichthys notatus*. Copeia 1965; 1965: 239-241.

Crane JM. Feeding and growth by the sessile larvae of the teleost *Porichthys notatus*. Copeia 1981; 1981: 895-897.

Cummins J. 2009. Sperm motility and energetics In: Sperm Biology: An Evolutionary Perspective (eds. Birkhead TR, Hosken DJ, Pitnick S) Academic Press, London, pp 185-206.

Firman RC, Simmons LW. Sperm midpiece length predicts sperm swimming velocity in house mice. Biol Let 2010; 6: 513-516.

Fitzpatrick JL, Desjardins JK, Milligan N, Montgomerie R, Balshine S. Reproductive-tactic-specific variation in sperm swimming speeds in a shell-brooding cichlid. Biol Reprod 2007; 77: 280-284.

Fitzpatrick JL. 2008. Sperm Competition in Fish. Ph.D. Thesis, McMaster University, Ontario, Canada.

Fitzpatrick JL, Nadella S, Bucking C, Balshine S, Wood CM. The relative sensitivity of sperm, eggs and embryos to copper in the blue mussel (*Mytilus trossulus*). Comp Biochem Phys C 2008; 147: 441-449.

Fitzpatrick JL, Montgomerie R, Desjardins JK, Stiver KA, Kolm N, Balshine, S. Female promiscuity promotes the evolution of faster sperm in cichlid fishes. P Natl Acad Sci USA 2009; 106: 1128-1132.

Fitzpatrick JL, Garcia-Gonzalez F, Evans JP. Linking sperm length and velocity: the importance of intramale variation. Biol Let 2010; DOI:10.1098/rsbl.2010.0231.

Gage MJG, Morrow EH. Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. Curr Biol 2003; 13: 754-757.

Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB, Parker GA. Spermatozoal traits and sperm competition in atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. Curr Biol 2004; 14: 44-47.

Gill ME, Spiropoulos J, Moss C. Testicular structure and sperm production in flounders from a polluted estuary: a preliminary study. J Exp Mar Biol Ecol 2002; 281: 41-51.

Gomendio M, Roldan ERS. Sperm competition influences sperm size in mammals. Proc R Soc Lond 1991; 243: 181-185.

Gomendio M, Roldan ERS. Implications of diversity in sperm size and function for sperm competition and fertility. Int J Dev Biol 2008; 52: 439-447

Harper RD, Case JF. Disruptive counterillumination and its anti-predatory value in the plainfin midshipman *Porichthys notatus*. Mar Biol 1999; 134: 529-540.

Haubruge E, Petit F, Gage MJ. Reduced sperm counts in guppies (*Poecilia reticulata*) following exposure to low levels of tributyltin and bisphenol A. Proc Biol Sci 2000; 267: 2333-2337.

Hewitt LM, Kovacs TG, Dubé MG, MacLatchy DL, Martel PH, McMaster ME, Paice MG, Parrott JL, van den Heuvel, MR, van der Kraak GJ. Altered reproduction in fish exposed to pulp and paer mill effluents: roles of the individual compounds and mill operating conditions. Enviorn Toxicol Chem 27; 2008: 682-697.

Humphries S, Evans JP, Simons LW. Sperm competition: linking form to function. BMC Evol Biol 2008; 8: 319-329.

Ibara RM, Penny LT, Ebeling AW, Van Dykhuizen G, Cailliet. 1983. The mating call of the plainfin midshipman fish, *Porichthys notatus* In: Predators and Prey in Fishes (eds. Noakes DLG, Lindquist DG, Helfman GS, Ward JA) Dr. W. Junk Publishers: The Hauge, pp 205-212.

Johnston TA, Miller LM, Whittle DM, Brown SB, Wiegand MD, Kapuscinski AR, Leggett WC. Effects of maternally transferred organochlorine contaminants on early life survival ina freshwater fish. Environ Toxicol Chem 2005; 24: 2594-2602.

Jørgensen EH, Bye BE, Jobling M. Influence of nutritional status on biomarker responses to PCB in the Arctic charr (*Salvelinus alpinus*). Aquat Toxicol 1999; 44: 233-244.

Keckeis H, Bauer-Nemeschkal B, Kamler E. Effects of reduced oxygen level on the mortality and hatching rate of *Chondrostoma nasus* embryos. J Fish Biol 1996; 49: 430-440.

Khan AT, Weis JS. Effects of methylmercury on sperm and egg viability of two populations of killifish *(Fundulus heteroclitus*). Arch Environ Contam Toxicol 1987; 16: 499-505.

Khan AT, Weis JS. Differential effects of organic and inorganic merury on the micropyle of eggs of *Fundulus heteroclitus*. Environ Biol Fishes 1993; 37: 323-327.

Kime DE. The effects of pollution on reproduction in fish. Rev Fish Biol Fisher 1995; 5: 52-96.

Kime DE, Ebrahimi M, Nysten K, Roelants I, Rurangwa E, Moore HDM, Ollevier F. Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to the effects of heavy metals Aquat Toxicol 1996; 36: 223-237.

Klumpp DW, Humphrey C, Huasheng H, Tao F. Toxic contaminants and their biological effects in coastal waters of Xiamen, China. II. Biomarkers and embryo malformation rates as indicators of pollution stress in fish. Mar Pollut Bull 2002; 44: 761-769.

Kunz YW. 2004. Development Biology of Teleost Fishes. (ed. Kunz YW) Springer: Dordrecht.

Labbe C, Martoriati A, Devaux A, Maisse G. Effect of sperm cryopreservation on sperm DNA stabliyt and progeny development in rainbow trout. Mol Reprod Dev 2001; 60: 397-404.

Lahnsteiner F, Berger B, Weismann T, Patzner RA. Determination of semen quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters, and spermatozoal metabolism. Aquaculture 1998; 163:.163-181.

Lahnsteiner F, Mansour N, Berger B. The effect of inorganic and organic pollutants on sperm motility of some freshwater teleosts. J Fish Biol 2004; 65: 1283-1297

Levitan DR. Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegates*. Proc R Soc Lond B 2000; 267: 531-534.

Lewis SEM, Aitken RJ. DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell Tissue Res 2005; 322: 33-41.

Lüpold S, Calhim S, Immler S, Birkhead TR. Sperm morphology and sperm velocity in passerine birds. Proc Biol Sci 2009; 276: 1175–1181.

Marentette JR, Fitzpatrick JL, Berger RG, Balshine S. Multiple male reproductive morphs in the invasive round goby (*Apollonia melanostoma*). J Great Lakes Res 2009; 35: 302-308.

Marconato A, Bisazza A, Fabris M. The cost of parental care and egg cannibalism in the river bullhead, *Cottus gobio* L. (Pisces, Cottidae). Behav Ecol Sociobiol 1993; 32: 229-237.

Milani D, Grapentine LC. 2006. Application of BEAST sediment quality guidelines to Hamilton Harbour, an Area of Concern. Environment Canada. NWRI Contribution No. 06-407.

McKibben JR, Bass AH. Behavioral assessment of acoustic parameters relevant to signal recognition and preference in a vocal fish. Journal of the Acoustical Society of America 1998; 104: 520-3533.

Moran AL, Woods HA. Oxygen in egg masses: interactive effects of temperature, age, and egg-mass morphology on oxygen supply to embryos. J Exp Biol 2007; 210: 722-731.

Mossman J, Slate J, Humphries S, Birkhead T. Sperm morphology and velocity are genetically codetermined in the zebra finch. Evolution 2009; 63: 2730-2737.

Neff BD, Fu P, Gross MR. Sperm investment and alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*). Behav Ecol 2003; 14: 634-641.

Oliveira RF, Taborsky M, Brockmann HJ. 2008. Alternative Reproductive Tactics: An Integrative Approach (eds. Oliveira RF, Taborsky M, Brockmann HJ) Cambridge University Press: New York.

Parker GA. Sperm competition and its evolutionary consequences in the insects. Biol Rev

Camb Philos Soc 1970; 45:525–567.

Parker GA, Ball MA. Sperm competition, mating rate and the evolution of testis and ejaculate sizes: a population model. Biol Lett 2005; 1: 235-238.

Pitcher TE, Beausoleil JJ, Abbott JA, Vandereerden JL. Sperm design and function in the redside dace *Clinostomus elongates*. Fish Biol 2009; 75: 924-931.

Rohwer S. Parent cannibalism of offspring and egg raiding as a courtship strategy. Am Nat 1978; 112: 429-440.

Sato T, Hirose M, Taborsky M, Kimura S. Size-dependent male alternative reproductive tactics in the shell-brooding cichlid fish *Lamprologus callipterus* in Lake Tanganyika. Ethology 2004; 110: 49-62.

Schoenfuss HL, Levitt JT, Van Der Kraak G, Sorensen PW. Ten-week exposure to treated sewage discharge has relatively minor, variable effects on reproductive behavior and sperm production in goldfish. Environ Toxicol Chem 2002; 21: 2185-2190.

Selevan SG, Borkovec L, Slott VL, Zudová Z, Rubes J, Evenson DP, Perreault SD. Semen quality and reproductive health of young Czech men exposed to seasonal air pollution. Environ Health Persp 2000; 108: 887-894.

Snook RR. Sperm in competition: not playing by the numbers. Trends Ecol Evol 2005; 20: 46-53.

Spies R. Benthic-pelagic coupling in sewage-affect marine ecosystems. Mar Environ Res 1984; 13: 195-230.

Stanley H.P. Electron microscopic observations on the biflagellate spermatids of the teleost fish *Porichthys notatus*. Anat Rec 1965; 151: 477.

Stockley P, Gage MJG, Parker GA, Møller AP. The evolution of testis size and ejaculate characteristics. Am Nat 1997; 149: 933-954.

Suk H, Neff BD, Fitzpatrick JL, Balshine S. Isolation and characterization of polymorphic microsatellite loci in plainfin midshipman fish. Hereditas 2009; 146: 204-207.

Taborsky M. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. Adv Study Behav 1994; 23: 1-100.

Taborsky M. Sperm competition in fish: 'bourgeois' males and parasitic spawning. Trends Ecol Evol 1998; 13: 222-227.

Tuset VM, Dietrich GJ, Wojtczak M, Slowinska M, de Monserrat J, Ciereszko A. Relationships between morphology, motility and fertilization capacity in rainbow trout (*Oncorhynchus mykiss*) spermatozoa. J Appl Ichthyol 2008; 24: 393–397.

Urbach D, Bittner D, Lenz TL, Bernet D, Wahli T, Wedekind C. Sperm velocity in an Alpine whitefish: effects of age, size, condition, fluctuating asymmetry and gonad abnormalities. J Fish Biol 2007; 71: 672-683.

VanLook KJW, Kime DE. Automated sperm morphology analysis in fishes: the effect of mercury on goldfish sperm. J Fish Biol 2003; 63: 1020-1033.

Vladić TV, Järvi T. Sperm quality in the alternative reproductive tactics of Atlantic salmon: the importance of the loaded raffle mechanism Proc R Soc Lond B 2001; 268: 2375-2381.

Westernhagen H von. 1988. Sublethal effects of pollutatnts on fish eggs and larvae. In: Fish Physiology (eds. Hoar WS, Randall DJ). Academic Press, London, pp 253-346.

Westernhagen H von, Dethlefsen V. The use of malformations in pelagic fish embryos for pollution assessment. Hydrobiologia 1997; 352: 241-250.

Westernhagen H von, Dethlefsen V, Haarich M. Can a pollution event be detected using a single biological effects monitoring method? Mar Pollut Bull 2001; 42: 294-297.

2.8 CONCLUDING REMARKS

2.8.1 LIMITED SUPPORT FOR CONTAMINANT IMPACTS ON SPERM

The results of my thesis provide little evidence for differences in sperm investment patterns between fish from clean versus contaminated areas. Sperm velocity, a primary determinant of fertilization success (Gage et al. 2004; Casselman et al. 2006), did not vary between round gobies collected from clean and contaminated areas and I must still determine whether observed differences in sperm morphology between clean and contaminated midshipman will be reflected in sperm velocity. Proportionately more dead eggs were observed in nests of midshipman nests from contaminated areas suggesting that fertilization may be impaired and this may be related to contaminant burdens. A recently completed standardized population census of midshipman nests (Cogliati et al. unpublished data) will provide further assurances that the high proportions of dead eggs in contaminated nests were not artefacts of sampling bias. Given my results and that study populations of round gobies and plainfin midshipman are abundant, with round goby catch efforts equal between clean and contaminated sites, both species appear resilient to harsh environmental conditions.

Consequently, thriving populations of contaminated fish will impact the ecosystems they inhabit. For round gobies, resiliency to polluted habitats has likely aided in the successful establishment of this invasive species in other contaminated areas of the Laurentian Great Lakes. Also, round gobies and plainfin midshipman are both important food sources for avian predators (double-crested cormorants, *Phalacrocorax auritus,* Somers et al. 2003; bald eagles, *Haliaeetus leucocephalus*, Gill & Elliot 2003,

respectively). Reproducing populations of contaminated fish will indeed provide plentiful prey for predators, potentially accelerating the bioaccumulation and movement of contaminants up trophic levels. Tolerance to aquatic contaminants including PAHs and PCBs, has also been documented in the mummichog (*Fundulus heteroclitus*, Weis 2002). Determining how contaminated populations of round goby and midshipman maintain themselves (e.g. increased reproductive output, overwinter survival, body condition) will be important in assessing the ecological impact of these species.

2.8.2 LIMITATIONS OF SOME METHODS AND EXPERIMENTAL DESIGN

Although striking contaminant related-differences in sperm velocity were not detected, more subtle alterations may indeed exist but simply masked or were not detectable based on the methods used in this study. First, parameters such as ejaculate volume, sperm number and sperm ATP content were not assessed but could obviously be valuable biomarkers for aquatic pollution. Second, I obtained round goby and plainfin midshipman sperm from dissected testes rather than stripped milt (sperm + accessory gland fluids). One of the many proposed roles of accessory gland fluid is to improve the viability of sperm (Eggert 1931). The motility of sperm may be altered by the presence or absence of accessory gland fluids (but see Hulak et al. 2008) and the behaviour of sperm in a natural spawning environment may vary from that which was observed under a microscope from testes stripped sperm. This may be especially true for round goby sperm that are released in sperm trails (see Discussion, Chapter 1 of this thesis). Third, the spawning environment (the water) in which sperm are activated was filtered and not always from the same environment as where the fish came from. In particular, round goby sperm (from fish collected from both clean and contaminated collection sites) was

activated using filtered water from the clean collection site in Hamilton Harbour. Thus, contaminated sperm, and to a lesser degree clean sperm, were swimming in a novel aquatic environment that could have differed in pH, ionic concentration, temperature, oxygen and toxicant concentration, all factors that alter sperm motility.

Finally, the social environment fish experienced after capture and prior to dissection could have affected sperm characteristics. Though the number of days in the lab did not influence round goby sperm velocity (Repeated Measures ANOVA: $F_{1,517}$=0.001, P=0.97), males were housed in aquaria that contained up to 5 other male fish. The presence of male competitors (Pilastro et al. 2002) and/or formation of dominance hierarchies (Rudolfsen et al. 2006) could also influence sperm investment patterns. In contrast, plainfin midshipman were always processed the day of collection and were housed in isolation. Accounting for these differences in housing social environment will be imperative when comparing round goby and plainfin midshipman sperm velocity.

2.8.3 SPECIES-SPECIFIC DIFFERENCES

The results perhaps hint that some environmental factors could be modifying sperm investment and that these factors appear to impact each species differently (Table 2). The area or part of the sperm that was influenced by contaminants varied between species. In male round gobies from contaminated areas, shorter midpieces were observed whereas plainfin midshipman males from contaminated areas had shorter sperm heads. A short midpiece can indicate reduced mitochondrial volume and reduced energy production to propel the sperm cell toward an egg (Cardullo & Baltz 1991). A short

TABLE 2

Morphological and ecological differences between round goby and plainfin midshipman.

	Round Goby (*Neogobious melanostomus*)	**Plainfin Midshipman** (*Porichthys notatus*)
TRAIT		
Relative testes size	Clean<Contaminated	Clean= Contaminated
Sperm head length	Clean= Contaminated	Clean> Contaminated
Sperm midpiece length	Clean> Contaminated	Clean= Contaminated
Sperm tail length: head length	Clean= Contaminated	Clean= Contaminated
Sperm velocity	Clean= Contaminated	not yet analysed
Ratio of SM:GM*	Clean> Contaminated	not recorded in this study
Parental care duration per clutch of eggs	14-20 days[a]	37 to 55 days[b]
Ratio of egg size: sperm size	131x	105x

*SM = sneaker male, GM = guarding male
a = Charlebois et al. 1997
b = Arora 1948

sperm head could indicate damaged DNA rendering sperm unviable or offspring of poorer quality. These impairments have the potential to influence sperm velocity and male reproductive success. A number of factors could drive these observed morphological differences.

First, plainfin midshipman sperm were activated in unfiltered water that was obtained from the collection site while in round gobies only clean filtered water was used to activate sperm from fish collected in both clean and contaminated areas. A balanced experimental design activating sperm from clean and contaminated male in water collected from clean and contaminated areas could reveal whether activation fluid alters sperm swimming speed. Also, compared to Slag Point, sediment from Hamilton Harbour has considerably higher levels of PAHs, copper, zinc, nickel, lead, cadmium, mercury and arsenic (Table 3). In addition to a more severe contaminant profile at Hamilton Harbour, exposure duration varies between species. Round gobies occupy a lake and are exposed to water and sediment constantly but are territorial for only short periods of time (2-3 weeks) compared to Type I midshipman that during the breeding season live under rocks in the tidal zone which are subjected to long periods of desiccation for up to 5 months. The ebb and flow of the tide may concentrate or dilute the strength of water and sediment contamination in the nearshore of Ladysmith Inlet (Figure 7). Both fish species migrate to deeper waters after the breeding season during the winter, and so both species may escape exposure to heavily contaminated sediments for part of the year. However, round gobies are thought to overwinter in deeper parts of Hamilton Harbour, but the Harbour as a whole may be still considered fairly contaminated. Midshipman move to deeper parts of the Pacific Ocean that are likely not

TABLE 3

Concentrations of PAHs, copper, zinc, nickel, lead, mercury, arsenic and cadmium in sediment collected from Hamilton Harbour, Lake Ontario (Milani & Grapentine 2006) and Slag Point, Ladysmith Inlet (B.C. Ministry of Agriculture and Lands 2005). All concentrations are presented in μg/g.

Toxicant	Hamilton Harbour	Slag Point
PAHs	157-499	<10
copper	>197	<116
zinc	>820	<156
nickel	>75	<63
lead	>250	<50
mercury	>2	<1
arsenic	>33	<17
cadmium	>3.5	<0.5

as polluted and where sperm production is not affected by contaminants. Thus, one may predict that compared to round gobies, midshipman sperm production is overall less impacted by the presence of contaminants in the waters they inhabit. Perhaps shortened sperm heads in midshipman is a consequence of a relatively shorter contaminant exposure (e.g. only during the breeding season) and reductions in round goby midpiece structure are a result of longer, more constant contaminant exposure (e.g. year round exposure). This hypothesis suggests that contaminants first influence DNA located in the sperm head and this can have major implications for the next generation. Then, only after prolonged exposure are problems within the mitochondria observed, the site of ATP production and many intricately synchronized physiological mechanisms. Whether site differences in midshipman sperm velocity will be similar to round gobies has yet to be determined as data analyses are in progress.

Verification of this hypothesis will be aided through 1) investigating whether site differences in midshipman sperm velocity will be similar to velocity differences found in round gobies, 2) comparisons of sperm head morphology between sperm of fish reared in clean versus contaminated water for shorter and relatively longer periods and 3) fertilizing and rearing eggs from these treatments to determine how reduced sperm head and midpiece size respectively influence offspring survival.

2.8.3 INTERACTIVE EFFECTS ON SPERM IN SPECIES WITH MARTs

In the studies in this thesis we did not detect a difference in sperm swimming speed in relation to tactics as has been reported in other studies (Burness et al. 2004; Fitzpatrick et al. 2009). Marentette et al. (2009) also did not find a tactic related sperm swimming speed difference in round gobies. Interactions between social environment

and sperm competition could potentially mask or counteract subtle site/contaminant related differences. Species demonstrating MARTs are commonly used model systems to study the theory of sperm competition, but such species with complex mating behaviour have not been previously studied in relation to contaminated-mediated effects on sperm. Studies on organisms with simpler mating systems based on laboratory exposures to a wide variety of contaminants have largely shown that contaminants reduce sperm swimming speed (Table 1). Males used in these studies are likely all exposed to similar levels of contaminants. In contrast, in our study the males adopting the different tactics were likely to be exposed to differing levels of contaminants based on differences in the mating behaviours adopted by different mating tactics. That no differences in sperm swimming speed were observed in naturally exposed guarding and sneaker round gobies implies that sperm from these males are modified by a complex interaction between the degree of contaminant exposure, the degree of sperm competition and possibly other factors (e.g. female choice). Reductions in sperm velocity due to contaminant exposure may be counteracted by increases in sperm velocity due to high sperm competition. Future research should be aimed at quantifying velocity of sperm from clean and contaminated sneaker male midshipman to further demonstrate whether mating tactics respond to contaminant exposure in similar or dissimilar ways. Given that this is the first study to explore the interactive effects of aquatic pollution and alternative mating strategies on sperm competitive ability, I strongly recommend and encourage further investigation of other species with MARTs that are directly (aquatic contamination) and indirectly (global warming) impacted by anthropogenic change.

2.8.5 CONCLUSION

Laboratory controlled studies investigating how exposures to aquatic toxicants impact physiology continue to be the prominent methodology in ecotoxicological research. However, the sperm velocity result of my thesis contradict results found in laboratory studies and brings to light the importance of examining how wild populations of animals are influenced by combinations of pollutants. By using animals collected from their contaminated habitats, I found that in contrast to predictions based on previous empirical research conducted using naïve animals exposed to single toxicants, sperm from these chronically exposed fish did not swim slower than unexposed fish, at least not in clean, filtered water. Two other studies also found that fish collected from contaminated areas did not have slower swimming sperm relative to fish collected from cleaner areas, rather sperm swam faster in exposed fish (Khan & Weis 1987; Kleinkauf et al. 2004). There is also laboratory evidence for toxicity of a single contaminant differing when it is in combination with another contaminant. For example, Kime et al. (1996) found that the toxicity of cadmium on sperm velocity was reduced when in the presence of zinc, compared to when sperm was exposed to cadmium alone. My research contributes to the growing gap between results from controlled laboratory single exposures and field studies that look at wild animals and real world cocktail exposures on sperm. From my review of the literature, studies (including my thesis) that investigate the competitive ability of sperm in naturally exposed fish are extremely limited (Table 4). There is a pressing need to expand our knowledge of the swimming dynamics of sperm from contaminated wild fish, especially while swimming in an environmental mileu that most closely resembles the conditions experienced in the wild.

TABLE 4

Summary of laboratory exposures (N=number of exposures; some studies had more than one exposure) and field studies (N=number of studies) that investigated how contaminant exposure alters sperm velocity in aquatic species (e.g. fish, sea urchins, oysters, mussels).

	Laboratory Exposures N=19	**Field Studies N=5**
Sperm Velocity		
Increased	**3**	**2**
No differences	**3**	**1**
Decreased	**13**	**2**

2.8.6 REFERENCES

Arora HL. Observations on the habits and early life history of the Batrachoid fish, *Porichthys notatus* Girard. Copeia 1948; 1948: 89-93.

Burness G, Casselman SJ, Schulte-Hostedde AI, Moyes CD, Montgomerie R. Sperm swimming speed and energetics vary with sperm competition risk in bluegill (*Lepomis macrochirus*). Behav Ecol Sociobiol 2004; 56: 65-70.

Cardullo RA, Baltz JM. Metabolic regulation in mammalian sperm: Mitochondrial volume determines sperm length and flagellar beat frequency. Cell Motil Cytoskel 1991; 19: 180-188.

Casselman SJ, Schulte-Hostedde AI, Montgomerie R. Sperm quality influences male fertilization success in walleye (*Sander vitreus*). Can J Fish Aquat Sci 2006; 63: 2119-2125.

Charlebois PM, Marsden JE, Goettel RG, Wolfe RK, Jude DJ. Rudnicka S. 1997. The round goby, *Neogobius melanostomus* (Pallas), a review of European and North American literature. Illinois-Indiana Sea Grant Program and Illinois Natural History Survey, Champaign. INHS Special Publication Number 20. 76 pages.

Fitzpatrick JL, Desjardins JK, Milligan N, Montgomerie R, Balshine S. Reproductive-tactic-specific variation in sperm swimming speeds in a shell-brooding cichlid. Biol Reprod 2007; 77: 280-284.

Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB, Parker GA. Spermatozoal traits and sperm competition in atlantic salmon: relative sperm velocity is the primary determinant of fertilization success. Curr Biol 2004; 14: 44-47.

Gill CE, Elliot JE. Influence of food supply and chlorinated hydrocarbon contaminants on breeding success of bald eagles. Ecotoxicology 2003; 12: 95-111.

Hulak M, Rodina M, Linhart O. Characteristics of stripped and testicular Northern pike (*Esox lucius*) sperm: spermatozoa motility and velocity. Aquat Living Resour 2008; 21: 207-212.

Khan AT, Weis JS. Effects of methylmercury on sperm and egg viability of two populations of killifish *(Fundulus heteroclitus*). Arch Environ Contam Toxicol 1987; 16: 499-505.

Kime DE, Ebrahimi M, Nysten K, Roelants I, Rurangwa E, Moore HDM, Ollevier F. Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish; application to the effects of heavy metals Aquat Toxicol 1996; 36: 223-237.

Kleinkauf A, Macfarlane C, Yeates S, Simpson MG, Leah RT. A biomarker approach to endocrine disruption in founder – estrogen receptors, hepatocyte proliferation, and

sperm motility. Ecotox Environ Saf 2004; 58: 324-334.

Marentette JR, Fitzpatrick JL, Berger RG, Balshine S. Multiple male reproductive morphs in the invasive round goby (*Apollonia melanostoma*). J Great Lakes Res 2009; 35: 302-308.

Pilastro A, Scaggiante M, Rasotto MB. Individual adjustment of sperm expenditure accords with sperm competition theory. P Natl Acad Sci USA 2002; 99: 9913-9915.

Rudolfsen G, Figenschou L, Folstad I, Tveiten H, Figenschou M. Rapid adjustments of sperm characteristics in relation to social status. Proc R Soc B 2006; 273: 325-332.

Somers CM, Lozer MN, Kjoss VA, Quinn JS. The invasive round goby (*Neogobius melanostomus*) in the diet of nestling double-crested cormorants (*Phalacrocorax auritus*) in Hamilton Harbour, Lake Ontario. J Great Lakes Res 2003; 29: 392-399.

Weis JS. Tolerance to Environmental Contaminants in the Mummichog, *Fundulus heteroclitus*. Hum Ecol Risk Assess 2002; 8: 933-953.